

RESEARCH ARTICLE



Novel approaches for the discovery of pharmacogenetic biomarkers of chemotoxicity in patients with colorectal cancer

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[Correction added on 11 July 2025, after first online publication: The affiliations of Pau Riera and David Páez have been updated in this version.]

Background and Purpose: Chemotherapeutic treatment for colorectal cancer (colorectal cancer) allows for increased patient overall survival. However, current therapeutic regimens are often associated with the development of adverse drug reactions, which represent a morbidity, mortality and economic issue. We propose to identify novel germline markers that allow us to predict adverse drug reactions development after colorectal cancer chemotherapy.

Experimental Approach: For that purpose, we selected 163 colorectal cancer patients with severe adverse drug reactions (CTCAE grades 3–4) and 52 controls and applied whole-exome sequencing (WES) to discover novel germline toxicity variants.

Key Results: We found 13 cases carrying actionable dihydropyrimidine dehydrogenase gene (*DPYD*) variants and a novel, potentially pathogenic *DPYD* variant – c.2071G > T, p.(V691L), rs202212118. Moreover, we found 30 novel rare, high-impact variants in 14 reported genes. We also identified seven patients carrying more than one variant in the same gene or pathway, with one patient hinting at a potential digenic inheritance. Using an exome-wide approach, we discovered and independently validated three novel candidate toxicity genes (*ALDH9A1*, *FAM83A* and *EPX*). Gene-based analyses also provided 14 genes significantly associated with neuropathy, skin toxicity and cardiotoxicity.

Conclusions and Implications: Overall, the present work has utilised state-of-the-art approaches to uncover several novel candidate toxicity variants/genes.

Abbreviations: ADR, adverse drug reaction; EGA, European Genome-phenome Archive; FOLFIRI, 5-FU plus LV with irinotecan; FOLFOX, 5-FU plus LV with oxaliplatin; gnomAD, Genome Aggregation Database; MAF, Minor Allele Frequency; NFE, non-Finnish Europeans; SKAT-O, SNP-set kernel association test (optimized); SNP, single nucleotide polymorphism; XELOX, capecitabine with oxaliplatin.

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KEYWORDS

adverse drug reactions, colorectal cancer, personalised medicine, pharmacogenomics

1 | INTRODUCTION

Colorectal cancer (CRC) constitutes a major health problem, ranking third in terms of incidence and second in terms of mortality by cancer. Its burden is expected to increase by more than 60% by 2040 (Morgan et al., 2023). The preferred treatment for colorectal cancer is surgery, but chemotherapy is also commonly used as is radiotherapy for rectal cases.

The chemotherapy for colorectal cancer is based on fluoropyrimidines (such as 5-fluorouracil or 5-fluorouracil plus leucovorin and capecitabine) that can be administered alone or in combination with other cytotoxic drugs, such as oxaliplatin or irinotecan (Argilés et al., 2020; Cervantes et al., 2022; Glynne-Jones et al., 2017). As both tumour and normal cells can metabolise chemotherapeutic drugs, these are often associated with adverse drug reactions (ADRs) that impact the quality of life of patients. Each patient presents a different toxicity profile, but the more common adverse drug reactions are digestive toxicities, haematological toxicities and non-specific ones, like asthenia (Food and Drug Administration, 2002a, 2002b, 2009, 2014, 2015, 2017).

Pharmacogenetics and pharmacogenomics study the genetic inter-variability in response and toxicity. They are essential to guide dosage and treatment options for each patient, allowing for a more personalised medicine. Toxicity is a result of the unwanted effects of therapy on the normal cells and would therefore depend on inherited genetic variation. Although the contribution of genetics to the development of adverse drug reactions is irrefutable, the toxicity variants reported so far have only been able to explain a reduced number of the cases and much of the heritability remains yet unexplained.

Therefore, the aim of this study was to identify and validate new toxicity biomarkers in colorectal cancer patients after treatment with chemotherapy, taking advantage of novel technological approaches. For that, we used whole-exome sequencing data from a cohort of extreme toxicity phenotypes and we applied several strategies of genomic analysis for the identification of both high- and low-penetrance toxicity variants, including a comprehensive assessment of variants in described pharmacogenes, as well as the discovery of novel rare toxicity variants/genes.

2 | METHODS

2.1 | Design and analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2025).

What is already known?

- Next-generation sequencing technologies have allowed the possibility to inspect the missing heritability in toxicity pharmacogenomics.

What does this study add?

- We comprehensively analysed next-generation sequencing-based pharmacogenomic data, successfully identifying several candidate toxicity variants and genes.

What is the clinical significance?

- Validation of these genes could offer pre-emptive treatment adjustments to avoid chemotoxicity.

2.2 | Patients and samples

We recruited stage II-IV colorectal cancer patients that had undergone a first line of fluoropyrimidine-based chemotherapy - **5-fluorouracil (5-FU)** plus **leucovorin (folinic acid; LV)**, **capecitabine**, 5-FU plus LV with **oxaliplatin (FOLFOX)**, 5-FU plus LV with **irinotecan (FOLFIRI)**, or capecitabine with oxaliplatin (XELOX). Patients were enrolled retrospectively (after first line treatment completion) from 10 recruitment centres in Spain and internationally (Mexico, Complejo Hospitalario Universitario de Ourense, Hospital Clínico Universitario de Santiago, Hospital Universitario Gregorio Marañón, Institute of Experimental Medicine, Czech Republic, Hospital Universitario Infanta Sofia, Biobank of Santiago de Compostela, Institut Català d'Oncology, Hospital de la Santa Creu i Sant Pau and EPICOLON II (Fernandez-Rozadilla et al., 2013) (Table S1) and an informed consent was obtained from all patients, according to the tenets of the Declaration of Helsinki (World Medical Association, 2013). We classified the patients from the discovery cohort into case and control groups, according to the following criteria: - Cases - patients with any extreme (grade 3/4) adverse drug reaction on the first three cycles of chemotherapy; Controls - patients who received ≥ 8 cycles and had mild or no adverse drug reactions (grade ≤ 1). We considered different toxicity groups, including single toxicities ($n > 5$) and systemic groups, including digestive, haematological and global toxicity. Altogether, we recruited 163 cases and 52 controls for the discovery phase (Table 1) and we compared the control group with 13 distinct case groups, each corresponding to a different toxicity group.

TABLE 1 Sample counts per phenotype for the discovery phase.

Phenotype	TOTAL
Diarrhoea	47
Neutropenia	42
Asthenia	31
Nausea	26
Mucositis	16
HFS	11
Leucopenia	8
Neuropathy	7
Skin toxicity ^a	6
Cardiotoxicity ^a	6
Anaemia	4
Allergy	3
Hepatotoxicity	3
Thrombopenia	2
Lymphopenia	1
Conjunctivitis	1
Enteritis	1
Digestive ^b	1
Haematological ^b	1
Control	52

^aAny Common Terminology Criteria for Adverse Events (CTCAE) adverse drug reaction (ADR) in this category.

^bUnspecified (ADR) subtype; HFS: hand-foot syndrome.

The patient clinical data were collected homogeneously using a Case Report Form, which included relevant clinical data: type of cancer, stage, chemotherapeutic regimen, number of cycles received, adverse drug reactions for each cycle and toxicity grade. The adverse drug reactions were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v3.

The list of high-impact variants of interest detected in the discovery cohort was validated in 144 additional samples from Hospital Clínico Universitario de Santiago, Hospital Universitario Gregorio Marañón, Hospital Universitario Infanta Sofia, Hospital de la Santa Creu i Sant Pau and EPICOLON II. These included patients with grade ≥ 2 toxicities happening up to chemotherapy cycle four. The control group was kept as in the discovery phase (Table 2).

We also ran a meta-analysis with the whole-exome sequencing data and the EGAS00001002763 cohort (European Genome-phenome Archive/EGA) (Traylor et al., 2018) (Table 3).

2.3 | DNA extraction

From each patient, a sample of peripheral blood was collected in an ethylenediaminetetraacetic acid (EDTA) tube. DNA was extracted with the *DNeasy Blood and Tissue* kit (Qiagen, Hilden, Germany). Genomic DNA quantity and quality were evaluated by measuring the DNA concentration with the *Qubit 3.0 Fluorometer* and the *Qubit DNA*

TABLE 2 Samples and phenotypes for the validation phase.

Phenotype	Total
Neutropenia	45
Diarrhoea	44
Mucositis	27
Nausea	19
Anaemia	11
Leucopenia	9
Thrombopenia	7
Neuropathy	4
Others	6
Asthenia	2
HFS	2
Cardiotoxicity	1
Enteritis	1
Controls	20

HS Assay kit (Thermo Fisher Scientific Inc, Massachusetts, USA). Genomic integrity control was performed with the *Agilent TapeStation 4200* using a *genomic DNA ScreenTape* (Agilent Technologies, California, USA). All samples had a minimum concentration of $13 \text{ ng } \mu\text{L}^{-1}$ and DNA Integrity Number (DIN) > 5 .

2.4 | Whole exome sequencing

Library preparation and whole exome sequencing were undertaken using 151-bp paired-end DNA libraries with the *SureSelect V6 + UTRs* kit (Agilent Technologies, California, USA). The exomes were sequenced on a *NovaSeq6000* platform (Illumina Inc, California, USA) to obtain an average depth of $72\times$ (calculated for targeted regions). The data were processed following the GATK best practices workflow for germline short variant (single nucleotide polymorphisms [SNPs] and indel) discovery (Depristo et al., 2011; Van der Auwera & O'Connor, 2020). Sequences were aligned to the reference genome hg19 and variants were annotated with *ANNOVAR* v2019.

2.5 | Variants in reported toxicity genes

We explored the genetic variation in genes that had already been related to adverse drug reaction toxicity in the literature (Table S1) (Simões et al., 2020).

2.5.1 | Variants described in the literature

We first inspected the list of described pharmacogenomic variants. We checked for the presence/absence of such variants in the sequenced exomes and performed statistical analysis using the Chi-squared test comparing cases and controls followed by Bonferroni

TABLE 3 Phenotypes and the respective sample counts for the meta-analysis.

Phenotype	WES ^a	EGA ^a	Total
HFS	9/51	13/241	22/292
Diarrhoea + mucositis	54/51	50/199	104/220
Diarrhoea + mucositis + neutropenia	85/51	133/369	218/420

Note: WES: whole-exome sequencing data from our study; EGA: toxicity cohort (Traylor et al., 2018).

^aNcases/Ncontrols.

correction for multiple testing. This was achieved by dividing the nominal threshold of 0.05 by the number of variants inspected (Table S1). A corrected *P* value of ≤ 0.05 was considered as statistically significant.

2.5.2 | Novel variants in reported genes

From the list, we also assessed other variants. Variant prioritisation was focussed on variants with higher probability of affecting functionality and thus, we selected rare (Genome Aggregation Database (gnomAD)_{NFE} ≤ 0.01) variation with predicted high impact: truncating variants (nonsense, frameshift), canonical splicing variants ($\pm 1/2$ -bp from exons) and high-impact missense variants (predicted by at least three out of four in silico tools: PolyPhen-2 probably/possibly deleterious, combined annotation-dependent depletion (CADD)_{phred} ≥ 15 , deleterious annotation of genetic variants using neural networks (DANN) ≥ 0.995 , genomic evolutionary rate profiling (GERP)₊₊ ≥ 2).

2.6 | Discovery of novel rare toxicity variants/genes

For exome-wide association analyses, we used the complete annotated vcf file and applied variant filtering for quality control, as suggested by Plink 1.9. Firstly, we performed per-individual filtering to remove individuals with excess missing genotypes ($-mind$ 0.2). Secondly, we undertook per-sample filtering to remove SNPs with $>20\%$ missing genotypes ($-geno$ 0.2). After the quality control, 198 patients remained for analysis.

We validated the candidate variants from the discovery phase by genotyping these at the Santiago node of the Spanish Genotyping Centre (CeGen, Spain) using the *iPLEX Pro* technology (Agena Bioscience, California, USA).

2.6.1 | Gene-based tests

We evaluated the possibility that toxicity may be caused by different variants in the same gene using the SNP-set kernel association test (optimized) (SKAT-O), from the Rare Variant tests (Rvtests) software v2.0.9 (Lee et al., 2012; Zhan et al., 2016). The different toxicity groups were compared with the controls. To evaluate the effect of

the variants profiles, we performed tests including different types of variants according to their impact: - (i) rare exonic variants; (ii) rare exonic variants, excluding synonymous; and (iii) rare truncating variants (nonsense, frameshift and splicing). Significance levels were calculated as $P \leq 0.05$ after performing FDR correction.

2.7 | Genome-Wide Association Studies (GWAS) meta-analyses

Additional quality control filtering for the GWAS included removal of SNPs that deviated from Hardy-Weinberg equilibrium ($-hwe$ 0.001) and extraction of common variants according to gnomAD frequencies ($NFE \geq 0.05$). For the whole-exome sequencing data, the Chi-squared test was applied for the grouped toxicities (global, digestive and haematological), for all toxicity groups with $n > 20$ (diarrhoea, neutropenia, asthenia and nausea) and for hand-foot syndrome (HFS), with the Bonferroni correction for exome-wide analyses (5.5×10^{-7}). We also calculated the genomic inflation factor and generated Q-Q plots Figure S1 and Manhattan plots, with RStudio (RStudio Team, 2020). The genomic inflation factor ranged from 1.07 (diarrhoea and asthenia) to 1.21 (hand-foot syndrome/HFS).

We estimate that with our maximum sample size of 147 cases and 50 controls in the global toxicity group, we have 80% power to detect variants with minor allele frequencies (MAFs) $>15\%$ and OR effect sizes of >3 2 (https://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/). For the meta-analysis, we could effectively detect variants with MAFs $>10\%$ and effect sizes OR >2 . This would correspond to identifying low to moderately penetrant common variants.

Data preparation and quality control guidelines for the EGA dataset were followed using the McCarthy Tools (<https://www.well.ox.ac.uk/~wrayner/tools/>). We used the Haplotype Reference Consortium (HRC)-1000G-check-bim-v4.2 to check the quality of the plink files before imputation, including the strand, alleles, position, Ref/Alt assignments and frequency differences. This removed ambiguous A/T and G/C SNPs if MAF > 0.4 , SNPs with differing alleles, SNPs with >0.2 allele frequency difference and SNPs not in the reference panel – the Haplotype Reference Consortium (HRC) panel v1.1 (McCarthy et al., 2016). We then performed imputation using the Michigan Imputation Server and the HRC EUROPEAN panel v1.1 as a reference panel. We have followed the imputation quality control guidelines, which indicate that, for common variants, the Rsq value must be ≥ 0.30 . Metal (Willer et al., 2010) was used for the meta-analysis of the two datasets, under a fixed effects

model. Bonferroni correction was applied for genome-wide analysis (5×10^{-8}).

2.8 | Materials

Details of materials and suppliers are provided in the specific sections.

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY <http://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24 (Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Davies, Amarosi, et al., 2023; Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Davies, Annett, et al., 2023).

3 | RESULTS

3.1 | Variants in reported toxicity genes

3.1.1 | Variants described in the literature

We found 13 cases that were heterozygous for the risk alleles of the four clinically actionable dihydropyrimidine dehydrogenase gene (DPYD) variants (rs3918290, rs67376798, rs558860621 and rs56038477). Amongst these, neutropenia and nausea were the most frequent severe adverse drug reactions (4/13, 31%), followed by asthenia and diarrhoea (3/13, 23%), then hand-foot syndrome (HFS; 2/13, 15%) and lastly, neuropathy, mucositis, skin rash, conjunctivitis and septic shock (1/13, 8%) (Table 4). As these patient toxicities were well justified by their risk genotypes, they were removed from further

TABLE 4 Clinically actionable DPYD variants.

rsID	Patient	Phenotype
rs3918290	A313	Diarrhoea
	A893	Neutropenia + asthenia
rs67376798	A326	Asthenia + nausea
	A339	Diarrhoea + nausea
	A853	Neutropenia
	A856	Mucositis
	B1559	Neutropenia
rs55886062	A310	HFS + skin rash
rs56038477	1220N	Conjunctivitis
	A334	Neuropathy
	A338	Diarrhoea + HFS
	20011763	Nausea + asthenia + septic shock
	20031358	Neutropenia + nausea
	293N	Control

Note: Table presents the patients in our cohort that were heterozygous for these variants, including their phenotype.

analyses. Interestingly, we also found one control individual carrying the rs56038477 variant. This could be because of the incomplete penetrance of this allele. This patient was also removed from further analyses. Note that 'decreased function' was inferred by detecting the exonic tag SNP rs56038477 and in rare cases, the causal decreased function variant c.1129-5923C > G may not be present despite having this tag SNP.

Two additional patients carried the **UGT1A1** rs4148323 variant in heterozygosity (A363 with asthenia and A366 with nausea), which is also actionable. Because this variant is described to be associated with toxicity to irinotecan and both patients had received 5-FU plus LV with oxaliplatin (FOLFOX), we could not initially justify the observed toxicity and hence decided to keep them in for further analysis.

Apart from the clinically actionable variants, we also found carriers (both cases and control patients) of five other common DPYD variants. These had been previously described in the literature, but with conflicting pathogenicity information. rs2297595, rs1801159 and rs1801160 (gnomAD_NFE $\geq 5\%$), and rs1801158 and rs17376848 (gnomAD_NFE $\geq 1\%$) are considered level of evidence 3 by PharmGKB and normal function by the Clinical Pharmacogenetics Implementation Consortium (CPIC; Amstutz et al., 2017; Whirl-Carrillo et al., 2012). The observed frequency of these variants in our cohort was similar to the gnomAD frequency.

We also found two previously reported rare DPYD variants: - rs114096998 and rs776973423. DPYD rs114096998 was found in one control, which is in line with a previously published work that showed that this variant causes the enzyme activity to increase to 138% when compared to the wild type (Offer et al., 2014). This variant is not yet included in the pharmacogenetics guidelines but considering its potentially increased enzymatic activity, it may be suggested that carriers of this allele could receive higher 5-FU dosages because of their higher tolerance. This would also explain our phenotyping of this patient as a control. DPYD rs776973423 was found in one case. This variant was previously predicted to be deleterious by DPYD-Varifier (Shrestha et al., 2018).

Besides DPYD, the literature reports a much larger set of toxicity genes. Thus, we also investigated these genes. We could not replicate any of the reported associations but found significant associations with other adverse drug reactions instead: - **ABCB1** rs2032582 was associated with skin toxicity ($p_{\text{corrected}} = 0.02$) and **SLCO1B1** rs2306283 with neuropathy ($p_{\text{corrected}} = 1 \times 10^{-3}$) (Table 5).

3.1.2 | Novel variants in reported genes

We also investigated additional, not previously reported variants in these reported toxicity genes. Briefly, we prioritised rare, truncating (nonsense, frameshift), canonical splicing and high-impact missense variants. For each gene, we analysed its relationship with any toxicity phenotype (not only with the reported adverse drug reaction). We found 31 variants in 14 genes, all in heterozygosity (Table 6).

Interestingly, one patient (A345) who developed neutropenia carried one novel rare variant in **ABCC5**. Previous reports showed

TABLE 5 Variants of interest in reported toxicity genes.

Gene	rsID (frequency ^a)	MAF (our cohort)	Reported adverse drug reaction (ADR)	Nominal P-value for reported ADR	OR (95% CI)	Best corrected P-value (ADR)
ABCB1	rs2032582 (0.64)	0.58	Global (Shrestha et al., 2018)	0.42	0.8 (0.52–1.27)	0.02 (skin toxicity)
	rs717620 (0.17)	0.23	Diarrhoea (Chen et al., 2015)	0.74	0.9 (0.46–1.66)	0.96 (leucopenia)
	rs3740066 (0.37)	0.38		0.89	1.1 (0.62–1.88)	0.96 (neuropathy)
CDA	rs2072671 (0.28)	0.35	Global (Di Martino et al., 2011; Loganayagam et al., 2013)	0.91	1.1 (0.61–1.52)	1 (diarrhoea)
	rs602950 (0.22)	0.36		0.72	0.9 (0.57–1.44)	0.88 (leucopenia)
DPVD	rs1801160 (0.05)	0.08	Global (Castro-Rojas et al., 2017; Lecomte et al., 2004;	0.63	1.5 (0.59–3.68)	0.14 (skin toxicity)
	rs1801159 (0.19)	0.22	Marsh et al., 2009; Pullarkat & Lenz, 2001; Rosmarin et al., 2015)	0.06	1.8 (0.99–3.23)	0.37 (haematological)
	rs1801158 (0.02)	0.05		0.31	0.7 (0.27–1.96)	1 (neutropenia)
	rs114096998 (2 × 10 ^{−5})	2 × 10 ^{−3}		1	NA (NA)	NA
	rs2297595 (0.09)	0.08		0.99	1.2 (0.49–3.05)	0.69 (nausea)
	rs17376848 (0.05)	0.03		0.15	4.0 (0.51–2.13)	1 (neutropenia)
	rs776973423 (1 × 10 ^{−4})	2 × 10 ^{−3}		1	NA (NA)	NA (NA)
ERCC1	rs11615 (0.49)	0.37	Neuropathy (Di Martino et al., 2011)	0.24	2 (0.65–6.16)	0.89 (leucopenia)
ERCC2	rs13181 (0.32)	0.32	Haematological (Di Martino et al., 2011)	0.77	1.1 (0.64–2.01)	0.98 (digestive)
MTHFR	rs1801133 (0.32)	0.35	Global (Di Martino et al., 2011)	0.16	1.4 (0.88–2.29)	1 (skin toxicity)
	rs1801131 (0.29)	0.31		0.62	0.9 (0.55–1.42)	1 (skin toxicity)
MIR27A	rs895819 (0.34)	0.28	Global (Rosmarin et al., 2015)	0.53	1.2 (0.73–1.99)	0.15 (HFS)
SLCO1B1	rs2306283 (0.40)	0.38	Digestive (Wu & Dolnick, 2003)	1	1.0 (0.59–1.64)	1 × 10 ^{−3} (neuropathy)
UGT1A1	rs4148323 (0.01)	4 × 10 ^{−3}	Global (Di Martino et al., 2011)	1	NA (NA)	0.95 (nausea)
UGT1A9	rs11692021 (0.35)	0.34	Global (Vasilou & Pappa, 2000)	0.64	1.1 (0.71–1.83)	0.81 (leucopenia)
UMPS	rs2279199 (0.47)	0.47	Global (Rosmarin et al., 2015)	0.82	1.1 (0.69–1.67)	0.98 (cardiotoxicity)
	rs1801019 (0.17)	0.14		0.15	1.7 (0.85–3.57)	0.21 (cardiotoxicity)

Note: Table shows the variants previously described as associated with toxicity, with their frequency in the general population and in our cohort, the ORs and P-value for the reported associated adverse drug reaction (ADR) and the best P-value across all analysed ADRs. We do not mention reported ORs because for most variants, there are multiple, often conflicting ones, depending on the reporting study. OR = odds-ratio; CI = confidence interval; a: gnomAD_NFE v2.1.1.

TABLE 6 Candidate toxicity variants present in the literature-reported genes.

Gene	pLI	Missense Z-score	Change	rsID	MAF	Patient	Phenotype
ABCB1	0	1.4	p.(Q1107P)	rs55852620	5×10^{-3}	A871 ^a 1158N C213	Diarrhoea Control Control
			p.(I261V)	rs36008564	7×10^{-4}	339N ^a A318 A852 C028	Diarrhoea Neutropenia + diarrhoea Control Allergic reaction
ABCC2	0	−0.9	p.(S281N)	rs56131651	7×10^{-3}	A881 C155 S11028	Asthenia Control Asthenia
			p.(K495E)	rs17222561	4×10^{-4}	1243N	Diarrhoea + mucositis + leucopenia
			p.(R1079Q)	rs145672804	7×10^{-4}	B1550 ^a	Neutropenia
			p.(R1100C)	rs142715085	8×10^{-4}	B1550 ^a	Neutropenia
			p.(R1181L)	rs8187692	4×10^{-4}	A330 A366	Diarrhoea + HFS Nausea
ABCC5	0	3.6	p.(K8N)	rs200090959	4×10^{-5}	A345	Neutropenia
ABCG1	0.1	2.1	p.(R65H)	rs959974308	1×10^{-5}	A343	Skin toxicity
			p.(S181L)	rs138056067	1×10^{-3}	A887	Asthenia + allergic reaction
			p.(T628M)	rs199627225	7×10^{-6}	A871 ^a	Diarrhoea
ABCG2	0	−0.9	p.(M131I)	rs759726272	4×10^{-5}	A880 ^a	Asthenia
			p.(P112Q)	rs199473672	6×10^{-4}	A356 ^a	Control
			p.(K652Pfs*3)	rs754196551	5×10^{-5}	339 N ^a	Asthenia
			p.(G262X)	rs200473953	3×10^{-5}	A357	Anaemia
			p.(R236X)	rs140207606	2×10^{-4}	A880 ^a	Asthenia
CES1	0	−2	p.(C87R)	rs149261413	3×10^{-4}	A876	Neutropenia
			p.(G224R)	rs768635294	3×10^{-3}	A356 ^a	Control
CES2	0	0.4	p.(R34Q)	rs140461033	2×10^{-3}	1268N ^a M085	Diarrhoea + mucositis Hepatotoxicity
			p.(G255V)	rs780237910	7×10^{-5}	S40588	Asthenia + diarrhoea
			p.(P260S)	rs147942040	1×10^{-4}	A862 C124	Asthenia + allergic reaction + nausea control
DPYD	0	−0.2	p.(V691L)	rs202212118	1×10^{-4}	1268N ^a	Diarrhoea + mucositis
ENOSF1	0	−0.3	p.(S376P)	rs766984806	3×10^{-5}	A372	Neutropenia
			p.(D75H)	rs377711094	1×10^{-4}	A980 ^a	Diarrhoea + mucositis
ERCC1	0	0.7	p.(A199T)	rs141811629	2×10^{-5}	A847	Control
			p.(V623fs*26)	rs756630156	9×10^{-5}	A897	Neuropathy + HFS
MTHFR	0	0.9	p.(R519C)	rs45496998	3×10^{-4}	A842 S270523	Control Neutropenia
SLC22A7	0	1.2	p.(I213V)	rs369251648	3×10^{-5}	A369	Thrombopenia
SLCO1B1	0	−0.9	p.(R580X)	rs71581941	1×10^{-5}	A868	Skin toxicity + diarrhoea
			c.481 + 1G > T	rs77271279	9×10^{-5}	A902	Nausea + diarrhoea
TYMS	0.7	2.6	p.(Q32E)	rs1034044510	2×10^{-5}	A980 ^a	Diarrhoea + mucositis

Note: For each gene, we include the gnomAD (Genome Aggregation Database) pLI (probability of being Loss-of-function Intolerant) and Z-scores, which assign gene-based constraint metrics and intolerance scores for loss of function and missense variants. pLI ranges from 0 to 1; with pLI ≥ 0.9 being considered extremely intolerant, whereas the Z-score ranges from −5 to 5, with a missense Z-score ≥ 3 considered excessively constrained or intolerant (Chen et al., 2017; Mizukami et al., 2019). All genes had a pLI ≤ 0.9 , meaning that they are predicted to be tolerant to loss of function variation. Only ABCC5 was predicted to be intolerant to missense changes. In bold: values of pLI and missense Z-score that pass the threshold for intolerance.

^aPatient with >1 candidate toxicity variant.

association of variants in this gene with neutropenia (Chen et al., 2015; Di Martino et al., 2011). *ABCC5* has a missense Z-score > 3, indicating that it is extremely intolerant to missense changes.

Remarkably, seven other patients carried two rare, high impact variants in the same gene, gene-family or pathway. Patient A880 carried two variants in *ABCG2* (rs759726272 and rs140207606) and patient B1550 carried two variants in *ABCC2* (rs142715085 and rs145672804). We also found patients with high-impact variants in two genes. Interesting novel variants included the *TYMS* rs1034044510 and the *ENOSF1* rs377711094 changes found in patient A980. Additionally, patient 339N carried one novel variant in *ABCB1* and *ABCG2* rs754196551 (stop-gain). Patient 1268N had a variant in *CES2* rs140461033 and one in *DPYD* rs202212118. Patient A871 had two variants in two ABC genes (*ABCB1* rs55852620 and *ABCG1* rs199627225). Lastly, a control patient (A356) had one variant in each *CES1* rs768635294 and *ABCG2* rs199473672.

3.2 | Discovery of novel rare toxicity variants/genes

Next-generation sequencing (NGS) offers comprehensive insights into the genetic makeup and has been proven useful to identify not only common but rare variation. Thus, we exploited this and employed various analytical strategies on our whole-exome sequencing data.

Complementarily, we evaluated the contribution of other genes/variants not previously linked to toxicity. For this, we first selected rare coding variants, truncating or splicing, and then shortlisted those that were present in ≥ 4 cases with a given phenotype but were absent in the controls. In the discovery cohort, we found 10 novel candidate variants of interest in 10 different genes (*HMGXB4* rs769388508, *INO80E* rs759306300, *BAGE2* rs541080822, *DNAJB7* rs200971397, *MDM1* rs776852538, *RP2* rs767564816, *ALDH9A1* rs201507277, *EPX* rs35617692, *FAM83A* rs148011353 and *USP6* rs200143577), potentially associated with diarrhoea, digestive, haematological and global toxicities.

For validation of these 10 variants of interest, we genotyped them in an additional cohort of 144 samples. The rs769388508 and the rs759306300 variants are short tandem repeats, thus different allelic variants are not possible to be distinguished by *Sequenom*. Variants rs200143577 and rs541080822 hybridise in more than one genomic region, so it was not possible to design specific extension primers. Therefore, these four variants were removed from the genotyping assay. Furthermore, variant rs776852538 presented low PCR efficacy, limiting the intensity of the amplified product, making it difficult to correctly assign genotypes.

We successfully genotyped in the validation cohort five variants identified in the discovery cohort. Of these, three were identified in nine cases, although the toxicities varied from the ones observed in the discovery: the *ALDH9A1* rs201507277 was present in two patients with mucositis, one with enteritis, one with diarrhoea and two with neutropenia. The *FAM83A* rs148011353 SNP was found in a patient with neutropenia, and another with neutropenia and leucopenia; and

the *EPX* rs35617692 was found in a patient with mucositis (Table 7). Variants rs200971397 and rs767564816 were not found in any of the cases. All genotyped controls were wild-type (wt).

3.2.1 | Gene-based tests

Regarding the gene-based SKAT-O tests, we identified 14 novel genes significantly associated with skin toxicity, cardiotoxicity and neuropathy (Table 8). Of these, *DGKI* and *SCN5A* genes seem the most interesting according to the scores of probability of intolerance to variation. Variants in *DGKI* and *SCN5A* are present in 50% of the cases with neuropathy ($n = 3/6$) whilst in less than 10% of the controls ($n = 2/51$ and $n = 5/51$, respectively). Interestingly, patient A331 had one variant in each of these two genes (Table S2). In fact, all cases with skin toxicity or neuropathy except one (A859) had rare variants in more than one gene significant for the analysed adverse drug reaction. Another example is patient A370, who developed neuropathy and had one rare variant in three of the SKAT-O genes associated with neuropathy (*MYBPC3* rs11570082, *PDZD7* rs142301501 and *SCN5A* rs6791924).

3.3 | Global toxicity genome-wide association studies (GWAS) meta-analyses

As to the GWAS on common variants, we did not find any exome-wide significant associations in any of the comparisons. However, the top variant identified from the GWAS) was *ITGAX* rs2230424) was borderline significant (Table 9). The Manhattan plots for each analysis are shown in Figure S2.

We also performed a meta-analysis of our cohort with the EGAS00001002763 dataset, for the phenotypic categories diarrhoea-mucositis-neutropenia (DMN), diarrhoea-mucositis (DM) and hand-foot syndrome (HFS). We observed no exome-wide significant SNPs in these analyses either (Table 10).

4 | DISCUSSION

In the early stages of pharmacogenetic studies, these were mainly based on candidate gene approaches, focussing on those considered a priori to be relevant to the drug pharmacokinetics and pharmacodynamics – pharmacogenes. Indeed, more than 200 genes have been proposed as pharmacogenes, such as *DPYD*, *TYMS*, *MTHFR* and *CDA* (Loganayagam et al., 2013). Despite efforts, these have not provided conclusive evidence to establish a clear and consistent link between these genes and toxicity, which is supported by our negative results from the analysis of previously reported variants. As of now, only two genes, *DPYD* and *UGT1A*, are included in the current pharmacogenetic guidelines for colorectal cancer treatment.

Next-generation sequencing has revolutionised genomic studies by providing a more comprehensive understanding of a patient

TABLE 7 Validation of rare, high impact variants.

Gene	SNP	Relevant adverse drug reaction (ADR) in discovery	Patient and ADR (discovery)	Patient – phenotype (validation)
ALDH9A1	rs201507277	Diarrhoea Digestive Global	A317 - diarrhoea A855 - diarrhoea + mucositis A868 - diarrhoea + skin toxicity A902 - diarrhoea + nausea 4/147 patients (2.7%)	7B825 - diarrhoea 15008 - mucositis V009 - enteritis DP46 - mucositis 2434486 - neutropenia 2144353 - neutropenia 6/124 patients (4.8%)
FAM83A	rs148011353	Diarrhoea Digestive Global	1,243 N - diarrhoea + mucositis + leucopenia 339N - diarrhoea A328 - diarrhoea A345 - neutropenia A761 - diarrhoea + nausea + hepatotoxicity M085 - hepatotoxicity S60194 - nausea 7/147 patients (4.8%)	8H739 - leucopenia + neutropenia DP52 - leucopenia + neutropenia 2/124 patients (1.6%)
EPX	rs35617692	Haematological Global	A861 - neutropenia A882 - neutropenia S51254 - neutropenia 1247N - leucopenia + diarrhoea A980 - diarrhoea + mucositis S20020714 - diarrhoea + nausea + asthenia 6/147 patients (4.1%)	DP40 - mucositis 1/124 patients (0.8%)

Note: Toxicities in bold (either single – diarrhoea, or grouped toxicity – digestive, haematological, global) are associated with the variant in the discovery phase.

genetic profile, as it allows for the inspection of both common and rare variation. In the context of pharmacogenomics, this may prove extremely helpful to unravel the missing pharmacogenomic heritability. In our study, we take advantage of this and apply different analytical strategies to our whole-exome sequencing data, to successfully identify several candidate toxicity variants and genes.

In 14 of the previously reported pharmacogenes, we have identified 31 novel, high-impact rare variants. Compellingly, we found one patient that presented one variant in *DPYD* (rs202212118), which is, as we have seen extensively, an extremely important pharmacogene. There are no publications reporting this variant yet. Therefore, functional studies would be necessary to determine if this variant does have a critical impact on the dihydropyrimidine dehydrogenase protein (DPD) function and, if so, it could potentially be included in the pharmacogenetic guidelines.

Moreover, we envisage the importance of ABC-transporters genes, because of their key role in pharmacokinetics and pharmacodynamics of chemotherapeutic drugs, particularly in drug influx and efflux. The *ABCC5* pharmacogene is involved in the flux of irinotecan. Hence, if its function is altered, it may impact drug concentration and lead to severe adverse drug reactions. Indeed, previous works have linked variants in this gene with irinotecan-related neutropenia (Chen et al., 2015; Di Martino et al., 2011). According to its missense Z-score, *ABCC5* is intolerant to missense changes, making our newly detected variant (rs200090959) a good candidate to explain the

toxicity in this patient. We also found a patient carrying two novel rare, high impact variants in *ABCC2*. According to the gnomAD *Variant Co-Occurrence* tool, the two variants in *ABCC2* are probably within different haplotypes, indicating that this patient may be a compound heterozygote for *ABCC2* variants. As oxaliplatin is eliminated from the liver cells largely by this protein, this could result in an increase in the toxic accumulation of oxaliplatin inside the cell (Marsh et al., 2009). Further experiments, including gene knockdowns, would be useful to verify the functionality of these variants and the implications of this gene in toxicity.

Interestingly, we also found one patient who presented novel rare variants in two genes of the same pathway. This patient carried the *TYMS* rs1034044510 and the *ENOSF1* rs377711094. *TYMS* encodes for the thymidylate synthase (TYMS), the main target of 5-FU. Two variants in this gene have been extensively studied: rs45445694 and rs11280056 (Castro-Rojas et al., 2017; Lecomte et al., 2004; Pullarkat & Lenz, 2001; Rosmarin et al., 2015). In 2015, a study reported an association of an intronic variant located in the *ENOSF1* (which overlaps partly with *TYMS*) capable of explaining the toxicity attributed to these two *TYMS* polymorphisms. These are in moderate linkage disequilibrium. Thus, the assessment of the exact impact of each variant is not straightforward, but the presence of both variants could potentially confer a higher risk. Although the biological function of *ENOSF1* is still unclear, it has been suggested that it can modify *TYMS* expression (Rosmarin et al., 2015; Wu & Dolnick, 2003).

TABLE 8 SKAT-O rare variant test for genes associated with toxicity.

Gene	pLI	Missense Z-score	Associated adverse drug reaction (ADR)	SKAT-O test (variants in test)	Variants in other ADRs	Corrected P-value
<i>ITPR3</i> ^a	0	4.55	Cardiotoxicity	Exonic (cases: 1 missense; controls: 2 missense, 9 syn)	Asthenia, diarrhoea, nausea, neutropenia, mucositis, HFS, leucopenia, neuropathy, skin toxicity	0.01
<i>INSYN2B</i>	0.98	1.78	Cardiotoxicity	Exonic (cases: 2 missense, 1 syn; controls: 1 missense)	Asthenia, diarrhoea, neuropathy, nausea, mucositis, leucopenia, anaemia, neutropenia	0.03
<i>SCMH1</i>	0	1.95	Skin toxicity	Exonic (cases: 1 missense ^b ; controls: 1 missense, 1 syn)	Neutropenia, diarrhoea	0.04
<i>MYBPC3</i> ^a	0	1.45	Neuropathy	Exonic (cases: 1 missense; 2 syn; controls: 3 missense, 2 syn)	Nausea, thrombopenia, neutropenia, asthenia, HFS, leucopenia, nausea, diarrhoea, cardiotoxicity	5×10^{-3}
<i>DGKI</i>	0.93	3.08	Neuropathy	Exonic (cases: 1 indel, 2 missense; controls: 2 syn)	Diarrhoea, nausea, HFS, asthenia, neutropenia, hepatotoxicity, mucositis, leucopenia	0.04
<i>STK31</i>	0.01	0.26	Skin toxicity	Exonic excl. syn (cases: 1 stopgain, 1 missense ^b ; controls: 3 missense)	Nausea, asthenia, leucopenia, cardiotoxicity, diarrhoea, neutropenia	5×10^{-4}
<i>ARAP3</i>	0	1.82	Skin toxicity	Exonic excl. syn (cases: 1 indel, 2 missense; controls: 3 missense)	Diarrhoea, neutropenia, cardiotoxicity, nausea, asthenia, HFS, linfopenia, neuropathy	8×10^{-3}
<i>CLPB</i>	0	1.05	Skin toxicity	Exonic excl. syn (cases: 1 missense ^b)	Asthenia, nausea, neutropenia, mucositis, asthenia, diarrhoea	0.03
<i>PARVB</i>	0	0.1	Skin toxicity	Exonic excl. syn (cases: 1 missense ^b)	Diarrhoea, nausea	0.03
<i>CPT1A</i>	0	1.64	Skin toxicity	Exonic excl. syn (cases: 2 missense ^b ; controls: 1 missense)	Neutropenia, asthenia, diarrhoea, HFS, neutropenia, asthenia	0.03
<i>ZBTB45</i>	0	1.36	Neuropathy	Exonic excl. syn (cases: 3 missense; controls: 1 missense)	Neutropenia, asthenia, nausea, cardiotoxicity, diarrhoea, mucositis	3×10^{-3}
<i>PDZD7</i>	0	-0.37	Neuropathy	Exonic excl. syn (cases: 1 missense, 1 missense ^b ; controls: 1 indel, 1 missense)	Diarrhoea, mucositis, skin toxicity, asthenia, HFS, hepatotoxicity, neutropenia, nausea	0.01
<i>SCN5A</i>	0.91	2.75	Neuropathy	Exonic excl. syn (cases: 1 missense, 1 missense ^b ; controls: 4 missense)	HFS, asthenia, diarrhoea, nausea, leucopenia, mucositis, skin toxicity, hepatotoxicity, neutropenia, thrombopenia	0.02
<i>COL4A2-AS2</i>	0	2.19	Skin toxicity	Truncating (cases: 1 indel; 1 stopgain)	Diarrhoea, nausea, mucositis, leucopenia, neutropenia	0.01

Note: In bold: values of pLI (probability of being Loss-of-function Intolerant) and missense Z-score that pass the threshold for intolerance. syn: synonymous.

^aThe frequency of rare variants in this gene was higher in controls than in cases.

^bVariant present in more than one patient.

Therefore, we hypothesise about a potential digenic inheritance and further studies on these two variants would be appealing to help understand the potential influence on each other and the interaction between both genes.

Although the candidate gene approach produced interesting results, its view is limited within a set of genes that have been previously described to be involved in the pharmacokinetics/pharmacodynamics of chemotherapeutic drugs. Thus, we expanded our analysis to an exome-wide, hypothesis-free approach. We discovered and independently validated three candidate rare, high impact variants (in *ALDH9A1*, *FAM83A* and *EPX*). *ALDH9A1* belongs to the

aldehyde dehydrogenase family of enzymes (ALDHs) that are responsible for the oxidation of toxic aldehydes and are up-regulated in many cancers. It has been shown that cancer treatments, such as chemo and radiotherapy, are related to the production of reactive oxygen species (ROS) that lead to aldehyde accumulation (Vasilou & Pappa, 2000; Zanon et al., 2022). Consequently, changes in this enzyme could impair the detoxification of cellular aldehydes and the protection against the oxidative stress caused by chemotherapy, ultimately leading to toxicity. *FAM83A* belongs to a family of eight oncogenes, which function as intermediaries in the *EGFR/RAS* signalling pathway, having a significant role in cell survival, proliferation,

TABLE 9 Results of the adverse drug reaction (ADR) toxicity GWAS (Genome-Wide Association Study).

Phenotype (Ncases/Ncontrols)	Variant	Gene	P-value
Global (147/51)	rs2230424	ITGAX	9×10^{-7}
Digestive (71/51)	rs10985765	GABBR2	1×10^{-5}
Haematological (52/51)	rs480963	MROH7	1×10^{-4}
Diarrhoea (44/51)	rs10985765	GABBR2	2×10^{-5}
Neutropenia (37/51)	rs11568187	MLC1	1×10^{-4}
Asthenia (28/51)	rs4577273	CROCC2	3×10^{-5}
Nausea (21/51)	rs11230346	OR4C11	1×10^{-5}
HFS (9/51)	rs7853758	SLC28A3	4×10^{-6}

Note: For each of the eight phenotypic categories, the top associated variant is shown. Only ITGAX rs2230424 was borderline exome-wide significant. Abbreviation: HFS, hand-foot syndrome.

TABLE 10 Results of the adverse drug reaction (ADR) toxicity meta-analysis.

Phenotype	Variant	Gene	P-value
DMN	rs3739672	ANP32B	1.3×10^{-5}
DM	rs59366579	FLII	9.3×10^{-5}
HFS	rs73264111	BPIFB4	3.6×10^{-5}

Note: For each of the three phenotypic categories, the top associated variant is shown. Abbreviations: DMN; diarrhoea-mucositis-neutropenia; DM, diarrhoea-mucositis; HFS, hand-foot syndrome.

invasion and immune evasion (Mizukami et al., 2019). The FAM83A protein has been identified as a diagnostic and prognostic biomarker for lung, breast and pancreatic cancer (Bartel et al., 2016; Chen et al., 2017), but no prior relationship to toxicity has been described. EPX encodes the eosinophil peroxidase. This enzyme has an oxidative inflammatory function and acts against parasites and bacteria (Acharya & Ackerman, 2014; Romano et al., 2000). For FAM83A and EPX, this is the first description of a possible association between these genes and toxicity. Ultimately, *in vitro* studies should be used to confirm the actual functional consequences of these two stop-gain variants possibly associated with the development of toxicity.

Because it is difficult to identify novel toxicity loci at the variant level because of the low frequency of these high-impact changes, we also performed gene-based tests. These assess the cumulative effects of multiple variants in the same gene, considerably reducing the multiple testing burden and increasing the power to detect novel toxicity genes. We identified 14 genes significantly associated with neuropathy, skin toxicity and cardiotoxicity. For instance, DGKI and SCN5A were associated with neuropathy. DGKI expresses in the brain and retina and encodes for an enzyme that belongs to the family of diacylglycerol (DAG) kinases (The Human Protein Atlas, n.d.-a). These convert DAG into phosphatidic acid regulating its cellular levels (Ding et al., 1998; Sato et al., 2013). The DGKI enzyme has synaptic functions and is involved in neural responses (Sato et al., 2013). SCN5A encodes for the sodium voltage-gated channel alpha subunit 5 that can be found in neuronal cells (The Human Protein Atlas, n.d.-b). Indeed, sodium voltage-gated channels have already been implicated in chemotherapy-related neuropathy (Adelsberger et al., 2000; Boyette-

Davis et al., 2018). It has been shown that oxaliplatin interferes with the activity of sodium voltage-gated channels and the altered flux of ions can lead to neuropathy. Hence, it is reasonable to argue that both DGKI and SCN5A might have a role in the development of neuropathy. We must, however, consider that our SKAT-O analysis only identified significant genes in the smaller phenotypic groups ($n < 10$); thus, these results should be further validated in larger datasets.

Although there is a huge amount of data supporting the key role of the inherited variability and its impact on development of adverse drug reactions from pharmacogenetic and omic studies, the findings are considerably restricted, as most studies are small and have produced conflicting results regarding candidate toxicity genes and variants, limiting the translation of such results into clinical practice. These discrepancies may be because of several reasons, for instance, lack of standardisation, complexity in adverse drug reaction phenotyping, need for novel research approaches and lack of functional studies. Moreover, as per our study, all patients were treated with fluoropyrimidines, but there is a degree of heterogeneity in terms of monotherapy or combinations with other drugs and the therapeutic regimens across the different recruiting centres and tumour staging that could also be contributing to low success rates in identifying novel pharmacogenes.

We propose a series of actions to address these limitations and to help facilitate the implementation of pharmacogenomic results within the clinical context. In order to improve homogenisation and consistency in the toxicity data collection, standardised case report forms (CRFs) as well as established grading scales, such as the Common Terminology Criteria for Adverse Events (CTCAE), should be used to enable rapid and reproducible phenotyping of patients. Then, using this standardised phenotyping, it is necessary to invest in the collection of prospective cohorts with adequate sample sizes based on the frequencies of each of the candidate variants (larger cohorts will be needed for rare variants, such as those identified by SKAT-O tests). After identifying strong toxicity candidates, validation studies should be conducted, along with functional toxicity studies in *in vitro* or *ex vivo* models (organoids) that prove the reduced activity of target genes in regard to the metabolism of fluoropyrimidines. Subsequently, for candidate genes, a risk scoring system would need to be developed based on the quantity/quality of rare variants to determine the

functional reduction threshold necessary for toxicity to occur (similar to the approach used with *DPYD*). Additionally, it is relevant to derive the incidence and penetrance of each risk variant based on the population data obtained from the above-mentioned cohorts. Following all these steps, a technology assessment would need to be carried out to identify the most practical methodology for identifying these variants and/or genes, which could be performed through genotyping with the open array, as is carried out for *DPYD* variants, or through DNA integrity number in case of genes. In addition to the need for clear evidence regarding the functional relevance of a pharmacogenetic biomarker, demonstrating its cost-effectiveness is essential for its integration into clinical practice and acceptance by health-care professionals and institutions. Ultimately, the translation from bench to bedside of these risk loci will also rely on specific guidelines and recommendations on how to adjust treatment based on the genetic test results. This will also allow the derivation of new and optimised functionality, penetrance and incidence data once they begin to be implemented as pre-emptive tests.

5 | CONCLUSIONS

Taking all these appealing results into account, we would be able to propose novel candidate variants/genes that might be responsible for the toxicity susceptibility in 36% of our patients. The toxicity presented by these patients is possibly being determined by one, two or a few rare high penetrance variants, following a monogenic inheritance (for instance, the patient carrying a variant in the *DPYD*), a digenic inheritance (patient with variants in *TYMS* and *ENOSF1*) or an oligogenic inheritance (patients that carried variants in more than one *SKAT-O* gene – for example, patient A331 who carried rare variants in *DGKI*, *ZBTB45*, *PDZD7*, *SCN5A* and *ARAP3*), respectively. Furthermore, we might argue that for the remaining 64% of patients, toxicity may therefore be because of the polygenic contribution of multiple common variants, each conferring a small increase in toxicity, or the interaction between genetics and the environment, although our cohort was largely underpowered to detect these effects. This is why we suggest that toxicity susceptibility is likely being influenced by a spectrum of different types of variants.

Although pharmacogenomics has been on the rise these last decades, there is still a long way to go to resolve the missing heritability problem and to fully understand the mechanism of action of toxicity variants. For that purpose, we believe that it would be pivotal to better characterise and functionally validate the rare high penetrance variants/genes that we have discovered in this study, possibly with enzymatic assays. Ultimately, the goal will be to translate these findings into clinical practice and improving pre-emptive pharmacogenetic testing as a standard of care.

AUTHOR CONTRIBUTIONS

A. R. Simoes: Data curation (equal); formal analysis (lead); investigation (lead); methodology (equal); validation (lead); visualisation (equal); writing—original draft (equal); writing—review and editing (equal).

S. Carlés González: Investigation (supporting). **M. d. C. Lopez-Doldan:** Resources (equal). **G. Duran:** Resources (equal). **A. Fernández-Montes:** Resources (equal). **M. Perfecta Fernandez:** Resources (equal). **M. Salgado Fernandez:** Resources (equal). **J. Garcia Mata:** Resources (equal). **D. Paez:** Resources (equal). **P. Riera:** Resources (equal). **E. Guino:** Resources (equal). **L. Lopez-Fernandez:** Resources (equal). **B. Garcia de Santiago:** Resources (equal). **E. López Aspiroz:** Resources (equal). **A. Rojas:** Resources (equal). **A. Castells:** Resources (equal). **S. Castellvi-Bel:** Resources (equal). **C. Ruiz Ponte:** Resources (equal). **V. Vymetalkova:** Resources (equal). **A. Carracedo:** Conceptualisation (equal); funding acquisition (lead); supervision (lead); writing—review and editing (equal). **C. Fernandez-Rozadilla:** Conceptualisation (equal); data curation (equal); formal analysis (lead); investigation (supporting); methodology (equal); supervision (lead); validation (supporting); visualisation (equal); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for [Design and Analysis](#) and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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REFERENCES

- Acharya, K. R., & Ackerman, S. J. (2014). Eosinophil granule proteins: Form and function. *Journal of Biological Chemistry*, 289, 17406–17415. <https://doi.org/10.1074/jbc.R113.546218>
- Adelsberger, H., Quasthoff, S., Grosskreutz, J., Lepier, A., Eckel, F., & Lersch, C. (2000). The chemotherapeutic oxaliplatin alters voltage-gated Na(+) channel kinetics on rat sensory neurons. *European Journal of Pharmacology*, 406, 25–32.
- Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Amarosi, L., Anderson, C. M. H., Beart, P. M., Broer, S., Dawson, P. A., Gyimesi, G., Hagenbuch, B., Hammond, J. R., Hancox, J. C., ... Verri, T. (2023). The Concise Guide to PHARMACOLOGY 2023/24: Transporters. *British Journal of Pharmacology*, 180(Suppl 2), S374–S469. <https://doi.org/10.1111/bph.16182>
- Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Annett, S., Boison, D., Burns, K. E., Dessauer, C., Gertsch, J., Helsby, N. A., Izzo, A. A., Ostrom, R., Papapetropoulos, A., ... Wong, S. S. (2023). The Concise Guide to PHARMACOLOGY 2023/24: Enzymes. *British Journal of Pharmacology*, 180(Suppl 2), S289–S373. <https://doi.org/10.1111/bph.16181>
- Amstutz, U., Henricks, L. M., Offer, S. M., Barbarino, J., Schellens, J. H., Swen, J. J., Klein, T. E., McLeod, H. L., Caudle, K. E., Diasio, R. B., & Schwab, M. (2017). Clinical pharmacogenetics implementation consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing: 2017 update. *Clinical Pharmacology & Therapeutics*, 13, 210–216.
- Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., Laurent-Puig, P., Quirke, P., Yoshino, T., Taieb, J., Martinelli, E., Arnold, D., & ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. (2020). Localised colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, 31, 1291–1305. <https://doi.org/10.1016/j.annonc.2020.06.022>
- Bartel, C. A., Parameswaran, N., Cipriano, R., & Jackson, M. W. (2016). FAM83 proteins: Fostering new interactions to drive oncogenic signaling and therapeutic resistance. *Oncotarget*, 7, 52597–52612. <https://doi.org/10.18632/oncotarget.9544>
- Boyette-Davis, J. A., Hou, S., Abdi, S., & Dougherty, P. M. (2018). An updated understanding of the mechanisms involved in chemotherapy-induced neuropathy. *Pain Management*, 8, 363–375. <https://doi.org/10.2217/pmt-2018-0020>
- Castro-Rojas, C. A., Esparza-Mota, A. R., Hernandez-Cabrera, F., Romero-Diaz, V. J., Gonzalez-Guerrero, J. F., Maldonado-Garza, H., Garcia-Gonzalez, I. S., Buenaventura-Cisneros, S., Sanchez-Lopez, J. Y., Ortiz-Lopez, R., Camacho-Morales, A., Barboza-Quintana, O., & Rojas-Martinez, A. (2017). Thymidylate synthase gene variants as predictors of clinical response and toxicity to fluoropyrimidine-based chemotherapy for colorectal cancer. *Drug Metabolism and Personalized Therapy*, 32, 209–218. <https://doi.org/10.1515/dmpt-2017-0028>
- Cervantes, A., Adam, R., Roselló, S., Arnold, D., Normanno, N., Taieb, J., Seligmann, J., De Baere, T., Osterlund, P., Yoshino, T., & Martinelli, E. (2022). Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Annals of Oncology*, 34, 10–32. <https://doi.org/10.1016/j.annonc.2022.10.003>
- Chen, S., Huang, J., Liu, Z., Liang, Q., Zhang, N., & Jin, Y. (2017). FAM83A is amplified and promotes cancer stem cell-like traits and chemoresistance in pancreatic cancer. *Oncogene*, 6, 1–10.
- Chen, S., Villeneuve, L., Jonker, D., Couture, F., Laverdière, I., Cecchin, E., Innocenti, F., Toffoli, G., Lévesque, E., & Guillemette, C. (2015). ABCC5 and ABCG1 polymorphisms predict irinotecan-induced severe toxicity in metastatic colorectal cancer patients. *Pharmacogenetics and Genomics*, 25, 573–583. <https://doi.org/10.1097/FPC.0000000000000168>
- Curtis, M. J., Alexander, S. P. H., Cortese-Krott, M., Kendall, D. A., Martemyanov, K. A., Mauro, C., Panettieri, R. A. Jr., Papapetropoulos, A., Patel, H. H., Santo, E. E., Schulz, R., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., Wang, X., & Ferdinandy, P. (2025). Guidance on the planning and reporting of experimental design and analysis. *Br J Pharmacol*, 182, 1413–1415. <https://doi.org/10.1111/bph.17441>
- Depristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., Del Angel, G., Rivas, M. A., Hanna, M., & McKenna, A. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, 43, 491–501.
- Di Martino, M. T., Arbitrio, M., Leone, E., Guzzi, P. H., Saveria Rotundo, M., Ciliberto, D., Tomaino, V., Fabiani, F., Talarico, D., Sperlongano, P., & Doldo, P. (2011). Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study. *Cancer Biology and Therapy*, 12, 780–787. <https://doi.org/10.4161/cbt.12.9.17781>
- Ding, L., Traer, E., McIntyre, T. M., Zimmerman, G. A., & Prescott, S. M. (1998). The cloning and characterization of a novel human

- diacylglycerol kinase, DGK α . *Journal of Biological Chemistry*, 273, 32746–32752. <https://doi.org/10.1074/jbc.273.49.32746>
- Fernandez-Rozadilla, C., Cazier, J. B., Moreno, V., Crous-Bou, M., Guinó, E., Durán, G., Lamas, M. J., López, R., Candamio, S., Gallardo, E., Paré, L., Baiget, M., Páez, D., López-Fernández, L. A., Cortejoso, L., García, M. I., Bujanda, L., González, D., Gonzalo, V., ... EPICOLON Consortium. (2013). Pharmacogenomics in colorectal cancer: A genome-wide association study to predict toxicity after 5-fluorouracil or FOLFOX administration. *Pharmacogenomics Journal*, 13, 209–217. <https://doi.org/10.1038/tpj.2012.2>
- Food and Drug Administration. (2002a). HIGHLIGHTS OF PRESCRIBING INFORMATION. Oxaliplatin.
- Food and Drug Administration. (2002b). HIGHLIGHTS OF PRESCRIBING INFORMATION. Xeloda.
- Food and Drug Administration. (2009). HIGHLIGHTS OF PRESCRIBING INFORMATION. Cetuximab.
- Food and Drug Administration. (2014). HIGHLIGHTS OF PRESCRIBING INFORMATION. Irinotecan.
- Food and Drug Administration. (2015). HIGHLIGHTS OF PRESCRIBING INFORMATION. Panitumumab.
- Food and Drug Administration. (2017). HIGHLIGHTS OF PRESCRIBING INFORMATION. Bevacizumab.
- Glynne-Jones, R., Wyrwicz, L., Tiret, E., Brown, G., Rödel, C., Cervantes, A., Arnold, D., & ESMO Guidelines Committee. (2017). Rectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, 28, iv22–iv40. <https://doi.org/10.1093/annonc/mdx224>
- Lecomte, T., Ferraz, J. M., Zinzindohoué, F., Lorient, M. A., Tregouet, D. A., Landi, B., Berger, A., Cugnenc, P. H., Jian, R., Beaune, P., & Laurent-Puig, P. (2004). Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. *Clinical Cancer Research*, 10, 5880–5888.
- Lee, S., Emond, M. J., Bamshad, M. J., Barnes, K. C., Rieder, M. J., Nickerson, D. A., NHLBI GO Exome Sequencing Project—ESP Lung Project Team, Christiani, D. C., Wurfel, M. M., & Lin, X. (2012). Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *American Journal of Human Genetics*, 91, 224–237. <https://doi.org/10.1016/j.ajhg.2012.06.007>
- Loganayagam, A., Arenas Hernandez, M., Corrigan, A., Fairbanks, L., Lewis, C. M., Harper, P., Maisey, N., Ross, P., Sanderson, J. D., & Marinaki, A. M. (2013). Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. *British Journal of Cancer*, 108, 2505–2515. <https://doi.org/10.1038/bjc.2013.262>
- Marsh, S., McLeod, H., Dolan, E., Shukla, S. J., Rabik, C. A., Gong, L., Hernandez-Boussard, T., Lou, X. J., Klein, T. E., & Altman, R. B. (2009). Platinum pathway. *Pharmacogenetics and Genomics*, 19, 563–564. <https://doi.org/10.1097/FPC.0b013e32832e0ed7>
- McCarthy, S., das, S., Kretschmar, W., Delaneau, O., Wood, A. R., Teumer, A., Kang, H. M., Fuchsberger, C., Danecek, P., Sharp, K., Luo, Y., Sidore, C., Kwong, A., Timpson, N., Koskinen, S., Vrieze, S., Scott, L. J., Zhang, H., Mahajan, A., ... Haplotype Reference Consortium. (2016). A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics*, 48, 1279–1283. <https://doi.org/10.1038/ng.3643>
- Mizukami, T., Izawa, N., Nakajima, T. E., & Sunakawa, Y. (2019). Targeting EGFR and RAS/RAF signaling in the treatment of metastatic colorectal cancer: From current treatment strategies to future perspectives. *Drugs*, 79, 633–645. <https://doi.org/10.1007/s40265-019-01113-0>
- Morgan, E., Arnold, M., Gini, A., Lorenzoni, V., Cabasag, C. J., Laversanne, M., Vignat, J., Ferlay, J., Murphy, N., & Bray, F. (2023). Global burden of colorectal cancer in 2020 and 2040: Incidence and mortality estimates from GLOBOCAN. *Gut*, 72, 338–344. <https://doi.org/10.1136/gutjnl-2022-327736>
- Offer, S. M., Fossum, C. C., Wegner, N. J., Stuflesser, A. J., Butterfield, G. L., & Diasio, R. B. (2014). Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Research*, 74, 2545–2554. <https://doi.org/10.1158/0008-5472.CAN-13-2482>
- Pullarkat, S. T., & Lenz, H. J. (2001). Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics Journal*, 1, 65–70. <https://doi.org/10.1038/sj.tpj.6500012>
- Romano, M., Baralle, F. E., & Patriarca, P. (2000). Expression and characterization of recombinant human eosinophil peroxidase. Impact of the R286H substitution on the biosynthesis and activity of the enzyme. *European Journal of Biochemistry*, 267, 3704–3711. <https://doi.org/10.1046/j.1432-1327.2000.01403.x>
- Rosmarin, D., Palles, C., Pagnamenta, A., Kaur, K., Pita, G., Martin, M., Domingo, E., Jones, A., Howarth, K., Freeman-Mills, L., Johnstone, E., Wang, H., Love, S., Scudder, C., Julier, P., Fernández-Rozadilla, C., Ruiz-Ponte, C., Carracedo, A., Castellvi-Bel, S., ... Tomlinson, I. (2015). A candidate gene study of capecitabine-related toxicity in colorectal cancer identifies new toxicity variants at DPYD and a putative role for ENOSF1 rather than TYMS. *Gut*, 64, 111–120. <https://doi.org/10.1136/gutjnl-2013-306571>
- RStudio Team. (2020). *RStudio: Integrated development for R*. RStudio, PBC.
- Sato, M., Liu, K., Sasaki, S., Kunii, N., Sakai, H., Mizuno, H., Saga, H., & Sakane, F. (2013). Evaluations of the selectivities of the diacylglycerol kinase inhibitors R59022 and R59949 among diacylglycerol kinase isozymes using a new non-radioactive assay method. *Pharmacology*, 92, 99–107. <https://doi.org/10.1159/000351849>
- Shrestha, S., Zhang, C., Jerde, C. R., Nie, Q., Li, H., Offer, S. M., & Diasio, R. B. (2018). Gene-specific variant classifier (DPYD-Varifier) to identify deleterious alleles of dihydropyrimidine dehydrogenase. *Clinical Pharmacology and Therapeutics*, 104, 709–718. <https://doi.org/10.1002/cpt.1020>
- Simões, A. R., Fernández-Rozadilla, C., Maroñas, O., & Carracedo, Á. (2020). The road so far in colorectal cancer pharmacogenomics: Are we closer to individualised treatment? *Journal of Personalized Medicine*, 10, 1–40.
- The Human Protein Atlas. (n.d.-a). <https://www.proteinatlas.org/ENSG00000157680-DGKI/single+cell+type>
- The Human Protein Atlas. (n.d.-b). <https://www.proteinatlas.org/ENSG00000183873-SCN5A/single+cell+type>
- Traylor, M., Walker, J. L., Corrigan, A. A., Hernandez, M. A., Newhouse, S. J., Folarin, A. A., Patel, H., Ross, P. J., Sanderson, J. D., Spicer, J., Prescott, N. J., Mathew, C. G., Marinaki, A. M., & Lewis, C. M. (2018). Exome array analysis of adverse reactions to fluoropyrimidine-based therapy for gastrointestinal cancer. *PLoS ONE*, 13, e0188911. <https://doi.org/10.1371/journal.pone.0188911>
- Van der Auwera, G. A., & O'Connor, B. D. (2020). *Genomics in the cloud: Using Docker, GATK, and WDL in Terra*. O'Reilly Media.
- Vasiliou, V., & Pappa, A. (2000). Polymorphisms of human aldehyde dehydrogenases. *Pharmacology*, 61, 192–198. <https://doi.org/10.1159/000028400>
- Whirl-Carrillo, M., McDonagh, E. M., Hebert, J. M., Gong, L., Sangkuhl, K., Thorn, C. F., Altman, R. B., & Klein, T. E. (2012). Pharmacogenomics knowledge for personalized medicine. *Clinical Pharmacology and Therapeutics*, 92, 414–417. <https://doi.org/10.1038/clpt.2012.96>
- Willer, C. J., Li, Y., & Abecasis, G. R. (2010). METAL: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, 26, 2190–2191. <https://doi.org/10.1093/bioinformatics/btq340>
- World Medical Association. (2013). World medical association declaration of Helsinki: Ethical principles for medical research involving human

- subjects. *Clinical Review & Education*, 310, 2191–2194. <https://doi.org/10.1001/jama.2013.281053>
- Wu, Q., & Dolnick, B. J. (2003). Detection of thymidylate synthase modulators by a novel screening assay. *Molecular Pharmacology*, 63, 167–173. <https://doi.org/10.1124/mol.63.1.167>
- Zanoni, M., Bravaccini, S., Fabbri, F., & Arienti, C. (2022). Emerging roles of aldehyde dehydrogenase isoforms in anti-cancer therapy resistance. *Frontiers in Medicine*, 9, 1–10.
- Zhan, X., Hu, Y., Li, B., Abecasis, G. R., & Liu, D. J. (2016). RVTESTS: An efficient and comprehensive tool for rare variant association analysis using sequence data. *Bioinformatics*, 32, 1423–1426. <https://doi.org/10.1093/bioinformatics/btw079>

SUPPORTING INFORMATION

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