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Effect of DPYD, MTHFR, ABCB1, XRCC1, ERCC1 and GSTP1 on chemotherapy related toxicity in colorectal carcinoma

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TITLE PAGE

TITLE

EFFECT OF DPYD, MTHFR, ABCB1, XRCC1, ERCC1 AND GSTP1 ON CHEMOTHERAPY RELATED TOXICITY IN COLORECTAL CARCINOMA

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Samples were provided by the Biobank of Andalusia (Collection Code: 19150007). The study was approved by the HUVN Ethics and Research Committee, and was conducted according to the Declaration of Helsinki.

CONSENT TO PUBLISH

I, on behalf of all the authors of this work, give our consent to publish

DATA AVAILABILITY

All data were confidentially recorded at University Hospital. We are not authorized to share our database or make it public.

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

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AUTHORS' CONTRIBUTIONS

The list of the coauthors includes all appropriate persons, and all persons listed have contributed in some way to the design, implementation and/or analysis, interpretation and reporting of the study.

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ABSTRACT

ABCB1, DPYD, MHTFR, XRCC1, ERCC1, GSTP1 and UGT1A1 genetic variants affect proteins related to CRC chemotherapy toxicity

A retrospective cohort study was conducted in 194 CRC patients.

In first line treatment, DPYD rs17376848 AG genotype was associated with hematological toxicity (OR= 4.85; p=0.03); GSTP1 G-allele (OR=3.01; p=0.005) and MTHFR rs1801133 T allele (OR=2.51; p=0.03) with respiratory toxicity; GSTP1 G-allele with cardiovascular toxicity (OR=4.05; p=0.01); ERCC1 rs11615 GG genotype with neurological toxicity (OR=3.98; p=0.01) and with asthenia (OR=2.91; p=0.08); XRCC1 rs1799782 T allele (OR=0.31; p=0.03) and GSTP1 G-allele (OR=1.81;p=0.01) with cutaneous toxicity. In second line treatment, XRCC1 rs1799782 T-allele was associated with asthenia (OR=0.17; p=0.03) and XRCC1 rs25487 T-allele with gastrointestinal toxicity (OR=3.03; p=0.005). After stratifying by treatment, in the 5-Fluorouracil group, the DPYD rs17376848 AG genotype was associated with hematological toxicity (OR=2.76; p=0.003), ABCB1 rs1045642 T-allele with the need of treatment adjustment due to toxicity (OR=3.06; p=0.01), and rs1045642 CC genotype with gastrointestinal toxicity (OR=5.80;p=0.03). In the capecitabine group, the MTHFR rs1801131 CC genotype was associated with asthenia (OR=3.48; p=0.009). In the oxaliplatin group, rs1045642 TT genotype was associated with the need to adjust treatment (OR=0.32; p=0.02), ERCC1 rs11615 GG genotype with asthenia (OR=3.01; p=0.01) and rs1615 GSTP1 GG genotype with respiratory toxicity (OR=5.07; p=0.009).

ABCB1 rs1045642 T-allele reduces the need for treatment modification with both 5FU and oxaliplatin. Although several biomarkers predicted different toxic effects, they cannot be considered as risk factors for severe toxicity.

INTRODUCTION

Surgery is the treatment of choice in colorectal cancer (CRC) early stages. From stage III, chemotherapy is recommended as adjuvant treatment after surgery, or as only treatment if surgery is not possible. The main drug in CRC chemotherapy treatment is 5-Fluorouracil (5FU) or Capecitabine, its oral prodrug, used either in monotherapy or more frequently, in combination with Oxaliplatin and/or Irinotecan [1]. Chemotherapy toxic effects are numerous and, in some cases, can compromise the patient's life. However, there is variability in the intensity of adverse reactions, being even mild or even non-existent in a significant percentage of patients [2–5]. As toxicity increases with the time of exposure to the chemotherapeutic agent, it seems reasonable that

genetic variants affecting the correct functioning of proteins involved in drug's ADME process (absorption, distribution, metabolism and elimination) are, in some way, related to drug's toxicity. Some genetic variants of the *DPYD* gene [6–13], that encodes dihydropyrimidine dehydrogenase (DPD), the enzyme responsible for the hepatic metabolism of 5FU and capecitabine, have been related to the fluoropyrimidines toxicity. The variants that deeply decrease the enzyme activity are rs3918290 (*DPYD**2A), rs55886062 (*DPYD**13) and rs67376798, while the effect of rs17376848 results in a moderate reduction [14].

The ATP-binding cassette sub-family B member 1 (*ABCB1*) is a transport protein, encoded by *ABCB1* gene [15], expels xenobiotics (as toxins or drugs) previously absorbed. Some tumor cells overexpress a large amount *ABCB1*, which is an important cause of the multiresistance of some tumors to chemotherapy [16,17]. The rs1045642 polymorphism of *ABCB1* [18–20] have been broadly related to the toxicity of these drugs.

The *MTHFR* gene encodes an enzyme called methylenetetrahydrofolate reductase. This enzyme, catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and plays an important role in DNA synthesis. The TT and CT genotypes of rs1801833 polymorphism in *MTHFR* have been associated with increased risk of diarrhea, mucositis and neutropenia [21], whereas the CC genotype has been described as a risk factor for severe toxicity after 5FU treatment in CRC patients [22].

X-ray repair cross complementing 1 (*XRCC1*) and Excision Repair Cross-Complementing group 1 (*ERCC1*) are proteins encoded by the gene of the same name whose function is to repair DNA damaged by ionizing radiation and alkylating agents such as oxaliplatin and irinotecan [23,24].

GSTP1 (Glutathione S-Transferase) is an enzyme that plays an important role in the detoxification of xenobiotics, encoded in humans by *GSTP1* gene [25]. Polymorphisms in genes *XRCC1*, *ERCC1* and *GSTP1* gene have been associated with toxicity to both oxaliplatin and irinotecan schemes [18,20,26–29]

The aim of this study was to investigate the influence of *DPYD*, *MTHFR*, *ABCB1*, *ERCC1*, *XRCC1* and *GSTP1* polymorphisms on the any kind of toxic effects induced by chemotherapy in CRC patients.

MATERIAL AND METHODS

A retrospective observational cohort study was conducted.

Study population

Data were obtained from 194 CRC patients treated at the Oncology Unit of the Hospital Universitario

Virgen de las Nieves (HUVN). New toxicity data were collected from 86 patients who received a second line of treatment at the same hospital. All patients were Caucasians older than 18 years. Exclusion criteria included incomplete clinical history or synchronous neoplasm different from CRC. The study was approved by the HUVN Ethics and Research Committee, and was conducted according to the Declaration of Helsinki. All data were confidentially recorded.

Clinical and sociodemographic variables

Variables collected were: chemotherapy treatment regimen, toxicity according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE v. 4.0) [30], diagnosis, date and age of diagnosis, sex, tumor size, TNM tumor staging according to the American Joint Committee of Cancer (AJCC) classification [31], histopathology, date of surgery, date of death (if applicable), Eastern Cooperative Oncology Group (ECOG) Performance Status and modification of chemotherapy treatment (dose reduction, delay or suspension).

Genetic variables

Samples of paraffin-embedded healthy tissue from surgical resection were provided by the Biobank of Andalusia (Collection Code: 19150007). Only healthy tissue was used, since we were looking for polymorphisms in the germline. DNA was extracted using the QIAamp DNA Mini Kit, following manufacturer's instructions and stored at -40°C. The following polymorphisms were analyzed by Real-Time PCR with TaqMan® probes: *DPYD* rs3918290, rs55886062, rs67376798 and rs17376848, *ABCB1* rs1045642, *MTHFR* rs1801133 and rs1801131, *XRCC1* rs25487 and rs1799782, *ERCC1* rs11615 and rs3212986, *GSTP1* rs1695 and *UGT1A1* rs4148323. As quality control of the methodology, twenty percent of samples were confirmed by Sanger sequencing.

Dependents variables

Toxicity grade (1-2 vs 3-4) and type (asthenia, neurological, hematological, cutaneous, gastrointestinal, cardiovascular, respiratory or infusional reaction) were analyzed. In the analysis stratified by treatment subgroups, the modification of treatment was also evaluated (dose reduction, delay or suspension due to toxicity).

Statistical analysis

The software used was R 2.3.0 (29). Shapiro-Wilks test determined the normality of the variables. Non-normally distributed quantitative variables were described by medians and interquartile range, qualitative variables as frequencies. Pearson chi-square/Fisher for qualitative and Wilcoxon tests for

quantitative were used for bivariate analysis. Bonferroni correction was applied (significance threshold: $p < (0.05/\text{number of SNPs})$). Significance level for any other test was $p < 0.05$. Multivariate analysis was carried out using a logistic regression model including all bivariate associations with $p < 0.1$ as potential confounders. Hardy-Weinberg equilibrium and disequilibrium linkage was performed using PLINK v1.90b4.5 [32]. Allelic population frequencies were compared with the Iberian Population in Spain [33]. The genetic models were defined as follows: genotypic (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes), allelic (minor allele vs. major allele), trend (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes), dominant (minor allele homozygotes plus heterozygotes vs. major allele homozygotes) and recessive (minor allele homozygotes vs. heterozygotes plus major allele homozygotes) [34]. In the total sample (194 first-line patients) and (86 second-line patients), toxicity to any CRC chemotherapy scheme was evaluated (Figure 1). A second analysis stratifying the sample according to treatment was performed. Toxicities included in this analysis were those associated with the main drug in the therapy scheme, according to the drug's prescribing information [2–5]. Only the genes associated with toxicity in previous studies were analyzed in each subgroup, according to the 'Variant Annotations' information provided by The Pharmacogenomics Knowledge Base [35]. In first line, 103 patients were treated with schemes containing 5FU, 84 patients with therapy based on capecitabine and 166 patients with oxaliplatin. In second line, 54 patients were treated with irinotecan based schemes (Figure 2).

RESULTS

Patients Characteristics

The total sample (first-line) included 194 patients that received 5FU, capecitabine, oxaliplatin or irinotecan based schemes. All patients were homozygous (no variant was found) for *DPYD* rs3918290, *DPYD* rs55886062 and *UGT1A1* rs4148323, therefore they were not included in the association analysis. No variants were found in the Spanish population (Iberian Population in Spain, IBS) of The 1000 Genomes Project for these single nucleotide polymorphisms (SNPs) either [33]. Minor allele frequencies (MAF) were calculated and results are shown in Supplementary Table 1. All genes were in linkage disequilibrium (Supplementary Table 2). Most of the SNPs were in Hardy-Weinberg equilibrium, with the exception of *ABCB1* rs1045642 ($p=0.0002852$) and *MTHFR* rs1801131 ($p=0.03836$). No statistical differences with those described for the Iberian population [33] were found for either of these variants (*ABCB1* rs1045642 T allele: 0.47 vs 0.46; $p=0.8873$;

MTHFR rs1801131 C allele: 0.35 vs 0.27; $p=0.2213$). Baseline characteristics of the total sample and each subgroup of treatment patients are summarized in Table 1.

Influence of clinical and genetic variables on treatment toxicity in first line of treatment

Bonferroni adjusted p -value of 0.005 (0.05/10 SNPs) was considered for bivariate analysis both in first and second line.

Hematological Toxicity. In the bivariate analysis, two variables tended to increase the toxicity risk: the *DPYD* rs17376848 AG genotype ($RR_{REC}=2.28$; CI95%, 1.36-3.82; $p=0.02665$) and tumor localization in colon ($RR=1.56$; CI95%, 0.90-2.70; $p=0.09761$). In the multivariate analysis, the *DPYD* rs17376848 AG genotype increased hematological toxicity risk ($OR_{REC}=4.85$; CI95%, 1.23-23.65; $p=0.0297$).

Cardiovascular Toxicity. Patients with *GSTP1* rs1695 G allele presented higher risk of cardiovascular toxicity ($RR_{TREND}=3.09$; CI95%, 1.13-8.48; $p=0.004486$), while the T allele of *XRCC1* rs25487 showed a trend ($RR_{TREND}=3.02$; CI95%, 1.03-8.83; $p=0.04015$). The independent association of *GSTP1* rs1695 G allele with higher cardiovascular toxicity risk was confirmed in multivariate analysis ($OR_{TREND}=4.05$; CI95%, 1.40-13.50; $p=0.0129$).

Neurological Toxicity. ECOG 0 patients had a greater risk of neurological toxicity ($RR=1.48$, CI95%, 1.05-2.09; $p=0.004287$). Other risk factors were adenocarcinoma ($RR=1.52$, CI95%, 1.09-2.13; $p=0.01639$) and younger age at diagnosis (61.01 [56.05-66.24] vs 70.21 [61.64-74.78] years; $p=1.8 \cdot 10^{-05}$). Patients with CC genotype for *MTHFR* rs1801133 ($RR_{DOM}=1.19$; CI95%, 0.98-1.43; $p=0.05093$) or *ERCC1* rs11615 GG genotypes ($RR_{REC}=1.20$; CI95%, 1.03-1.39; $p=0.07193$) showed higher toxicity risk trends. Multivariate analysis confirmed the protective effect of older age ($OR=0.92$, CI95%, 0.89-0.97; $p=0.000417$) and the adenocarcinoma histopathology as risk factor for neurological toxicity ($OR=4.22$, CI95%, 1.45-12.45; $p=0.007827$). An interaction between histopathology and age was found, showing that patients with adenocarcinoma and older age had a lower risk of neurological toxicity ($OR=0.89$, CI95%, 0.82-0.97; $p=0.00798$). *ERCC1* rs11615 GG genotype ($OR_{REC}=3.98$; CI95%, 1.17-19.38; $p=0.047893$) was also associated with an increased toxicity.

Cutaneous Toxicity. Women ($RR=1.42$; CI95%, 1.02-1.98; $p=0.04153$) and patients with tumor localization in colon ($RR=1.87$; CI95%, 1.15-3.03; $p=0.004654$) showed higher cutaneous toxicity risk. The *GSTP1* rs1695 G allele ($RR_{TREND}=1.54$; CI95%, 1.13-2.10; $p=0.008009$) and the GG genotype of *ERCC1* rs3212986 ($RR_{DOM}=1.50$; CI95%, 1.07-2.10; $p=0.01798$) showed a trend to increased cutaneous toxicity. The *XRCC1* rs1799782 T allele ($RR_{TREND}=0.50$, CI95%, 0.23-1.10; $p=0.04551$)

showed a trend to reduced cutaneous toxicity. Tumor location in colon (OR=2.63; CI95%, 1.30-5.54; p=0.00847), as well as G allele of *GSTP1* rs1695 (OR_{TREND}=1.81; CI95%, 1.52-2.89; p=0.01113), were confirmed as risk factors for cutaneous toxicity in the multivariate analysis, while the T allele of *XRCC1* rs1799782 was confirmed as protective factor (OR_{TREND}=0.31; CI95%, 0.10-0.82; p=0.029).

Asthenia. The GG genotype for *ERCC1* rs11615 and the T allele for *MTHFR* rs1801133 showed a tendency to increase the risk of suffering from asthenia (RR_{REC}=1.77, CI95%, 1.23-2.54; p=0.007205); (RR_{TREND}=1.24; CI95%, 0.96-1.60; p=0.09112). In multivariate analysis, only the effect of *ERCC1* rs11615 GG genotype on asthenia was confirmed (OR_{REC}=2.91, CI95%, 1.32-6.64; p=0.00894).

Respiratory Toxicity. It occurred more frequently in women (RR=2.76; CI95%, 1.05-7.27; p=0.03176), in younger patients (57.57 [53.08-61.56] vs 63.09 [57.02-69.72] years; p=0.0342), and those carrying the G allele of *GSTP1* rs1695 (RR_{TREND}=2.63; CI95%, 1.36-5.08; p=0.004281) or the G allele of *DPYD* rs17376848 (RR_{TREND}=5.97; CI95%, 1.43-24.85; p=0.00363). *MTHFR* rs1801133 T allele showed a tendency to increase the risk of respiratory toxicity (RR_{TREND}=1.88; CI95%, 0.97-3.67; p=0.04628), as did the TT genotype of *ERCC1* rs3212986 (RR_{REC}=2.93; CI95%, 1.05-8.18; p=0.06406). In the multivariate analysis, *MTHFR* rs1801133 T allele (OR_{TREND}=2.51; CI95%, 1.32-6.64; p=0.00894), as well as the G allele of *GSTP1* rs1695 (OR_{TREND}=3.01; CI95%, 1.43-6.72; p=0.00469), were confirmed as risk factors for increased respiratory toxicity risk.

Bivariate analysis in first treatment line are shown in Supplementary Table 3 (clinicopathological characteristics) and Supplementary Table 4 (SNPs). Table 2 shows the multivariate analysis.

Clinical and genetic variables influence on treatment toxicity in second line of treatment

Asthenia. Patients in stages III-IV (RR=0.17; CI95%, 0.03-0.83; p=0.014) or carrying the *XRCC1* rs1799782 T allele (RR_{TREND}=0.35; CI95%, 0.09-1.27; p=0.05764) had less risk of asthenia. These protective effects were both confirmed in the multivariate analysis (OR=0.08; CI95%, 0.004-0.43; p=0.0168); (OR_{TREND}=0.17; CI95%, 0.03-0.69; p=0.0291).

Neurological Toxicity. Patients with larger tumor size experienced more toxicity (5.50 [3.87-7.17] vs 4.15 [3.05-5.00] cm; p=0.02061), while being female was a protective factor (RR=0.42, CI95%, 0.17-1.00; p=0.045). In the multivariate analysis, only larger tumor size was confirmed as a risk factor for neurological toxicity (OR=1.36, CI95%, 1.07-1.80; p=0.01652).

Gastrointestinal Toxicity. *XRCC1* rs25487 C allele increased toxicity risk (RR_{TREND}=2.19; CI95%, 1.25-3.84; p=0.003465), while *GSTP1* rs1695 G allele (RR_{TREND}=0.79; CI95%, 0.60-1.03; p=0.04375) and *MTHFR* rs1801131 CC genotype showed a tendency to decrease it (RR_{REC}=0.64; CI95%, 0.33-1.24; p=0.08628). In the multivariate analysis, the *XRCC1* rs25487 T allele was confirmed as a risk factor

for gastrointestinal toxicity.

Cutaneous Toxicity. The AA genotype of *ERCC1* rs11615 showed a tendency to increased cutaneous toxicity ($RR_{DOM}= 1.56$; CI95%, 0.90-2.72; $p=0.09519$), which was not confirmed in multivariate analysis.

Infusional Reaction. Patients with T allele for *XRCC1* rs1799782 experienced an increased risk of infusional reaction, but without being significant after Bonferroni correction ($RR_{TREND}= 3.87$; CI95%, 1.38-10.84; $p=0.0157$).

Cardiovascular Toxicity. The AA genotype of *ERCC1* rs11615 showed a tendency to decrease the risk of cardiovascular toxicity ($RR_{DOM}= 0.14$; CI95%, 0.02-1.15; $p=0.07654$).

Hematological toxicity. The CC genotype of *XRCC1* rs1799782 increased the risk of hematological toxicity ($RR_{DOM}=2.09$; CI95%, 1.06-4.12; $p=0.09903$), although not significantly after Bonferroni correction.

Bivariate analysis in patients in second-line of treatment are shown in Supplementary Table 5 (clinicopathological characteristics) and Supplementary Table 6 (SNPs). Multivariate analysis for second line of treatment is shown in Table 2.

Clinical and genetic variables influence on treatment toxicity with 5-Fluorouracil in first line

Bonferroni adjusted p-value of 0.01 (0.05/5 SNPs) was considered as significant for bivariate analysis in both 5FU and capecitabine treatment groups.

Grade 3-4 Toxicity. Tumor location in rectum showed a tendency to increase risk of grade 3-4 toxicity during treatment with 5FU ($RR=2.45$; CI95%, 0.81-7.41; $p=0.07505$).

Modification of treatment. Women ($RR=1.32$; CI95%, 1.02-1.72; $p=0.0428$) and patients whose tumors were located in rectum ($RR=1.86$; CI95%, 1.11-3.12; $p=0.001912$) needed more modifications during treatment. The T allele of *ABCB1* rs1045642 tended to decrease the need of treatment modifications with 5FU ($RR_{TREND}=0.86$; CI95%, 0.71-1.05; $p=0.0720$). This association ($OR_{TREND}=3.06$; CI95%, 1.32-7.69; $p=0.012073$) was confirmed in multivariate analysis, along with rectal cancer ($OR=6.51$, CI95%, 2.24-20.67; $p=0.000844$).

Gastrointestinal Toxicity. In the bivariate analysis, only trends were obtained; older patients and those with *ABCB1* rs1045642 CC genotype showed a trend to increased gastrointestinal risk (60.9 [56.4-64.5] vs 58.4 [51.5-63.6] years; $p=0.09109$); ($RR_{DOM}=1.29$; CI95%, 1.05-1.59; $p=0.0643$). Age of patients and *ABCB1* rs1045642 CC genotype were furtherly confirmed as gastrointestinal toxicity risk factors during 5FU treatment by multivariate analysis ($OR= 1.06$; CI95%, 1.01-1.13; $p=0.0369$); ($OR_{DOM}= 5.80$; CI95%, 1.40-40.37; $p=0.0320$).

Respiratory toxicity. Larger tumor size and different histopathology from adenocarcinoma were risk factors for respiratory toxicity during treatment with 5FU (7.0 [6.0-10.0] vs 4.5 [3.5-6] cm; $p=0.02117$); (RR=0.10, CI95%, 0.02-0.42; $p=0.009267$). In multivariate analysis, both histopathology adenocarcinoma (OR=0.11, CI95%, 0.01-1.10; $p=0.036079$) and smaller tumor size (OR=0.63, CI95%, 0.39-0.95; $p=0.036079$) were confirmed as protective factors for respiratory toxicity.

Infusional reaction. Tumor size was related to experiencing an infusional reaction risk during 5FU treatment (7.0 [4.0-8.7] vs 4.5 [3.5-5.9]; $p=0.03833$).

Hematological Toxicity. The *DPYD* rs17376848 AG genotype increased the risk of experiencing haematological toxicity during treatment with 5FU (RR_{REC}=2.76; CI95%, 2.11-3.62; $p=0.00322$).

Bivariate analysis is shown in Supplementary Table 7 and multivariate analysis in Table 3.

Clinical and genetic variables influence on treatment toxicity with Capecitabine in first line

Grade 3-4 Toxicity. Women experienced less high-grade toxicities during treatment with capecitabine (RR=0.37; CI95%, 0.14-0.98; $p=0.02582$). Patients carrying the CC genotype in *ABCB1* rs1045642 had lower risk of toxicity 3-4 (RR_{DOM}= 0.31; CI95%, 0.08-1.19; $p=0.04444$). Female gender was associated with grade 3-4 toxicity during treatment with capecitabine in multivariate analysis (OR=0.27; CI95%, 0.07-0.85; $p=0.037$).

Modification of treatment. Only two trends were obtained: the TT genotype of *ABCB1* rs1045642 reduced the risk of treatment modifications (RR_{REC}=0.58; CI95%, 0.28-1.20; $p=0.0767$), whereas the CC genotype of *MTHFR* rs1801131 increased it (RR_{REC}=1.50; CI95%, 1.05-2.15; $p=0.07181$).

Respiratory Toxicity. Being a woman (RR=4.10; CI95%, 1.15-14.66; $p=0.03115$) and being younger (58.0 [56.2-60.5] vs 69.5 [62.1-73.0] years; $p=0.003536$) were risk factors for respiratory toxicity during treatment with capecitabine. Patients carrying the T allele of *MTHFR* rs1801133 showed a tendency to increased risk (RR_{TREND}=1.81; CI95%, 0.79-4.14; $p=0.02704$). Female gender and age were confirmed, in the multivariate analysis, as risk factors for respiratory toxicity during treatment with capecitabine (OR=4.78; CI95%, 1.11-25.54; $p=0.0435$); (OR=1.10; CI95%, 1.02-1.20; $p=0.0148$).

Cutaneous toxicity. Patients with colon cancer experienced more cutaneous toxicity than those with rectal cancer (RR=1.88; CI95%, 0.97-3.61; $p=0.04013$).

Infusional reaction. Women experienced infusional reaction more frequently during treatment (RR=2.76; CI95%, 1.20-6.34; $p=0.01269$).

Cardiovascular toxicity. Women experienced more cardiovascular toxicity than men (RR=2.96; CI95%, 2.17-4.05; $p=0.01922$).

Asthenia. The CC genotype of *MTHFR* rs1801131 was found to be a risk factor for asthenia

(RR_{REC}=3.48; CI95%, 1.81-6.66; p=0.009395).

Bivariate and multivariate analyses can be found in Supplementary Table 8 and Table 3, respectively.

Clinical and genetic variables influence on treatment toxicity with Oxaliplatin in first line

Bonferroni adjusted p-value of 0.008 (0.05/6 SNPs) was considered as significant threshold for bivariate analysis.

Grade 3-4 Toxicity. An advanced ECOG showed a trend to lower toxicity grade 3-4 (RR=0.55; CI95%, 0.32-0.94; p=0.05074) and the GG genotype of *GSTP1* rs1695 with the increased risk (RR_{DOM}=1.61; CI95%, 0.98-2.64; p=0.05566), although none of them were significant.

Modification of treatment. Patients with colon cancer needed more treatment modifications than those with rectal cancer (RR=1.59; CI95%, 1.16-2.19; p=0.0005318). The TT genotype of *ABCB1* rs1045642 showed a tendency to reduce the need to adjust the treatment (RR_{REC}=0.67; CI95%, 0.43-1.04; p=0.02556). In multivariate analysis, patients with colon cancer needed more modifications in their treatment than those patients with rectal cancer (OR=3.74; CI95%, 1.79-7.96; p=0.000495), whereas patients who presented the TT genotype of *ABCB1* rs1045642 needed fewer changes (OR_{REC}=0.32; CI95%, 0.12-0.82; p=0.018370). The percentage of patients carrying the TT genotype that required modification was not different regardless the use of FOLFOX or XELOX in first line (53.48 vs 40%; p=0.5).

Infusional Reaction. Female gender was associated with increased risk of infusional reaction during treatment with oxaliplatin (RR=1.62; CI95%, 0.92-2.87; p=0.09564), although it was not significant.

Respiratory Toxicity. Age (60.9 [55.1-66.2] vs 64.6 [57.6-70.2]; p=0.02921), female gender (RR=3.25; CI95%, 1.16-9.05; p=0.01681) and the G allele of *GSTP1* rs1695 (RR_{TREND}=2.04; CI95%, 0.96-4.37; p=0.003047) were associated with an increased risk of respiratory toxicity. The GG genotype of *GSTP1* rs1695 (RR_{REC}=3.94; CI95%, 1.52-10.25; p=0.01503) and the TT genotype of *ERCC1* rs3212986 showed a tendency to increase the risk of respiratory toxicity (RR_{REC}=2.87; CI95%, 1.02-8.07; p=0.04715), while the AA genotype of *GSTP1* rs1695 showed a tendency to decrease it (RR_{DOM}=0.24; CI95%, 0.07-0.83; p=0.0127). In multivariate analysis, the GG genotype of *GSTP1* rs1695 was found to be a risk factor for respiratory toxicity during treatment with oxaliplatin (OR_{REC}=5.07; CI95%, 1.41-16.8; p=0.00879).

Asthenia. Only trends were obtained; the diagnosis of colon cancer (RR=1.52; CI95%, 0.93-2.46; p=0.07184), the GG genotype of *ERCC1* rs11615 (RR_{REC}=1.74; CI95%, 1.21-2.52; p=0.009538) and the TT genotype of *ERCC1* rs3212986 (RR_{REC}=1.61; CI95%, 1.05-2.46; p=0.06031) tended to increase the risk of asthenia. In the multivariate analysis, patients homozygous for *ERCC1* rs11615 G allele

experienced increased risk of asthenia compared to heterozygous or homozygous for the A allele ($OR_{REC}=3.01$; CI95%, 1.30-7.32; $p=0.0118$).

Neurological Toxicity. A histopathology of adenocarcinoma tumor showed a tendency to increase the risk of neurological toxicity ($RR=1.26$; CI95%, 0.90 -1.76; $p=0.0659$).

Bivariate analysis for patients who included oxaliplatin in their treatment are detailed in Supplementary Table 9, while multivariate analysis is shown in Table 3.

Clinical and genetic variables influence on treatment toxicity with Irinotecan in second line

Five SNPs were analyzed: *ABCB1* rs1045642, *XRCC1* rs25487, *XRCC1* rs1799782, *ERCC1* rs11615 and *ERCC1* rs3212986. Therefore, a p-value adjusted by Bonferroni of 0.01 (0.05/5) was taken for bivariate analysis. In multivariate analysis, the threshold for a significant p-value was 0.05.

In the bivariate analysis of this treatment subgroup (54 patients), only tendencies were found (Supplementary Table 10). No independent association was found in multivariate analysis.

Grade 3-4 Toxicity. Patients homozygous for the T allele of *XRCC1* rs25487 ($RR_{REC}=2.44$; CI95%, 0.99-6.04; $p=0.0842$) tended to experience more grade 3-4 toxicity, while those with the G allele of *ERCC1* rs11615 showed a tendency to experience lower grade toxicities ($RR_{TREND}=0.51$; CI95%, 0.22-1.16; $p=0.0761$).

Modification of treatment. Patients with the TT genotype of *XRCC1* rs25487 showed a tendency to more treatment modifications ($RR_{REC}=1.67$; CI95%, 1.31-2.12; $p=0.04032$).

Gastrointestinal Toxicity. Patients in advanced stages of disease showed a tendency to experience less gastrointestinal toxicity ($RR=0.56$; CI95%, 0.24-1.35; $p=0.0914$).

Hematological Toxicity. Patients carrying the T allele of *ABCB1* rs1045642 showed a tendency to experienced less hematological toxicity during treatment with irinotecan ($RR_{TREND}=0.55$; CI95%, 0.27-1.12; $p=0.06919$).

Asthenia. The CC genotype of *XRCC1* rs1799782 showed a tendency to increase the risk ($RR_{DOM}=4.74$; CI95%, 0.72-31.34; $p=0.06542$), whereas female gender showed a tendency to protect from suffering asthenia ($RR=4.74$; CI95%, 0.72-31.34; $p=0.06542$).

Neurological Toxicity. Patients with the T allele of *XRCC1* rs25487 showed a tendency to have a lower risk of neurological toxicity ($RR_{TREND}=0.18$; CI95%, 0.02-1.35; $p=0.07869$).

DISCUSSION

Toxicity degree 3-4 is de most frequently outcome in the literature. We analyzed all grades of toxicity

and modification of treatment was also evaluated (dose reduction, delay or suspension due to toxicity) to make sure that no toxicity that could influence the treatment was left out.

DPYD is the gene that encodes dihydropyrimidine dehydrogenase (DPD), the enzyme responsible for the hepatic metabolism of 5FU and capecitabine. The variants that deeply decrease the enzyme activity are rs3918290 (*DPYD**2A), rs55886062 (*DPYD**13) and rs67376798, while the effect of rs17376848 results in a moderate reduction [14]. In the Spanish population (Iberian Population in Spain, IBS) represented in The 1000 Genomes Project, variant alleles for rs3918290, rs55886062 and rs67376798 are not found and the frequency of the rs17376848 variant is nearly rare (0.0187, 4/214) [33]. In our study, none of the 194 patients were carriers of rs3918290 and rs55886062 variants, whereas we found two patients with the rs67376798 variant; unfortunately, this finding was insufficient to find associations with the risk of toxicity. However, the nine carriers found for rs17376848 variant had an increased risk of hematological toxicity, both in the overall first line group ($OR_{REC}=4.85$; CI95%, 1.23-23.65; $p=0.03$) and in the subgroup treated with 5FU ($RR_{REC}=2.76$; CI95%, 2.11-3.62; $p=0.003$). Among the six patients treated with FOLFOX, two developed neutropenia, one thrombocytopenia and another one experienced both neutropenia and thrombocytopenia during treatment. Only in one case of neutropenia, the grade was considered severe (3). No association was obtained in the capecitabine subgroup, maybe because only two of the nine carriers found for rs17376848 variant allele were in this group. One of them received neither 5FU nor capecitabine in their treatment, so it was not taken into account. Our findings are in agreement with those obtained by other authors. A study of 64 Italian patients found the AG genotype of rs17376848 associated with grade 3-4 toxicity to capecitabine, irinotecan and oxaliplatin (COI scheme), ($OR=14.53$; CI95%, 1.36-155.20; $p=0.03$)[7]. Teh et al. [9] also associated the G allele of rs17376848 with the risk of neutropenia during treatment with 5FU in 26 patients of Asian origin ($OR=2.3$; CI95%, 1.01-5.09; $p=0.02$). In Asian countries, the frequency of this variant is higher (0.1212 for the Chinese population and 0.1202 for the Japanese population) [33], which could explain the result despite the small sample. Kristensen [8] obtained a similar result in 68 European colon cancer patients where the variant was related to higher risk of toxicity to 5FU and capecitabine ($RR=6$; $p=0.01$). On the other hand, subsequent studies conducted in the populations of two clinical trials (CAIRO2, 508 patients and TOSCA, 100 patients) did not associate the AG genotype of rs17376848 with the risk of severe toxicity during treatment with 5FU or capecitabine ($RR=0.9$; CI95%, 0.3-3.0; $p=0.9$)[36]; ($OR=1.34$; CI95%, 0.73-2.49; $p=0.5$)[6]. The disparity in the results may be due to more rigid criteria to define a toxic event as severe used in clinical trials, compared to smaller studies, resulting probably in

leaving out toxic events due to the moderate effect of *DPYD*-rs17376848 on DPD activity.

The ATP-binding cassette sub-family B member 1 (*ABCB1*) is a transport protein, encoded by *ABCB1* gene [15], expels xenobiotics (as toxins or drugs) previously absorbed. Some tumor cells overexpress a large amount *ABCB1*, which is an important cause of the multiresistance of some tumors to chemotherapy [16,17]. In our study, patients treated with 5FU carrying the T allele of rs1045642 required treatment adjustment due to toxicity (OR=3.06; CI95%, 1.32-7.69; p=0.01), whereas in the group treated with Oxaliplatin, a smaller number of patients with the TT genotype needed adjustment of the treatment (OR=0.32; CI95%, 0.12-0.82; p=0.02). This result, a priori, may be meaningless, since in the treatment group with 5FU, 97% (100/103) of the patients were treated with both drugs in combination (FOLFOX). However, in the oxaliplatin treatment group, in addition to the 100 patients undergoing treatment with FOLFOX, 64 patients under treatment with XELOX were also analyzed and this "protective effect" seemed to be related to XELOX scheme, since in the Capecitabine group (where there was no FOLFOX), a similar result was obtained (RR=0.58, CI95%, 0.28-1.20; p=0.08) although did not become significant. To verify this hypothesis, we also compared the percentage of treatments in the first line with FOLFOX or with XELOX of patients carrying the TT genotype that required modification (53.48 vs 40%; p=0.5), although there was no difference, so to elucidate this, a larger sample would be needed. There was also an association between the CC genotype and the risk of gastrointestinal toxicity during treatment with 5FU (OR=5.80, 95% CI, 1.40-40.37; p=0.03). Other authors [19,20] have linked the CC genotype with the lower incidence of diarrhea during treatment based on irinotecan (RR=0.27; CI95%, 0.16-1.03; p=0.04); (OR=0.162; CI95%, 0.031-0.844; p=0.03).

In theory, by reducing the activity of the enzyme that is encoded, polymorphisms in the *MTHFR* gene would enhancing both the effect and the toxicity of fluoropyrimidines [37]. Our results showed an association between the TT genotype of *MTHFR* rs1801133 and the higher risk of presenting a respiratory toxicity episode during the treatment in the first line (OR=2.51; CI95%, 1.10-6.10; p=0.03). This result was only reflected in the subgroup of capecitabine treatment as a trend (RR=1.81; CI95%, 0.79-4.14; p=0.02). No study has associated the rs1801133 polymorphism with capecitabine toxicity [37–39]. In agreement with this, the CT + TT genotypes were related to increased diarrhea risk (OR=2.14; CI95%, 1.01-4.56; p=0.04), mucositis (OR=8.17; CI95%, 1.25-53.61; p=0.03) and neutropenia (OR=3.03; CI95%, 1.39-6.60; p=0.005) during treatment with 5 FU in 161 patients of Asian origin [21]. Korean patients who carried the TT genotype were at greater risk of experiencing severe toxicity with FOLFOX than the CT and CC genotypes (OR=2.32; CI95%, 1.19-4.55;

p=0.01)[29]. In contrast, in a study of 130 Italian patients, the CC genotype was associated with an increase in non-specific severe toxicity (OR=3.10; CI95%, 1.21-7.94; p=0.04) [22]. Patients included in our study who carried the CC genotype of the *MTHFR* variant rs1801131 had a higher risk of asthenia during treatment with capecitabine (OR=3.48; CI95%, 1.81-6.66; p=0.009), consistent with the development of some episode of grade 2-3 toxicity during treatment with capecitabine (OR=0.06; CI95%, 0.01-0.3, p < 0.05), according to a small study of 54 patients [39]. Other studies have not found an effect of rs1801131 on the risk of experiencing severe toxicity (grades 3-4) (22,37).

X-ray repair cross complementing 1 (XRCC1) and Excision Repair Cross-Complementing group 1 (ERCC1) are proteins encoded by the gene of the same name whose function is to repair DNA damaged by ionizing radiation and alkylating agents such as oxaliplatin and irinotecan [23,24]. Our results showed an association of the T allele of *XRCC1* rs1799782 with the reduction of the risk of experiencing cutaneous toxicity in the first line of treatment (OR=0.31, CI95%, 0.10-0.82; p=0.03). This finding was not replicated in the stratification by treatment. In second line, the T allele of *XRCC1* rs1799782 protected from asthenia (OR=0.17, CI95%, 0.03-0.69; p=0.03) and the T allele of *XRCC1* rs25487 increased gastrointestinal toxicity (OR=3.03; CI95%, 1.47-7.01; p=0.005). Although over 60% of the second line treatments contained irinotecan, results in this subgroup remained as trends, probably due to the small sample size: the CC genotype of *XRCC1* rs1799782 was associated with an increased risk of asthenia (RR=4.74; CI95%, 0.72-31.34; p=0.006542), the presence of the T allele of *XRCC1* rs25487 with severe toxicity (RR_{REC}=2.44; CI95%, 0.99-6.04; p=0.08) and with less neurological toxicity (RR_{TREND}=0.18; CI95%, 0.02-1.35; p=0.08). The CC genotype of rs25487 had been associated with a lower risk of neuropathy during treatment with FOLFOX in 292 Asian patients (HR=0.56, CI95%, 0.32-0.99; p=0.04) [29]. However, McLeod and colleagues found no association between the *XRCC1* variants and 114 patients treated with irinotecan, leucovorin and 5FU [18]. This is the first study to evaluate variant rs1799782 in colorectal cancer, so the results cannot be compared with previous studies. In our study, patients with the GG genotype of *ERCC1* rs11615 had a greater risk of asthenia, both in the first line (OR=2.91; CI95%, 1.32-6.64; p=0.009), as the subgroup whose treatment contained oxaliplatin (OR=3.01; CI95%, 1.30-7.32; p=0.01). First-line patients also had a higher risk of neurological toxicity (OR=3.98; CI95%, 1.17-19.38; p=0.04), although this result was not reflected in any treatment subgroup. In the literature, *ERCC1* rs11615 had been linked to the risk of neutropenia during treatment with oxaliplatin in Asian population (AA genotype; OR=4.58; CI95%, 1.20-17.40; p=0.026)[29] and in Spanish population (GG genotype; OR=0.20; CI95%, 0.06-

0.69; p=0.01)[20].

GSTP1 (Glutathione S-Transferase) is an enzyme that plays an important role in the detoxification of xenobiotics, encoded in humans by *GSTP1* gene [25]. Thierry Lecomte associated rs1615 polymorphism to grade 3 neurotoxicity during oxaliplatin-based treatment in 64 patients of European origin (AA genotype; OR=5.54; CI95%, 1.1-28.02; p=0.03)[26] and there are other studies that have also suggested this association [18,28]. In our study, the GG genotype showed an association with higher risk of respiratory toxicity in the oxaliplatin group (OR=5.07; CI95%, 1.41-16.8; p=0.009), in agreement with the association of the G allele with cardiovascular toxicity (OR=4.05; CI95%, 1.40-13.50; p=0.01), cutaneous (OR=1.81; CI95%, 1.52-2.89; p=0.01) and respiratory (OR=3.01; CI95%, 1.43-6.72; p=0.005) in the overall population treated in first line.

The majority of studies have been focused on the analysis of severe toxicity (grades 3-4), since they are the most clinically relevant toxic episodes and therefore the ones that are most interesting to predict. Hence the importance of finding genetic biomarkers, which can be easily assessed at the time of diagnosis, as they are constant over time. In the present study, both severe and any degree of toxicity experienced have been considered. None of the molecular biomarkers investigated were capable to predict grade 3-4 toxicity; however, some tendencies were shown, such as the AA genotype of rs1695 of *GSTP1* in the oxaliplatin subgroup; the TT genotype of rs25487 of *XRCC1* and the G allele of *ERCC1* rs11615 in the irinotecan subgroup; the CC genotype of *ABCB1* rs1045642 in the capecitabine subgroup. Regarding the need for treatment modification, patients with the *ABCB1* T allele for rs1045642 required fewer treatment modifications in both the 5FU and the oxaliplatin group. Apart from the obtained associations, we observed some tendencies, like the TT genotype of *ABCB1* rs1045642 and the CC genotype of *MTHFR* rs1801131 in the capecitabine group and the TT genotype of *XRCC1* rs25487 in the irinotecan subgroup. These data could serve as a basis for future studies with larger sample size that can elucidate whether these trends reflect true associations masked by a lack of power.

The main limitation of this study was the heterogeneity of treatment schemes given to patients, which is inherent to CRC therapy. The stratification in treatment subgroups, although provided a more homogeneous evaluation of the toxicities derived from each drug, supposed a loss of statistical power that may have derived in the lack of some associations. A larger sample size may have helped in this regard. Also, as any other retrospective study, the accuracy of the information was highly dependent on the exhaustiveness of the clinical records.

CONCLUSION

ABCB1 rs1045642 T allele reduces the need for treatment modification with both 5FU and oxaliplatin. *DPYD* rs17376848, *MTHFR* rs1801131 and rs1801133, *XRCC1* rs1799782 and rs25487, *ERCC1* rs11615 and *GSTP1* rs1695 polymorphisms are associated with different toxic effects during the chemotherapy treatment of CRC, although they cannot be considered as risk factors for severe toxicity (grades 3 and 4). No associations were found between gene polymorphisms *DPYD* rs67376798 and *ERCC1* rs3212986 and chemotherapy toxicity. Our results show that although the genes studied cannot be considered as markers of severe toxicity, they are associated with toxicity and perhaps it would be convenient to take this into account

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FIGURE LEGEND

Figure 1. Study population divided by chemotherapeutic treatment line.

Figure 2. Study population stratifying by treatment subgroups and SNP analyzed in each subgroup.