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Toxicity and therapy outcome associations in *LIG3*, *SLCO1B3*, *ABCB1*, *OPRM1* and *GSTP1* in high-grade serous ovarian cancer

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Abstract

Adverse effects are the major limiting factors in combinatorial chemotherapies. To identify genetic associations in ovarian cancer chemotherapy-induced toxicities and therapy outcomes, we examined a cohort of 101 patients receiving carboplatin-paclitaxel treatment with advanced high-grade serous ovarian cancers. Based on literature and database searches, we selected 19 candidate polymorphisms, designed a multiplex single nucleotide polymorphism-genotyping assay and applied Cox regression analysis, case–control association statistics and the log-rank Mantel−Cox test. In the Cox regression analysis, the *SLCO1B3* rs1052536 AA-genotype was associated with a reduced risk of any severe toxicity (hazard ratio = 0.35, $p = 0.023$). In chi-square allelic test, the *LIG3* rs1052536 T-allele was associated with an increased risk of neuropathy (odds ratio [OR] = 2.79, $p = 0.031$) and *GSTP1* rs1695 G allele with a poorer response in the first-line chemotherapy (OR = 2.65, $p = 0.026$). In Kaplan–Meier survival analysis, *ABCB1* rs2032582 TT-genotype was associated with shorter overall survival (uncorrected $p = 0.025$) and *OPRM1* rs544093 GG and GT genotypes with shorter platinum-free interval (uncorrected $p = 0.027$) and progression-free survival (uncorrected $p = 0.012$). Results suggest that *SLCO1B3* and *LIG3* variants are associated with the risk of adverse effects in patients receiving carboplatin-paclitaxel treatment, the *GSTP1* variant may affect the treatment response and *ABCB1* and *OPRM1* variants may influence the prognosis.

KEYWORDS
chemotherapy, epithelial ovarian cancer, genetic association, therapy response, toxicity

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1 | BACKGROUND

Ovarian cancer is one of the deadliest gynecologic malignancies worldwide. In 2018, it was the ninth leading cancer diagnosis (295 000 cases) and the eighth leading cause of cancer mortality (185 000 deaths) among women.[1] High-grade serous ovarian cancer (HGSOC) is the most prevalent subtype and accounts for over 70% of epithelial ovarian cancers.[2] HGSOC is usually diagnosed in the advanced stage and has a poor 5-year survival rate of 43%.[3] While 80% of HGSOC patients respond initially to first-line treatment, almost 80% of these patients experience multiple and finally chemoresistant relapses.[4] In the advanced stage, epithelial ovarian cancers are treated with debulking surgery followed by chemotherapy. When primary debulking surgery (PDS) is not feasible, the treatment is started with neoadjuvant chemotherapy to reduce tumour burden, followed by interval debulking surgery (IDS). The standard first-line chemotherapy of HGSOC includes a platinum agent and taxane, commonly carboplatin and paclitaxel, respectively.

While platinum derivatives and taxanes both target cell proliferation, they have distinct mechanisms of action. Platinum compounds bind covalently to DNA forming intrastrand and interstrand cross-links, which interferes with DNA replication and ultimately leads to apoptosis of proliferating cells. On the other hand, taxanes stabilize microtubules, which arrest cell division and cause cytotoxicity. For this reason, several rapidly renewing organ systems such as the bone marrow, the central and peripheral nervous system and the gastrointestinal tract are affected by platinum derivatives and taxanes besides the intended target. Especially, the hematologic adverse effects are rather common in carboplatin-paclitaxel combination treatment.[5–9] Severe hematologic toxicities have become less frequent with short paclitaxel infusions compared with former 24-h infusions,[10] but paclitaxel dose can be limited by neuropathy.

The development of toxicities is often associated with altered drug pharmacokinetics, leading to increased drug exposure. Mielke et al. showed that peripheral neuropathy was associated with increased paclitaxel exposure.[11] Similarly, non-haematological toxicities increased along with higher total drug exposure over time.[12] In addition, the severity of adverse events was associated with drug exposure.[13,14] Variation in genes encoding drug-metabolizing enzymes and transport proteins could influence the pharmacokinetics of chemotherapeutics and thus induce toxicities.[7] In addition, genes related to DNA repair may affect the cytotoxicity and response to the treatment. Many pathways, genes and even specific variants and genotypes are shared between toxic effects and drug efficacy, which may affect a patient's prognosis, such as progression-free survival (PFS) and overall survival (OS).

The purpose of this study was to investigate the genetic association between selected candidate polymorphisms and chemotherapy-induced toxicity and clinical parameters in Finnish patients with advanced HGSOC.

2 | MATERIALS AND METHODS

2.1 | Study population

The study patients participated in a prospective clinical Epithelial Ovarian Cancer-Staging and Response to Chemotherapy Evaluated by PET/CT (MUPET) trial (ClinicalTrials.gov identifier: NCT01276574) and were treated at the Department of Gynaecology and Obstetrics at Turku University Hospital, Finland, during the period of 2010 to 2019. Approval (ETMK 145/1801/2015) was obtained from the institutional Ethics Committee of the Hospital District of Southwest Finland, and all subjects signed an informed consent form. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[15]

The cohort consisted of 101 patients with histologically confirmed HGSOC (Table 1), operated and treated in a single hospital and who had completed their primary carboplatin-paclitaxel therapy. Moreover, they had detailed clinical records available, including toxicity scorings and adequate quality DNA extracted from whole blood. The stage of disease was determined according to the guideline of the International Federation of Gynaecology and Obstetrics.[16]

Forty patients underwent PDS and received 4–12 (median 6) cycles of adjuvant chemotherapy. In 61 patients, acceptable debulking was not achievable at

TABLE 1 Summary of the patient information.

Number of patients	101
Age at diagnosis, years	
Median	67
Range	38–81
Interquartile range	62–72
Histology, number of patients (%)	
High-grade serous	101 (100%)
Stage, number of patients (%)	
II	1 (1%)
III	64 (63%)
IV	36 (36%)
Treatment strategy, number of patients (%)	
Primary debulking surgery (PDS)	40 (40%)
Neoadjuvant chemotherapy (NACT)	61 (60%)
Chemotherapy regimen, number of patients	
Carboplatin + paclitaxel	77
Carboplatin + paclitaxel + bevacizumab	24
Total number of chemotherapy cycles, number of patients (%)	
3 cycles	5 (5%)
4 cycles	1 (1%)
5 cycles	2 (2%)
6 cycles	55 (54%)
7 cycles	9 (9%)
8 cycles	6 (6%)
9 cycles	20 (20%)
10 cycles	2 (2%)
12 cycles	1 (1%)
Therapeutic outcome, median in days (range, interquartile range)	
Progression-free survival (PFS)	487 (68–1716, 324–783)
Platinum-free interval (PFI)	313 (14–1569, 154–648)
Overall survival (OS)	1088 (233–3559, 716–1419)
Initial drug response, number of patients (%)	
Complete response	53 (52%)
Partial response	33 (33%)
Stable disease	2 (2%)
Progressive disease	12 (12%)
Died during chemotherapy	1 (1%)

the time of diagnosis, and the patients were first treated with 3–4 (median 3) cycles of neoadjuvant chemotherapy (NACT) followed by IDS and adjuvant chemotherapy (0–7 cycles, median 4). All patients were treated with carboplatin (area under the concentration-time curve 5 mg/mL/min, AUC5) combined with paclitaxel (175 mg/m^2) administered intravenously in 3-week cycles. In 24 of 101 patients, the anti-vascular endothelial growth factor antibody bevacizumab (7.5–15 mg/kg) was added to the adjuvant chemotherapy. According to the institutional treatment guideline, the whole blood count, neutrophils, liver enzymes and serum creatinine levels were analysed before each cycle of chemotherapy, and the presence of neurological, gastrointestinal and dermatological symptoms was registered. The severity grade (1–5) of the first-line chemotherapy-related toxicities and action taken (change of regimen, dose or schedule or discontinuation of the treatment) due to toxicity were collected retrospectively from patient records (Table 2). The severity grade of the toxicity was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0. Evaluated toxicities were hepatic, gastrointestinal, hearing, haematological (neutropenia, anaemia and thrombocytopenia), skin and neurological (see supporting information Table S1, which contains toxicity and prognosis phenotypes). Grades 3–5 and grade 2 toxicities that resulted in the change of chemotherapy regimen of the patients were considered clinically relevant. Additionally, genetic association to OS, PFS, platinum-free interval (PFI) and primary treatment response were evaluated. OS was defined as the time from diagnosis to death from any cause, PFS as the time from diagnosis to progression and PFI as the time from the last primary chemotherapy to the first progression. The patients' treatment response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[17] and CA12-5 criteria of The Gynecological Cancer Intergroup (GCIC).[18]

2.2 | DNA sampling, single nucleotide polymorphism (SNP) selection and genotyping methods

To test for the association of common germline SNPs to chemotherapy-induced toxicity and treatment response, we genotyped 22 candidate variants (see supporting information Tables S2 and S3, which describe the details of investigated SNPs). These SNPs were selected based on their previous associations with severe chemotherapy adverse effects (anaemia, neutropenia, thrombocytopenia, infections, skin toxicity, neuropathy, hepatotoxicity, gastrointestinal toxicities and hearing toxicity), the pharmacokinetics of taxane and platinum compounds, clinical outcome (drug response, OS, PFS and PFI) or association with serous ovarian cancer.

The SNP selection was made using literature searches and the PharmGKB (RRID:SCR_002689),[19] ClinVar (RRID:SCR_006169),[20] and DisGeNET (RRID:SCR_006178)[21] databases. In PharmGKB, we searched for variant-drug annotations using "platinum compounds," "carboplatin," "cisplatin," "oxaliplatin," "taxanes," "cabazitaxel," "docetaxel," "paclitaxel," "cyclophosphamide" and

TABLE 2 Summary of studied toxicities, their abundance and severity.

	Anaemia	Neutro-penia	Thrombo-cytopenia	Infections	Skin toxicity	Neuro-pathy	Hepato-toxicity	Gastrointestinal toxicities	Hearing toxicities
Grade 1	7 (6.9%)	1 (1%)	3 (3%)	1 (1%)	-	13 (12.9%)	1 (1%)	-	-
Grade 2	23 (22.8%)	7 (6.9%)	2 (2%)	2 (2%)	1 (1%)	5 (5%)	1 (1%)	1 (1%)	1 (1%)
Grade 3	-	24 (23.8%)	-	5 (5%)	2 (2%)	10 (9.9%)	-	-	-
Grade 4	-	6 (5.9%)	-	-	-	-	-	-	-
Grade 5	-	-	-	1 (1%)	-	-	-	-	-
Unidentified grade	-	1 (1%)	-	-	-	7 (6.9%)	1 (1%)	-	-
Total	30 (29.7%)	39 (38.6%)	5 (5%)	9 (8.9%)	3 (3%)	35 (34.7%)	3 (3%)	1 (1%)	1 (1%)

"bevacizumab" as keywords. We included significant associations for toxicity, dosage, efficacy and metabolism. In ClinVar, we included pathogenic and likely pathogenic variants associated with ovarian cancer or carcinoma. In DisGeNET, we searched associated SNPs for Ovarian Serous Adenocarcinoma. The two latter databases were used as supporting evidence for the PharmGKB search. The resulting primary candidate genes and variants were systematically evaluated and annotated for associated drugs, chemotoxicities, drug responses and associated neoplasms. The final annotations for the set of SNPs included in the final analyses were derived from the PharmGKB database and using the Ensembl Variant Effect Predictor (VEP) online tool (RRID: SCR_007931).[22] Allele frequencies in the Finnish and study population are reported in supporting information Tables S4 and S5.

2.3 | DNA purification

DNA was purified from a frozen buffy coat of EDTA blood in Auria biobank, Turku University Hospital, Turku, Finland. Isolation was performed using the Chemagic™ 360/MSM I instrument (PerkinElmer, Waltham, Massachusetts, USA) with Chemagic™ DNA Blood Kit special (PerkinElmer, Waltham, Massachusetts, USA) according to the manufacturer's instructions.

2.4 | Assay design

We applied Agena Bioscience MassARRAY System (previously known as Sequenom MassARRAY iPLEX), which provides an accurate and reliable custom SNP genotyping platform with flexible multiplexing options optimal for this study. The system has three main steps: locus-specific amplification, locus-specific primer extension and locus separation by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry incorporating a mass-modified terminator base.[23] Our custom assay was designed with the software ADS Assay Design Suite (Agena Bioscience, San Diego, California, USA) according to the manufacturer's instructions (see supporting information Table S6 for the details of designed primers). We started the computational design with 36 candidate SNPs and were able to fit 22 priority SNPs in two pools with predicted high success scores. Efficient multiplexing of the rest of the SNPs was not possible, and therefore, they were excluded.

Genotyping of the selected SNPs was performed by the Genotyping laboratory of the Institute for Molecular Medicine Finland (FIMM) Technology Centre, University of Helsinki. All the assays showed excellent or good

separation of the genotype clusters indicating highly accurate genotype calls. The genotype calls were further validated by comparing to whole-genome sequencing (WGS)-based genotypes available for 55 patients (100% accordance between the genotyping assay and WGS genotypes, data not shown). Briefly, the genotypes were called with GATK v4.1.4.1 following best practices.[24,25] This analysis was performed using Anduril 2.[26] Non-variant sites were force-genotyped in GenotypeGVCFs.[24] The selected SNPs passed filtering otherwise. We detected 100% concordance between these methodologies (data not shown).

2.5 | Statistical methods

Various methods were used to analyse the associations of genetic variants with chemotherapy-induced toxicities (17 phenotypes in total) and treatment response (five phenotypes) (see supporting information Table S1 for the toxicity and prognosis phenotypes). The toxicity phenotypes were evaluated with Cox regression analysis (IBM SPSS Statistics 29, IBM Corporation, Armonk, New York, USA). Multiple testing corrected *p*-value of below 0.05, obtained with Bonferroni correction where the raw *p*-value was multiplied by the number of investigated SNPs, was considered statistically significant. The effect of genotypes on PFI, PFS and OS was evaluated using Kaplan–Meier survival analysis and the log-rank Mantel–Cox test using GraphPad Prism 8.4 (GraphPad Software, San Diego, California, USA), with an uncorrected $p < 0.05$ considered statistically significant. Finally, the standard case–control association analysis was performed using the chi-square allelic test. Genotypic analysis was carried out with Cochran–Armitage trend, genotypic, dominant gene action, recessive gene action, additive effects of allele dosage and dominance deviation test. The allelic and genotypic association analyses were applied to further evaluate both toxicity and treatment response phenotypes and were conducted with PLINK version 1.90b6.17.[27] Here, multiple testing corrected *p*-value of below 0.05 obtained with Bonferroni correction (multiplication by the number of investigated SNPs) was considered statistically significant.

Hardy–Weinberg equilibrium was tested with an exact test, and the linkage-disequilibrium (LD) between SNPs was examined using PLINK.[28] None of the polymorphisms deviated from the Hardy–Weinberg equilibrium (See supporting information Table S7 for Hardy–Weinberg equilibrium data). Three variants were excluded from the further analysis as rs1934951 (*CYP2C8*) and rs4149117 (*SLCO1B3*) were in high or complete LD with rs1113129 (*CYP2C8*, $r^2 = 0.97$) and rs7311358 (*SLCO1B3*, $r^2 = 1.00$), respectively, and rs79085477 (*BMP7*) was not polymorphic in our cohort (See supporting information Tables S8 and S9 for LD data).

3 | RESULTS

In the present study, 101 patients who received carboplatin and paclitaxel as first-line treatment and fulfilled all other selection criteria were included in the genetic analysis (Table 1). The summary of toxicity characteristics is summarized in Table 2. In short, grades 3 and 4 neutropenia was present in 30 patients (29.7%), grades 3–5 infections in 6 patients (6%), grade 3 neuropathy in 10 patients (9.9%) and grade 3 skin adverse effects in 2 patients (2%). In total, 43 patients (42.6%) underwent grade 3 or higher toxicity during their treatment. In the patients with an unidentified grade, grade 2, or less severe toxicities ($n = 18$), 61.1% of treatment regimens were changed. The median follow-up time of participants was 1159 days (95% CI = 1021–1271), with disease progression in 93 (92.1%) and death in 67 (66.3%) patients.

We studied 19 gene variants for their association with hepatic, gastrointestinal, hearing, haematological, skin and neurological toxicity in the first-line treatment (see supporting information Tables S10–S12 for detailed data of toxicity association results). In the Cox regression analysis, *SLCO1B3* rs7311358 (c.699G>A, p.Met233Ile, GRCh38.p12_chr12: 20862826G>A) was associated with a reduced risk of any kind of grades 3–5 toxicity (hazard ratio [HR] = 0.35, 95%CI 0.19–0.66, Bonferroni-corrected $p = 0.023$; see Table 3). *LIG3* rs1052536 (c.*50C>T, GRCh38.p12_chr17: 35004556C>T) was associated with an increased risk of neuropathy of any grade, but the effect did not reach statistical significance after multiple testing correction (Bonferroni-corrected $p = 0.061$). In the chi-square allelic test, *LIG3* rs1052536 was significantly associated with an increased risk of neuropathy of any grade (odds ratio [OR] = 2.79, 95%CI = 1.46–5.35, Bonferroni-corrected $p = 0.031$) and *SLCO1B3* rs7311358 associated with a reduced risk of grade 3–5 toxicities (OR = 0.36, 95%CI = 0.18–0.71, Bonferroni-corrected $p = 0.047$). The associations of these two variants followed the gene-dosage model according to the Cochran–Armitage trend test.

A significant allelic association was observed between *GSTP1* rs1695 (c.313A>G, p.Ile105Val, GRCh38.p12_chr11: 67585218A>G) and poorer response to the first-line chemotherapy (OR = 2.65, 95%CI = 1.46–4.81, Bonferroni-corrected $p = 0.024$) (See supporting information Tables S13–S15 for complete results of prognosis associations). In addition, this association followed the gene-dosage model (Bonferroni-corrected $p = 0.026$).

TABLE 3 An overview of the significant genetic associations.

Gene variant	The effect of alternative allele	Cox regression analysis		Chi-square allelic test		Genotypic analysis
		Hazard ratio (95% CI)	*p*-value[a]	Odds ratio (95% CI)	Allelic association, *p*-value[a]	Genotypic association, *p*-value[a,b]
LIG3 rs1052536 c.*50C>T	Increased risk of any neuropathy	CC *versus* CT *versus* TT n.a.	$p = 0.061$	C *versus* T 2.79 (1.46–5.35)	$p = 0.031$*	$p = 0.020$*
SLCO1B3 rs7311358 c.699G>A	Decreased risk of any grade 3–5 toxicities	GG/AG *versus* AA 0.35 (0.19–0.66)	$p = 0.023$*	G *versus* A 0.36 (0.18–0.71)	$p = 0.047$*	$p = 0.021$*
SLCO1B3 rs7311358 c.699G>A	Decreased risk of any grade 3–5 toxicities compared with no toxicities at all	GG/AG *versus* AA 0.39 (0.21–0.74)	$p = 0.073$	G versus A 0.29 (0.12–0.69)	$p = 0.074$	$p = 0.026$*
GSTP1 rs1695 c.313A>G	Decreased rate of complete response to the first-line chemotherapy	n.a.	n.a.	A *versus* G 2.65 (1.46–4.81)	$p = 0.026$*	$p = 0.026$*

Abbreviation: n.a., not applicable.
[a]Adjusted with Bonferroni correction.
[b]Cochran−Armitage trend test.
*p-value < 0.05.

In the Kaplan–Meier survival analysis, the associations of *ABCB1* rs2032582 (c.2677G>T, p.Ala893Ser, GRCh38.p12_chr7: 87531302C>A) with OS (log-rank Mantel–Cox test, uncorrected $p = 0.025$) and *OPRM1* rs544093 (g.130858G>T, GRCh38.p12_chr6: 154136358G>T) with PFI (log-rank Mantel–Cox test, uncorrected $p = 0.027$) and PFS (log-rank Mantel–Cox test, uncorrected $p = 0.012$) were observed (Figures 1 and 2). For full results of Kaplan–Meier analysis, see supporting information Table S16.

4 | DISCUSSION

We investigated the impact of 19 genetic variants on paclitaxel-carboplatin-based chemotherapy-induced adverse effects and therapeutic outcomes. Two SNPs showed association with toxicity phenotypes, namely, rs1052536 in *LIG3* with the risk of neuropathy of any grade and rs7311358 in *SLCO1B3* with the risk of severe (grades 3–5) toxicities in general. Three SNPs were associated with prognostic measures: rs1695 in *GSTP1* with response to the first-line therapy, rs2032582 in *ABCB1* with OS and rs544093 in *OPRM1* with PFI and PFS, but the associations of *ABCB1* and *OPRM1* were not statistically significant after multiple testing correction. The strength of our study is that it focused exclusively on the advanced stages III–IV ovarian cancers that were verified to represent high-grade serous histology, unlike in most previous pharmacogenetic studies in ovarian cancer. In addition, the patients were operated and treated in a single hospital according to similar general practices and we had direct access to the patients' fully comparable clinical information and medical records, including details of medications used and descriptions of toxicity symptoms, prognostic parameters as well as laboratory test results. Due to the relatively limited sample size, the study should be considered exploratory and the results interpreted with caution. Furthermore, the study was conducted in the Finnish population and may not be directly applicable to other populations.

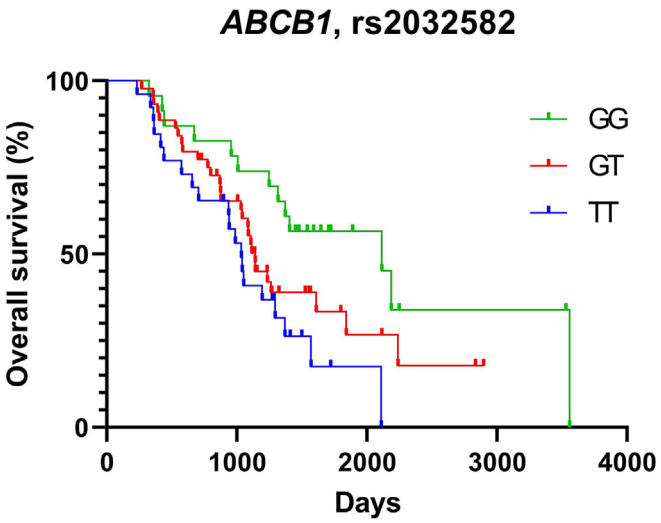

FIGURE 1 Kaplan–Meier survival analysis of ABCB1 rs2032582 (GRCh38.p12_chr7:87531302C>A) in overall survival. According to the analysis, rs2032582 was significantly associated with the OS (log-rank Mantel-Cox test, uncorrected $p = 0.0245$). ABCB1 rs2032582 GG, GT and TT genotypes consisted of 23, 4, and 26 patients, respectively. The overall survival (OS) time (days from diagnosis to death) is on the *x*-axis, and the proportion of surviving patients overall at each time point is on the *y*-axis. Steps on the lines indicate the time of death of individual patients.

To our knowledge, this is the first report of the association between rs1052536 (c.*50C>T), a SNP in the 3′ untranslated region of *LIG3* and chemotherapy-induced neuropathy in any common cancer. In the chi-square allelic test, neuropathy of any grade was significantly more frequent after multiple testing corrections within the patients with *LIG3* c.*50T allele than those with C allele (OR = 2.79, Bonferroni-corrected *p*-value = 0.03). *LIG3* encodes nuclear and mitochondrial DNA ligase III polypeptides, which play a vital part in DNA repair,[29] but their role in the paclitaxel- and carboplatin-induced toxicity in ovarian cancer patients has not been reported before. In cisplatin and cyclophosphamide-treated ovarian cancer patients, the c.*50TT genotype has been previously associated with a reduced risk of grades 3 and 4 neutropenia,[30] but in our data, the association did not reach statistical significance (uncorrected $p = 0.06$).

This is also, to our knowledge, the first report of the association of *SLCO1B3* missense variant rs7311358 (c.699G>A, p.Met233Ile) with the incidence of severe adverse effects in ovarian cancer patients, specifically in the high-grade serous histotype. In our study, the patients with the rs7311358 A allele had fewer grades 3–5 adverse effects than those with the G allele. The effect of *SLCO1B3* variation on chemotherapy-induced toxicities is poorly understood. A *SLCO1B3* variant rs4149117 (c.334T>C, p.Ser112Pro), which is in a complete LD with rs7311358 in our data and European population,[31] has been found to reduce, increase, or not affect the risk for toxicities.[7,32,33] Similarly, the mechanism explaining our finding of a reduced risk of severe adverse effects associated with the rs7311358 remains elusive. In vitro study has shown that this variant reduces the transport of paclitaxel and carboplatin and could therefore increase their systemic exposure and the occurrence of adverse effects.[32] However, in two clinical studies, the rs7311358 was not associated with altered clearance of paclitaxel.[34,35] Alternatively, the association between the *SLCO1B3* variation and reduced risk of toxicity could be explained by its LD

with another transporter gene *SLCO1B1*, which encodes a liver-specific uptake transporter for paclitaxel and possibly also for platinum drugs.[36,37] The well-characterized *SLCO1B1* no-function variant rs4149056 (c.521 T>C, p.Val174Ala)[38] is in LD with the *SLCO1B3* rs7311358 reference allele in the Finnish population ($r^2 = 0.35$, $D' = 0.68$).[39] Therefore, patients with the rs7311358 G allele and a higher incidence of adverse effects are more likely to harbour the rs4149056 C allele resulting in reduced SLCO1B1 function. However, further studies are required to confirm the role of *SLCO1B1* variation in carboplatin-paclitaxel chemotherapy.

In our study, the *OPRM1* intronic variant rs544093 G>T showed genotypic association with PFI and PFS but not with OS. In the Kaplan–Meier survival analysis (Figure 2), the patients with TT genotype had significantly longer PFI and PFS than those with GT and GG genotypes. However, the results should be treated with caution, as they were not significant after multiple testing corrections. *OPRM1* encodes mu-opioid receptor (MOR), which participates in tumour proliferation and ultimately metastasis.[40,41] The genetic variation and expression of MOR have been associated with the survival and progression of various cancers.[42–44] It appears that the present study is the first to report an association of *OPRM1* with the prognosis of ovarian cancer. On the other hand, the rs544093 has been previously linked with taxane-platinum-based chemotherapy-induced neurotoxicity,[45] but such an association was not observed in our data.

ABCB1 missense variant rs2032582 (c.2677G>T, p.Ala893Ser) was associated with the OS in our cohort. The patients harbouring GG phenotype had longer OS (median survival 2115 days) than those with GT (1140 days) or TT (1035 days) genotypes, but the association (uncorrected $p = 0.0245$) was not significant after multiple testing correction. Earlier studies in Scottish and Korean ovarian cancer patients have not observed any significant associations between *ABCB1* c.2677G>T variant with progression-free and OS.[46,47] Unlike our present study, the previous studies included various tumour subtypes, and some patients were treated with carboplatin-docetaxel or cisplatin-paclitaxel instead of carboplatin-paclitaxel. In contrast, a study with non-small-cell lung cancer patients treated with carboplatin and paclitaxel observed that patients with GT and TT genotypes had longer PFS than those with GG phenotype,[32] but no statistical difference in OS was observed.

The *ABCB1* c.2677G>T variant reduces the in vitro transport of fluorescent-labelled paclitaxel compared with wild-type protein,[48,49] but the effect of common *ABCB1* genetic variations on drug pharmacokinetics, paclitaxel clearance and exposure is inconsistent.[14,50–52] However, pharmacogenetic studies conducted in cancer patients have associated the 2677T allele with increased paclitaxel-induced gastrointestinal toxicity and neutropenia.[47,53] Furthermore, paclitaxel-treated patients co-administered with medications that inhibited the function of ABCB1 protein had a higher risk of dose modification due to peripheral neuropathy.[54] The reduced ABCB1 function can result in increased intracellular accumulation of paclitaxel, which may be the cause of, for instance, paclitaxel-induced peripheral neuropathy.[55] Thus, more frequent adverse events could then have contributed to our observed poorer survival.

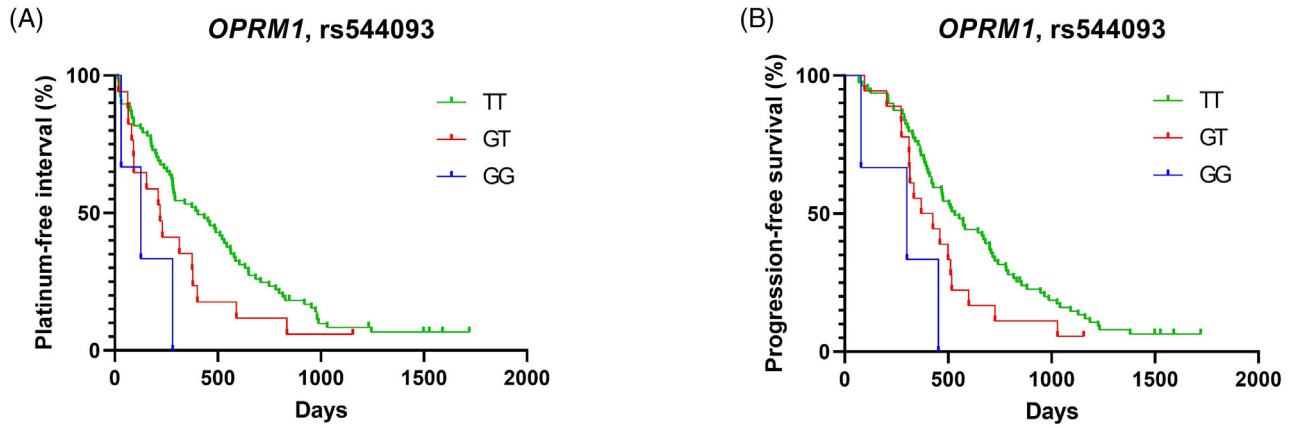

FIGURE 2 Kaplan–Meier survival analysis of OPRM1, rs544093 (GRCh38.p12_chr6:154136358G>T) in platinum-free interval (panel A) and progression-free survival (panel B). According to the analysis, rs544093 was significantly associated with the PFI (log-rank Mantel–Cox test, uncorrected $p = 0.0267$) and PFS (log-rank Mantel–Cox test, uncorrected $p = 0.0119$). In panel A, the number of patients with TT, GT and GG genotypes was 77, 17 and 3, respectively. In panel B, the number of patients with TT, GT and GG genotypes was 79, 18 and 3, respectively. The survival time from the last primary chemotherapy to the first progression (panel A) and from diagnosis to progression (panel B) is on the *x*-axis, and the proportion of surviving patients overall at each time point is on the *y*-axis. Steps on the lines indicate the time of progression of individual patients.

GSTP1 missense variant rs1695 (c.313A>G, p.Ile105Val) was, after multiple testing corrections, significantly associated with the response to the first-line treatment, as the patients with G allele had poorer response than those with A allele. The variant leads to the improved rate of cisplatin conjugation with glutathione in vitro and thus to higher clearance.[56,57] In another previous study, *GSTP1* c.313A>G was not associated with the drug response in ovarian cancer patients.[47] However, the study was conducted with Korean patients with mixed tumour histology, and some of the patients received chemotherapy consisting of carboplatin-docetaxel or cisplatin-paclitaxel. Moreover, c.313A>G has been previously studied for association with PFS and OS. In a recent study, patients that received tri-weekly carboplatin and paclitaxel treatment and harboured AA-genotype had better PFS and OS than those carrying the AG or GG genotype.[58] However, the study was conducted with Japanese patients, and only 48% of the patients had uncategorized serous ovarian cancer, which may explain the divergence in different studies. No associations were found either in our study between *GSTP1* c.313A>G and PFS or OS, thus being in agreement with previous studies in ovarian cancer.[7,46,47]

In conclusion, we detected three statistically significant associations in 19 candidate polymorphisms related to taxane-platinum-based chemotherapy-induced adverse effects and therapeutic outcomes. Two genetic variants associated with *LIG3* and *SLCO1B3* genes contributed to the risk of toxicities, whereas the variant in *GSTP1* influenced the response. Furthermore, according to the Kaplan–Meier survival analysis, the variants of *ABCB1* and *OPRM1* affected the OS, PFI and PFS. Further studies with a larger number of patients are warranted to verify the clinical relevance of these findings.

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CONFLICT OF INTEREST STATEMENT

Authors declare no competing interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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