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ORIGINAL ARTICLE

Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients

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Platinum drugs are among the most active and widely used agents in the treatment of different cancers. However, the great individual variability in both outcome and toxicity of platinum chemotherapy requires the identification of genetic markers that can be used to screen patients before treatment. In this study, 21 polymorphisms in 10 genes, the protein activities of which may be addressed in different aspects of cisplatin metabolism, were tested for correlations with efficacy and toxicity of cisplatin–cyclophosphamide regimen in 104 ovarian cancer patients. The glutathione S-transferase P1 (GSTP1) Ile105Val polymorphism was strongly associated with progression-free survival ($\chi^2 = 12.12$, $P = 0.002$). The allelic status of the *GSTA1* –69 C>T polymorphism correlated with the overall survival: patients with T/T genotype survived longer than C/C carriers ($P = 0.044$). Thrombocytopenia, anemia and neuropathy were less frequent among patients with the *GSTM1*-null or *GSTM3* intron 6 AGG/AGG genotypes. Severe neutropenia was associated with the TP53 72 Pro/Pro, XPD 312 Asp/Asn and XRCC1 399 Arg/Arg genotypes. A higher risk of nephrotoxicity was noted for patients with the heterozygous *ERCC1* 19007 T/C and 8092 C/A genotypes. No correlations were found between genotypes and complete tumor responses.
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Introduction

Platinum-based drugs are among the most widely used cytotoxic agents for the treatment of many types of cancer. However, their effective clinical use is impeded by significant variations in both the response rate and the rate of adverse reactions.[1,2] The possibility of improving drug efficacy and reducing toxic adverse effects, including the identification of individuals at the risk of drug resistance or toxicity, involves the exploration of interindividual variations at the DNA sequence level (nucleotide repeats, insertions, deletions and single-nucleotide polymorphisms) that result in altered expression levels and/or activities of the encoded proteins. These may account for up to 95% of the variability in drug disposition and effects.[3,4]

Some polymorphisms have been described as important for the activity of platinum drugs, the most common of which occur in DNA repair and glutathione S-transferase (GST) genes. By their effects on mRNA levels or mRNA

stability, polymorphisms in genes for DNA repair (for example, *ERCC1* 19007 T>C and 8092 C>A, XPD Asp312Asn and Lys751Gln) can modulate the total DNA repair capacity[5–8] and influence the removal of platinum–DNA adducts, the persistence of which underpins the antitumor potential of platinum drugs.[9] GSTs, especially GSTP1, can substantially limit the amount of free platinum drugs available for interaction with DNA, by catalyzing their binding to tripeptide glutathione, one of the most abundant molecules in cells.[10,11] *GSTP1* is usually overexpressed in a wide variety of tumors. *In vitro* experiments have shown the importance of the allelic status of *GSTP1* for platinum metabolism. The variants of the enzyme with Val at codon 105 have shown higher activity against cisplatin and carboplatin than enzymes with Ile at this codon.[12,13] There have been numerous studies of the roles of these and other polymorphisms in the response to treatment and survival of different cancers, including ovarian cancer.[14–22] However, many of these studies have been limited, testing only a few markers. Furthermore, these markers frequently vary from one study to another, which clearly affects any comparison of the data and might result in different inferences about the role of any polymorphism. In this study, we evaluated the possible correlations between a panel of 21 polymorphisms (Table 1) in both GST and DNA repair genes, and *TP53* and cytochrome P450 2E1 (*CYP2E1*), and the efficacy and toxicity of cisplatin-based chemotherapy in patients with ovarian cancer.

Patients and methods

Patients

The study population comprised women with epithelial ovarian cancer who had received no previous chemotherapy or radiation therapy. Adequate organ function was a criterion for inclusion, including creatinine clearance $\geqslant 60\,ml\,min^{-1}$, hemoglobin level $\geqslant 10\,g$ per 100 ml, leukocyte count $\geqslant 4.0 \times 10^9$ per liter and platelet count $\geqslant 100 \times 10^9$ per liter. The upper age limit was 65 years. To obtain a group of homogeneous ethnicity, only patients of eastern Slavonic origin were included. Patients with serious concomitant diseases (diabetes, uncontrolled hypertension, myocardial infarction within the last 6 months and so on) and clinically significant hearing impairment (grade 2 or higher) were excluded.

All patients signed an informed consent form and underwent standard clinical and laboratory assessments, including CA-125. Before the initiation of chemotherapy, venous blood samples (10 ml) were collected for genetic studies. The chemotherapy regimen was intravenous cisplatin ($100\,mg\,m^{-2}$) and cyclophosphamide ($600\,mg\,m^{-2}$) on day 1, every 3 weeks, for a maximum of six cycles. The therapy was terminated earlier in cases of disease progression or unacceptable toxicity. Intraperitoneal chemotherapy and radiotherapy were not permitted.

Toxicity was assessed according to standard National Cancer Institution criteria. Maximum attention was paid to neutropenia, anemia, thrombocytopenia, nephrotoxicity, neuropathy, ototoxicity and emesis. When grade 2–4 neutropenia, thrombocytopenia or grades 3 and 4 anemia was observed, the next treatment was postponed until recovery to grade 1 or 0. With prolonged cytopenia, the doses of cisplatin and cyclophosphamide were reduced by 25%. When creatinine clearance decreased to within the range of 59–41 ml min^{-1}, the dose of cisplatin was reduced by 25%; when it decreased to $\leqslant 40\,ml\,min^{-1}$, cisplatin was discontinued. Ototoxicity was evaluated audiometrically after the first two cycles and after the completion of treatment. With grade 2 ototoxicity, cisplatin was stopped. With grades 3 and 4 emesis, the doses of both drugs were reduced by 25%. To evaluate the associations between the genotypes and treatment toxicity, the patients were classified as having good or poor tolerance (Table 2).

The tumor response was assessed after every two cycles. After the completion of chemotherapy, the patients were followed-up for disease relapse and survival. After progression, most patients received a second-line treatment, which was generally taxane based.

Genotyping

DNA was isolated from leukocytes by proteinase K treatment, followed by extraction using phenol–chloroform.[23] Most polymorphisms were genotyped using a PCR–restriction fragment length polymorphism-based technique,[24–33] except for variants of *CYP2E1* (96 bp),[34] *TP53* (16 bp),[32] *GSTM3* (3 bp),[35] *GSTM1* and *GSTT1* (the two latter gene polymorphisms were determined simultaneously using a multiplex PCR protocol, amplification of the fourth-exon region of the guanosine triphosphate-cyclohydrolase 1 gene serving as an internal control (the primers 5′-gtccttttgttttat gaggaaggc-3′ and 5′-ggtgatgcactcttataatctcagc-3′).[36]

To visualize the resulting fragments, the amplified and enzyme-digested samples were run on 6% polyacrylamide gel (*GSTM3*) or 2.5% agarose gel and stained using ethidium bromide. The details of the genetic variants studied, primer sequences and restriction enzymes are summarized in Table 1.

Statistical analysis

The primary end point of the study was to analyze the association between the genetic polymorphisms and clinical outcome (objective (tumor) response, overall survival (OS) and progression-free survival (PFS) and toxicity). The two-sided χ^2 test and Fisher's exact test were used to determine the relationship between each categorical variable and the genotypes tested. Survival curves were generated using the Kaplan–Meier product limit method. Survival curves were compared by the log-rank test and χ^2 test (for two curves and multiple curves, respectively). Statistical significance was set at $P<0.05$. The statistical analyses were performed using Statistica software (version 6.0, StatSoft, Inc, Tulsa, OK, USA) and GraphPad InStat (version 3.00, GraphPad Software, Inc, San Diego, CA, USA).

Table 1 Characteristics of the polymorphisms studied, with primer sequences and restriction enzymes

Site location	*Type of polymorphism*	*Genotype*	*Enzyme*	*Primer sequences*
GSTA1 (5′ flanking)	SNP, rs3957357	C/T	*Eam*1104I	5′-tgttgattgtttgcctgaaatt-3′, 5′-gttaaacgctgtcaccgtcct-3′
GSTM1 (whole gene)	Gene deletion	+/0[a]	—	5′-gaactccctgaaaagctaaagc-3′, 5′-gttgggctcaaatatacggtgg-3′
GSTM3 (intron 6)	3-bp deletion, rs1799735	AGG/−	—	5′-ggggaaaaggtaggaagaagggaa-3′, 5′-gatgcttaggtctgaggagtagta-3′
GSTM3 (exon 8)	SNP, rs7483	G/A, Val^{224}Ile	*Hin*1II	5′-ccagtggggcaacaagcat-3′, 5′-ggacaccagtaacataagtg-3′
GSTP1 (exon 5)	SNP, rs1695	A/G, Ile^{105}Val	*Alw*26I	5′-gtagtttgcccaaggtcaag-3′, 5′-agccacctgaggggtaag-3′
GSTP1 (exon 6)	SNP, rs1138272	C/T, Ala^{114}Val	*Bsh*1236I	5′-acaggatttggtactagcct-3′, 5′-agtgccttcacatagtcatccttgcgc-3′
GSTT1 (whole gene)	Gene deletion	+/0[a]	—	5′-ttccttactggtcctcacatctc-3′, 5′-tcaccggatcatggccagca-3′
ERCC1 (exon 4)	SNP, rs11615	T/C, Asn^{118}Asn	*Tai*I	5′-gcagagctcacctgaggaac-3′, 5′-gaggtgcaagaagaggtgga-3′
ERCC1 3′ UTR	SNP, rs3212986	C/A	*Mbo*II	5′-cagagacagtgccccaagag-3′, 5′-gggcaccttcagctttcttt-3′
XPD (exon 10)	SNP, rs1799793	G/A, Asp^{312}Asn	*Eco*130I	5′-cagctcatctctccgcaggatcaa-3′, 5′-gtcggggctcaccctgcagcacttcct-3′
XPD (exon 23)	SNP, rs13181	A/C, Lys^{751}Gln	*Pst*I	5′-ctgctcagcctggagcagctagaatcagaggagacgctg-3′, 5′-aagaccttctagcaccaccg-3′
XRCC1 (exon 6)	SNP, rs1799782	C/T, Arg^{194}Trp	*Msp*I	5′-ggtaagctgtacctgtcactc-3′, 5′-gacccaggaatctgagcc-3′
XRCC1 (exon 9)	SNP, rs25489	G/A, Arg^{280}His	*Rsa*I	5′-tggggcctggattgctgggtctg-3′, 5′-cagcaccactaccacaccctgaagg-3′
XRCC1 (exon 10)	SNP, rs25487	G/A, Arg^{399}Gln	*Msp*I	5′-caagtacagccaggtcctag-3′, 5′-ccttccctcatctggagtac-3′
TP53 (intron 3)	16-bp duplication rs17878362	−/dupl	—	5′-gcagagacctgtgggaagcga-3′, 5′-accgtagctgccctggtaggt-3′
TP53 (exon 4)	SNP, rs1042522	G/C, Arg^{72}Pro	*Bsh*1236I	5′-caatggatgatttgatgctg-3′, 5′-tggtaggttttctgggaagg-3′
TP53 (intron 6)	SNP, rs1625895	G/A	*Msp*I	5′-aggtctggtttgcaactggg-3′, 5′-gaggtcaaataagcagcagg-3′
CYP2E1 (5′ flanking)	96-bp insertion	−/ins	—	5′-gtgatggaagcctgaagaaca-3′, 5′-ctttggtggggtgagaacag-3′
CYP2E1 (5′ flanking)	SNP, rs2031920	C/T	*Rsa*I	5′-ccagtcgagtctacattgtca-3′, 5′-ttcattctgtcttctaactgg-3′
CYP2E1 (intron 6)	SNP, rs6413432	T/A	*Dra*I	5′-gacagggtttcatcatgttgg-3′, 5′-agtcgacatgtgatggatcca-3′
CYP2E1 (intron 7)	SNP, rs2070676	C/G	*Taq*I	5′-gggctttcatcttcatttcga-3′, 5′-caaaatgtgggctttcatctg-3′

Abbreviations: CYP2E1, cytochrome P450 2E1; GST, glutathione S-transferase; SNP, single-nucleotide polymorphism; UTR, untranslated region.
[a]The genotype was defined as positive if at least one copy of the gene was present.

Results

From May 2003 to June 2007, a total of 104 patients entered the study. The median age was 52 years (range 23–65 years). The majority of patients (74 women) had stage III disease, and stages I, II and IV were detected in 13, 7 and 10 patients, respectively. Twenty-one patients were receiving adjuvant chemotherapy (that is, they had no residual disease after surgery).

A total of 506 cycles of chemotherapy were delivered, and 60 patients received all six cycles. In 44 patients, the therapy was terminated early: 23 patients had severe toxicity, the cancer of 11 patients had progressed, the treatment of 4 patients was limited to four cycles because their prognoses were favorable and 6 refused to complete the therapy.

The genotype frequencies for the polymorphisms studied are shown in Table 3.

Correlation between polymorphisms and tumor response. In total, 83 patients were evaluable for their responses to treatment. There were 37 complete and 32 partial responses, with an overall response rate of 83%; the disease of six patients was stable and the cancers of eight had progressed. As the prognosis of patients with partial response in terms of overall survival was not better than ones with stable or progressive disease ($P=0.99$), partial responders were combined with non-responders for analysis. No significant correlations were found between genotypes and the proportion of patients who achieved a complete response.

Correlation between polymorphisms and survival. In all, 100 patients were assessable for PFS and OS. The median duration of follow-up was 32 months. To the date of analysis, 63 patients had progressed and 27 patients had died. The median PFS was 11.6 months and the median OS was not reached. The estimated 3-year survival rates were 27% for PFS and 55% for OS.

The polymorphism GSTP1 Ile105Val was associated with treatment outcome. Patients with a homozygous Ile/Ile genotype had an increased PFS compared with that of patients with one or two Val alleles (log-rank test, $P=0.0001$; Figure 1). The median PFS for patients with a homozygous Ilel/Ile genotype was 15 months, compared with 8.0 and 8.5 months in patients with the Val/Val and Ile/Val genotypes, respectively. The association remained significant in a further analysis after the exclusion of patients treated with adjuvant therapy (log-rank test, $P=0.005$). An analysis restricted to patients without adjuvant therapy also showed an association between PFS and the XPD Asp312Asn and Lys751Gln polymorphisms. In both cases, patients with the heterozygous genotype fared better (Figure 2), especially compared with patients with the homozygous 312 Asp/Asp or 751 Lys/Lys genotypes

Table 2 Groups of graded toxicities

Side effect	*Grades of toxicity considered as tolerable for patients*	*Grades of toxicity considered as severe for patients*
Neutropenia	0–2 (43)[a]	3–4 (59)
Anemia	0–1 (49)	2–4 (54)
Thrombocitopenia	0 (87)	1–4 (16)
Nephrotoxicity	0 (67)	1–4 (36)
Ototoxicity	0 (62)	1–4 (38)
Neuropathy	0–1 (64)	2–3 (31)
Emesis	0–2 (78)	3–4 (26)

[a]Number of patients in a group.

Table 3 Frequencies of polymorphic genotypes in patients

Polymorphism	*Genotypes (no. of patients)*		
GSTA1 –69C>T	C/C (43)	C/T (49)	T/T (12)
GSTM1 gene deletion	0/0 (47)	+/0[a] (57)	—
GSTM3 AGG deletion	AGG/AGG (83)	AGG/– (16)	–/– (5)
GSTM3 Val^{224}Ile	Val/Val (39)	Val/Ile (57)	Ile/Ile (8)
GSTP1 Ile^{105}Val	Ile/Ile (41)	Ile/Val (53)	Val/Val (10)
GSTP1 Ala^{114}Val	Ala/Ala (80)	Ala/Val (24)	—
GSTT1 gene deletion	0/0 (18)	+/0[a] (86)	—
ERCC1 19007T>C	T/T (43)	T/C (46)	C/C (15)
ERCC1 8092C>A	C/C (61)	C/A (37)	A/A (6)
XPD Asp^{312}Asn	Asp/Asp (34)	Asp/Asn (50)	Asn/Asn (20)
XPD Lys^{751}Gln	Lys/Lys (28)	Lys/Gln (54)	Gln/Gln (22)
XRCC1 Arg^{194}Trp	Arg/Arg (94)	Arg/Trp (10)	—
XRCC1 Arg^{280}His	Arg/Arg (95)	Arg/His (9)	—
XRCC1 Arg^{399}Gln	Arg/Arg (49)	Arg/Gln (45)	Gln/Gln (10)
TP53 16bp duplication	–/– (72)	–/dupl (29)	dupl/dupl (3)
TP53 Arg^{72}Pro	Arg/Arg (52)	Arg/Pro (40)	Pro/Pro (12)
TP53 13494G>A	G/G (75)	G/A (27)	A/A (2)
CYP2E1 96-bp insertion	–/– (100)	–/ins (4)	—
CYP2E1 –1053C>T	C/C (102)	C/T (2)	—
CYP2E1 7632T>A	T/T (91)	T/A (12)	A/A (1)
CYP2E1 9896C>G	C/C (82)	C/G (20)	G/G (2)

Abbreviations: CYP2E1, cytochrome P450 2E1; GST, glutathione S-transferase.
[a]The genotype was defined as positive if at least one copy of the gene was present.

(log-rank test, $P=0.027$ or $P=0.017$, respectively). When the XPD and GSTP1 polymorphisms were analyzed together, patients with the GSTP1 Val-containing genotype, combined with the XPD 312 Asp/Asp or 751 Lys/Lys genotype, had shorter median PFS compared with those of patients with other genotype combinations (6.5–7.0 months vs 8.0 months or more, respectively). However, the XPD and GSTP1 polymorphisms seemed to be independently associated with PFS. No significant correlations were found when the distributions of the XPD 312 and 751 genotypes were compared between patients with the GSTP1 105 Ile/Ile genotype and patients with one or two Val alleles.

Only one polymorphism (*GSTA1* –69 C>T) was significantly associated with OS. Patients with the homozygous T/T genotype had significantly better survival than patients with the C/C genotype (log-rank test, $P=0.044$ for all patients (Figure 3), and $P=0.025$ if patients with adjuvant chemotherapy were excluded).

Correlation between polymorphisms and toxicity. A significant difference in severe neutropenia was observed according to the XRCC1 Arg399Gln polymorphism ($\chi^2=7.279$, $P=0.026$). Grade 3 or 4 neutropenia developed in 71.4% of patients (35 of 49 patients) with a homozygous Arg/Arg genotype compared with 45.3% of patients (24 of 53) with an Arg/Gln or Gln/Gln genotypes (odds ratio (OR) = 3.02, 95% confidence interval (CI) 1.33–6.88; Fisher's exact test, $P=0.009$; Supplementary Table). Severe neutropenia was also more frequent among patients with a homozygous TP53 72 Pro/Pro genotype (10 of 11 patients, 90.9%; OR = 8.57, 95% CI 1.05–69.8; $P=0.023$) or with a heterozygous XPD 312 Asp/Asn genotype (33 of 48, 68.8%; OR = 2.33, 95% CI 1.05–5.33; $P=0.045$). The XPD Asp312Asn polymorphism also seemed to be important for bone marrow toxicity. Patients with the Asp/Asn genotype were more prone to thrombocytopenia (OR = 4.05, 95% CI 1.21–13.58; $P=0.027$) and anemia (OR = 2.32, 95% CI 1.05–5.13; $P=0.048$). Thrombocytopenia and anemia were also associated with the *GSTM1* and *GSTM3* polymorphisms. Patients with a homozygous *GSTM1* gene deletion (*GSTM1* null) had a lower risk of these adverse effects than carriers of functional *GSTM1* variants: OR = 0.13

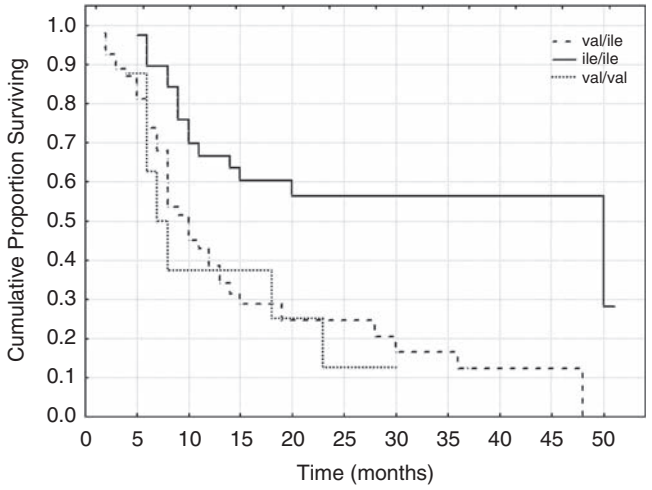

Figure 1 Kaplan–Meier function for progression-free survival in women with ovarian cancer, according to the glutathione S-transferase P1 (GSTP1) 105 polymorphism.

for thrombocytopenia (95% CI 0.03–0.62; $P=0.005$) and OR = 0.29 for anemia (95% CI 0.13–0.66; $P=0.003$). The *GSTM3* AGG/AGG genotype was more common in patients without thrombocytopenia (85.1 vs 56.3% with cytopenia; OR = 0.23, 95% CI 0.07–0.71; $P=0.014$) or anemia (91.8 vs 70.4% with anemia; OR = 0.21, 95% CI 0.07–0.69; $P=0.007$). Patients with *GSTM1* null or *GSTM3* AGG/AGG genotypes also had a decreased risk of neuropathy (OR = 0.37, 95% CI 0.15–0.92; $P=0.031$ and OR = 0.34, CI

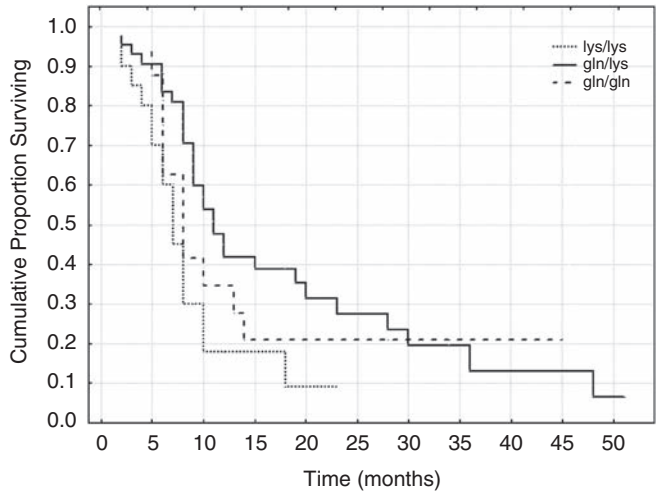

Figure 2 Kaplan–Meier function for progression-free survival in women with ovarian cancer without adjuvant chemotherapy, according to the XPD 751 polymorphism.

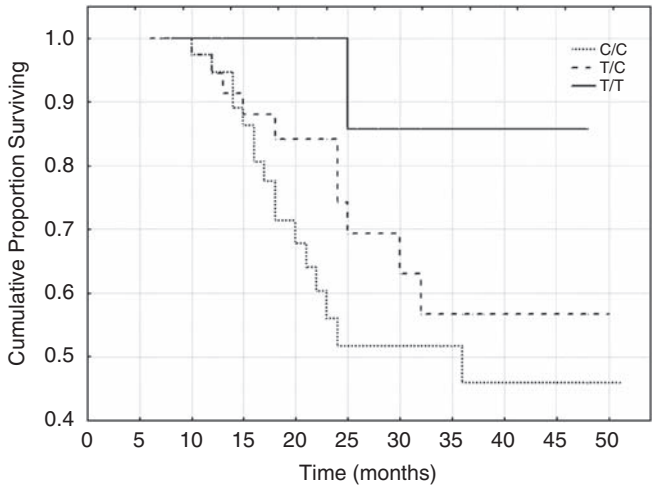

Figure 3 Kaplan–Meier function for overall survival in women with ovarian cancer, according to the glutathione S-transferase A1 (*GSTA1*) –69 C>T polymorphism.

0.12–0.96; $P=0.055$, respectively). The risk of severe emesis (grades 3 and 4) was higher in patients with the *GSTT1*-null genotype than in patients with a functional *GSTT1* variant (50 vs 11.5%, respectively; OR = 4.06, 95% CI 1.40–11.78; $P=0.014$). An association was noted between nephrotoxicity and only the *ERCC1* gene polymorphisms, and cases of renal dysfunction were more prevalent among patients with the heterozygous T/C (46.7%) and C/A (52.8%) genotypes compared with the homozygous variants (OR = 2.51, 95% CI 1.09–5.57; $P=0.037$, and OR = 3.29, 95% CI 1.40–7.73; $P=0.009$, respectively). Ototoxicity was not significantly associated with any of the polymorphisms studied.

Considering that different polymorphisms were associated with the same side effects, some of the genotype combinations were also tested for correlations with the risk of toxicity. Two combinations (*GSTM3* + XPD 312 for thrombocytopenia and anemia, and XRCC1 399 + XPD 312 for neutropenia) were found to be more powerful (in terms of ORs) compared with the corresponding single polymorphism analysis (see above). But only the association between combined XRCC1 399 Arg/Arg + XPD 312 Asp/Asn genotype and severe neutropenia (OR = 7.93, 95% CI 2.189–28.798; $P=0.0004$) remained significant after Bonferroni correction for multiple testing.

Discussion

Our results show that the GSTP1 Ile105Val polymorphism may influence cisplatin efficacy (in terms of survival) in patients with ovarian cancer. The patients with a homozygous Ilel/Ile genotype had significantly better PFS than did patients with one or two Val alleles. Their OS did not differ significantly, but patients with the Ile/Ile genotype tended to live longer than patients with the Ile/Val genotype (log-rank test, $P=0.14$). Our findings, together with existing data on the prevalent expression of GSTP1 in cancer cells,[11] are in good agreement with the results of an *in vitro* experiment in which the human 105 Val variant of the GSTP1 enzyme was significantly more active against cisplatin than was the enzyme containing the Ile residue.[12] However, our results are not universally consistent with those of clinical studies. Several studies have confirmed the beneficial role of the GSTP1 105 Ile allele in platinum-containing chemotherapy.[15,16] However, other studies have reported opposite associations or no relationship between the GSTP1 Ile105Val polymorphism and survival.[14,17,19,37] These contradictions may be partly attributable to differences in the chemical structures (the bulky cyclohexane ring of oxaliplatin) and reaction kinetics (the more stable leaving group of carboplatin) of platinum drugs.[38] Furthermore, different studies have used different agents together with the platinum drugs. From this perspective, the fate of our small group of 10 patients with the GSTP1 105 Val/Val genotype is informative. All of them failed to achieve a good result with first-line chemotherapy, but subsequent treatments with different medications (taxanes) were quite successful.

Together with the GSTP1 Ile105Val polymorphism, *GSTT1* and *GSTM1* gene deletions have been associated with a better survival.[17,19] However, there were no significant differences in the survival times of patients with homozygous *GSTT1* and *GSTM1* gene deletions and those with functional variants in this study.

Another GST polymorphism with a possible affect on survival in our study was *GSTA1* –69 C>T. Functionally, it results in reduced *GSTA1* expression and related enzyme activity.[24,39] There is little information about the role of the *GSTA1* –69 C>T polymorphism in chemotherapy. GSTA1 has the greatest catalytic activity for the conjugation of glutathione to alkylating agents, including the metabolites

of cyclophosphamide.[40,41] This polymorphism was associated with survival after breast cancer treatment with cyclophosphamide-containing drugs, and a significantly reduced risk of death was observed in patients with the *GSTA1* –69 T/T variant.[42] In our study, patients with the homozygous T/T genotype also showed better survival.

In some studies, associations between the GSTP1 Ile105Val polymorphism and the adverse effects of platinum-containing regimens have also been found. It has been shown that the GSTP1 105 Val allele significantly reduced the risk of developing severe oxaliplatin-induced cumulative neuropathy[43] and that homozygosity for GSTP1 105 Val was even more protective against cisplatin-related ototoxicity.[44] We observed no such associations. Moreover, severe hearing impairment was observed more frequently among patients with a homozygous Val/Val genotype (in six of nine patients; OR = 3.69, 95% CI 0.86–15.74; $P = 0.08$). The protective capacity of the GSTP1 105 Val allele against oxaliplatin-induced neurotoxicity was also not confirmed in another recent study with a FOLinic acid-Fluororuracil-OXaliplatin regimen,[45] in which carriers of the GSTP1 105 Val/Val genotype were more susceptible to grade 3 neurotoxicity than were patients with the Ile/Val or Ile/Ile genotype. An association with ototoxicity has also been reported for the *GSTM3* polymorphism in intron 6.[33] The *GSTM3**B allele, which has a three-base deletion creating a recognition site for the YY1 transcription factor, was proposed to be protective against cisplatin-induced hearing loss. This association was not confirmed in our study. To evaluate the possible functional significance of GSTM3 enzyme activity in the efficacy of treatment, the Val/Ile polymorphism at codon 228 was examined.[25] However, no significant association was found.

We found no association between treatment efficacy and the *ERCC1* 19007 T/C or 8092 C/A polymorphism. Our data, showing the correlations between the XPD Asp312Asn and Lys751Gln polymorphisms and survival, are generally consistent with the results of a study of ovarian cancer treated using a carboplatin–paclitaxel combined therapy, in which carriers of at least one variant allele (312 Asn or 751 Gln) had a significantly reduced risk of death compared with patients with Asp/Asp or Lys/Lys genotype.[20] Our results are also similar to data reported for patients with non-small-cell lung cancer treated using cisplatin–gemcitabine therapy, among whom the time to disease progression was significantly longer in patients with the heterozygous 751 Lys/Gln genotype than in those with the homozygous 751 Lys/Lys genotype.[46]

Except for its association with severe neutropenia, the XRCC1 Arg399Gln polymorphism did not correlate significantly with the other parameters tested. However, patients treated with nonadjuvant chemotherapy who were homozygous or heterozygous for Arg lived markedly longer without progression than did patients with Lys/Lys genotype (medians PFS was 8.5–9.0 months vs 6.5 months, respectively; χ^2 test, $P = 0.11$). The lack of statistical significance can be attributed to the insufficient sample size. This tendency is in good agreement with studies of patients with colorectal cancer, non-small-cell lung cancer, esophageal cancer and gastric cancer.[47]

In contrast to GST and the DNA repair proteins, the p53 protein does not seem to directly affect cisplatin metabolism or transformation. However, it has a crucial role in mediating cellular responses to DNA damage.[9] Three of the most common *TP53* gene polymorphisms (the 16-bp duplication in intron 3 and the two single-nucleotide polymorphisms in exon 4 (codon 72) and intron 6) have been shown to be important for p53 function.[48] Moreover, the Arg72Pro polymorphism is located in the proline-rich region of p53 and the substitution of Arg with Pro can directly affect the structure of the putative SH3-binding domain. The Arg72 protein is more efficient in apoptosis induction, whereas the Pro72 form induces more G1 arrest and is better at activating p53-dependent DNA repair.[49] In some studies, no correlation between the functional p53 Arg72Pro polymorphism and the results of ovarian cancer chemotherapy has been found.[37,50] In two other studies, an association with survival time was observed but the inferences were opposite.[18,51] No associations between either the Arg72Pro polymorphism or the two other polymorphisms in introns 3 and 6 and survival time were observed in our study.

There is no evidence that cisplatin can be bioactivated by CYP2E1. However, a role for CYP2E1 in cisplatin-induced nephrotoxicity has been proposed as a site for the generation of reactive oxygen species and a significant source of catalytic iron.[52,53] We have tested some widely known polymorphisms in the promoter region and introns of the *CYP2E1* gene, but no associations with either nephrotoxicity or any other characteristic were found.

Our results, taken in the context of other published data, generally suggest that some genetic polymorphisms can be considered as predictive tools for the pretreatment evaluation of the efficacy and toxicity of platinum-containing chemotherapies. We tested 21 polymorphisms in 10 genes, the protein activities of which may be addressed in different aspects of cisplatin metabolism. Our results suggest that the GSTP1 Ile105Val polymorphism may be considered as an important locus for predicting cisplatin efficacy (in terms of PFS and possibly OS). Unfortunately, none of the polymorphisms was associated with ototoxicity, one of the most frequent reasons for cisplatin discontinuation in our study. At the same time, the *ERCC1* polymorphisms are possible predictors of nephrotoxicity, although further studies are required to validate this finding and the association between *GSTA1* polymorphism and OS.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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