Research Article

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Pharmacogenomics

Pharmacogenetic score predicts overall survival, progression-free survival and platinum sensitivity in ovarian cancer

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Aim: To define the impact of polymorphisms in genes involved in platinum-taxane and estrogen activity in the outcome of platinum-based treated ovarian cancer patients (OCP). **Patients & Methods:** Two hundred and thirty OCP were analyzed for 124 germ-line polymorphisms to generate a prognostic score for overall survival (OS), progression-free survival (PFS) and platinum-free interval (PFI). **Results:** *ABCG2* rs3219191D>I, *UGT1A* rs10929302G>A and *UGT1A* rs2741045T>C polymorphisms were significantly associated with all three parameters (OS, PFS and PFI) and were used to generate a score. Patients in high-risk group had a poorer OS (hazard ratio [HR]: 1.8; 95% CI: 1.3–2.7; p = 0.0019), PFS (HR: 2.0; 95% CI: 1.4–2.9; p < 0.0001) and PFI (HR: 1.9; 95% CI: 1.4–2.8; p = 0.0002) compared with those in low-risk group. **Conclusion:** The prognostic-score including polymorphisms involved in drug and estrogen pathways stratifies OCP according to OS, PFS and PFI.

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Keywords: estrogen • ovarian cancer • pharmacogenetics • platinum therapy • polymorphism • survival

Epithelial ovarian cancer (EOC) represents the main cause of death from gynecologic malignancy in Western countries and ranks as the seventh most incident cancer worldwide [1]. The asymptomatic nature of the early stages of the disease hinders its diagnosis, which occurs at advanced stages in most cases when the survival probability drop off [2]. The standard therapeutic approach for advanced EOC consists of primary debulking surgery (PDS) followed by platinum-based chemotherapy or, in patients who cannot undergo PDS, neoadjuvant chemotherapy (NACT) before interval debulking surgery (IDS) [3]. Despite the recent innovations in first-line therapy with the introduction of targeted agents like anti-VEGF antibodies and poly-ADP ribose polymerase inhibitors, platinum-based therapy coupled with paclitaxel or doxorubicin is still the irreplaceable backbone chemotherapy.

Even though this disease shows high chemosensitivity to first-line therapy, in particular in patients with high-grade serous histology, most patients become resistant to platinum-based chemotherapy [4].

Genetic interindividual variability in platinum and taxane pharmacodynamics (PD) and pharmacokinetics (PK), in other words, drug metabolism, distribution, transport, elimination, could have the potential to influence treatment outcome, exposing patients to suboptimal dose of drugs. Moreover, endogenous processes linked to estrogen activity play a role in the onset and progression of the disease. Many experimental data show that estrogen and progesterone exert different effects on gynecological cancers like ovarian, breast, endometrium and uterine

cancer through their receptor-dependent signaling pathways. Current evidence suggests that the elevated estrogens and decreased progesterone levels have a correlation in the progression of ovarian cancer [5], thus it could be speculated that genetic individual differences regulating estrogen activities may impact the outcome of the disease.

Genetic variants affecting drug therapy (pharmacogenetics [PGx] variants) could have a predictive and/or a prognostic value: they can predict treatment efficacy (e.g., response to therapy) or they can be prognostic markers of outcome, whereby the relationship between the biomarker and clinical outcome may be indirect and independent from the treatment. Although many of the studies conducted so far have mainly focused on predictive biomarkers [6,7], over the years, evaluations of the relationships between PGx variants and disease prognosis have been pursued [8].

The research of predictive and prognostic PGx biomarkers in EOC has so far been mostly inconsistent [9]. One possible explanation may be searched on the applied approaches. On one side, most candidate–gene studies have been focused on the coding regions of the genome, investigating functional genetic polymorphisms (i.e., missense, frameshift, truncating genetic variations, etc.,) which, affecting the structure of the protein they encode for, are more likely to have a major impact in drug response and disease outcome. Nonetheless, noncoding DNA regions, once considered 'junk DNA,' lately have attracted increasing attention, as they are likely to be involved in regulatory processes [10,11] that may indirectly impact the outcome of the disease. On the other side, the research has been concentrated on the effect of single polymorphisms on treatment and disease outcome to obtain clinically implementable single biomarkers. Unfortunately, this strategy may not be the most appropriate when it comes to polymorphisms, since in most cases the effect of single genetic variants is likely to be modest, especially if they do not have a direct structural impact on the encoded protein. Conversely, a wider approach considering [12,13] synergic/addictive effects of different genetic variants in the same pathway could have a better predictive/prognostic value.

For the aforementioned reasons, research in this field may benefit from different strategies that combine investigation on both coding and noncoding DNA regions with a more comprehensive approach that evaluates the possible synergic effect of multiple genetic variants involved in the same process, such as response to therapy and disease outcome. Thus, the combination of the small-size deleterious effects of polymorphisms on genes involved in different pathways regulating the same process may enhance the predictive and prognostic power of PGx biomarkers. In our previous work [14], this strategy was successfully applied in a population of 230 EOC patients, leading to the definition of an immunogenetic score able to stratify patients according to their overall survival (OS), progression-free survival (PFS) and platinum-free interval (PFI).

Here, we propose to apply this combinational approach (which considers the additive effect of unfavorable genotypes of single genetic variations mostly located in noncoding DNA regions) to the same EOC population considering a panel of 124 polymorphisms in candidate genes involved in platinum and taxanes derivatives PK and PD, including drug metabolism, transport, detoxification, DNA synthesis and repair, estrogen pathways, cell regulation processes and angiogenesis. The prognostic impact of these polymorphisms, in terms of OS, PFS and PFI, was explored.

Methods

Patients

Two hundred and thirty high-stage (III–IV), high-grade (G2–G3) advanced EOC patients were retrospectively included in this study. Clinical and demographic characteristics of the study population were previously described [14] and are reported in Table 1. Briefly, all patients were aged ≥18 years, had histologically confirmed diagnosis of EOC and provided written informed consent for the use of peripheral blood and clinical data for research purposes. Patients were treated with PDS or IDS and received a platinum-based therapy. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of our Institute (Institutional Review Board no. CRO-2014-43). Blood samples were collected at the time of primary surgery.

Gene variants & genotyping

Target genes coding for proteins involved in the PK and PD of platinum-based therapy and their potential modulation of chemotherapeutics response or disease outcome were selected according to a combination of PubMed-MEDILINE search and a cross-check in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, obtaining a final list of 60 genes.

Table 1. Patients' clinical and demographic characteristics.

Patients and tumor characteristics	n (%)
Total	230
Age (years)	
– Median (range)	58 (23–81)
Stage at diagnosis	
– III	174 (76%)
– IV	56 (24%)
Therapy	
– PDS + first line	154 (67%)
– Neoadjuvant + IDS	76 (33%)
Residual disease	
– R0	123 (53.5%)
– R >0	107 (46.5%)
Tumor grade	
– G3	177 (77%)
– G2–3	53 (23%)
Histology	
– Papillary serous	188 (81.7%)
– Endometrioid	9 (3.9%)
– Mixed serous/endometrioid	7 (3.0%)
– Undifferentiated	5 (2.2%)
– Clear cells	3 (1.3%)
– Mucinous	3 (1.3%)
– Mixed serous/other	2 (0.9%)
– Transitional cells	1 (0.4%)
– Unknown	12 (5.3%)
Platinum-based therapy	
– Carboplatin + taxol containing†	182 (79.2%)
– Carboplatin + caelyx	20 (8.7%)
– Carboplatin	26 (11.3%)
– Cisplatin	1 (0.4%)
– PAC‡	1 (0.4%)
Platinum sensitivity	
– Refractory (0–1 months)	27 (12.3%)
– Resistant (1–6 months)	54 (24.5%)
– Partially sensitive (6–12 months)	51 (23.2%)
– Sensitive (>12 months)	88 (40.0%)
OS (months)	
– Evaluable patients	230
– Total events	186 (80.9%)
– Within 60 months	148 (64%)
– Missing	0
– Median OS (range)	44.6 (2.2–222.3)
PFS (months)	
– Evaluable patients	223
– Total events	203 (88.3%)
– Within 36 months	178 (79.8%)
– Missing	7 (3%)
– Median PFS (range)	16.30 (1.6–161.6)

The table reports the main clinical and demographic characteristics of the patients included in the study. All patients were treated with surgery and platinum-based chemotherapy.
†Carboplatin + taxol containing regimens include weekly carboplatin + taxol and carboplatin + taxol switched to: carboplatin + caelyx; carboplatin + cyclophosphamide; cisplatin + taxol, PEC (ciplatin, epirubicin and cyclophosphamide) or combinations thereof.
‡PAC = cisplatin, doxorubicine and cyclophosphamide.
IDS: Interval debulking surgery; OS: Overall survival; PDS: Primary debulking surgery; PFI: Platinum-free interval; PFS: Progression-free survival.

Table 1. Patients' clinical and demographic characteristics (cont.).

Patients and tumor characteristics	n (%)
PFI (months)	
– Evaluable patients	220
– Total events	203 (88.3%)
– Within 36 months	178 (79.8%)
– Missing	10 (4.3%)
– Median (range)	9.0 (0.0–156.9)

The table reports the main clinical and demographic characteristics of the patients included in the study. All patients were treated with surgery and platinum-based chemotherapy.
†Carboplatin + taxol containing regimens include weekly carboplatin + taxol and carboplatin + taxol switched to: carboplatin + caelyx; carboplatin + cyclophosphamide; cisplatin + taxol, PEC (ciplatin, epirubicin and cyclophosphamide) or combinations thereof.
‡PAC = cisplatin, doxorubicine and cyclophosphamide.
IDS: Interval debulking surgery; OS: Overall survival; PDS: Primary debulking surgery; PFI: Platinum-free interval; PFS: Progression-free survival.

In particular, the biological processes and related genes evaluated were: drug metabolism (*CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP3A4* and *CYP3A5*), membrane transport (*ABCB1, ABCC1, ABCC2, ABCG2, SLC46A1, SLC19A1* and *SLCO1B1*), detoxification (*GSTA1, GSTM1, GSTM3, GSTP1* and *GSTT1*), DNA synthesis and catabolism (*ATIC, DHFR, DYPD, FOLR1, FPGS, GGH, MTHFD1, MTHFR, MTR, MTRR, SHMT* and *TYMS*), DNA repair (*APEX, ATM, ERCC1, ERCC2, ERCC5, hEXO1, hMLH1, hMSH2, hMSH6, hOGG1, MDM4, MGMT, RAD51, XRCC1, XRCC3* and *TP53*), estrogen activity (*COMT, CYP17A1, CYP19A1, CYP1B1, ESR1, ESR2, PRDM2* and *UGT1A*), factors involved in cell cycle regulation (*CCDN1, EGF, EGFR, FGFR4* and *p21*) and angiogenesis (*VEGFA*). A set of 124 polymorphisms both in coding and noncoding DNA regions (PubMed-MEDLINE search) of genes that encoded proteins involved in platinum and taxanes PK and PD-related pathways and estrogen activity were considered. Genes and polymorphisms investigated are listed in Supplementary Table 1. A 3 mL blood sample was obtained from each patient at the time of surgery and genomic DNA was automatically extracted with BioRobot EZ1 (Qiagen SPA, Milan, Italy).

Polymorphisms were genotyped with Automated Fragment Analysis on Genetic Analyzer ABI PRISM 3100 (Applied Biosystems, CA, USA), TaqMan® Assays on 7500 Real-Time PCR System (Applied Biosystems), PSQ96 MA Pyrosequencing (Biotage AB, Uppsala, Sweden) or a custom-designed Illumina GoldenGate Assay on a BeadXpress® Reader (Illumina, CA, USA) as previously reported [14]. PCR amplifications were performed in an Eppendorf Mastercycler gradient (Eppendorf, Milan, Italy), with TaqGold DNA Polymerase (Applied Biosystems). Analyses were performed according to manufacturer's instructions including negative and positive controls. The details of genotyping assays, primer sequences and PCR conditions are available upon request.

Statistical analyses

The end points evaluated in this study were: OS, calculated as the lapse of time from diagnosis to death for any causes or the last follow-up; PFS, determined as the lapse of time from diagnosis to recurrence/progression or death/last follow-up and PFI, considered as the time from the last platinum administration to recurrence or progression/last follow-up. Patients' follow-up was truncated at 5 years. Age, stage, grade, residual tumor (R) and therapy setting were tested for association with OS, PFS and PFI and the variables significantly associated were included as covariates in the multivariate analysis.

In order to evaluate the impact of polymorphisms on the outcome, a stepwise selection of the prognostic markers was performed. Initially, a Cox proportional hazard model adjusted for the two clinical variables significantly associated with OS, PFS and PFI in the univariate analysis (Table 2) was used to test the associations between the polymorphisms and the three end points. Results were reported as adjusted hazard ratios (HRs) with corresponding 95% CIs. Three genetic models were tested, specifically, additive, dominant and recessive; the best-fitting model according to the Wald test was selected. Unadjusted differences in OS, PFS and PFI according to genotypes were plotted as Kaplan–Meier curves and the statistical significance was established by the log-rank test.

As a second step, only the polymorphisms significantly associated with each of the three end points ($p < 0.05$) were selected to be combined. Supplementary Table 2 lists the HR, 95% CI and p-value of the multivariate models for the selected polymorphisms. For each of them, a 'risk genotype' was identified and a score was assigned to each individual genotype (for additive models) or groups of genotypes (for dominant models) according to its risk of death, progression/recurrence and resistance to platinum-based therapy. The global score attributed to each patient

Table 2. Univariate associations of relevant clinical variables with platinum-free interval, progression-free survival and overall survival.

Patients and tumor characteristics	n (%)	PFI (HR 95% CI), p-value	PFS (HR 95% CI), p-value	OS (HR 95% CI), p-value
Total	230			
Age (years)		1.01 (0.99–1.02), p = 0.251	1.01 (0.99–1.02), p = 0.269	1.01 (0.99–1.02), p = 0.522
– Median (range)	58 (23–81)			
Stage				
– III	174 (76%)	Ref.		
– IV	56 (24%)	0.84 (0.59–1.19), p = 0.323	0.84 (0.59–1.19), p = 0.325	0.71 (0.49–1.03), p = 0.073
Therapy setting				
– PDS + first line	154 (67%)	Ref.	Ref.	Ref.
– Neo-adjuvant + IDS	76 (33%)	2.02 (1.46–2.79), $p = 2.01 \times 10^{-5}$ †	1.66 (1.20–2.29), p = 0.00198†	2.18 (1.53–3.10), $p = 1.47 \times 10^{-5}$ †
Residual disease				
– R0	123 (53.5%)	Ref.	Ref.	Ref.
– R >0	107 (46.5%)	2.30 (1.69–3.13), $p = 1.05 \times 10^{-7}$ †	2.21 (1.63–3.00), $p = 3.62 \times 10^{-7}$ †	2.22 (1.59–3.11), $p = 3.38 \times 10^{-6}$ †
Tumor grade				
– G3	177 (77%)	Ref.	Ref.	Ref.
– G2–3	53 (23%)	1.08 (0.76–1.54), p = 0.658	1.12 (0.77–1.60), p = 0.530	1.19 (0.81–1.74), p = 0.379

The associations between known prognostic variables (age, stage, therapy setting, residual disease and tumor grade) and the three end points tested are reported in this table. Therapy setting and residual disease were the only variables significantly associated with the three end points. These clinical variables were used in the multivariate associations between polymorphisms and the three end points as covariates.
† Statistically significant associations.
G: Grade; HR: Hazard ratio; IDS: Interval debulking surgery; OS: Overall survival; PDS: Primary debulking surgery; PFI: Platinum-free interval; PFS: Progression-free survival; Ref: Reference category.

Table 3. Scheme for score construction.

Polymorphism	Patient's genotype	Type of allele	Risk points attributed	Genetic model
ABCG2_CTCAdel rs3219191	II	I = protective allele	0	Additive
ABCG2_CTCAdel rs3219191	ID		1	
ABCG2_CTCAdel rs3219191	DD		2	
*UGT1A1*93_-3156A/G* rs10929302	GG	A = risk allele	0	Dominant
*UGT1A1*93_-3156A/G* rs10929302	AG or AA		1	
UGT1A9_-440T/C rs2741045	CC	T = risk allele	0	Additive
UGT1A9_-440T/C rs2741045	TC		1	
UGT1A9_-440T/C rs2741045	TT		2	

A risk score was generated grouping the three polymorphisms associated with OS, PFS and PFI. A point was assigned to each genotype of the three polymorphisms according to its risk of death, progression and platinum resistance as shown in the table. According to the genotype of each polymorphisms, patients had a total score derived by the sum of the assigned points, ranging from 0 to 5.
OS: Overall survival; PFI: Platinum-free interval; PFS: Progression-free survival.

derived from the sum of the scores assigned to each genotype across the selected polymorphisms and ranged from 0 to 5 (Table 3). The global score stratifies patients into two distinct prognostic groups (low and high risk) according to the genotypes they bore. The score classes were defined taking into account the score risk trend and the samples sizes balance across groups. All the analyses were performed using R statistical software v. 3.5.2 (www.r-project.org).

Results

Patients & clinical outcome

As previously described [14], there were 230 recruited subjects, all of Caucasian origin (self-reported). The majority of patients (76%) was affected by a stage III high-grade serous disease. One hundred and fifty-four patients (67%) had a PDS followed by first-line treatment, while 76 (33%) received NACT + IDS. Optimal cytoreduction (defined as no visible residual disease, R0) was achieved in 53.5% of cases. A platinum-based therapy was administered to all patients and it was associated with paclitaxel in 79.2% of cases. Clinical and demographic characteristics of recruited patients are reported in Table 1.

Patients subjected to NACT + IDS treatment were older than those treated with PDS (median age: 60 vs 56 years; p = 0.007), were more prone to be refractory or resistant to platinum therapy (42 vs 28%; p = 0.005), to experience disease progression (89 vs 75%; p = 0.014), death (79 vs 57%; p = 0.001) and to have a shorter OS (36 vs 44 months; p = 0.001). Nonetheless, NACT + IDS patients more likely achieved optimal cytoreduction (Supplementary Table 3). Clinically relevant variables (age, stage, grade, residual disease and therapy setting) were tested for association with OS, PFS and PFI in univariate analysis in the entire cohort of 230 patients. Residual disease (R0 or R >0) and therapy setting (NACT + IDS or PDS + first line) were significantly associated with each end point, in particular patients with R >0 or undergoing NACT + IDS treatment had shorter OS, PFS and PFI (Table 2).

Genotyping

One hundred and twenty-four polymorphisms in 60 genes involved in drug metabolism and transport, estrogen activity, in DNA synthesis, repair and catabolism, as well as factors involved in important regulatory cell processes were identified. Genotyping was successful in more than 207 patients for 118/124 analyzed polymorphisms. The allele frequencies are reported in Supplementary Table 1 and were consistent with those previously reported (https://www.ncbi.nlm.nih.gov/snp). Positive and negative controls were included, with 100% concordance rate for replicated samples.

Polymorphisms' association with the clinical outcomes

Sixteen out of 124 analyzed polymorphisms were significantly associated with OS, PFS or PFI (Table 4): *VEGF* rs2010963, *ERCC1* rs11615, *TP53* rs1042522, *XRCC1* rs3213239, *XRCC3* rs1799796, *XRCC3* rs861539, *FPGS* rs10106, *ABCG2* rs2046134, *ABCG2* rs3219191, *COMT* rs4680, *CYP2C9* rs1057910, *CYP3A4* rs2740574, *UGT1A* rs3064744, *UGT1A* rs4124874, *UGT1A* rs10929302 and *UGT1A* rs2741045. The number of patients effectively genotyped, the obtained genotype frequencies and the distribution according to the genetic models applied are reported in Supplementary Table 4 for each of the 16 polymorphisms significantly associated with the end points.

In particular, the following 6/124 polymorphisms were associated with both PFS and PFI: *VEGF* rs2010963, *XRCC1* rs3213239, *ABCG2* rs2046134, *COMT* rs4680, *CYP2C9* rs1057910 and *CYP3A4* rs2740574. The polymorphisms *TP53* rs1042522, *XRCC3* rs1799796, *XRCC3* rs861539 and *FPGS* rs10106 were associated only with PFS, *ERCC1* rs11615 only with PFI while *UGT1A* rs3064744 and *UGT1A* rs4124874 only with OS.

Among the 16 polymorphisms associated with at least one of the considered end points, five of them were involved in the DNA repair pathway and displayed an effect on PFS or PFI, but not on OS. Regarding the drug metabolism pathway, two polymorphisms on *CYP2C9* and *CYP3A4* genes were significantly associated with worse PFS and PFI, with the most significant association showed by *CYP3A4* rs2740574. Precisely, *CYP3A4* rs2740574 patients carrying at least one G variant allele were at increased risk of progression and platinum-resistance compared with AA genotype-bearing patients (HR: 2.03; 95% CI: 1.38–3.96; p = 0.0016 for PFS and HR: 2.47; 95% CI: 1.46–4.20; p = 0.0008 for PFI) according to a dominant model. Correspondingly, the median PFS and PFI were 17 (16–19) versus 12 (10–23) months and 10 (9–13) versus 6 (2–18) months, with the worst times showed by patients carrying at least one G allele.

It is noteworthy that both the polymorphisms emerged in the analysis of the drug transport pathway were on the *ABCG2* gene, with *ABCG2* rs3219191 being associated with all the tested end points and the *ABCG2* rs2046134 demonstrating a detrimental effect both in PFS (HR: 2.05; 95% CI: 1.04–4.04; p = 0.0371) and PFI (HR: 2.47; 95% CI: 1.25–4.88; p = 0.0091).

Five polymorphisms from estrogen pathway were associated with at least one of the end points tested: *COMT* rs4680, *UGT1A* rs3064744, *UGT1A* rs4124874, *UGT1A* rs10929302 and *UGT1A* rs2741045. Interestingly, the

Table 4. SNPs significantly associated with at least one end point.

Pathway	Gene	Base change	rsID	Function	Model	OS		PFS		PFI	
						HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Angiogenesis	*VEGF*	G>C	rs2010963	5′-UTR	Dom	1.08 (0.77–1.51)	0.6675	1.45 (1.06–1.99)	0.0191†	1.48 (1.08–2.02)	0.0139†
DNA repair	*ERCC1*	T>C	rs11615	Synonymous (Asn118Asn)	Dom	1.32 (0.91–1.92)	0.1474	1.35 (0.97–1.89)	0.0797	1.43 (1.02–2.01)	0.0364†
DNA repair	*TP53*	G>C	rs1042522	Missense (Pro72Arg)	Dom	1.22 (0.88–1.70)	0.2382	1.38 (1.02–1.87)	0.0357†	1.34 (0.99–1.82)	0.058
DNA repair	*XRCC1*	delGGCC	rs3213239	2 Kb upstream	Dom	1.32 (0.94–1.85)	0.1044	1.37 (1.01–1.87)	0.0411†	1.39 (1.03–1.90)	0.034†
DNA repair	*XRCC3*	A>G	rs1799796	Intron	Add	0.83 (0.65–1.06)	0.1277	0.79 (0.63–0.98)	0.0339†	0.82 (0.66–1.02)	0.0785
DNA repair	*XRCC3*	C>T	rs861539	Missense (Thr241Met)	Dom	1.16 (0.82–1.62)	0.3993	1.37 (1.01–1.87)	0.0459†	1.32 (0.97–1.8)	0.078
DNA synthesis	*FPGS*	A>G	rs10106	3′-UTR	Rec	0.88 (0.58–1.33)	0.5483	0.89 (0.65–1.24)	0.0474†	0.89 (0.64–1.23)	0.0518
Drug transport	*ABCG2*	A>G	rs2046134	Intron	Dom	1.43 (0.67–3.07)	0.3578	2.05 (1.04–4.04)	0.0371†	2.47 (1.25–4.88)	0.0091†
Drug transport	*ABCG2*	CTCAdel (D > I)	rs3219191	Intron	Add	0.77 (0.60–0.98)	0.036†	0.77 (0.62–0.97)	0.0251†	0.74 (0.59–0.93)	0.0091†
Metabolism	*CYP2C9*	A>C	rs1057910	Missense (Ile359Leu)	Dom	1.28 (0.86–1.91)	0.2174	1.47 (1.03–2.11)	0.0331†	1.49 (1.04–2.13)	0.0284†
Metabolism	*CYP3A4*	A>G	rs2740574	2 Kb upstream	Dom	1.69 (0.90–3.18)	0.1005	2.34 (1.38–3.96)	0.0016†	2.47 (1.46–4.20)	0.0008†
Estrogen	*COMT*	A>G	rs4680	Missense (Val158Met)	Dom	1.32 (0.89–1.95)	0.1624	1.44 (1.01–2.05)	0.0456†	1.43 (1.00–2.03)	0.0492†
Estrogen	*UGT1A*	TA (6 >7)	rs3064744	Intron	Add	1.51 (1.18–1.94)	0.0013†	1.17 (0.94–1.46)	0.1631	1.18 (0.94–1.47)	0.1456
Estrogen	*UGT1A*	A>C	rs4124874	Intron	Add	1.29 (1.01–1.66)	0.0423†	1.19 (0.95–1.49)	0.1277	1.18 (0.95–1.48)	0.1434
Estrogen	*UGT1A*	G>A	rs10929302	Intron	Dom	1.87 (1.32–2.63)	0.0004†	1.87 (1.36–2.56)	0.0001†	1.86 (1.36–2.56)	0.0001†
Estrogen	*UGT1A*	T>C	rs2741045	Intron	Add	1.40 (1.08–1.81)	0.0098†	1.27 (1.01–1.59)	0.0444†	1.27 (1.01–1.60)	0.0421†

Significant multivariate associations between polymorphisms and at least one among OS, PFS and PFI are shown in the table as HRs and 95% CI. The following genetic models were applied: Add, Dom and Rec. Data were adjusted for residual disease and therapy setting.
†Significant associations.
Add: Additive; D: Deletion; Dom: Dominant; HR: Hazard ratio; I: Insertion; OS: Overall survival; PFI: Platinum-free interval; PFS: Progression-free survival; rsID: Reference single nucleotide polymorphism identification number; Rec: Recessive.

100% (4/4) *UGT1A* locus polymorphisms tested showed a deleterious effect on OS (*UGT1A* rs3064744: HR: 1.51; 95% CI: 1.18–1.94; p = 0.0013; *UGT1A* rs4124874: HR: 1.29; 95% CI: 1.01–1.66; p = 0.0423; *UGT1A* rs10929302: HR: 1.87; 95% CI: 1.32–2.63; p = 0.0004; *UGT1A* rs2741045: HR: 1.40; 95% CI: 1.08–1.81; p = 0.0098) and two of them demonstrated a concordantly poorer PFS and PFI.

Three polymorphisms belonging to the drug transport and estrogen metabolism pathways were associated with all the evaluated end points: *ABCG2* rs3219191, *UGT1A* rs10929302 and *UGT1A* rs2741045. *ABCG2* rs3219191 was associated with a decreased risk of death (HR: 0.77; 95% CI: 0.60–0.98; p = 0.036), progression (HR: 0.77; 95% CI: 0.62–0.97; p = 0.0251) and platinum resistance (HR: 0.74; 95% CI: 0.59–0.93; p = 0.0091). Accordingly, its CTCA insertion allele was linked to an increasingly improved median survival time (median OS: Del/Del = 36 [27–48] months, Ins/Del = 52 [43–not reached] months, Ins/Ins = 56 [48–not reached] months), to a longer PFS (median PFS: Del/Del = 16 [12–19] months, Ins/Del = 17 [15–22] months, Ins/Ins = 19 [17–29] months) and also to an increased PFI (median PFI: Del/Del = 5 [4–10] months, Ins/Del = 10 [8–16] months, Ins/Ins = 13 [10–23] months). The other two polymorphisms were both located in the *UGT1A* locus and were associated with a higher risk of death, progression and platinum resistance. Specifically, patients bearing at least one G allele of *UGT1A* rs10929302, according to a dominant model showed an 87% increased risk of death (HR: 1.87; 95% CI: 1.32–2.63; p = 0.0004) and progression (HR: 1.87; 95% CI: 1.36–2.56; p = 0.0001) and an 86% increased risk of platinum resistance (HR: 1.86; 95% CI: 1.36–2.56; p = 0.0001) compared with AA patients. The median survival

times decreased accordingly, with patients carrying at least one G allele having a median OS of 37 (32–43) months (compared with 55 [51–not reached) months of the AA patients), a median PFS of 14 (13–16) months (vs 20 [17–24] months of AA patients) and a poorer PFI of 8 (6–9) months (compared with 13 [10–18] months of AA patients). This polymorphism showed the most significant association with OS. Similarly, also the C variant allele of *UGT1A* rs2741045 was associated with a progressively higher risk of death (HR: 1.40; 95% CI: 1.08–1.81; p = 0.0098), progression (HR: 1.27; 95% CI: 1.01–1.59; p = 0.0444) and platinum resistance (HR: 1.27; 95% CI: 1.01–1.60; p = 0.0421), according to an additive model. Median OS, PFS and PFI were the following for TT, TC and CC patients, respectively: OS: 53 (48–not reached) months versus 43 (36–54) months versus 35 (29–51) months; PFS: 19 (16–23) months versus 16 (13–21) months versus 16 (13–20) months.

Finally, of note, most of the polymorphisms (11/16) showing a significant association with the end points lied in introns or upstream gene regulatory regions, including the three significantly associated with all the end points. For all the associations found, the risk correlated with these polymorphisms was consistent across the three different outcomes.

Combined effect of unfavorable genotypes

With the aim of evaluating whether the combination of multiple genetic alterations in the considered pathways (metabolism, transport and activity of platinum- and taxane derivatives, drug detoxification, DNA repair, synthesis and catabolism, estrogen activity and cell cycle) would have an additive effect, we generated a score based on polymorphisms significantly associated with all the three end points. Precisely, a score with increasing value was attributed to each genotype of *ABCG2* rs3219191 (Ins/Ins vs Ins/Del vs Del/Del) and *UGT1A* rs2741045 (CC vs TC vs TT) as the number of risk alleles increased (0 vs 1 vs 2). Regarding *UGT1A* rs10929302, which was associated with the end points with a dominant model, a score was attributed to the two groups of genotypes of the genetic model (GG = 0 vs AG + AA = 1). A global score, deriving from the sum of the scores of each polymorphism and ranging from 0 to 5, was calculated for each patient. Details on the construction of this score are provided in Table 3. The score was applied to 201 cases successfully genotyped for the three polymorphisms and its application resulted in the stratification of the patients into two distinct prognostic groups (low risk and high risk) (Figure 1).

Patients in the high-risk group (4–5), compared with those in the low-risk group (0–3), had a 1.8-fold (95% CI: 1.3–2.7; p = 0.0019) increased risk of death in a multivariate model. The median survival time was 52.1 months for the low-risk group (95% CI: 46.5–57.1 months), decreasing at 32.8 months (95% CI: 25.3–38.0) for patients in the high-risk group (log-rank p = 0.0012) (Table 5).

This significant difference was observed also for PFS and PFI (Figure 1B & C), both in the univariate and in multivariate models. Compared with patients in the low-risk group, subjects in high-risk group had a twofold (95% CI: 1.4–2.9; p < 0.0001) and 1.9-fold (95% CI: 1.4–2.8; p = 0.0002) increased risk of progression and platinum resistance, respectively. Median PFS were 13.5 (95% CI: 11.8–16.8) and 18.5 (95% CI: 16.3–20.7) months for the high-risk and the low-risk groups, respectively (log-rank p = 0.001), while median PFI were 4.5 (95% CI: 3.4–9.9) and 11.6 (95% CI: 8.7–13.6) months (log-rank p = 0.00039) for the high-risk and the low-risk groups, respectively (Table 5).

Moreover, we assessed the potential clinical impact of the score, adjusting for known clinical prognostic variables (such as R and therapy setting). We found that the score is still highly significant and it is able to improve patients stratification according to the outcome rather than using R and therapy setting alone.

Discussion

PGx predictive and prognostic biomarkers remain an unsolved issue in EOC patients treated with a platinum-based therapy. As recently reported [15], the analysis of polymorphisms derived from candidate genes is still taken into consideration as an attractive strategy, but its implementation in EOC may benefit from wider approaches that consider both coding and noncoding candidate gene regions and integrates polymorphisms data in order to amplify the chances of detecting synergic or additive effects on treatment and disease outcome.

With the aim of trying to deepen the predictive and prognostic role of germ-line polymorphisms in EOC, this study assessed 124 genetic variants from 60 candidate genes involved in platinum, taxane and estrogen pathways in 230 patients. A combinational strategy based on the investigation of polymorphisms in both coding and noncoding DNA combined among each other to increase the possibility to better stratify patients according to their outcome was applied. Sixteen polymorphisms on the aforementioned pathways were significantly and independently associated with OS, PFS or PFI. In particular, the pathways emerged from our analysis were angiogenesis (one

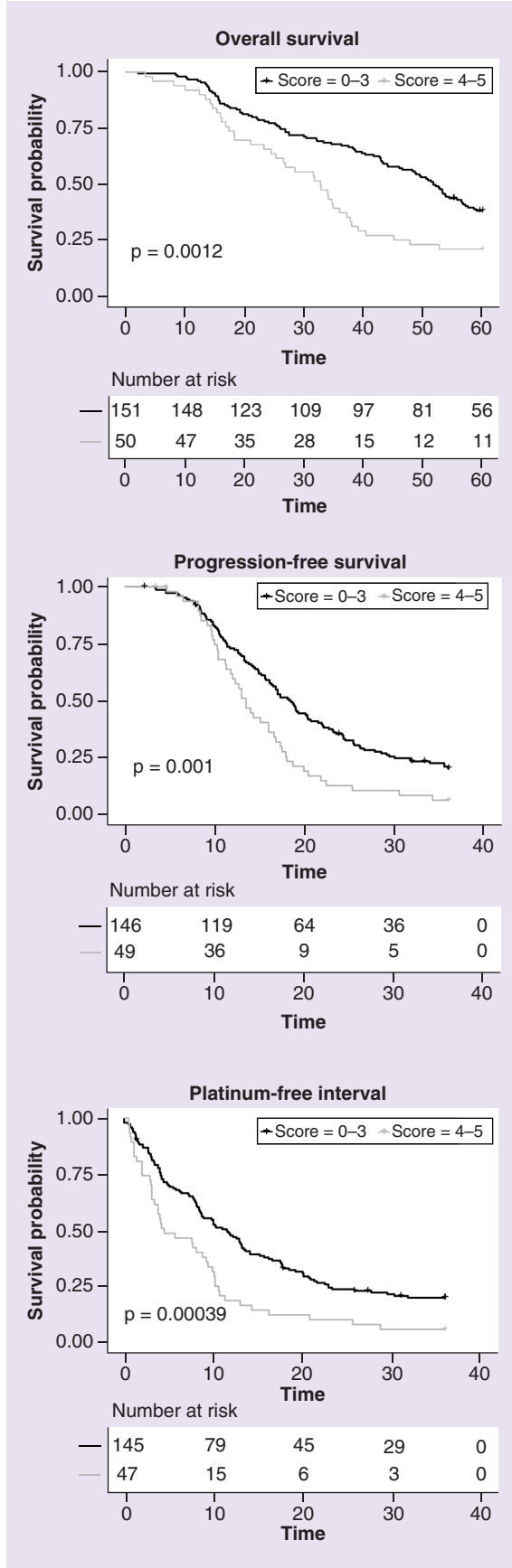

Figure 1. Kaplan–Meier estimates for survivals and log-rank p-values of the univariate associations according to the score for overall survival (A), platinum-free interval (B) and progression-free survival (C). Patients were divided into two groups according to the genotypes they carried. The low-risk group included patients with 0–3 points, and the high-risk group patients with 4–5 points. OS (**A**, p = 0.0012), PFS (**B**, p = 0.001) and PFI (**C**, p = 0.00039) had a significantly worsening trend as the score increased. At the bottom of each panel is reported a table with the number of patients still at risk at the corresponding time-point. OS: Overall survival; PFI: Platinum-free interval; PFS: Progression-free survival.

Table 5. Median survival times and hazard ratios.

Risk group	Score	Patients (n)	Events, n (%)	Median, months (95% CI)	HR (95% CI)	p-value
Overall survival						
Low	0–3	151	92	52.1 (46.5–57.1)	Ref.	
High	4–5	50	39	32.8 (25.3–38.0)	1.8 (1.3–2.7)	0.002†
NACT + IDS	–		163	–	1.9 (1.4–2.8)	0.0003†
RD >0	–		93	–	2.1 (1.5–3.0)	<0.0001†
Progression-free survival						
Low	0–3	146	114	18.5 (16.3–20.7)	Ref.	
High	4–5	49	44	13.5 (11.8–16.8)	2.0 (1.4–2.9)	<0.0001†
NACT + IDS	–		163	–	2.0 (1.4–2.7)	<0.0001†
RD >0	–		93	–	2.2 (1.6–3.0)	<0.0001†
Platinum-free interval						
Low	0–3	145	114	11.6 (8.7–13.6)	Ref.	
High	4–5	47	44	4.5 (3.4–9.9)	1.9 (1.4–2.8)	0.0002†
NACT + IDS	–		163	–	1.6 (1.2–2.2)	0.005†
RD >0	–		93	–	2.1 (1.5–3.0)	<0.0001†

The table reports the multivariate associations between the score and OS, PFS and PFI. Patients were grouped into two groups (low and high risk) according to their genotypes (0–3; 4–5, respectively). As the score increased, the median survival times decreased for all three end points. Accordingly, the risk associated (HR) increased as the score raised. Under the solid lines are reported the associations of the three end points with the clinical variables used as covariates.
†Statistically significant associations.
HR: Hazard ratio; IDS: Interval debulking surgery; NACT: Neoadjuvant chemotherapy; OS: Overall survival; PFI: Platinum-free interval; PFS: Progression-free survival; RD: Residual disease; Ref: Reference.

polymorphism), DNA synthesis and repair (six polymorphisms), drug metabolism (two polymorphisms) and transport (two polymorphisms) and estrogen metabolism (five polymorphisms).

Regarding the angiogenesis pathway, our study highlighted that the *VEGF* rs2010963 C allele carriers had a poorer PFS and PFI. The C allele of this polymorphism, located in the 5′-untranslated region (UTR) of the gene, is associated with an overexpression of the protein, in turn associated with poor prognosis [16]. Moreover, this variant produces a longer VEGF isoform whose function has not been fully elucidated yet, but was found to be overexpressed in tumors. Our work confirms that patients carrying a variant associated with VEGF overexpression had a poorer outcome.

DNA synthesis and repair pathway has been largely investigated in ovarian cancer since cisplatin and carboplatin, used in first-line treatment, are DNA-damaging agents. DNA repair mechanisms are fundamental for recognition and removal of platinum adducts. It has been hypothesized that enhanced DNA repair ability can lead to a lower sensitivity to platinum-based chemotherapy, which is linked to a worse prognosis [17]. Our findings related to this pathway concerned the polymorphisms *ERCC1* rs11615, *TP53* rs1042522, *XRCC1* rs3213239, *XRCC3* rs1799796, *XRCC3* rs861539 and *FPGS* rs10106, which, with the exception of *ERCC1* rs11615, had an effect on PFS. Among them, *XRCC1* rs3213239, an indel located 2 Kb upstream the gene whose functional effect is still unknown, was associated also with PFI, reinforcing the hypothesis that germ-line alteration in DNA repair system have a role in the sensitivity to platinum-based therapy and outcome. *ERCC1* rs11615 was previously suggested as a potential biomarker of platinum sensitivity [18] and our results, highlighting a significant association with PFI, are in line with this hypothesis.

Regarding the drug metabolism pathway, two polymorphisms emerged as significantly associated with both PFS and PFI: *CYP2C9* rs1057910 and *CYP3A4* rs2740574. Among them, *CYP3A4* rs2740574 showed one of the most significant correlations found in this study, with the variant G allele significantly predicting poorer PFS and PFI. It was also associated with poorer OS, but not significantly, and, for this reason it was not included in the prognostic score. CYP3A4 is a Phase I metabolic enzyme, which counts paclitaxel among its substrates. The polymorphism rs2740574 (*CYP3A4*1B*) consists in A to G transition in the promoter region at position -392 [19] and its variant (G) allele is a validated biomarker of ovarian cancer risk [20]. It was also found to affect the OS of EOC patients treated with standard (carbo/cisplatin and paclitaxel) first-line chemotherapy [19]. This result is in line with what we found in our population, although the association with OS was not significant. The G allele seems to increase the *CYP3A4* expression due to a reduced binding of a transcriptional repressor [21], although this biological effect

is still controversial [22,23]. It should be considered that the frequency of the G allele is quite low (4%) and no homozygous GG patients were found in our cohort, so our results came from the comparison between 208 AA patients versus 16 AG patients in a dominant model. It is possible that a larger population, where the variant allele is present in a higher number of subjects may be needed to clarify the role of this polymorphism.

It is noteworthy that five polymorphisms (*COMT rs4680, UGT1A rs3064744, UGT1A rs4124874, UGT1A rs10929302* and *UGT1A rs2741045*) of the estrogen pathway displayed significant association with at least one end point and all of them were associated with a poorer outcome. Even more remarkable, is that the polymorphisms clustered on genes deputized to estrogen catabolism (*COMT* and *UGT1A*). It is well known that polymorphisms within genes responsible for estrogen catabolism could alter cellular levels of genotoxic 4-hydroxylated catechol estrogens and antiangiogenic 2-methoxyestradiol, thus influencing the risk of developing ovarian cancer [24]. Female steroid hormones have been correlated to EOC pathogenesis and to epithelial-to-mesenchymal transition of ovarian cancer cells, a phenomenon that is noted to cause drug resistance [5]. In addition, epidemiological data show that ovarian cancer initiation and its biology are related to life-time estrogen exposure [25]. As a consequence, a modified pathway in estrogen metabolism could affect the levels of these hormones or their carcinogenic metabolites in blood and this could impact on outcomes and prognosis of EOC patients. Based on the prognostic effect of the polymorphisms identified from our study, we hypothesize that alterations in the clearance of estrogens and their hydroxylated carcinogenic metabolites may also have a role in EOC prognosis definition.

The remaining results are related to two polymorphisms (*ABCG2* rs2046134 and *ABCG2* rs3219191) in drug transporters. Both these polymorphisms were associated with PFI. Of note, the only two polymorphisms emerged from drug transporters clustered on the *ABCG2* gene, an efflux pump whose substrates include carboplatin and paclitaxel, suggesting that genetic alterations in this gene could be involved in drug resistance, especially to platinum-based chemotherapy.

Among the 16 polymorphisms that emerged, three genetic variants, one on *ABCG2* gene and two in the *UGT1A* locus, were associated with all three end points (OS, PFS and PFI), demonstrating a concordant prognostic effect. From their combination, we generated a prognostic score based on the genotypes carried by each patient, identifying two risk groups (high- and low-risk), characterized by an explicit and significant worse OS, PFS and PFI as the score increased (32.8 vs 52.1 months for OS, 13.5 vs 18.5 months for PFS, 4.5 vs 11.6 months for PFI, respectively). It is remarkable that the score was able not only to discriminate patients according to their platinum sensitivity but also to identify patients with higher probability of progression and death.

These results suggested an additive impact of polymorphisms within different pathways and the score allowed to better stratify patients according to their clinical outcome compared with the single SNPs, in particular when considering PFS and PFI (Tables 4 & 5). Moreover, the proposed score maintained its prognostic significance also when adjusted for presence of residual disease and neoadjuvant therapy, the clinical variables significantly associated with poor prognosis in our EOC population (Table 5).

The polymorphisms included in the score lied on *ABCG2* gene and *UGT1A* locus. *ABCG2* encodes for the BRCP, a member of the ATB-binding cassette (ABC) *trans*-membrane transporters [26,27]. Its activity consists in the energy-mediated transport of a variety of drugs out of the cell against concentration gradient, including several drugs commonly used for the treatment of EOC, such as cisplatin, carboplatin, paclitaxel, doxorubicin, gemcitabine and topotecan [28]. This transporter, besides being involved in drug adsorption, distribution and elimination, also contributes to multidrug resistance [29]. In fact, associations with clinical outcomes of patients treated with *ABCG2* substrates have been previously reported [27,30]. Yoh *et al.* [31] assessed the expression of several ABC transporters in patients with advanced non-small-cell lung cancer reporting a better response to platinum derivatives and improved PFS and OS in *ABCG2*-negative patients compared with that of *ABCG2*-positive patients.

Although up to date the functional effects of *ABCG2* rs3219191 indel has not been clarified, several researches suggest that polymorphisms in this gene may alter the ATPase activity or reduce the efflux activity of the transporter, resulting in an enhanced drug sensitivity [32–34]. A large study conducted by the Gynecologic Oncology Group on 511 EOC patients with the aim of evaluating associations between functional polymorphisms in several ABC transporters and patients' clinical outcome, obtained statistically significant results only for a polymorphism on the *ABCG2* gene, the rs2231142, whose variant allele was associated with median PFS and a reduced risk of progression compared with patients bearing the wild-type gene [28]. However, the same study failed to demonstrate an association with OS. Although that study did not considered the *ABCG2* rs3219191, the effect showed by this polymorphism in our population has a comparable effect. Patients carrying the variant allele (Insertion) of *ABCG2* rs3219191 indel showed an increasingly improved PFS (Ins/Del = 17.0 vs Ins/Ins = 19.1 months) compared

with those bearing the wild-type variant (Del/Del = 16 months). In addition, our study demonstrated that this polymorphism had a significant effect also in OS and PFI. The variant allele was associated also with a progressively prolonged OS (Del/Del = 36 vs Ins/Del = 52.1 vs Ins/Ins = 55.75 months) and PFI (Del/Del = 4.5 months vs Ins/Del = 9.9 vs Ins/Ins = 13.1 months). It can be hypothesized that alterations in noncoding portions of this gene may have consequences in its expression, which may lower its efflux activity of platinum and taxane derivatives, enhancing the drug action inside tumor cells and delaying the onset of chemoresistance. This can affect PFS and PFI as observed in our study. The effect on OS may be explained by the monocentric nature of our study that allowed to decrease the disparity in patients' treatment after the failure of platinum-based therapies, a factor that may affect, at least in part, the OS.

The other two polymorphisms included in the score (*UGT1A* rs2741045 and *UGT1A* rs10929302) belonged to the *UGT1A*, a complex locus encoding for several Uridine 5′-diphospho -glucuronosyltransferases (UGTs), that are identified as *UGT1A1-10*. The locus includes several exons: some of them are characteristics of one specific protein, others are in common among the different isoenzymes encoded. The genetic construction of this locus maintains its complexity also in the sequences between exons that are shared among different isoforms. In fact, all the *UGT1A* polymorphisms investigated in the present study were reported to be present in the introns/promoter regions of multiple isoforms. UGTs are a superfamily of Phase II enzymes that conjugate endogenous compounds, such as bilirubin or estrogens, and xenobiotics with glucuronic acid to make them more hydrosoluble and, therefore, easier to excrete. Although the functional effect of the polymorphism included in the score remains to be elucidated, both *UGT1A* rs2741045 and *UGT1A* rs10929302 were associated with bilirubin-circulating levels, underlying a possible effect of these genetic variations in influencing the enzyme activity [35–38].

The *UGT1A* locus is highly polymorphic, and its genetic variations have shown to affect the global activity of UGTs, acknowledged as very important pharmacogenes [39]. As aforementioned, these genes are deputized to the catabolism of several carcinogenic substances, including estrogens and their carcinogenic derivatives. In fact, polymorphisms on this gene were also linked to the risk of developing several types of cancer, due to the decreased capacity to glucuronidate carcinogens and other types of cancer-promoting molecules (e.g., sex hormones). In particular, increased risk of developing breast, bladder, colorectal, endometrial, esophageal, head and neck, liver, lung, prostate and thyroid cancers was reported [40,41], and, regarding ovarian cancer, the association between the *UGT1A1*28* rs3064744 polymorphism and the mucinous histological subtype was also previously described [42]. Besides the two polymorphisms included in the score (*UGT1A* rs2741045 and *UGT1A* rs10929302), even the two other *UGT1A* polymorphisms investigated in the study (*UGT1A* rs3064744 and *UGT1A* rs4124874) were associated with poorer OS, further confirming the prognostic role of *UGT1A* genetic variants. At best of our knowledge, this finding was never previously reported and the biological explanation for the association between the two *UGT1A* polymorphisms (*UGT1A* rs2741045 and *UGT1A* rs10929302) with OS, PFS and PFI in EOC is to be defined since these genes do not have a known direct effect in platinum or taxane metabolism, but, as mentioned before, are involved in estrogen detoxification. However, the effect of *UGT1A* polymorphisms on sex hormones in EOC and the resulting prognostic consequence deserve to be deepened with further studies.

An intriguing finding derived from this work is that most of the significant associations (11/16, nearly the 70%) between polymorphisms and OS, PFS and PFI mainly concern intronic or upstream gene regulatory regions and this suggests that these regions should be more deeply investigated. It is a common approach in the candidate gene studies to focus mainly on functional polymorphisms that alter the protein-coding sequence or have a direct effect in the protein structure. However, the coding fraction of a genome represents only the 2% of its entire sequence that actually comprehends regulatory elements and/or it is transcribed into noncoding RNAs [43]. Very little attention has been paid to this part of the genome that has been disregarded as 'junk-DNA' for many years. Thanks to the advances in the sequences technology, a huge amount of sequence data became available, both from germ-line and tumor tissues, that provided the chance to shed new light in the role of this noncoding part of the genome. Polymorphisms in these regions can alter the binding sites of transcription factors, by creating new binding sites or by disrupting existing ones. In this regard, the intronic *UGT1A* rs2741045C>T polymorphism included in our score was predicted by Haploreg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php [44]) to change the motif bound by AP-1 in liver and lung cell lines. AP-1 represents a transcription factor subunit whose human counterpart is called JUN, a putative transforming protein that directly interacts with specific target DNA sequences to regulate gene expression. Also the *UGT1A* rs10929302G>A intronic polymorphism is predicted to alter the motif bound by MXI1, an oncogenic transcription factor tightly regulated in normal cells but frequently deregulated in human cancers. In particular, it acts as a transcriptional repressor thought to negatively regulate

MYC function and therefore it is considered a potential tumor suppressor. In addition, structural aberrations such as insertions or deletions may alter the proper interaction of regulatory elements with their controlled genes [43]. Interestingly, the *ABCG2* rs3219191 indel polymorphism, also included in our score, was predicted to change the motif bound from Zfp410 in several cell lines. Alterations in this motif make the site no more recognizable from DNA-binding factors, which may result in transcription modification.

The potential clinical impact of the obtained results underlines how the type and the number of germ-line polymorphisms in drug and estrogen associated pathways may contribute to the patient-specific capability of coping with the disease and produce an adequate response to therapy. In particular, this new score, whether validated in an external cohort of patients, should be tested as soon as possible in patient' clinical course, such as at surgery or at biopsy, when the EOC diagnosis occurs. We can speculate that this would allow to identify those patients that are at higher risk of worse prognosis and could benefit from insertion in clinical trials with treatment dose escalation or experimental therapies. As regards the limits of this study, the main one is the lack of the validation of the proposed prognostic score in an external cohort of patients, that is an essential step to demonstrate its validity. In the absence of the possibility to reproduce our results in an independent set of patients, we adopted a statistical approach (adjusted multivariate analyses) and a selection strategy to include polymorphisms into the score (i.e., all those associated with all the three end points) to produce sufficiently reliable hypothesis-generating results. Another issue to be reported is that the presented dataset was already used to define another prognostic signature [14]. In our opinion, this is not in contrast with the prognostic score defined within this work, since these signatures reflect different aspects of patients' background (i.e., on the one hand, the immune system profile and, on the other hand, drugs PK/PD and sexual hormone status) that may contribute and cooperate to determine the outcome of the disease. Moreover, our study population included only self-reported Caucasian patients. As already well known the frequency and the impact of polymorphisms in different ethnicities are very heterogeneous. This will limit the generalizability of our prognostic score to Caucasian patients, leaving its impact on other ethnicities still unknown and unpredictable: for this reason, it would be interesting to test it in groups of patients of different origins from Caucasian. Finally, we could not take into account the patients' *BRCA* status, an important predictive factor for response to therapy, since the study has retrospectively enrolled patients managed in a time in which somatic and germ-line *BRCA* testing were not performed in a widespread manner. However, it would be essential to understand the impact of *BRCA* patient's status on the proposed prognostic score.

Conclusion

In conclusion, this study demonstrated the potentiality of polymorphisms in platinum-taxane transport and estrogen pathways in predicting ovarian cancer patients survival and platinum sensitivity, allowing to elaborate a PGx score, based on three genetic variants (*ABCG2* rs3219191, *UGT1A* rs2741045 and *UGT1A* rs10929302), with a prognostic value. Although these findings must be regarded as exploratory, if independently and clinically validated, they could be exploited to select high-risk patients and to help the oncologist in addressing therapeutic decision making.

Future perspective

The role of PGx in EOC is still controversial. In recent years, new approaches such as genome-wide association studies and technologies such as next-generation sequencing allowed to generate a huge amount of genetic information. Still, gene candidate approach, the more easily translatable into the clinics, may be applied if wider approaches are used. Our study prompted the investigation of that part of the genome which for decades has been neglected as considered useless: the noncoding sequences. The deeper investigation of this part of the genome may give new tools and perspectives in future researches.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.4155/pgs-2020-0049

Author contributions

S Gagno contributed to the conceptualization, data curation, investigation, methodology, project administration, visualization, writing – original draft and writing – review and editing. M Bartoletti was responsible for the conceptualization, investigation, resources and data curation. C Romualdi contributed to the methodology, formal analysis and validation. E Poletto performed

investigation, data curation, resources and supervision. S Scalone was responsible for the resources and investigation. R Sorio was responsible for the resources, supervision and conceptualization. M Zanchetta helped in writing – review and editing. E De Mattia contributed to the software, data curation and writing – review and editing. R Roncato performed investigation. E Cecchin helped in supervision. G Giorda was responsible for the resources. G Toffoli contributed to the conceptualization, project administration, resources, funding acquisition and supervision.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval (internal code CRO-2014-43) and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. In addition, informed consent has been obtained from the participants involved.

Summary points

- The role of pharmacogenetics in ovarian cancer is still unclear.
- The discovery of predictive and prognostic biomarkers would help clinicians to guide their treatment decisions.
- A combinational strategy based on the investigation of polymorphisms in both coding and noncoding DNA combined among each other to increase the possibility to better stratify patients according to their outcome was applied.
- A panel of 124 polymorphisms in 60 genes involved in drug metabolism, membrane transport, detoxification, DNA synthesis and catabolism, DNA repair, estrogen activity and metabolism, cell cycle regulation and angiogenesis was investigated in 230 advanced ovarian cancer patients.
- Sixteen polymorphisms in angiogenesis (one), DNA synthesis and repair (six), drug metabolism (two), drug transport (two) and estrogen detoxification (five) pathways were significantly associated with overall survival (OS), progression-free survival (PFS) or platinum-free interval (PFI), suggesting a role of these pathways in epithelial ovarian cancer (EOC) prognosis definition.
- Three polymorphisms were able to predict each end point with a concordant effect and were used to generate a score, which allowed patients to be divided into two prognostic groups according to their outcome.
- All the *UGT1A* polymorphisms tested in our study showed a significant association with OS, supporting a role of *UGT1A* in EOC prognosis as observed for other types of cancer, possibly due to its involvement in estrogen catabolism.
- A polymorphism on another estrogen metabolic enzyme (*COMT*) has also a significant effect on PFS and PFI.
- Most of the polymorphisms significantly associated with OS, PFS and PFI were present within intronic/upstream gene regulatory regions suggesting that a deeper exploration of these regions may be considered.
- The combination of multiple intronic polymorphisms into a score allowed to exploit the candidate gene approach to stratify EOC patients for OS, PFS and PFI.

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