ABCB1 (*MDR 1*) Polymorphisms and Progression-Free Survival among Women with Ovarian Cancer following Paclitaxel/Carboplatin Chemotherapy

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Abstract

Purpose: The human *ABCB1* gene encodes P-glycoprotein, which transports a broad range of anticancer drugs, including paclitaxel. Although the functional consequences of *ABCB1* polymorphisms have been the subject of numerous studies, few have assessed the association with clinical outcome.

Experimental Design: We assessed the association between the 2677G>T/A, 3435C>T, and 1236C>T *ABCB1* polymorphisms and progression-free and overall survival in 309 patients from the Australian Ovarian Cancer Study treated with paclitaxel/carboplatin and subsequently tested significant observations in an independent validation set.

Results: Women who carried the minor T/A alleles at the 2677G>T/A polymorphism were significantly less likely to relapse following treatment compared with homozygote GG carriers ($P_{\text{Log-rank}} = 0.001$) in the Australian Ovarian Cancer Study cohort. Subgroup analyses showed that this effect was limited to cases with residual disease ≤1 cm ($P_{\text{Log-rank}} = 0.0004$), not for those with residual disease >1 cm ($P_{\text{Log-rank}} = 0.3$). This effect was not confirmed in an independent validation set of carboplatin/paclitaxel-treated patients ($n = 278$) using a higher residual disease cut point (≤2 cm). However, analysis of the unrestricted data set expanded to include docetaxel-treated patients ($n = 914$) did support an effect of the 2677T/A allele in patients with no macroscopic residual disease (hazard ratio, 0.70; 95% confidence interval, 0.46-1.04; $P_{\text{one-sided}} = 0.039$).

Conclusion: Our findings indicate that there is an effect of the 2677G>T/A polymorphism on progression-free survival in ovarian cancer patients who are treated with a taxane/carboplatin, which is dependent on the extent of residual disease, with a better prognosis for patients with the 2677T/A allele and minimal residual disease.

Ovarian cancer is the seventh leading cause of cancer mortality among women globally, accounting for 4.2% of cancer deaths (1, 2). Treatment of advanced ovarian cancer typically involves cytoreductive surgery followed by chemotherapy with a combination of a platin (usually carboplatin) and a taxane (usually paclitaxel; refs. 3, 4). Although response rates to initial chemotherapy are high, not all patients respond, and the basis of varying tumor responsiveness and toxicity between patients with otherwise ostensibly similar tumors and apparently standardized chemotherapy protocols remains largely unknown (5). Hypotheses for interindividual differences in drug levels (pharmacokinetics) and/or drug metabolism (pharmacodynamics) have been postulated. Unfortunately, even after a good initial response to chemotherapy, the majority of patients will eventually develop drug-resistant disease and succumb (6).

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Received 3/5/08; revised 5/15/08; accepted 5/22/08.

Grant support: U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0729, National Health and Medical Research Council of Australia, Cancer Council Victoria, Cancer Council Queensland, Cancer Council New South Wales, Cancer Council South Australia, The Cancer Foundation of Western Australia and Cancer Council Tasmania, NIH grant R21 CA113491 (St. Louis and Glasgow), and Cancer Research UK programme grant C536/A2662 (Glasgow).

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Note: The full AOCS Study Group is listed on http://www.aocstudy.org/.

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doi:10.1158/1078-0432.CCR-08-0606

Translational Relevance

Current "standard of care" for advanced ovarian cancer typically involves cytoreductive surgery followed by chemotherapy with paclitaxel and carboplatin. Although initially effective in the majority of patients, tumor response to chemotherapy in individual patients can vary widely. Inherited differences that alter the expression or function of proteins that are involved in drug disposition could contribute significantly to this variation in individual response. We report a significant effect of the *ATP-binding cassette transporter B1* (*ABCB1*; *multidrug resistance 1*) 2677G›T/A genotypes on progression-free survival in women with ovarian cancer, particularly among women with optimally debulked tumors. If our findings are confirmed, there could be significant clinical implications because the ability to predict chemotherapy response could (*a*) influence optimal dosing based on individual *ABCB1* genotypes, (*b*) allow selection of alternative drugs that are not phosphoglycoprotein substrates, and (*c*) identify patients who might be exposed to the toxic effects of chemotherapy without significant benefit. Furthermore, the clinical implications could extend to any chemotherapeutic agents that are phosphoglycoprotein substrates and to other cancers treated with such drugs. In addition, this study might generate further interest in the delineation of this and other pharmacogenetic markers of relevance to chemotherapeutic outcomes and ultimately to individualization of treatment according to genotype.

Paclitaxel is a potent anticancer drug that is effective against a wide range of solid tumors (7). Several mechanisms of drug resistance to paclitaxel have been hypothesized, one of the most widely studied being the amplification and expression of phosphoglycoprotein (PGP), an energy-dependent drug efflux pump (8). PGP is a transmembrane transporter belonging to the superfamily of ATP-binding cassette (ABC) transporters and is encoded by the human *ABCB1* [also known as *multidrug resistance 1* (*MDR1*)] gene (9). It has both an excretory and protective function and plays a major role in drug disposition by limiting intracellular uptake of substrates from the gastrointestinal tract and contributing to their excretion via the liver, kidneys, and intestine. PGP transports a broad range of compounds, including most natural anticancer drugs such as paclitaxel (10, 11). *In vitro* studies have shown that high levels of PGP expression are correlated with multidrug resistance in several cell lines and that the degree of overexpression correlates with the degree of resistance (12). More recently, clinical studies of ovarian cancer patients showed that increased PGP expression correlates unfavorably with response and progression-free survival following chemotherapy (13).

It is plausible that variation in response to chemotherapy may be explained by interindividual variability in PGP expression, which may in part be influenced by *ABCB1* single nucleotide polymorphisms (SNP). The first *ABCB1* SNP to be identified was the triallelic 2677G>T/A SNP (alanine>serine or threonine; rs2032582; ref. 14), which is one of the most common of the coding region variants known, the most common being the synonymous 1236C>T (rs1128503) and 3435C>T (rs1045642) SNPs (15).

There have been a limited number of studies assessing the success or failure of drug therapy for ovarian cancer in relation to *ABCB1* genotypes. A study of 53 ovarian cancer patients assessed the 2677G>T/A and 3435C>T SNPs and response to chemotherapy (16). A significant association was observed between homozygosity for the minor T/A allele of the 2677G>T/A SNP and a good response to paclitaxel treatment (P = 0.03). In contrast, Marsh et al. (17, 18) reported no significant association with 2677G>T/A and progression-free survival in 914 women. We analyzed the 2677G>T/A, 3435C>T, and 1236C>T *ABCB1* SNPs and their association with progression-free and overall survival in 309 women with epithelial ovarian cancer to determine whether *ABCB1* genotypes influence response to chemotherapy.

Materials and Methods

Patient cohort. This study was approved by the Human Research Ethics Committees at the Queensland Institute of Medical Research, Peter MacCallum Cancer Centre, University of Melbourne, and all participating hospitals and cancer registries. The Australian Ovarian Cancer Study[11] (AOCS) is a population-based case-control study previously described (19). Between January 2002 and June 2006, eligible women ages 18 to 79 y with a suspected diagnosis of epithelial ovarian, fallopian tube, or primary peritoneal cancer were recruited through gynecologic oncology units across all Australian states. Additional cases missed at the major treatment centers were identified through cancer registries and invited to participate. Information on primary site of tumor, histologic subtype, invasiveness, and grade was abstracted independently from histology reports by two researchers, and discrepancies were resolved by consensus. Detailed clinical and follow-up data were obtained at predefined intervals: after surgery, after primary chemotherapy, at 6 monthly intervals to 5 y, and annually thereafter. Treatment and clinical assessments were abstracted from medical records using case report forms, following Good Clinical Practice guidelines,[12] and included details of chemotherapy regimen, tumor imaging, and serial serum CA125 levels.

Cases were included in the current analyses if they met the following criteria: a diagnosis of primary invasive ovarian (n = 262), peritoneal (n = 35), or fallopian tube (n = 12) cancer; collection of primary treatment data was complete at the time of genotyping; and treatment with paclitaxel at a dose of 175 or 135 mg/m^2 and carboplatin (area under the curve, 5 or 6) for at least four cycles at 3 weekly intervals. The majority of women had undergone laparotomy for diagnosis, staging, and tumor debulking before chemotherapy, but 31 women who had neoadjuvant chemotherapy before surgery were also included.

Clinical definitions. Surgical staging was assessed in accordance with International Federation of Gynecologists and Obstetricians (FIGO) classification. For the AOCS cohort, optimal debulking was defined as ≤1 cm (diameter) residual disease, and suboptimal debulking was >1 cm (diameter) residual disease. Progression-free survival was defined as the time interval between the date of histologic

[11] http://www.aocstudy.org

[12] ICH E6: U.S. Food and Drug Administration; Good Clinical Practice: Consolidated Guidance. 1996. http://www.fda.gov/oc/gcp/guidance.html.

diagnosis and the first confirmed sign of disease recurrence, or progression, based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria modified for ovarian cancer as defined by the Gynecologic Cancer Intergroup (20). Briefly, in the majority of cases (71%), the date of progression was assigned using CA125 criteria: In patients with elevated CA125 pretreatment and normalization of CA125, progression was defined as an increase in CA125 to greater than, or equal to, two times the upper normal limit. In patients with elevated CA125 pretreatment, which never normalized, progression was defined as an increase in CA125 greater than, or equal to, two times the nadir value. Elevated values were confirmed by two separate measurements obtained at least 1 wk apart. The upper normal limit for each individual CA125 reading was used, as the value can vary depending on the assay type. CA125 progression was assigned as the date of the first measurement that meets the criteria as noted above. In cases where CA125 was not a marker, or progression preceded an increase in CA125, relapse was based on imaging (appearance of new lesion), or, in a minority of cases, global deterioration in health status attributable to the disease, or death.

DNA extraction and genotyping. DNA was extracted from peripheral blood and *ABCB1* SNPs were genotyped using matrix-assisted laser (MALDI-TOF) desorption/ionization time-of-flight mass spectrophotometric mass determination of allele-specific primer extension products using MassARRAY system and iPLEX technology. The design of amplification and extension oligonucleotides was carried out according to Sequenom guidelines using MassARRAY Assay Design software (version 1.0). PCR amplification of amplicons containing SNP(s) of interest was done using Qiagen HotStart Taq Polymerase on a Perkin-Elmer GeneAmp 2400 thermal cycler with 5 ng genomic DNA. Genotyping of the 2677G>T/A SNP was carried out in a uniplex PCR but 3435C>T and 1236C>T were amplified together. Case and control samples were plated randomly with 10% duplicated samples. The overall call rate was >98% and the duplicate concordance rate was >99%. We genotyped 770 controls from the AOCS study to establish allelic frequencies in our population and to further assess the genotyping quality by analysis of Hardy-Weinberg equilibrium. *ABCB1* genotype frequencies for each SNP did not deviate from expected proportions under Hardy-Weinberg assumptions ($P \geq 0.5$), and allele frequencies were concordant with published HapMap data for Caucasians.

Validation data set. The Scottish Randomised Trial in Ovarian Cancer (SCOTROC1) pharmacogenetic data published and described by Marsh et al. (18) was used as a validation set to test the significant observations from this analysis of the AOCS data. This set is completely independent of the AOCS data.

Statistical analyses. The genotype data for all SNPs were assessed for deviations from Hardy-Weinberg proportions among controls using the χ^2 goodness-of-fit test. The Kaplan-Meier product limit method was used to estimate and plot the overall and residual disease-specific (optimal versus suboptimal) progression-free and overall survival probabilities. Equality of survivor functions among *ABCB1* genotypes was assessed using the log-rank test and the *P* values for trend in survival probabilities for increasing minor alleles were derived from χ^2 test on one degree of freedom. Time to event (months) was calculated from the date of histologic diagnosis of ovarian cancer until documented progression of disease or death from ovarian cancer. Women were censored at the date of last follow-up or death due to unknown, or other, reasons unrelated to ovarian cancer. Cox proportional hazards models were used to obtain hazard ratios (HR) and 95% confidence intervals (95% CI) for the association between genotype and outcome, both unadjusted and adjusted for the effects of FIGO stage and residual disease (none versus ≤1 cm versus >1 cm). Statistical tests and *P* values derived from the AOCS data were two tailed and statistical significance was assessed at the conventional level of <0.05. Statistical analyses were done using STATA 9.0 (Stata Corp.). Similar techniques were used in the validation set, except that

Table 1. Clinical characteristics and 2677G>T/A genotype distribution of the AOCS cohort

	Cases (*n* = 309)	2677G>T/A genotypes*			*P* †
		GG (*n* = 91)	GA/GT (*n* = 141)	TT/TA (*n* = 68)	
Median age (range)	58.4 (31.3-80.5)	58.3 (35.5-78.5)	58.5 (35.5-78.5)	58.5 (42.7-76.5)	0.4
Primary site					
Ovary	262 (84.8)	76 (83.5)	116 (82.3)	63 (92.6)	0.3
Fallopian tube	12 (3.9)	3 (3.3)	7 (4.5)	2 (2.9)	
Peritoneal	35 (11.3)	12 (13.2)	18 (12.8)	3 (4.4)	
FIGO stage					
I	42 (13.6)	7 (7.7)	20 (14.2)	13 (19.1)	0.5
II	25 (8.1)	7 (7.7)	12 (8.5)	5 (7.3)	
III	212 (68.6)	68 (74.7)	94 (66.7)	45 (66.2)	
IV	30 (9.7)	9 (9.9)	15 (10.6)	5 (7.34	
Histology					
Serous	217 (70.2)	66 (72.5)	98 (69.5)	46 (67.7)	0.7
Clear cell	27 (8.7)	8 (8.8)	12 (8.5)	6 (8.8)	
Endometrioid	22 (7.1)	4 (4.4)	10 (7.1)	7 (10.3)	
MMMT	13 (4.2)	7 (7.7)	4 (2.8)	2 (2.9)	
Mucinous	6 (1.9)	1 (1.1)	4 (2.8)	1 (1.5)	
Other	24 (7.8)	5 (5.5)	13 (9.3)	6 (8.8)	
Residual disease					
Nil/microscopic	133 (43.1)	36 (39.6)	67 (47.5)	27 (39.7)	0.2
≤1 cm	94 (30.4)	27 (29.7)	43 (30.5)	20 (29.4)	
>1 to ≤2 cm	14 (4.5)	4 (4.4)	6 (4.3)	4 (5.9)	
>2 cm	47 (15.2)	13 (14.3)	22 (15.6)	11 (16.2)	
Macroscopic, size unknown	21 (6.8)	11 (12.0)	3 (2.1)	6 (8.8)	

Abbreviation: MMMT, malignant mullerian mixed tumor.
**n* does not sum to 309 because of missing genotype data.
† *P* values are based on χ^2 test for significant differences in frequencies between genotypes.

Table 2. Association between *ABCB1* 2677G>T/A genotypes and progression-free and overall survival

ABCB1 genotype	Cases, *n* (%)*	Relapse, *n* (%)	Died, *n* (%)	Progression-free survival, HR (95% CI), *P* value	
				Unadjusted	Adjusted ‡
2677G>T/A					
GG	91 (30.3)	66 (36.7)	28 (36.4)	1.00	1.00
GA/GT	141 (47.0)	77 (42.8)	32 (41.5)	0.62 (0.44-0.86), *P* = 0.004	0.74 (0.52-1.05), *P* = 0.1
TT/TA	68 (22.7)	37 (20.6)	17 (22.1)	0.60 (0.40-0.89), *P* = 0.013	0.74 (0.48-1.14), *P* = 0.2
GA/GT + TT/TA	209 (69.7)	114 (63.3)	49 (63.6)	0.61 (0.45-0.83), *P* = 0.002	0.74 (0.54-1.03), *P* = 0.07
3435C>T					
CC	80 (26.0)	44 (23.7)	20 (26.0)	1.00	1.00
CT	148 (48.0)	91 (48.9)	32 (41.5)	1.13 (0.79-1.62), *P* = 0.5	1.00 (0.68-1.46), *P* = 1.0
TT	80 (26.0)	51 (27.4)	25 (32.5)	1.22 (0.81-1.82), *P* = 0.3	1.06 (0.69-1.63), *P* = 0.8
1236C>T					
CC	94 (30.6)	55 (29.6)	26 (33.8)	1.00	1.00
CT	156 (50.8)	92 (49.4)	36 (46.7)	1.00 (0.71-1.39), *P* = 1.0	1.18 (0.83-1.68), *P* = 0.4
TT	57 (18.6)	39 (21.0)	15 (19.5)	1.14 (0.75-1.72), *P* = 0.5	1.03 (0.66-1.61), *P* = 0.9

*n does not sum to 309 due to missing genotype data.
† χ^2 test for trend in progression-free or overall survival by increasing number of T/A alleles.
‡ Adjusted for FIGO stage and residual disease.

one-tailed tests were used to test hypotheses where the direction of the association was prespecified.

Results

Of the 309 AOCS women with invasive epithelial cancer, 262 (85%) had primary ovarian tumors, 242 (78.3%) had FIGO stage III or IV disease, 217 (70%) had serous tumors, and 227 (73%) had optimal debulking (Table 1). Disease progression occurred in 186 women and 84 died during a median follow-up of 24.3 months. In 77 (91.7%) women, death was due to ovarian cancer. The remaining seven women died from unknown or other causes.

Analyses of the 3435C>T and 1236C>T SNPs revealed no significant associations with progression-free or overall survival in either unadjusted or adjusted models (Table 2). Allele frequencies for the G, T, and A alleles of the 2677G>T/A SNP in cases were 0.53, 0.45, and 0.02, respectively. The T and A alleles were defined collectively as the "minor allele." There were no significant associations between 2677G>T/A genotype and patient age at diagnosis, primary site, FIGO stage, histology, or residual disease following debulking laparotomy (Table 1). However, the HRs suggested a 40% reduction in risk of disease progression among heterozygous (P = 0.004) and homozygous (P = 0.013) carriers of the minor 2677T/A allele (Table 2; Fig. 1A). The effect was seen both in the co-dominant model comparing (GA/GT) heterozygotes and minor (TT/TA) homozygotes versus the common (GG) homozygotes ($P_{\text{Log-rank}}$ = 0.006) and in a model comparing the combined minor allele carriers (GA/GT + TT/TA) versus the common (GG) homozygotes ($P_{\text{Log-rank}}$ = 0.001; see Fig. 1B). A similar association was observed after adjustment for FIGO stage and residual disease (adjusted $HR_{\text{T/A carriers}}$, 0.74; 95% CI, 0.5-1.03; P = 0.07). Adjustment for stage in particular attenuated the associations, and the separate estimates for heterozygote and homozygote carriers of the minor T/A allele were no longer significant. Further adjustment for histologic subtype did not appreciably alter the association (data not shown). This suggests that the effect of 2677G>T/A genotype on progression is independent of other known clinical prognostic factors, although this did not achieve the prespecified significance level of $P < 0.05$ (Table 2).

Marsh et al. (17) reported no association between 2677G>T/A genotype and response to treatment. However, the distribution of size of residual disease varied considerably between the AOCS cohort and the SCOTROC1 patients described by Marsh et al. We therefore did subgroup analysis based on extent of residual disease. This analysis showed that cases with optimal debulking showed a significant association between 2677G>T/A genotype and disease progression in both unadjusted and adjusted models (Table 3; Fig. 1C and D). The HR indicated a 49% reduction in risk (Table 3) of disease progression for heterozygote and homozygote carriers of the minor 2677T/A allele compared with GG homozygotes in the optimally debulked group ($P_{\text{Log-rank}}$ = 0.0004; Fig. 1D) but not for patients with suboptimal debulking ($P_{\text{Log-rank}} > 0.3$; Fig. 1E and F). This difference by debulking status was statistically significant ($P_{\text{interaction}}$ = 0.01).

Analyses of the 2677G>T/A SNP with regard to overall survival revealed no significant survival advantage associated with this SNP in either crude or residual disease subgroup analysis (Tables 2 and 3).

To validate the observation of a better prognosis in patients with the 2677T/A allele and residual disease ≤1 cm, we assessed this effect in a validation data set derived from the SCOTROC1 previously described by Marsh et al. (18). Because the aim of this analysis was to see whether the 2677T/A allele was associated with a reduction in the risk of disease progression among patients with optimally debulked tumors from a different population, we decided *a priori* to conduct one-sided significance testing on the validation cohort. Because different categories of residual disease (none or microscopic, macroscopic ≤2 cm, and macroscopic >2 cm) were available in this data set, we chose, *a priori*, to restrict our analysis to patients with ≤2 cm residual disease that were treated with paclitaxel (the SCOTROC1 included patients also treated with docetaxel). The resulting "validation" set of 278

Table 2. Association between *ABCB1* 2677G>T/A genotypes and progression-free and overall survival (Cont'd)

P trend †	Overall survival, HR (95% CI), *P* value		*P* trend †
	Unadjusted	Adjusted ‡	
	1.00	1.00	
	0.70 (0.42-1.16), *P* = 0.2	0.92 (0.53-1.60), *P* = 0.7	
0.006	0.74 (0.41-1.36), *P* = 0.3	1.00 (0.51-1.93), *P* = 1.0	0.3
	0.71 (0.45-1.14), *P* = 0.1	0.93 (0.56-1.58), *P* = 0.8	
	1.00	1.00	
	0.83 (0.47-1.45), *P* = 0.5	0.84 (0.46-1.56), *P* = 0.6	
0.3	1.25 (0.70-2.26), *P* = 0.4	1.37 (0.71-2.66), *P* = 0.3	0.4
	1.00	1.00	
	0.74 (0.45-1.45), *P* = 0.5	0.86 (0.50-1.48), *P* = 0.6	
0.6	0.88 (0.46-1.66), *P* = 0.7	0.78 (0.39-1.59), *P* = 0.5	0.5

patients in whom 144 events were observed had 96% and 82% power (one-sided test, 5% level of statistical significance) to detect the unadjusted 49% and adjusted 40% risk reductions, respectively, that were observed in analyses of the AOCS data. The progression-free advantage associated with the 2677T/A allele among patients with low residual disease in the AOCS data set was not seen in the validation analysis (Table 4).

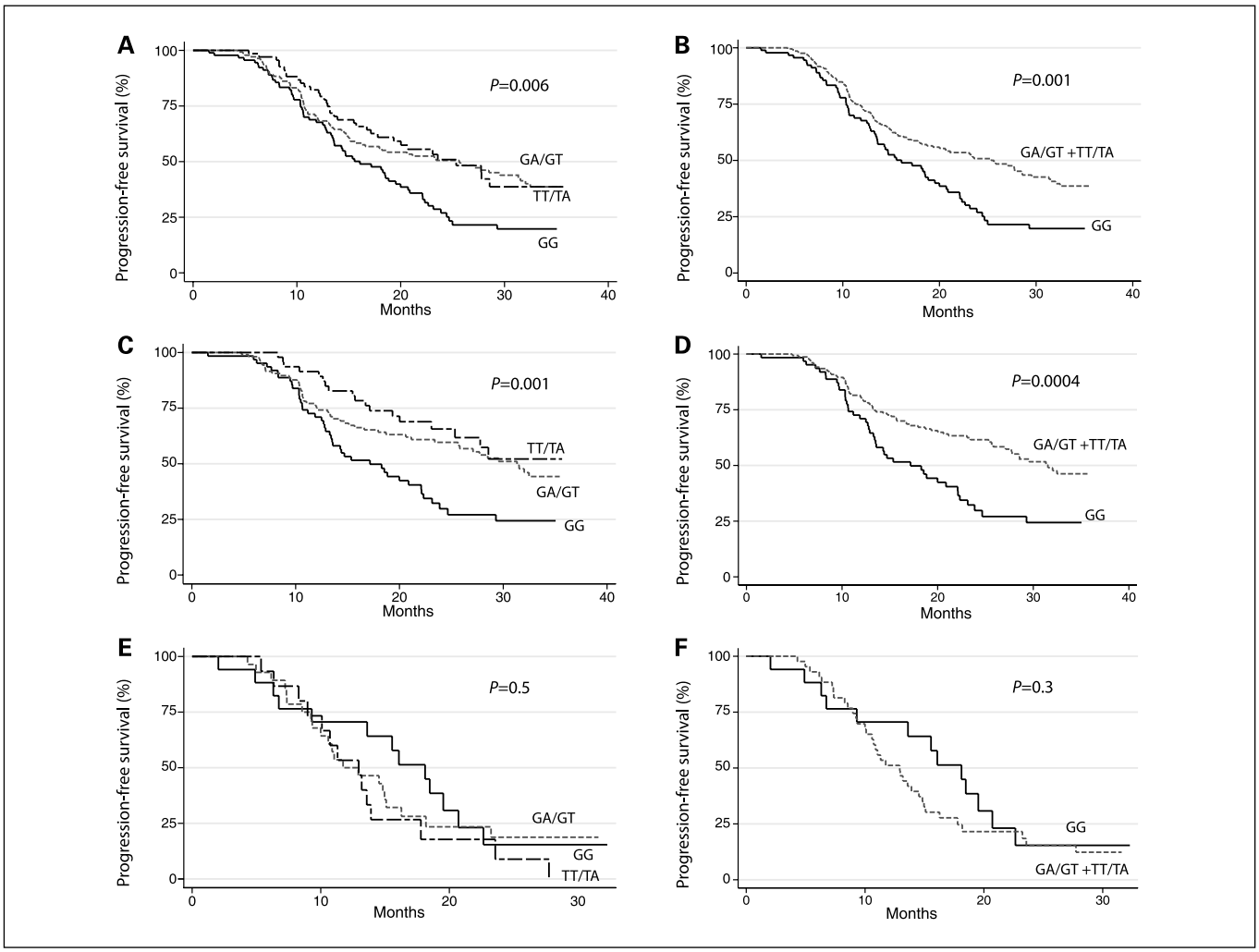

Fig. 1. Kaplan-Meier ovarian cancer progression-free survival curves for *ABCB1* 2677G>T/A genotypes for all (*A* and *B*), optimally debulked (*C* and *D*), and suboptimally debulked (*E* and *F*) tumors. *A, C,* and *E,* common homozygotes (GG), compared with heterozygotes (GA/GT), and minor homozygotes (TT/TA). *B, D,* and *F,* common homozygotes (GG) compared with all carriers of minor T/A alleles (GA/GT + TT/TA).

Table 3. Association between *ABCB1* 2677G>T/A genotypes and progression-free and overall survival by extent of residual disease

ABCB1 genotype	Cases, *n* (%)	Relapse, *n* (%)	Died, *n* (%)	Progression-free survival, HR (95% CI), *P* value	
				Unadjusted	Adjusted †
Optimally debulked (residual disease ≤1 cm)					
GG	63 (28.6)	44 (38.3)	16 (35.6)	1.00	1.00
GA/GT	110 (50.0)	52 (45.2)	20 (44.4)	0.55 (0.37-0.82), *P* = 0.004	0.64 (0.43-0.96), *P* = 0.03
TT/TA	47 (21.4)	19 (16.5)	9 (20.0)	0.43 (0.25-0.74), *P* = 0.002	0.51 (0.30-0.89), *P* = 0.02
GA/GT + TT/TA	157 (71.4)	71 (61.7)	29 (64.4)	0.51 (0.35-0.75), *P* = 0.001	0.60 (0.41-0.88), *P* = 0.01
Suboptimally debulked (residual disease >1 cm)					
GG	17 (28.3)	13 (26.0)	16 (50.0)	1.00	1.00
GA/GT	28 (46.7)	23 (46.0)	10 (31.2)	1.24 (0.62-2.48), *P* = 0.5	1.23 (0.62-2.46), *P* = 0.5
TT/TA	15 (25.0)	14 (28.0)	6 (18.7)	1.61 (0.75-3.45), *P* = 0.2	1.61 (0.75-3.45), *P* = 0.2
GA/GT + TT/TA	43 (71.7)	37 (74.0)	16 (50.0)	1.36 (0.72-2.59), *P* = 0.3	1.35 (0.71-2.57), *P* = 0.3

*χ^2 test for trend in progression-free or overall survival by increasing number of T/A alleles.
† Adjusted for FIGO stage and residual disease.

However, exploratory analysis of the unrestricted data set reported by Marsh et al. (18), expanded to include patients treated with docetaxel (n = 914), did reveal a similar interaction between the bulk of residual disease and the 2677G>T/A genotype ($P_{interaction}$ = 0.031, unadjusted; $P_{interaction}$ = 0.046, adjusted for stage). In patients with no or microscopic residual disease (281 patients, 103 events), an effect of the 2677T/A allele on progression-free survival was observed (unadjusted $HR_{T/A\ carriers}$, 0.70; 95% CI, 0.46-1.04; $P_{one\text{-}sided}$ = 0.039; stage-adjusted $HR_{T/A\ carriers}$, 0.62; 95% CI, 0.37-1.03; $P_{one\text{-}sided}$ = 0.033). Although these results are based on patients treated with either docetaxel or paclitaxel, we found no evidence that these associations depend on taxane type (test for interaction between allele and taxane; unadjusted P = 0.46; stage-adjusted P = 0.48).

Discussion

Women with ovarian cancer treated with at least four cycles of paclitaxel and carboplatin, and who are carriers of the minor T/A allele of the 2677G>T/A SNP, had a significantly longer relapse-free interval following treatment compared with similarly treated GG homozygous women in the AOCS cohort. Subgroup analyses showed that this effect was limited to cases with residual disease ≤1 cm but not for those with residual disease >1 cm. This association was not observed in a set of 278 women treated with paclitaxel that was prespecified to match the AOCS cohort as closely as possible. This set could not be defined as a true validation set because their classification of residual disease was slightly different. However, subsequent expansion of this set to include patients treated with docetaxel did reveal a longer progression-free interval in carriers of the 2677T/A allele with no macroscopic residual disease. Overall, our findings indicate that there is an effect of the 2677G>T/A polymorphism on progression-free survival in ovarian cancer patients who are treated with a taxane and carboplatin, which is dependent on the extent of residual disease, with the TT/TA genotype being associated with markedly better prognosis among patients with minimal residual disease.

We observed no significant overall survival benefit in the AOCS cohort associated with this SNP, but this will need to be reevaluated in the future because the median follow-up time in this cohort is only 24 months. In addition, it will be necessary to take into account in these analyses the type of chemotherapy the patients were given after their first relapse because we would hypothesize that an effect on overall survival would be strongest among patients who were given additional PGP substrates in subsequent rounds of chemotherapy.

Resistance to multiple, unrelated cytotoxic agents is the most serious cause of chemotherapy failure and has been associated with high levels of expression of PGP, which induces drug efflux and decreased drug absorption. The 2677G>T/A SNP results in an amino acid change from alanine to serine or threonine, but *in vitro* studies attempting to delineate the

Table 4. Association between *ABCB1* 2677G>T/A genotypes and progression-free survival in the validation data set from the SCOTROC1

ABCB1 genotype	Cases, *n* (%)	Relapse, *n* (%)	Progression-free survival, HR (95% CI), *P* value	
			Unadjusted	Adjusted*
Optimally debulked (residual disease ≤2 cm) treated with paclitaxel and carboplatin				
GG	67 (24.1)	34 (23.6)	1.00	1.00
GA/GT + TT/TA	211 (75.9)	110 (76.4)	1.07 (0.72-1.57), *P* = 0.625 †	0.99 (0.68-1.57), *P* = 0.485 †

*Adjusted for FIGO stage and residual disease.
† One-sided hypothesis test for better progression-free survival among T/A allele carriers compared with GG homozygotes.

Table 3. Association between *ABCB1* 2677G>T/A genotypes and progression-free and overall survival by extent of residual disease (Cont'd)

P trend*	Overall survival, HR (95% CI), *P* value		*P* trend*
	Unadjusted	Adjusted †	
	1.00	1.00	
	0.73 (0.38-1.41), *P* = 0.3	0.89 (0.46-1.73), *P* = 0.7	
0.001	0.73 (0.32-1.64), *P* = 0.4	0.96 (0.42-2.21), *P* = 0.9	0.4
	0.73 (0.39-1.34), *P* = 0.4	0.91 (0.49-1.69), *P* = 0.8	
	1.00	1.00	
	1.06 (0.38-2.91), *P* = 0.9	1.07 (0.39-2.96), *P* = 0.9	
0.2	1.15 (0.37-3.61), *P* = 0.8	1.08 (0.34-3.41), *P* = 0.9	0.8
	1.09 (0.42-2.79), *P* = 0.8	1.08 (0.42-2.77), *P* = 0.9	

functional impact of the 2677G>T/A SNP have been controversial (21, 22). Similarly, there is no clear evidence whether this SNP is associated with PGP levels (23–28). It is likely that the progression-free advantage observed among carriers of the 2677T/A allele in our study, all of whom received a minimum of four cycles of paclitaxel in combination with carboplatin every 3 weeks, is the result of decreased elimination of paclitaxel because carboplatin is a substrate of the multidrug resistance-associated protein-2 (MRP2) (29) but not of PGP. Pharmacokinetic studies have shown wide variation in the rate of drug clearance and plasma concentrations of paclitaxel following administration of paclitaxel and carboplatin at standard dosing schedules (30). Wong et al. (31) compared hepatic drug elimination with *ABCB1* variation in cancer patients and noted that patients with the GG genotype for the 2677G>T/A SNP had a significantly higher rate of PGP-mediated drug clearance than patients with the TT genotypes. This finding was further supported by a recent study of the PGP substrate, imatinib, and *ABCB1* genotype, which showed significantly decreased imatinib clearance among carriers of the minor T/A and T alleles of the 2677G>T/A and 3435C>T SNPs (32). These findings are consistent with the expression studies that suggest that the T/A alleles of the 2677G>T/A SNP are associated with reduced PGP-mediated drug efflux, higher intracellular drug concentrations, and consequently better responses to chemotherapy (23). A small change in effective drug concentration mediated by the 2677G>T/A SNP may be sufficient to observe a difference in response to chemotherapy when the volume of disease is small but may not be apparent in patients with bulky residual disease. However, further investigation is required to determine the mechanisms underlying the observed effect of the 2677G>T/A allele on progression-free survival and the relationship with extent of residual disease.

The role of the 3435C>T SNP in drug pharmacokinetics has also been the subject of much investigation but the conclusions are inconsistent (33–39). Discordance among studies may reflect differences in the type of functional assay or marker used to assess expression, PGP substrates, and ethnic differences between studies assessing tissue samples. Because the silent 3435C>T SNP is part of a common haplotype, and is in linkage disequilibrium with 2677G>T/A and 1236C>T (40), putative functional associations with the 3435C>T have also been attributed to linkage disequilibrium with the 2677G>T/A SNP. We did not find any association between the 3435C>T and the 1236C>T SNPs and either progression-free or overall survival, but without doing a full evaluation of the *ABCB1* locus, we cannot ascribe our findings directly to a functional effect of the 2677G>T/A SNP, although some studies have observed higher PGP activity among GG homozygotes of this SNP (23).

Although our data support a potential clinical role for PGP in determining progression-free survival in ovarian cancer patients, the effect is not large and is restricted to patients with minimal residual disease. Clinical trials of modulators of PGP in solid tumors have had limited success. Although such trials can be confounded by many factors that influence therapeutic index and may therefore mask effects on patient survival, our data suggest that subgroup analysis of patients based on residual disease and SNPs in trials of PGP modulators may be warranted.

In conclusion, our study shows an association between the *ABCB1* 2677G>T/A SNP and progression-free survival among women with ovarian cancer treated with paclitaxel and carboplatin, particularly among those with optimally debulked tumors. Given that paclitaxel, but not carboplatin, is a substrate of PGP (29), this effect is likely to be due to response to the taxane. Our study failed to show an association between the 2677G>T/A SNP and overall survival, but this may be due to our relatively short follow-up period and to the confounding effect of combined chemotherapy, with the platin drug thought to have a greater effect on outcome than the taxane. Clinical follow-up and data collection is ongoing for the full AOCS cohort, and further analyses may better assess the benefit associated with the *ABCB1* genotype. If we, or others, confirm the findings from our current analysis, the clinical implications could extend to any chemotherapeutic agents that are PGP substrates, to other cancers treated with such drugs, and potentially lead to individualized dosing schedules based on a patient's *ABCB1* genotype, and the rational choice of alternative non-PGP substrate drugs for second-line treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all the participating institutions for their cooperation and the women who participated in this study for their contributions.

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Clinical Cancer Research

Correction: *ABCB1 (MDR1)* Polymorphisms and Progression-Free Survival among Women with Ovarian Cancer following Paclitaxel/Carboplatin Chemotherapy

The authors of this article (Clin Cancer Res 2008;14:5594-601), which was published in the September 1, 2008, issue of *Clinical Cancer Research* (1), wish to inform the scientific community that the results of the analysis of the *ABCB1* 3435C>T and 1236C>T SNPs presented were incorrectly reported due to a corrupted analysis file. The corrected reporting of these results in the Results section, beginning at line 9 of page 5597, should be as follows:

Analysis of the 1236C>T SNP revealed a significant association with progression-free survival with a similar effect size and direction as 2677G>T/A, though these associations were likewise attenuated in adjusted models. Analysis of this SNP according to the extent of residual disease likewise showed a stronger association with progression-free survival in both adjusted and unadjusted models, with a significant trend in better progression-free survival for each additional T allele (*P*-trend <0.03). The 3435C>T, however, showed no significant association with progression-free survival in adjusted models ($P > 0.14$) (Table 2), with a marginal significantly increased risk of disease progression in adjusted models among cases that were optimally debulked ($P = 0.05$). Neither SNP were associated with overall survival in either the overall sample or the optimally debulked subset ($P_{adjusted} > 0.1$).

The corrected Table 2 appears below.

Table 2. Association between *ABCB1* SNP genotypes and progression-free and overall survival

ABCB1 genotype	Cases,[a] *N* (%)	Relapse, *N* (%)	Died, *N* (%)	Progression-free survival				Overall survival			
				HR (95% CI)	$P_{unadjusted}$	HR (95% CI)	$P_{adjusted}$[b] P_{trend}[c]	HR (95% CI)	$P_{unadjusted}$	HR (95% CI)	$P_{adjusted}$[b] P_{trend}[c]
2677G>T/A											
GG	91 (30.3)	66 (36.7)	28 (36.4)	1.00		1.00	0.006	1.00		1.00	0.3
GA/GT	141 (47.0)	77 (42.8)	32 (41.5)	0.62 (0.44–0.86)	0.004	0.74 (0.52–1.05)	0.1	0.70 (0.42–1.16)	0.2	0.92 (0.53–1.60)	0.7
TT/TA	68 (22.7)	37 (20.6)	17 (22.1)	0.60 (0.40–0.89)	0.013	0.74 (0.48–1.14)	0.2	0.74 (0.41–1.36)	0.3	1.00 (0.51–1.93)	1.0
GA/GT + TT/TA	209 (69.7)	114 (63.3)	49 (63.6)	0.61 (0.45–0.83)	0.002	0.74 (0.54–1.03)	0.07	0.71 (0.45–1.14)	0.1	0.93 (0.56–1.58)	0.8
3435C>T											
TT	87 (28.2)	46 (24.9)	22 (26.2)	1.00		1.00	0.02	1.00		1.00	0.2
TC	150 (48.7)	90 (48.6)	38 (45.2)	1.15 (0.80–1.64)	0.45	1.25 (0.86–1.83)	0.2	1.01 (0.60–1.71)	0.9	1.21 (0.68–2.15)	0.5
CC	71 (23.1)	49 (26.5)	24 (28.6)	1.60 (1.07–2.40)	0.02	1.38 (0.90–2.11)	0.1	1.44 (0.81–2.57)	0.2	1.40 (0.74–2.63)	0.3
1236C>T											
CC	98 (31.92)	70 (37.8)	32 (38.1)	1.00		1.00	0.006	1.00		1.00	0.1
CT	141 (45.93)	79 (42.7)	37 (44.0)	0.66 (0.48–0.91)	0.012	0.80 (0.57–1.13)	0.2	0.72 (0.45–1.15)	0.2	0.91 (0.55–1.52)	0.7
TT	68 (22.15)	36 (19.5)	15 (17.9)	0.60 (0.40–0.89)	0.012	0.75 (0.49–1.15)	0.2	0.60 (0.33–1.12)	0.1	0.75 (0.38–1.46)	0.4

[a] *N* does not sum to 309 because of missing genotype data.
[b] Adjusted for FIGO stage and residual disease.
[c] Test for trend in progression-free or overall survival by increasing number of T/A alleles.

In light of this discovery and correction, the authors wish to withdraw the statement in the Discussion on page 5600, "We did not find any association between the 3435C>T and the 1236C>T SNPs and either progression-free or overall survival", and replace it with the following: The observed association between the silent SNPs and progression-free survival is very likely explained by the pair-wise r-square between the 2677G>T/A and 1236C>T SNPs (both HapMap and 1000 Genomes $r^2 = 0.9$), and 2677G>T/A and 3435C>T SNPs (HapMap and 1000 Genomes $r^2 = 0.5$).

Reference

1. Johnatty SE, Beesley J, Paul J, Fereday S, Spurdle AB, Webb PM, et al. *ABCB1 (MDR1)* polymorphisms and progression-free survival among women with ovarian cancer following paclitaxel/carboplatin chemotherapy. Clin Cancer Res 2008;14:5594–601.

Published online January 3, 2012.
doi: 10.1158/1078-0432.CCR-11-2827

Clinical Cancer Research

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Clin Cancer Res 2008;14:5594-5601.