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Troels K. Bergmann, Henrik Gréen, Charlotte Brasch-Andersen, Mansoor R. Mirza, Jørn Herrstedt, Berit Hølund, Andreas Bois, Per Damkier, Werner Vach, Kim Brosen, et al.

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TK Bergmann*(1) H Gréen*(2) C Brasch-Andersen(1,6) MR Mirza(3) J Herrstedt(3) B Hølund(4) A du Bois (5) P Damkier(6) W Vach(7) K Brosen(1) C Peterson(2)

(1) – Clinical Pharmacology, Institute of Public Health, University of Southern Denmark, Odense, Denmark
(2) – Clinical Pharmacology, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden
(3) – Department of Oncology, Odense University Hospital, Odense, Denmark
(4) – Department of Pathology, Odense University Hospital, Odense, Denmark
(5) – Gynecology & Gynecological Oncology, Dr.Horst Schmidt Clinic, Wiesbaden, Germany
(6) – Department of Clinical Chemistry & Pharmacology, Odense University Hospital, Odense, Denmark
(7) – Clinical Epidemiology, Institute of Medical Biometry and Medical Informatics, University Medical Center Freiburg, Germany

*H Gréen and TK Bergmann contributed equally to the work

Corresponding author:

TK Bergmann
Clinical Pharmacology, Inst of Public Health
University of Southern Denmark
J.B. Winsleows vej 19, 2nd floor
5000 Odense C.
Denmark
Fax: +45 65 91 60 89
Phone +45 61 70 47 11
mail: tbergmann@health.sdu.dk

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Abstract

Purpose

Paclitaxel has a broad spectrum of anti tumor activity and is useful in the treatment of ovarian, breast and lung cancer. Paclitaxel is metabolized in the liver by CYP2C8 and CYP3A4 and transported by P-glycoprotein. The dose limiting toxicity is neuropathy and neutropenia, but the inter-individual variability in toxicity and also survival is large. The main purpose of this study was to investigate the impact of genetic variants in *CYP2C8* and *ABCB1* on the toxicity and survival.

Methods

The 182 patients previously treated for ovarian cancer with carboplatin and paclitaxel in either the AGO-OVAR-9 or the NSGO-OC9804 trial in Denmark or Sweden were eligible for this study. Genotyping was carried out on formalin fixed tissue. The patients' toxicity profiles and survival data were derived from retrospective data. *CYP2C8**3, *ABCB1* C1236T, G2677T/A and C3435T were chosen pre hoc for primary analysis; a host of other variants entered an exploratory analysis.

Results

Clinical data and tissue was available from a total of 119 patients. Twenty-two single nucleotide polymorphism(SNPs) in 10 genes were determined. Toxicity registration was available from 710 treatment cycles. Primary analysis: no statistical significant correlation was found between *CYP2C8**3, *ABCB1* C1236T, G2677T/A and C3435T and neutropenia, sensoric neuropathy and overall survival.

Conclusion

*CYP2C8**3 and the *ABCB1* SNPs C1236T, G2677T/A and C3435T are not statistically significantly correlated to overall survival, sensoric neuropathy and neutropenia in 119 patients treated for ovarian cancer with paclitaxel/carboplatin.

Keywords: paclitaxel, CYP2C8, ABCB1, ovarian cancer, neutropenia, neuropathy

Introduction

Paclitaxel is a highly active anticancer drug useful in the treatment of patients with e.g. breast, lung or ovarian cancer. It was first isolated in the seventies from the bark of the western yew, *Taxus brevifolia*[1]. In advanced ovarian cancer the initial response rate is almost 80 % but unfortunately most patients eventually relapse[2]. Patients are allocated to treatment according to the FIGO stage (International Federation of Gynecology and Obstetrics) with post-surgery chemotherapy with a taxane and a platin being the widely accepted treatment for stage II-IV. Paclitaxel is dosed normalized to body surface area (BSA) and in most regimens infused over 1, 3 or 24 hours. Dose limiting toxicities are neutropenia and neuropathy; other toxicities include arthralgia, myalgia and alopecia whereas nausea and vomiting are experienced by less than 30 % of the patients. Toxicity and clinical effects of paclitaxel vary greatly among patients and remain a clinically relevant problem with implications on survival and quality of life. Toxicity can result in dose delay, dose reduction or even early cessation of the treatment. It has been proposed and investigated that single nucleotide polymorphism (SNPs) could explain the variability in toxicity and effect. Sequence variants in the genome might have an impact on clinical outcome either indirectly by altering the elimination and/or disposition or directly by changes in the drug concentration in the micro environment of the cells in the different tissues.

Paclitaxel is metabolized to inactive compounds by CYP2C8 and CYP3A4 in the liver[3-5], and is also a substrate of the ATP driven efflux pump P-glycoprotein encoded by the *ABCB1* gene[6]. This study was initiated based on reports on *CYP2C8*3* as having decreased paclitaxel metabolism[7, 8] and reports of a functional significance of *ABCB1* SNPs with particular interest in C1236T, G2677T/A and C3435T[9-11]. In the study design these four 'highly implicated' and relatively frequent SNPs were selected (pre hoc) for the primary analysis; in addition other candidate genes linked to the pharmacodynamics and pharmacokinetics of paclitaxel were chosen for an exploratory analysis namely *CYP3A5*, *ABCC1, ABCC2, ABCG2, ABCC10*, *CYP1B1* and *SLCO1B3*[12-18]. These are all drug transporters except *CYP3A5* which has a substrate range similar to *CYP3A4*, and *CYP1B1* which has been implied to play a role in taxane metabolism and therapeutic effects[12].

The aim of the study was primarily to evaluate the impact of the *CYP2C8*3, ABCB1* C1236T, G2677T/A and C3435T variants on paclitaxel toxicity and survival and secondarily to explore the role of the other variants in *CYP2C8* and *ABCB1* as well as the variants in *CYP1B1, CYP3A4/5, SLCO1B3, ABCC1, ABCC2, ABCG2* and *ABCC10*. We present a retrospective pharmacogenetic study of 119 Scandinavian Caucasian patients who were treated with paclitaxel and carboplatin in either the AGO-OVAR-9 trial (ClinicalTrials.gov identifier: NCT00052468) or the NSGO-OC9804 trial (NCT00004934) (phase III trials of first-line treatment of ovarian cancer).

Patients and methods

Patients

In this study clinical data and tissue was collected from 119 patients (Table 1). The patients were all previously treated with paclitaxel (175mg/m^2) and carboplatin (AUC 5-6, using Calvert´s formula) in either the AGO-OVAR-9 trial (OVAR-9) or the NSGO-OC9804 trial (TEC). Both trials compared a three compound regimen to the standard treatment of paclitaxel/carboplatin in patients with primary epithelial ovarian cancer. In the OVAR-9 trial the primary and secondary endpoints were overall survival and progression free survival, response rate, duration of response, toxicity and quality of life. The study closed for inclusion in April 2004. A total of 83 patients in Denmark and Sweden were randomized to paclitaxel and carboplatin. In the TEC trial the primary and secondary endpoints were progression free survival and overall survival, toxicity and quality of life. The study closed for inclusion October 2001. A total of 99 patients in Denmark and Sweden were randomized to paclitaxel and carboplatin. Treatment records and follow-up data for OVAR-9 and TEC are kept by the AGO Ovarian Cancer Study Group in Wiesbaden, Germany and by the NSGO in Odense, Denmark. Formalin fixed paraffin embedded tissue samples from the patients were collected from departments of pathology at the participating centers in Denmark and Sweden. Eligibility criteria for this study were: 1) participation in either the OVAR-9 or the TEC study in either Denmark or Sweden, 2) randomization to paclitaxel and carboplatin treatment, 3) availability of formalin fixed paraffin embedded tissue (any type available) and 4) availability of treatment records and follow-up data. No patients were contacted. The study was approved by the regional ethics committee of Southern Denmark and the regional ethics committee in Linkoping, Sweden.

Toxicity

Patients were originally evaluated for toxicity using Common Toxicity Criteria (CTC-NCIC). The two studies used revisions from March 1998 and December 1994 which have only insignificant differences regarding the chosen toxicities. Generally, grade 0 was used for absence of the toxicity, 1, 2, 3 and 4 were assigned for mild, moderate, severe toxicities and very severe toxicity. The principal characteristic of grade 3 and 4 is interference with normal daily activity as opposed to grade 1 and 2.

Summary statistics of toxicities were evaluated pre hoc with regard to frequency distribution and cross-wise correlation. Overall survival and two toxicities with no indication of being strongly correlated (Spearman correlation coefficient = 0.07) were picked for primary analysis: 1) Sensoric neuropathy: grades seemed to be increasing with increasing number of cycles, and so time-to-event analysis was used, with time being the cycle number (0 to 9) and event time being the first cycle with reported increase in sensoric neuropathy score (CTC) of two from baseline, 2) Neutrophil depression calculated as difference between baseline and nadir divided by the baseline value in %. Nadir was defined as the lowest observed value between two cycles regardless of the actual sampling time.

Seven other toxicities of which none seemed to be strongly cross-wise correlated (Spearman coefficient ≤ 0.40) were left for explorative analysis: myalgia, arthralgia, mucositis, hearing loss, vomiting/nausea, motor neuropathy and compliance. For all toxicities the highest grade observed for any cycle was used. Nausea and vomiting seemed to be correlated (Spearman coefficient of 0.66) and the highest grade for either of the two was used. The grading for compliance was constructed using the actual dose of paclitaxel administered. Grade 1 was defined as at least one episode of reduced paclitaxel dose (range of 50 % - 90 % of initial dose) and grade 2 as occurrence of at least one cycle where paclitaxel was not given. In two patients who experienced an allergic reaction during paclitaxel infusion only the subsequent cycles were evaluated for compliance. Evaluation of all toxicity data were completed and inserted in the database, which was locked, before the analyses of genetic variants were disclosed. Summary of the toxicity is presented in Table 2.

Genotyping procedures

Genomic DNA was extracted from formalin fixed paraffin embedded tissue using QIAamp DNA Mini Kit, tissue protocol (VWR International A/S, Denmark) according the manufacturers protocol. SNPs in *CYP2C8, CYP3A4, SLCO1B3* (except G767C) and *ABCB1*(except A-1G) were determined using Pyrosequencing as previously described[19-21]. The variants in *CYP1B1, CYP3A5, ABCC1, ABCC10* and G767C in *SLCO1B3,* A-1G in *ABCB1* and rs2273697 in *ABCC2* were genotyped using TaqMan® pre-designed SNP genotyping assays by Applied Biosystems (Foster city, CA). SNPs in *ABCG2* and rs17222723 and rs8187710 in *ABCC2* were genotyped using SYBRgreen real time PCR assays as previously described[22].

Statistics

In order to overcome the problem of multiple testing we split the analysis into a primary and an exploratory analysis. For the primary analysis two sided P-values with a significance level of 0.05 was used. P-values were calculated for the exploratory analysis in similar fashion and reported without any correction for multiple testing - the results should therefore be interpreted with caution. Briefly, we assumed that heterozygocity is an intermediate between the homozygotes and that CTC scores are on an ordinal scale we thus used a non parametric test for trend (STATA10 command: *nptrend,* 2009, StataCorp, Texas, USA) for SNPs with three genotypes and similarly the Wilcoxon rank sum test for those with two genotypes. For the tri-allelic SNP G2677T/A in *ABCB1* patients carrying an A allele was omitted from the analysis. The Kaplan-Meier method with Log-rank test was used to test the risk of developing sensoric neuropathy (STATA10 command: *sts test*, with *trend* option). A Spearman correlation coefficient > 0.5 was arbitrarily set to determine if different toxicities were correlated. Overall survival analysis was carried out fitting a Cox model under the assumption of proportional hazard. This assumption was tested visually and by Schoenfeld residuals (data not shown). Classical prognostic variables[2] were tested one at a time in a univariate analysis (Table 3). Age (≤65 or >65 years), CA125 levels at baseline (≤35 or >35 U/ml) and FIGO stage (trend from stage II to IV) were

picked for the final model in which the SNPs were tested for trend. All calculations were carried out in STATA 10 (2009, StataCorp, Texas, USA).

Results

Patients

One hundred nineteen of 182 patients were eligible for analysis; 56 patients from the OVAR-9 trial and 63 from the TEC trial. Twenty and 31 patients were ineligible from the OVAR-9 and TEC trial respectively, because of oncology departments not participating. Tissue was unavailable from a total of 12 patients from the participating departments. Characteristics are summarized in Table 1.

Results of genotyping

Twenty-two single nucleotide polymorphisms (SNPs) were analyzed in 10 genes. For 57 patients both tumor and non cancerous tissue was available. The total call rate (across all SNPs) was 94.7 % evenly distributed (data not shown). Mismatching genotypes were found evenly distributed in 2.3 % of the cases where both tumor and non cancerous tissue was available. In these cases no final genotype was called. In one patient all calls from the tumor sample were discarded because the DNA quality was obviously very poor with a call rate of 41 % across the 22 SNPs. Allele frequencies were all similar to frequencies reported for Caucasians on the NCBI SNP database (dbSNP). The genotype distributions were all in Hardy-Weinberg equilibrium except *CYP2C8*4*, *1B and *1C, and *ABCB1* A61G.

Toxicity

Toxicity registration was available from 710 treatment cycles (Table 2). Twenty-three patients received treatment beyond the 6^{th} cycle. One patient did not receive chemotherapy (no cause stated). Toxicity registration for this patient was not done, but survival data was included in the final analysis (intention to treat).

Primary analysis

No statistical significant correlations was found between the SNPs: *CYP2C8**3, *ABCB1* C1236T, G2677T/A and C3435T and neutrophil depression and sensoric neuropathy (Table 4).
For overall survival the calculated hazard ratio for the individual genotype as a covariate in the Cox model is reported along with the 95% confidence interval (Table 4). None of the SNPs selected for primary analysis were statistically significantly associated with overall survival of the patients. *ABCB1* G2677T/A which is a tri-allelic SNP was tested excluding patients carrying an A allele. For completeness analyses treating the A allele as a T allele and using the five genotypes as a categorical variable were also carried out.

Exploratory analysis

Among the 190 tests of association between particular toxicities and SNPs, seven were found to have

a P-value ≤ 0.05 (data not shown). *ABCB1* G1199A was associated with increased risk of death with a hazard ratio of 2.03 (95% ci: 1.08-3.83), P-value = 0.03 (Figure 1).

Discussion

Definite results linking frequent *ABCB1* variants to taxane toxicity and effects in vivo have been elusive. This study retrospectively investigates possible correlations between a wide selection of candidate genes all with a reputed role in either the transport or the metabolism of paclitaxel and the toxicity and survival of patients treated with paclitaxel. Clinical data and formalin fixed paraffin embedded tissue was gathered from 119 patients with ovarian cancer who were treated with paclitaxel and carboplatin in either the OVAR-9 or the TEC trial. The genetic variant *CYP2C8*3* (a paclitaxel metabolizing enzyme) and three variants in the drug transporter *ABCB1:* C1236T, G2677T/A and C3435T were picked pre hoc for primary analysis; no statistical significant correlation for sensoric neuropathy, neutrophil depression and overall survival was found. This finding contrasts the correlation reported by Nakajima et al[23] for leukocytopenia and C3435T in 23 Japanese women with ovarian cancer and the report of Sissung et al[24] who found a trend between neutrophil decrease and the *ABCB1* C3435T/G2677T/A compound genotype in 26 patients with advanced solid tumors. Interestingly Green et al[25] recently reported a correlation between *CYP2C8*3* and hematological toxicity.

We also conducted a wide exploratory analysis of 19 SNPs in a total of 10 genes and both sensoric neuropathy, neutrophil depression and overall survival and other toxicities that are related to paclitaxel treatment i.e. myalgia, arthralgia, mucositis, hearing loss, motor neuropathy, vomiting/nausea and compliance. This analysis encompasses some 190 tests (data not shown) of which seven had a P-value ≤ 0.05. This finding is likely due to chance alone, given the multiple testing. This multitude of statistical testing can serve a purpose for future investigations but have no value without being confirmed in other studies. None of the seven possible associations have to our knowledge been reported in the literature before. In the explorative analysis of overall survival, an association with the non synonymous G1199A SNP in the *ABCB1* transporter was found with a P-value of 0.03 (uncorrected) and a hazard ratio of 2.03 [95 % c.i. 1.08-3.83] favoring patients with 1199GG genotype (Figure 1). This is in accordance with the shorter progression free survival for patients carrying the 1199GA genotype found by Green et al[26]. Similarly Johnatty et al[27] found a better progression-free survival for patients carrying the 2677TA alleles and Gréen et al[28] found an association for the same variant and good response to paclitaxel treatment. The finding should be confirmed in other studies before any conclusions can be made. *CYP1B1*3* has previously been associated with survival after paclitaxel treatment in breast cancer [29, 30]; but this finding was not reproduced in our material of ovarian cancer.

In 2007 Marsh et al[31] reported a very comprehensive retrospective pharmacogenetic study of toxicity and response to treatment with a taxane in 914 ovarian cancer patients. No significant

association was found for a selection of variants in the candidate genes: *ABCB1, ABCC1, ABCG2, CYP1B1, CYP2C8* and *CYP3A4/5* and hematologic toxicity, neurotoxicity and gastrointestinal toxicity. While this study included far more patients than our study and other previous studies it also raised a debate concerning power and the interpretation of P-values[32-34]. Our results however, do agree with the conclusions made by Marsh et al; but are again contrasted by a very recent paper by Leskelä et al[35] who demonstrates an association between *CYP2C8*3* and *CYP3A5* in a mixed retrospective/prospective study.

In this study we investigate candidate genes with putative impact on the pharmacokinetics of paclitaxel under the assumption that these might influence on toxicity or survival either directly through impact on clearance or the distribution of paclitaxel or indirectly through the paclitaxel concentration in the micro environment in the tumor and susceptible tissues (bone marrow, peripheral nerves etc.). We have thus not addressed the possibility that the susceptibility of the mentioned targets by themselves is determined by genetic variation. Ideally, future pharmacogenetic studies of chemotherapy should try to combine the pharmacokinetic and the pharmacodynamic components.

In conclusion this study primarily investigated the notion that the genetic variants *CYP2C8**3 and *ABCB1* C1236T, G2677T/A and C3435T explain some of the observed variability in toxicity and survival of patients treated with paclitaxel and carboplatin in ovarian cancer. No statistical significant associations were found in the primary analysis. The study also involved an explorative analysis of other candidate genes which might serve a purpose for studies in the future.

Table 1 – Summary of the 119 patients in the study

N=119 evaluable patients	Value – median (range)
Age (years)	57.8 (26.1-77.4)
BSA[a] (m^2)	1.71 (1.4 – 2.24)
	Value – N (%)
WHO/ECOG[b] Performance status	
0	64 (53.8 %)
1	52 (43.7 %)
2	3 (2.5 %)
Tumor type	
Ovary	113 (95 %)
Peritoneal	2 (1.7 %)
Fallopian tube	4 (3.4 %)
FIGO[c] stage	
IIb	10 (8.4 %)
IIc	8 (6.7 %)
IIIa	8 (6.7 %)
IIIb	8 (6.7 %)
IIIc	63 (52.9 %)
IV	22 (18.5 %)
Tumor grade (G)	
Well differentiated (G1)	13 (10.9 %)
Moderately differentiated (G2)	30 (25.2 %)
Poorly or undifferentiated (G3)	55 (46.2 %)
Undetermined	21 (17.7 %)

a) Body surface area (unavailable for 1 patient).

b) Eastern Cooperative Oncology Group

c) International Federation of Gynecology and Obstetrics

Table 2 - Common Toxicity Criteria (CTC) grades for N=119[a] patients

CTC grade	**0**		**1**		**2**		**3**	
	N		N		N		N	
Sensoric neuropathy	37	31 %	58	49 %	20	17%	3	3 %
Arthralgia	55	47 %	37	31 %	24	20%	2	2 %
Myalgia	52	44 %	34	29 %	29	25 %	3	3 %
Febrile neutropenia	117	99 %	na[f]		na[f]		1	1 %
Mucositis	87	74 %	27	23 %	4	3 %	na[f]	
Hearing loss	106	90 %	10	8 %	2	2 %	na[f]	
Nausea/vomiting	41	35 %	53	45 %	20	17 %	4[e]	3 %
Neuropathy (ataxia)	83	70 %	25	21 %	9	8 %	1	1 %
Compliance[b,c,d]	99[b]	84 %	14[c]	12 %	5[d]	4 %	na[f]	

a) One patient did not receive chemotherapy

b) No paclitaxel reduction

c) Paclitaxel dose reduced to <90% of initial dose

d) Paclitaxel discontinued

e) One patient had grade 4 nausea/vomiting

f) Na = not defined

Table 3 – Cox proportional hazard model for overall survival for N=119[a] patients. FIGO stage, age and Cancer antigen 125 were included as covariates in the final model.

Covariate		Patients		Univariate analysis		Multivariate analysis		
		N	%	HR[b]	P-value	HR[b]	95%ci	P-value
FIGO[c] stage	trend			2.3	<0.0001	1.92	1.26-2.93	0.002
	by category							
	II	18	15	1				
	III	79	66	2.28	0.032			
	IV	22	18	5.29	<0.001			
Histology	Serous	78	66	1			na	
	Mucinous	8	7	3.95	<0.001			
	Clear cell	2	2	3.36	0.096			
	Endometroid	14	12	0.72	0.426			
	Undifferentiated	2	2	0.66	0.676			
	Undetermined	15	13	1.76	0.081			
Tumor grade[d]	G1	13	13	1			na	
	G2	30	31	2.03	0.158			
	G3	55	56	2.41	0.065			
Residual tumor	<1 cm	50	42	1.96	0.005		na	
	≥ 1cm	69	58					
Age (years)	≤ 65	83	70	2.49	0.0003	2.36	1.47-3.80	<0.001
	>65	36	30					
Performance status	0	64	54	1.35	0.18		na	
	1-2	55	46					
Cancer antigen 125	≤35 U/ml	24	20	2.52	0.005	1.96	1.01-3.83	0.05
	>35 U/ml	95	80					

a) One patient did not receive treatment but were not excluded from the survival analysis (intention to treat)

b) Hazard ratio

c) International Federation of Gynecology and Obstetrics

d) Patients with missing grade were excluded from the analysis

Table 4 – Association tests (non parametric trend tests) of toxicity and overall survival versus SNPs in 119[a] patients.

Gene variant			Genotype[a]			Toxicity[b] and overall survival P-values and hazard ratio[d] with 95% confidence interval		
Gene	dbSNP ID	Variant	Wildtype	Heterotype	Variant	Neutrophil decrease%	Neuropathy logRank test	Overall survival Hazard ratio (95% ci)
CYP2C8	rs10509681	A1196G(*3)	95	21	1	0.64	0.98	1.13 (0.66-1.92)
ABCB1	rs1128503	C1236T	31	55	26	0.66	0.10	1.18 (0.84-1.66)
	rs2032582	G2677T/A[c]	35	49(GT) 1(GA)	28 (TT) 1(AA)	0.77	0.51	1.16 (0.84-3.5)
	rs1045642	C3435T	27	48	39	0.54	0.48	0.95 (0.71-1.26)

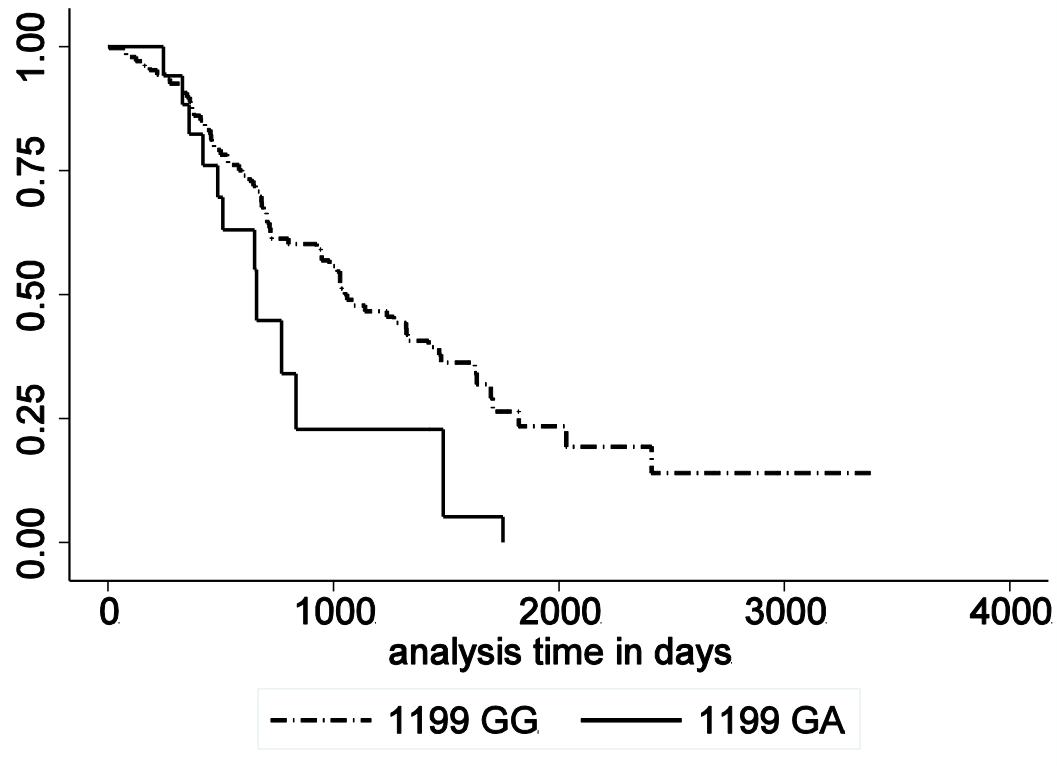
a) Genotyping data missing for some patients

b) One patient did not receive chemotherapy and was excluded from the toxicity analysis but included in the survival analysis (intention to treat)

c) Two patients with the A allele were excluded from the analysis

d) Hazard ratios and 95% confidence intervals for each SNP added to the final Cox model with FIGO stage, age and CA125 as covariates

Figure 1 - Overall survival function[a] (fraction) for 119 patients as function of *ABCB1* G1199A genotype. Hazard ratio 2.03 (95%c.i 1.08;3.83), P-value 0.03.



a) Survival curves for the Cox model of a typical patient i.e. FIGO[b] stage III, CA125 ≤35 U/ml and age >65 years.

b) International Federation of Gynecology and Obstetrics

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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