ANTICANCER RESEARCH *38*: 1455-1459 (2018)
doi:10.21873/anticanres.12370

Polymorphisms of *ABCB1, CYP3A4* and *CYP3A5* Genes in Ovarian Cancer and Treatment Response in Poles

ŁUCJA FISZER-MALISZEWSKA[1], ŁUKASZ ŁACZMAŃSKI[1], ANETA DOLIŃSKA[1], MARIA JAGAS[2], EWELINA KOŁODZIEJSKA[3], MAGDALENA JANKOWSKA[3] and PIOTR KUŚNIERCZYK[1]

[1]*Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland;*
[2]*Lower Silesian Oncology Center, Wroclaw, Poland;*
[3]*Lower Silesian Regional Specialist Hospital, Wroclaw, Poland*

Abstract. *Background/Aim: Ovarian cancer is a lethal gynecological malignancy, with 5-year survival of only about one third of patients. The ABCB1 gene encodes the P-glycoprotein which is one of the multidrug efflux pumps. Its decreased activity may result in multidrug resistance of cancer cells. Drug-metabolizing enzymes Cyp3A4 and Cyp3A5 may affect success of chemotherapy. In this study we attempted to examine the effects of 12 single nucleotide polymorphisms (SNPs) of the ABCB1 gene and one SNP in each of CYP3A4 and CYP3A5 genes on the incidence of ovarian cancer in Polish women and their response to treatment. Materials and Methods: Our study included 276 patients and 369 healthy control women. Results: The results showed no significant differences between patients and controls in allele frequencies of the tested SNPs, with one exception: rs2157926T allele decreased cancer risk by 99.4% (odds ratio, 0.006). Moreover, rs2032582T increased fourfold the risk of metastasis. Finally, rs1128503CC genotype prolonged survival (p=0.024778). Conclusion: These findings may contribute to a better prediction of therapy outcome.*

Ovarian cancer is a lethal gynecologic malignancy, with a mean age-standardized 5-year survival of 34.5% in Poland, and similar values for other European countries and in U.S.A. (1, 2). The patients' survival depends on many factors like, *e.g.*, cancer stage and class, metastasis and the response to treatment. The *ABCB1* gene (ATP-binding cassette, subfamily B, member 1; alternative name: *MDR1*, multidrug resistance 1; located in humans on chromosome 7q21.12) encodes the P-glycoprotein (Pgp), which functions as an ATP-dependent multidrug efflux pump, exporting exogenous and endogenous substrates from the cell (3). High Pgp levels are associated with a poor response of cancer cells to chemotherapeutic agents. In contrast, low Pgp levels characterize tumors that are sensitive to chemotherapy. Several single nucleotide polymorphisms (SNPs) in *ABCB1* have been described in Caucasians. Association of these polymorphisms with the response of ovarian cancer to chemotherapy has been investigated in recent years, but results are conflicting (4-7).

Like cytochrome *P450 3A4*, P-glycoprotein is non-specific for a large variety of drugs, and there is a wide overlap with the drug-metabolizing enzyme CYP3A4, making P-glycoprotein and CYP3A4 a synergistic defense mechanism against the intrusion of xenobiotics (3). Therefore, we attempted to examine the effects of twelve SNPs (rs10245483, rs2157930, rs10246878, rs2157926, rs3213619, rs2214102, rs9282564, rs868755, rs2229109, rs1128503, rs1045642, rs2032582) of the *ABCB1* gene and two polymorphisms of cytochrome P450 (*CYP3A4, CYP3A5*) on response to chemotherapy, metastasis and patients' survival in Polish women with ovarian cancer.

Materials and Methods

Study subjects. Two hundred and seventy-six women diagnosed with ovarian cancer and treated, as shown in Table I, in Lower Silesian Oncology Center, Wrocław or in WSS Hospital, Wrocław, were enrolled in our study. The inclusion criteria comprised histologically confirmed diagnosis of ovarian cancer and treatment with surgery and platinum-based chemotherapy; borderline tumor patients were excluded. For a control, we included two populations: Control #1 consisted of healthy blood donors. However, most of them were below 50 years of age, in contrast to our patients. Therefore, Control #2 was added, consisting of WSS Hospital patients with non-malignant diseases. Since SNP frequencies in both controls were

Correspondence to: Piotr Kuśnierczyk, Laboratory of Immunogenetics and Tissue Immunology, The Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Rudolfa Weigla 12, 53-114 Wrocław, Poland. Tel: +48 713371172, Fax: +48 713371382, e-mail: pkusnier@iitd.pan.wroc.pl; and Łaczmański Łukasz, The Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Rudolfa Weigla 12, 53-114 Wrocław, Poland. Tel: +48 713371124, Fax: +48 713371382, e-mail: lukasz.laczmanski@iitd.pan.wroc.pl

Key Words: Ovarian cancer, genetics, ABCB1, CYP3A4, CYP3A5.

very similar (results not shown), these two controls were combined in further analyses.

Our study has been approved by a Biomedical Committee of the Medical University in Wrocław, Poland.

DNA isolation and SNP typing. Genomic DNA was extracted from fresh or frozen blood samples using a QIAamp® DNA blood mini kit (Qiagen, Hilden, Germany). DNA concentration was adjusted to 20 μg/ml with the kit's AE solution (10 mM Tris, 0.5 mM EDTA, pH 9).

Genotyping of *ABCB1* SNPs was based on PCR (polymerase chain reaction) amplification of gene fragments containing specific polymorphisms and an amplicon analysis using either RFLP (restriction fragment length polymorphism) or HRM (high resolution melting). 150-300 bp fragments containing the SNPs of interest were amplified with primers either described in the literature or designed using Primer3 program and either *ABCB1* genomic reference sequences NG_011513.1 and NC_000007.13, *CYP3A4* sequence NG_008421.1, or *CYP3A5* sequence NG_007938.1. To overcome some limitations of conventional HRM (8), a modification by Zhou *et al*. 2004 (9) with unlabeled oligonucleotide probes was used. PCR followed by HRM were performed in the presence of 3'-end blocked ~30-nt probes and DNA saturating fluorescent dye EvaGreen (Biotium, Fremont, USA) in the Eco Real-Time PCR System (Illumina, San Diego, USA). The Eco system operations and HRM data processing were carried out using Eco System software v.5.0, which allowed the melting curves to be normalized and directly compared.

Statistical methods. Allele frequencies were assessed by gene counting and distribution of the polymorphic variants was tested with the Hardy-Weinberg equilibrium. Backward stepwise multivariate regression model was used to determine odds ratios (OR) and 95% confidence intervals (CI) for evaluating the risk association of *ABCB1, CYP3A4, CYP3A5* and *BRCA1* polymorphisms with the incidence rate of ovarian cancer. Discrepancies between controls and cases were determined using χ^2 Pearson test. Survival time model was performed by multiple regression and confirmed by median test.

Statistical analysis was performed using Statistica ver. 10 (StatSoft) with Medical Package. The differences were considered significant when p-value <0.05.

Table I. *Characteristics of patients and control subjects*.

Trait	Total	N	%
Patients			
Age (54,0/19-84 years)[1]	276		
≤50 years		88	31.9
>50 years		188	68.1
FIGO	275		
I		53	19.3
II		26	9.5
III		164	59.4
IV		32	11.6
Histopathological type of ovarian cancer	275		
Serous		121	44.0
Endometrioid		71	25.8
Undifferentiated		41	14.9
Mucous		19	6.9
Other		23	8.4
Grade	226		
1		30	13.3
2		83	36.7
3		113	50.0
Chemotherapy	276		
Cisplatinum-cyclofosfamide[2]		81	29.3
Cisplatinum-Taxol		176	63.8
Other[3]		19	6.9
Control #1*, age (32/18-84 years)[1]	166		
≤50 years		130	78.3
>50 years		36	21.7
Control #2**, age (61/20-87 years)[1]	203		
≤50 years		18	8.9
>50 years		185	91.1

[1]Median/range. [2]Adriamycin was added to cisplatinum-cyclofosfamide in the case of 7(3,5%) patients. [3]Treatment protocol was changed during chemotherapeutic procedure, *e.g*., cisplatinum-cyclophosphamide treatment was substituted with cisplatinum-taxol or another cytostatic drug was included (alkeran, etoposide, or megachemotherapy with iphosphamide + carboplatinum + etoposide) for single patients. *Control #1: a sample of healthy Polish population, consisting of 166 female blood donors from Lower Silesian Blood Transfusion Center (age, 28.0/18-58) and 51 women working at the Institute of Immunology and Experimental Therapy (age, 54/29-84). **Control #2: WSS Hospital non-malignancy patients, age-matched with ovarian cancer patients.

Results

ABCB1, CYP3A4, CYP3A5 polymorphisms were tested in 276 women with ovarian cancer and 370 healthy volunteers. There was no evidence of any deviations from the Hardy-Weinberg equilibrium in the distribution of *ABCB1, CYP3A4, CYP3A5* genotypes in the study and control groups (Table II).

There was no evidence of any deviation from the Hardy–Weinberg equilibrium (p=0.3589 and p=0.5441, chi-squared goodness of fit test) in the distribution of PTPN13 genotypes in either the control or the study group (Table II).

There was no evidence of any deviation from the Hardy–Weinberg equilibrium (p=0.3589 and p=0.5441, chi-squared goodness of fit test) in the distribution of PTPN13 genotypes in either the control or the study group (Table II).

We didn't observe any statistically significant differences between cancer and control group for rs10245483, rs2157930, rs10246878, rs2157926, rs3213619, rs2214102, rs9282564, rs868755, rs2229109, rs1128503, rs1045642, rs2032582 allele frequencies analyzed by χ1 Pearson test (data not shown). Backward stepwise multivariate regression model showed that "T" allele occurrence in the rs2157926 polymorphism reduced cancer risk by 99.4% (p-value=0.0000, OR=0.006, 95%CI=0.002-0.021; Table III).

Furthermore, backward stepwise multivariate regression model showed that "T" allele of the rs2032582 polymorphism increases metastasis risk four times (p-value=0.038, OR=3.925, 95%CI=1.076-14.318; Table IV).

Table II. *Distribution of genotypes of ABCB1, CYP3A4, CYP3A5 in the study and control groups.*

	Study group					Control group				
	NN observed (expected)	Nn observed (expected)	nn observed (expected)	χ^2	*p*-Value	NN observed (expected)	Nn observed (expected)	nn observed (expected)	χ^2	*p*-Value
rs2740574	259 (259)	17 (16.5)	0 (0.3)	0.279	0.5975	340 (340)	28 (26.9)	0 (0.5)	0.575	0.4480
rs776746	250 (251)	26 (24.8)	0 (0.6)	0.674	0.4115	326 (327.3)	43 (40.5)	0 (1.3)	1.412	0.2346
rs10245483	79 (80.4)	140 (137.1)	57 (58.4)	0.121	0.7274	112 (109.5)	178 (183)	79 (76.5)	0.278	0.5980
rs2157930	184 (183.9)	81 (81.1)	9 (8.9)	0.001	0.9812	238 (235.8)	114 (118.3)	17 (14.8)	0.492	0.4831
rs10246878	184 (185.1)	84 (81.9)	8 (9.1)	0.184	0.6677	245 (243.9)	110 (112.2)	14 (12.9)	0.141	0.7070
rs2157926	259 (258.3)	16 (17.4)	1 (0.3)	1.817	0.1776	332 (330.1)	34 (37.8)	3 (1.1)	3.786	0.052
rs3213619	258 (258.3)	17 (16.5)	0 (0.3)	0.280	0.5969	343 (342.5)	25 (26.0)	1 (0.5)	0.559	0.4548
rs2214102	241 (240.2)	33 (34.5)	2 (1.2)	0.535	0.4645	325 (326.3)	44 (41.4)	0 (1.3)	1.483	0.2232
rs9282564	214 (213.1)	57 (58.9)	5 (4.1)	0.278	0.5981	271 (269.8)	89 (91.5)	9 (7.8)	0.273	0.6016
rs868755	105 (109.1)	137 (128.9)	34 (38.1)	1.099	0.2945	134 (130.0)	170 (178.0)	65 (61.0)	0.754	0.3852
rs2229109	255 (254.4)	20 (21.1)	1 (0.4)	0.780	0.3770	347 (347.3)	22 (21.3)	0 (0.3)	0.348	0.5550
rs1128503	104 (103.5)	130 (131.0)	42 (41.5)	0.017	0.8955	127 (119.5)	166 (181.0)	76 (68.5)	2.527	0.1119
rs1045642	68 (73.1)	148 (137.9)	60 (65.1)	1.486	0.2229	98 (97.8)	184 (184.3)	87 (86.8)	0.001	0.9721

NN: No. of the major homozygotes; Nn: no. of the heterozygotes; nn: no. of the minor homozygotes.

Multiple regression showed that "CC" genotype in the rs1128503 polymorphism statistically significantly increases survival time (Table V). This result was confirmed by median test (χ^2=7.602, *p*-value=0.0224) (Table VI).

Discussion

Multiple polymorphisms have been described for the *ABCB1* gene. For some of them, their effects on mRNA and protein expression in different tissues as well as on the ABCB1 transporter activity have been observed *in vitro*. However, these effects so far seem too weak for reliable predictions with regard to prevalence of disease and a patient's response to treatment (3, 7), therefore further studies are necessary. Here, among two SNPs in *CYP3A4* and *CYP3A5* and 12 SNPs in *ABCB1* gene we observed an association with ovarian cancer risk for *ABCB1* rs2157926 SNP only. This SNP is located in intron 1 (-40106A>T) and has not been extensively studied so far (3, 7), therefore its effect on mRNA or protein expression or on treatment response is not established.

rs2032582 allele frequencies did not differ in our patients and controls. Similarly, in a large study of over 4,600 ovarian cancer patients from the Ovarian Cancer Association Consortium and The Cancer Genome Atlas, Johnatty *et al*. (5) did not observe any association of rs2032582 or any other SNP of the *ABCB1* gene with the overall survival or progression-free survival, although in an earlier study with a much lower number of patients, the same authors observed an effect of rs2032582 on progression-free survival (10). However, we found that rs2032582T allele increased metastasis risk about fourfold. This SNP has three alleles (G, T and A) and encodes an amino acid change in position 893 from alanine to serine or threonine, respectively. Notably, the different alleles were described to have similar expression in

Table III. *Backward stepwise multivariate regression model on ovarian cancer risk.*

Effect	Genotype	*p*-Value	OR	Lower 95%CI	Upper 95%CI
Intercept		0.000	85.333	27.340	266.342
rs2157926	TT	0.000	0.006	0.002	0.021

Table IV. *Backward stepwise multivariate regression model on risk of the metastasis.*

Effect	Genotype	*p*-Value	OR	Down 95%CI	Upper 95%CI
Intercept		0.007	0.287	0.116	0.712
BRCA1 mutation (Yes/No)	Yes	0.084	2.260	0.896	5.699
rs2032582	GT	0.038	3.925	1.076	14.318
rs2032582	GA	0.850	1.251	0.122	12.786
rs2032582	TT	0.107	6.009	0.680	53.095
rs1045642	CT	0.174	0.497	0.181	1.363
rs1045642	TT	0.325	0.530	0.150	1.875
rs10245483	GT	0.121	0.494	0.203	1.205
rs10245483	TT	0.928	1.042	0.425	2.560
rs1128503	CT	0.100	0.340	0.094	1.232
rs1128503	TT	0.170	0.266	0.040	1.762

Table V. *Multiple regression model on survival time.*

	Genotype	*p*-Value	Beta (β)	St.Err.β
Intercept		0.003405		
rs2214102	GG	0.790532	0.024994	0.093999
rs2214102	AG	0.721047	–0.032272	0.090285
rs2229109	GG	0.889958	–0.013300	0.096034
rs2229109	AG	0.537055	0.059716	0.096614
rs1128503	CC	0.024778	0.256215	0.113472
rs1128503	CT	0.986014	–0.001679	0.095707
rs1045642	CC	0.285461	–0.091043	0.085060
rs1045642	CT	0.304539	0.077290	0.075128
rs868755	CC	0.119192	–0.166473	0.106486
rs868755	AC	0.689034	0.038041	0.094956
BRCA1 mutation (Yes/No)	No	0.582646	–0.034311	0.062359

Table VI. *Median test model on survival time.*

Dependent: survival time [M]	Median Test. rs1128503. χ^2=7.601913. p-value=0.0224			
	CC	CT	TT	Total
<= Median: observed	53.00	58.00	29.000	140.00
Expected	52.44	66.18	21.382	
Obs.-exp.	0.56	–8.18	7.618	
>Median: observed	50.00	72.00	13.000	135.00
Expected	50.56	63.82	20.618	
Obs.-exp.	–0.56	8.18	–7.618	
Total: observed	103.00	130.00	42.000	275.00

normal tissues, unselected cell lines, and untreated malignant lymphomas, but there was loss of heterozygosity for rs2032582G>T in a number of selected cell lines and relapsed malignant lymphomas (3). The T allele (893Ser) was observed to increase V_{max} for vincristine (3) and increase the response to imanitib in chronic myelocytic leukemia (7). However, many studies on rs2032582G>T-A role in anticancer chemotherapy of different types of malignancy brought conflicting results, and generally the effects of all *ABCB1* polymorphisms were weak (7).

On the other hand, rs1128503CC genotype prolonged survival of our patients. This SNP is located in exon 12, but is synonymous (Gly412Gly). Its role was recently studied in several human diseases and their treatment outcome, but frequently with ambiguous results, depending on disease or ethnicity (11-15). No effect of rs1128503 or any of the SNPs tested here, but only effects of some other SNPs on treatment outcome were found in U.S. ovarian cancer patients (16).

Also, we did not detect any effect of rs1045642 on ovarian cancer risk or treatment outcome. This is a silent variant (Ile1145Ile), but nevertheless was suggested by some authors to affect Pgp protein expression, although it was questioned by others (3, 7). It was not examined in American ovarian cancer patients (16).

Recently, Tęcza *et al.* [2015] (17) published results showing associations of rs2032582 and rs1045642 with ovarian cancer, but only in patients positive and negative for *BRCA1* mutations, respectively. In contrast, we have not observed these relations in similar numbers of patients and controls. Grzybowska *et al.* [2002] (18) described the role of the germline mutations in the BRCA1 gene in predisposition to breast and ovarian cancer in Upper Silesian population. They showed a very high frequency of single *BRCA1* 5382insC mutation and *BRCA2* 9631delC. They suggested that this particular mutation in *BRCA2* gene is limited to the Southern part of Poland (18). We observed a significantly lower frequency in these two mutations in Lower Silesian population (data not shown). This can be a cause for differences between those two populations.

Altogether, our results suggest that genotyping for rs2157926, rs2032582 and rs1128503 may help predict ovarian cancer outcome.

Acknowledgements

This work was supported by a grant N N401 063735 from the Polish National Science Centre. The Authors would like to express their gratitude to all the patients and control volunteers for kindly donating their blood and their agreement to participate in this study.

References

1 De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, Pierannunzio D and Trama A: Cancer survival in Europe 1999-2007 by country and age: results of EUROCARE-5 – a population-based study. Lancet Oncol *15*: 23-34, 2014.

2 Siegel RL, Miller KD and Jemal A: Cancer Statistics, 2017. CA Cancer J Clin *67*: 7-30, 2017.

3 Cascorbi I: P-glycoprotein: Tissue distribution, substrates, and functional consequences of genetic variations. *In*: Drug Transporters, Handbook of Experimental Pharmacology, M.F. Fromm and R.B. Kim, (eds.). Springer-Verlag, Berlin, Heidelberg, 2011.

4 Grimm C, Polterauer S, Zeillinger R, Tong D and Heinze G: Two multidrug-resistance (ABCB1) gene polymorphisms as prognostic parameters in women with ovarian cancer. Anticancer Res *30*: 3487-3492, 2010.

5 Johnatty SE, Beesley J, Gao B, Chen X and Lu Y: ABCB1 (MDR1) polymorphisms and ovarian cancer progression and survival: A comprehensive analysis from the Ovarian Cancer Association Consortium and The Cancer Genome Atlas. Gynecol Oncol *131*: 8-14, 2013.

6 Tian C, Ambrosone CB, Darcy KM, Krivak TC and Armstrong DK: Common variants in ABCB1, ABCC2 and ABCG2 genes and clinical outcomes among women with advanced stage ovarian cancer treated with platinum and taxane-based chemotherapy: A Gynecologic Oncology Group study. Gynecol Oncol *124*: 575-581, 2012.
7 Wolking S, Schaeffeler E, Lerche H, Schwab M and Nies AT: Impact of genetic polymorphisms of ABCB1 (MDR1, P-glycoprotein) on drug disposition and potential clinical implications: update of the literature. Clin Pharmacokinet *54*: 709-735, 2015.
8 Wittwer CT: High-resolution DNA melting analysis: advancements and limitations. Hum Mutat *30*: 857-859, 2009.
9 Zhou L, Myers AN, Vandersteen JG, Wang L and Wittwer CT: Closed-tube genotyping with unlabeled oligonucleotide probes and a saturating DNA dye. Clin Chem *50*: 1328-1335, 2004.
10 Johnatty SE, Beesley J, Paul J, Fereday S, Spurdle AB, Webb PM, Byth B, Marsh S, McLeod H, AOCS StudyGroup, Harnett PR, Brown R, DeFazio A and Chenevix-Trench G: ABCB1 (MDR1) polymorphisms and progression-free survival among women with ovarian cancer following paclitaxel/carboplatin chemotherapy. Clin Cancer Res *14*: 5594-5601, 2008; Correction: Clin Cancer Res *18*: 319-320, 2012.
11 Liu S-G, Gao C, Zhang R-D, Zhao X-X, Cui L, Li W-J, Chen Z-P, Yue ZX, Zhang YY, Wu MY, Wang JX, Li ZG and Zheng HY: Polymorphisms in methotrexate transporters and their relationship to plasma methotrexate levels, toxicity of high-dose methotrexate, and outcome of pediatric acute lymphoblastic leukemia. Oncotarget *8*: 37761-37772, 2017.
12 Chen X, Jiang M, Rui-Ke Zhao R-K and Gu G-H: Quantitative assessment of the association between ABC polymorphisms and osteosarcoma response: a meta-analysis. Asian Pac J Cancer Prev *16*: 4659-4664, 2015.
13 Su J, Yu Q, Zhu H, Li X, Cui H, Du W, Ji L, Tong M, Zheng Y, Xu H, Zhang J, Zhu Y, Xia Y, Liu T, Yao Q, Yang J, Chen X and Yu J: The risk of clopidogrel resistance is associated with ABCB1 polymorphisms but not promoter methylation in a Chinese Han population. PLoS ONE *12*: e0174511, 2017.
14 Yan R-J, Lou T-T, Wu Y-F and Chen W-S: Single nucleotide polymorphisms of ABCB1 gene and response to etanercept treatment in patients with ankylosing spondylitis in a Chinese Han population. Medicine *96*: e5929, 2017.
15 Han S-S, Xu Y-Q, Lu Y, Gu X-C and Wang Y: A PRISMA-compliant meta-analysis of MDR1 polymorphisms and idiopathic nephrotic syndrome. Susceptibility and steroid responsiveness. Medicine *96*: 24e7191, 2017.
16 Peethambaram P, Fridley BL, Vierkant RA, Larson MC, Kalli KR, Elliott EA, Oberg AL, White KL, Rider DN, Keeney GL, Cunningham JM, Hartmann LC and Goode EL: Polymorphisms in ABCB1 and ERCC2 associated with ovarian cancer outcome. Int J Mol Epidemiol Genet *2*: 185-195, 2011.
17 Tęcza K, Pamula-Pilat J, Kolosza Z, Radlak N and Grzybowska E: Genetic polymorphisms and gene-dosage effect in ovarian cancer risk and response to paclitaxel/cisplatin chemotherapy. J Exp Clin Cancer Res *34*: 2, 2015.
18 Grzybowska E, Siemińska M, Zientek H, Kalinowska E, Michalska J, Utracka-Hutka B, Rogozińska-Szczepka J and Kaźmierczak-Maciejewska M: Germline mutations in the BRCA1 gene predisposing to breast and ovarian cancers in Upper Silesia population. Acta Biochim Pol *49*: 351-356, 2002.

Received January 9, 2018
Revised January 23, 2018
Accepted January 24, 2018