Cancer Chemother Pharmacol (2017) 80:251–260
DOI 10.1007/s00280-017-3354-1

ORIGINAL ARTICLE

Genetic variability in the regulation of the expression cluster of MDR genes in patients with breast cancer

Matvey M. Tsyganov[1] · Maxim B. Freidin[3] · Marina K. Ibragimova[1] · Irina V. Deryusheva[1] · Polina V. Kazantseva[2] · Elena M. Slonimskaya[2] · Nadezhda V. Cherdyntseva[1] · Nikolai V. Litviakov[1]

Received: 27 October 2016 / Accepted: 2 June 2017 / Published online: 10 June 2017

Abstract
Purpose We aimed to investigate the association between the polymorphism and expression patterns of multiple drug resistance genes (MDR) in breast cancer (BC).
Materials and methods The MDR gene expression levels were measured in tumor tissues of 106 breast cancer patients using quantitative real-time PCR. Affymetrix CytoScan™ HD Array chips were used to assess genotypes. Pairwise correlation analysis for *ABCB1, ABCC1, ABCC2* and *ABCG2* gene expression levels was carried out to reveal co-expression clusters. Associations between SNPs of MDR genes and their preoperative expression levels were assessed using analysis of covariance adjusting for covariates.
Results The SNPs associated with the expression of the *ABCB1, ABCC1, ABCC2* and *ABCG2* genes before NAC were detected. In addition, 21 SNPs associated with the expression of four ABC-transporter genes and involved in the expression regulation were identified. Validation in an independent sample confirmed the association between the MDR cluster genes and 11 SNPs.
Conclusions Four MDR genes: *ABCB1, ABCC1, ABCC2* and *ABCG2* were shown to form the functional expression cluster in breast tumor. Further studies are required to discover precise mechanisms of the cluster regulation, thereby providing new approaches and targets to combat the development of the MDR phenotype during chemotherapy.

Keywords Breast cancer · Single nucleotide polymorphism · Multiple drug resistance · Neoadjuvant chemotherapy · Gene expression

Electronic supplementary material The online version of this article (doi:10.1007/s00280-017-3354-1) contains supplementary material, which is available to authorized users.

✉ Matvey M. Tsyganov
TsyganovMM@yandex.ru

Maxim B. Freidin
mfreidin@medgenetics.ru

Marina K. Ibragimova
imk1805@yandex.ru

Irina V. Deryusheva
Irkin_097@mail.ru

Polina V. Kazantseva
polydoctor@yandex.ru

Elena M. Slonimskaya
slonimskaya@rambler.ru

Nadezhda V. Cherdyntseva
nvch@tnimc.ru

Nikolai V. Litviakov
nvlitv72@yandex.ru

[1] Department of Experimental Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, 5, Kooperativny Street, 634050 Tomsk, Russian Federation

[2] Department of General Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, 5, Kooperativny Street, 634050 Tomsk, Russian Federation

[3] Laboratory of Population Genetics, Research Institute of Medical Genetics, Tomsk National Research Medical Center, Russian Academy of Sciences, 10 Nabereznaya Ushaiky Str., 634050 Tomsk, Russian Federation

Introduction

Chemotherapy is an important component of breast cancer treatment. However, the frequency of tumor regression varies from 10–15% to 40–50%, and neoadjuvant chemotherapy (NAC) appears to be ineffective for most of the patients. The frequency of partial regression permitting organ-preserving treatment is 45–55% [1]. One of the main reasons for the lack of the efficacy of chemotherapy is the development of multiple drug resistance (MDR) phenotype due to the expression of ATP-dependant proteins of the ABC-transporter family (*ABCB1, ABCB11, ABCC1/2/3/4/5/6/10/11, ABCG1, ABCG2*) ejecting drugs from tumor cells against a concentration gradient [2–4].

Our previous studies showed that the efficacy of NAC for BC is independent of the basal levels of the ABC-transporter expression, but is rather influenced by the change in their expression during the course of treatment. In case of decreased *ABC* gene expression in tumors during NAC, patients exhibit clinical response to therapy, while in case of increased *ABC* gene expression, patients develop MDR phenotype and exhibit no response to therapy [5]. In 75% of preoperative BC patients, we observed an unidirectional change in expression levels of five MDR genes, *ABCB1*, *ABCC1*, *ABCC2*, *ABCG1,* and *ABCG2*, in accordance with the effect of NAC which we called "the gradient phenomenon"—the reduced expression coincides with good clinical response to NAC, while the increased expression coincides with the lack of clinical response to NAC [6]. Despite the fact that all studied ABC genes were located in different chromosomes, we suggested the presence of a single functional expression cluster of the ABC-transporter genes, which was responsible for a unidirectional change in their expression in tumors of the majority of BC patients who received chemotherapy.

It should be noted that the MDR phenotype is developed due to the action of several key ABC-transporter genes [6–8]. Only a few studies attempted to consider the group of MDR genes in particular, rather than separate members of the ABC-transporter family [9–11]. In the vast majority of cases, the studies of MDR were focused on the analysis of association between genes such as *ABCB1*, *ABCC1* and *ABCG2* and different clinical and morphological traits [12–17].

In our opinion, the major weakness of the vast majority of studies of associations between ABC gene expression and genetic variation was the fact that the genetic polymorphisms were assessed in normal tissue DNA, while the gene expression was analyzed in tumors. Such approach ignores allelic imbalance in tumors due to the loss of heterozygosity. This phenomenon can take place in 60–70% of all heterozygotic loci [18]. Thus, we believe that the analysis of both the gene expression and genetic variation must be carried out using tumor tissues.

In the current study, we aimed to investigate the association between polymorphisms and expression patterns of MDR genes in preoperative BC tumors. Experimental design for the current study involved the analysis of correlations between MDR gene expression levels in breast tumors to establish groups of ABC-transporter co-expression followed by the analysis of association between SNPs in the studied genes and their preoperative expression levels in patients with BC.

Materials and methods

The study group

A total of 106 patients aged 28–68 years (mean age ± SD 53.4 ± 0.8) with BC were enrolled into the study (Table 1), sixty-eight patients comprised the main study group (enrolled in 2006–2012) and 38 patients comprised the validation group (enrolled in 2015–2016). Major and validation groups did not differ by the main clinical and morphological parameters except for lymph node status and molecular subtype ($p = 3e{-}3$).

The diagnosis of BC was verified morphologically. The tumor stages were IIA-IIIB. In accordance with the «Consensus Conference on NAC in Carcinoma of the Breast, April 26–28, 2003, Philadelphia, Pennsylvania» [19], all patients underwent 2–4 courses of NAC by FAC scheme (5-fluorouracil, doxorubicin, cyclophosphamide), CAX scheme (cyclophosphamide, doxorubicin, xeloda). Imaging of the primary breast lesion was performed with mammography and/or ultrasonography. Clinical and imaging responses were categorized according to International Union against Cancer criteria [20]. A complete response (CR) was defined as complete disappearance of primary tumor and lymph node metastasis. A partial response (PR) was determined as >50% reduction in tumor size and stable disease (SD) as ≤50% reduction or <25% increase in tumor size. Progressive disease (PD) was described as >25% increase in tumor size. Surgery (radical resection or sectoral resection or mastectomy) was performed 1–2 weeks after completion of chemotherapy in patients who responded to therapy. After surgery, adjuvant chemotherapy or hormonal therapy was given.

The study was carried out in accordance with Helsinki Declaration of 1964 (amended in 1975 and 1983) and was approved by the Ethical Committee of the Cancer Research Institute. Signed informed consent was obtained from all participants.

Table 1 Demographics of the breast cancer patients

Trait	Value	Basic group patients $n = 68$ (%)	Validation group $n = 38$ (%)	p level
Age (year)	≤45	21 (30.9)	17 (44.7)	0.20
	>45	47 (69.1)	21 (55.3)	
Menstrual status	Premenopausal	36 (52.9)	22 (57.9)	0.68
	Postmenopausal	32 (47.1)	16 (42.1)	
Histological type	Invasive ductal carcinoma	58 (85.3)	33 (86.8)	0.76
	Invasive lobular carcinoma	3 (4.4)	2 (5.3)	
	Medullary carcinoma	2 (2.9)	0 (0)	
	Others	5 (7.4)	3 (13.2)	
Tumor size	T_1	9 (13.2)	1 (2.6)	0.26
	T_2	52 (76.5)	32 (84.2)	
	T_3	3 (4.4)	1 (2.6)	
	T_4	4 (5.9)	4 (10.5)	
Lymph node status	N_x	0 (0)	11 (28.9)	**2e−4**
	N_0	27 (39.7)	13 (34.2)	
	N_1	31 (45.6)	11 (28.9)	
	N_2	4 (5.9)	1 (2.6)	
	N_3	6 (8.8)	2 (5.3)	
Estrogen receptor	Positive	33 (48.5)	37 (97.4)	**1e−5**
	Negative	31 (42.6)	1 (2.6)	
	No data	4 (5.9)	0 (0)	
Progesterone receptor	Positive	35 (51.5)	34 (89.5)	**3e−3**
	Negative	29 (42.3)	4 (10.5)	
	No data	4 (5.9)	0 (0)	
HER2	0/+	47 (69.1)	37 (97.4)	**0.01**
	++	10 (14.7)	1 (2.6)	
	+++	6 (8.8)	0 (0)	
	No data	5 (7.4)	0 (0)	
Ki-67	Ki-67 >20	52 (76.5)	37 (97.4)	**0.03**
	Ki-67 <20	11 (16.2)	1 (2.6)	
	No data	5 (7.4)	0 (0)	
Molecular subtype	Luminal A	0 (0)	1 (2.6)	**3e−3**
	Luminal B	41 (60.3)	36 (94.7)	
	Triple negative	17 (25.0)	0 (0)	
	HER2-positive	10 (14.7)	1 (2.6)	
Histological form	Unicentric	45 (66.2)	24 (63.2)	0.83
	Multicentric	23 (33.8)	14 (36.8)	
NAC regimen	CAX	28 (41.2)	14 (36.8)	0.68
	FAC	40 (58.8)	24 (63.2)	
NAC response	Complete response	9 (13.2)	6 (15.8)	0.39
	Partial response	38 (55.9)	15 (39.5)	
	Stable disease	16 (23.5)	17 (44.7)	
	Progressive disease	5 (7.4)	0 (0)	

p value significance level; shown in bold statistically significant level ($p < 0.05$)

RNA extraction

Tumor tissues (~10 mm^3) were obtained before treatment using ultrasound-controlled biopsy. Samples with a content of tumor parenchyma of 40% and higher were analyzed. Tumor samples with a secondary-edematous infiltrative form were excluded. The tissues were placed in RNAlater (Ambion, USA), incubated for 24 h at a room

temperature and stored in −80 °C until DNA and RNA extraction. RNA was extracted from 106 matched tissues (before NAC) using RNeasy Plus mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA concentration and purity were assessed using NanoDrop 2000 instrument (Thermo Scientific, USA). The concentration varied between 80 and 250 ng/μl and A_{260}/A_{280} and A_{260}/A_{230} ratios were 1.85–2.10 and 1.90–2.05, respectively. The RNA integrity was assessed using TapeStation instrument and R6K ScreenTape kit (Agilent Technologies, USA). The RIN values were 5.6–7.8.

Expression profiling of the ABC genes

Expression profiling of the *ABCB1*, *ABCC1*, *ABCC2*, *ABCC5*, *ABCG1*, and *ABCG2* genes was carried out using quantitative real-time PCR (qPCR) using custom fluorescent labeled probes and RotorGene-6000 instrument (Corbett Research, Australia). The RNA was reverse transcribed to cDNA using RevertAid™ kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's instructions. qRT-PCR was performed in triplicate in a volume of 15 μl containing 250 lM dNTPs (Sibenzyme, Russia), 300 nM forward and reverse primers, 200 nM probe, 2.5 mM $MgCl_2$, 19 SE buffer (67 mM Tris–HCl pH 8.8 at 25 °C, 16.6 mM $(NH_4)2SO_4$, 0.01% Tween-20), 2.5 U Hot Start Taq polymerase (Sibenzyme, Russia), and 50 ng of cDNA template. Samples were heated for 10 min at 95 °C, followed by 40 cycles of amplification for 10 s at 95 °C and 20 s at 60 °C. Primer and probe (FAM-BHQ1) sequences were designed using Vector NTI Advance 11.5, Oligo 7.5, and the NCBI Nucleotide Database (https://www.ncbi.nlm.nih.gov/gene/) (Supplement Table 1). Primers/probes were synthesized by the DNA-Synthesis Company (Russia). PCR products were visualized by 1.5% agarose gel electrophoresis with 0.02% ethidium bromide. The mean expression level of each target gene was calculated for tumor tissue normalized to *GAPDH*. The average Ct (cycle threshold) was estimated for both the gene of interest and *GAPDH*. Relative expression was evaluated using the Pfaffl method [21]. The relative expression level was also normalized to a calibrator consisting of a pool of normal breast tissue specimens. For this purpose, specimens of adjacent normal breast tissues from 10 BC patients (NAC free) were used as a source of normal RNA. The results were articulated as n-fold differences in *ABCB1, ABCC1, ABCC2, ABCC5, ABCG1* and *ABCG2* gene expression relative to *GAPDH* and normal breast tissue. As the present study was conducted on the same group of patients as in 2013 [5], we used the one gene-referee, to interpret the results more correctly, both for the main group of patients, and for the validation group.

DNA extraction

DNA was extracted from 106 biopsy specimens of tumor tissues using QIAamp DNA mini Kit (Qiagen, Germany). The DNA concentration and purity were assessed by NanoDrop-2000 (Thermo Scientific, USA). The concentration varied from 50 to 150 ng/μl and A_{260}/A_{280} and A_{260}/A_{230} ratios were 1.95–2.15 and 2.15–2.40, respectively. The integrity of DNA was assessed using TapeStation instrument (Agilent Technologies, USA); the fragments of the DNA were as little as 48 kbp, thus suggesting its high integrity.

Microarray analysis

The CytoScan™ HD Array chip (Affymetrix, USA) was used for genotyping. It contained probes for 2,670,000 markers including 1,900,000 non-polymorphic markers for the analysis of copy number variations (CNVs) and more than 750,000 single nucleotide polymorphisms (SNPs). The sample processing, array hybridization and scanning were performed according to the manufacturer's protocols for Affymetrix GeneChip® Scanner 3000 7G, as described in the article [22]. The results were analyzed using "Chromosome Analysis Suite 3.1" software (Affymetrix, USA). Microarray study was carried out using DNA from 68 biopsies from the main patients group. Validation genotyping was carried out for 21 SNPs and revealed 100% consistency.

Validation of microarray study

Validation genotyping was carried out using real-time PCR in 96-well plate in CFX96 instrument (Bio-Rad Lab, United Kingdom). A two-step PCR was performed: 5 min denaturation at 94C followed by 42 cycles of 15 s at 94 °C, 30 s at primer-specific annealing temperature (58–64 °C). Automatic genotypes calling were done using the CFX96 instrument software. Primers and probes (FAM-BHQ1; ROX-BHQ2) were designed using Vector NTI Advance 11.5, Oligo 7.5, and the NCBI Nucleotide Database (https://www.ncbi.nlm.nih.gov/snp/) (Supplement Table 2). Primers/probes were synthesized by the DNA-Synthesis Company (Russia). qRT-PCR was performed in triplicate reactions in a volume of 15 μl containing 250 lM dNTPs (Sibenzyme, Russia), 300 nM forward and reverse primers, 100 nM probe 1 and 100 nM probe 2, 2.5 mM $MgCl_2$, 19 SE buffer (67 mM Tris–HCl pH 8.8 at 25 °C, 16.6 mM $(NH_4)2SO_4$, 0.01% Tween-20), 2.5 U Hot Start Taq polymerase (Sibenzyme, Russia), and 50 ng of template DNA.

Statistical analysis

Co-expression of the ABC-transporter genes was assessed by Spearman's correlation analysis. For the analysis of association between the levels of gene expression before the NAC and SNPs we used log-linear regression models. From the total of 749,158 SNPs genotyped, we excluded those significantly deviated from the Hardy–Weinberg equilibrium ($p < 1e{-}6$) and those with the minor allele frequency below 3%. After the filtering, 258,586 SNPs remained for the analysis. Log-linear regression models were ran assuming additive, codominant, dominant, and recessive effects of alleles. According to Akaike information criterion, recessive models appeared to be the best, so we report results only for recessive models (additive models AIC = 94.6; codominant models AIC = 95.4; dominant models AIC = 94.2; recessive models AIC = 90.3). The analysis was carried out in R v. 3.3.1 statistical environment. Bonferroni correction for multiple tests was applied and the significance level was set up at $p < 0.05$ after the correction. Wilcoxon–Mann–Whitney test was used to assess the differences between the studied groups as appropriate.

Results

At the first stage of the study, we tried to find groups of co-expressing ABC-transporter genes in breast tumors before NAC using correlation analysis. Expression of four genes, *ABCB1, ABCC1, ABCC2* and *ABCG2*, were found to be statistically significantly correlated before NAC (Spearman's $r = 0.27$–0.81, $p < 0.05$; Table 2), thus suggesting shared mechanisms of regulation of expression of these genes. In addition, statistically significant correlations were identified between *ABCC5* and *ABCG2* and between *ABCG1* and *ABCB3* genes. Data on correlations between expressions of the ABC-transporters are limited and controversial, possibly due to ignorance of additional factors that can affect gene expression, such as genetic heterogeneity. Taking this into account, we attempted to link the gene expression with the genetic variability before the NAC using genome-wide microarray analysis of SNPs. We assumed that if a group of genes shared SNPs associated with their expression levels, this would suggest the presence of a functional expression cluster of the genes.

With the use of log-linear regression approach, we analyzed genome-wide associations between SNPs and expression levels of the ABC-transporter genes before NAC assuming recessive model of the SNPs effects.

This analysis revealed an association between the expression levels of the *ABCB1, ABCC1, ABCC2* and *ABCG2* genes and 21 SNPs: *DISP1* (rs17535305), *DISP1* (rs61840266), *SPAG16* (rs35945601), *NAF1* (rs17571991), *ECHDC1* (rs6569487), *TXLNB* (rs9495425), *ZNF890P* (rs62442010), *AMPH* (rs12701634), *LOXL2* (rs13272093), *C8orf37-AS1* (rs12549485), *KCNQ3* (rs7818112), *ST3GAL1/ZFAT* (rs13255060), *ZBED5* (rs10840501), *DAOA-AS1* (rs4771495), *DHRS4L1* (rs10147475), *CHD2* (rs28458425), *RGMA* (rs7165938), *STXBP4* (rs9303363), *ADCYAP1* (rs304400), *FHOD3* (rs12965274), and *RALGAPA2* (rs3827963) (Fig. 1). It was established that the mutant alleles homozygotes for the above mentioned SNPs, except for the *SPAG16* (rs35945601), exhibited a 1.5- to 2-fold increase of expression levels as compared to alternative genotypes carriers ($p < 0.001$; Table 3). Notably, the four correlated genes, *ABCB1, ABCC1, ABCC2* and *ABCG2* (Table 2), also had the highest number of shared associated SNPs.

These data may confirm the presence of a functional expression cluster of the *ABCB1, ABCC1, ABCC2* and *ABCG2* genes in breast tumors before NAC treatment.

The results were validated in an independent sample of patients ($n = 38$), for which we analyzed *ABC* gene

Table 2 Correlation between ABC-transporter gene expression levels in breast tumors before NAC

Genes	*ABCB1*	*ABCC1*	*ABCC2*	*ABCG2*	*ABCC5*	*ABCG1*
ABCB1	–					
ABCC1	**0.46** $p = 4\mathbf{e{-}}4$	–				
ABCC2	**0.67** $p = 7\mathbf{e{-}}8$	**0.49** $p = \mathbf{e{-}}4$	–			
ABCG2	**0.68** $p = 2\mathbf{e{-}}8$	**0.33** $p = \mathbf{e{-}}3$	**0.68** $p = 3\mathbf{e{-}}10$	–		
ABCC5	**0.33** $p = 2\mathbf{e{-}}3$	0.15 $p = 0.29$	0.20 $p = 0.09$	**0.39** $p = \mathbf{e{-}}3$	–	
ABCG1	−0.07 $p = 0.89$	0.16 $p = 0.14$	0.05 $p = 0.75$	−0.16 $p = 0.18$	−0.08 $p = 0.48$	–

Spearman's rank correlation coefficients were calculated. Statistically significant correlations ($p < 0.05$) are highlighted in bold

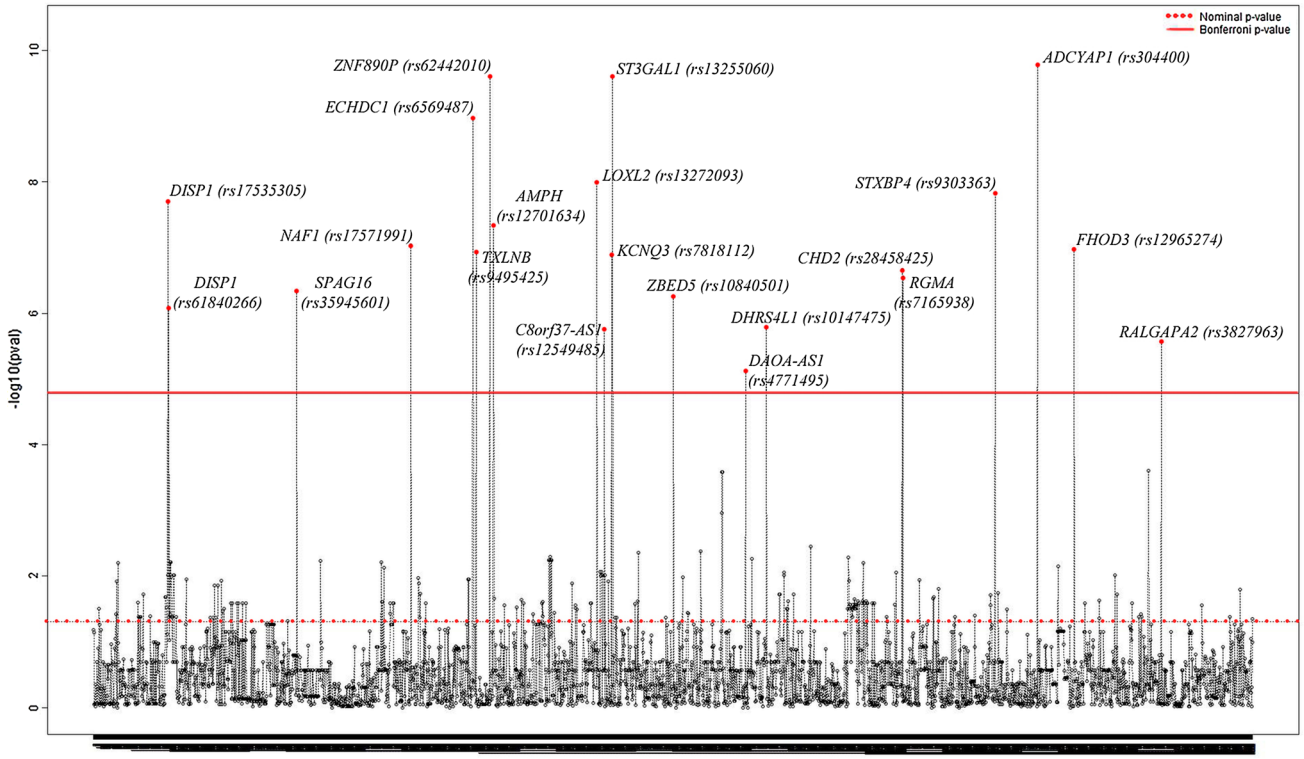

Fig. 1 *p* values (−log 10) for associations between SNPs and expression of the *ABCB1, ABCC1, ABCC2* and *ABCG2* before NAC treatment. A total of 258,586 SNPs was analyzed; *dotted red-line* corresponds to raw 5% significance level; *solid red-line* corresponds to 5% significance levels with Bonferroni correction

expression and genotypes for 21 associated SNPs in tumor biopsies obtained prior to NAC. We found that in this group, the cluster of four MDR genes was associated with 11 SNPs: *NAF1* (rs17571991), *ECHDC1* (rs6569487), *TXLNB* (rs9495425), *TXLNB* (rs9495425), *ZNF890P* (rs62442010), *AMPH* (rs12701634), *KCNQ3* (rs7818112), *ZBED5* (rs10840501), *DHRS4L1* (rs10147475), *CHD2* (rs28458425), *RGMA* (rs7165938), *STXBP4* (rs9303363), and *RALGAPA2* (rs3827963) (Table 4). This confirms that the ABC genes form a functional cluster with shared genetic regulation. For other ten SNPs, no statistically significant association was established. At least two of the SNPs not associated with the gene expression, *LOXL2* (rs13272093) and *ST3GAL1* (rs13255060) were very rare.

Discussion

Several studies showed the correlation between expression levels of the *ABCB1* and *ABCC1* genes [23–25]. A significant positive correlation between expression of the ABC-transporter genes, especially for *ABCB1/ABCG2*, *ABCB1/ABCC1* and *MVP/ABCC1* genes was also reported, and a co-regulation of the expression of these genes was proposed [26]. However, a recent study provided the data on co-expression between proteins of the ABC-transporters in BC and reported no significant correlation between Pgp, Bcpr and Mrp1 proteins (encoded by *ABCB1, ABCG2*, and *ABCC1,* respectively) before and after NAC [27]. Moreover, the analysis of tumors from 171 BC patients revealed no correlation between expression levels of *ABCB1* and *ABCC1* genes [28].

The mechanisms of the influence of the identified associated SNPs on the gene expression are presently unknown. However, some speculations can be put forward for several genes. Experiments with tumor cell lines resistant to Adriamycin have recently demonstrated that the *ST3GAL1* gene associated with the expression of the ABC-transporter gene cluster in our study (rs13255060) directly participates in the regulation of the expression of the *ABCB1* and *ABCC1* genes [29]. Decreased expression of the *AMPH* gene resulted in resistance of tumor cells to cisplatin [30]. The rs12701634 SNP in this gene affected transcription regulation according to F-SNP database (http://compbio.cs.queensu.ca/F-SNP/). In our study, the rs12701634 was associated with the increased expression of the *ABCB1, ABCC1, ABCC2* and *ABCG2* genes in breast tumors before NAC (Table 3).

Table 3 Average expression levels (±SE) for *ABCB1, ABCC1, ABCC2* and *ABCG2* genes in breast tumors before NAC treatment depending on associated genetic polymorphisms

Gene (SNP)	Genotype	The expression level of MDR genes before NAC (Mean ± SE)				Bonferroni-corrected *p* value	*p* value
		ABCB1	*ABCC1*	*ABCC2*	*ABCG2*		
DISP1 (rs17535305)	*CC/CG*	3.58 ± 0.67	1.08 ± 0.18	2.74 ± 0.61	1.94 ± 0.32	**2e−4**	7**e**−10
	GG	5.55 ± 3.05	3.51 ± 1.55	5.59 ± 2.23	4.84 ± 1.74		
DISP1 (rs61840266)	*AA/AT*	3.69 ± 0.69	1.09 ± 0.19	2.80 ± 0.63	1.93 ± 0.33	**3e−4**	**e**−9
	TT	5.55 ± 3.05	3.51 ± 1.55	5.59 ± 2.23	4.84 ± 1.74		
SPAG16 (rs35945601)	*TT/CT*	4.03 ± 1.00	1.44 ± 0.36	3.07 ± 0.66	3.00 ± 0.60	**3e−3**	**e**−8
	CC	3.60 ± 0.91	1.15 ± 0.30	2.95 ± 0.96	1.57 ± 0.36		
NAF1 (rs17571991)	*GG/GA*	3.61 ± 0.67	1.11 ± 0.18	2.76 ± 0.62	1.92 ± 0.33	**0.02**	6**e**−8
	AA	5.25 ± 2.93	3.23 ± 1.64	5.40 ± 1.97	5.09 ± 1.55		
ECHDC1 (rs6569487)	*GG/GA*	3.52 ± 0.66	1.07 ± 0.18	2.78 ± 0.62	1.90 ± 0.32	**1e−3**	4**e**−9
	AA	6.19 ± 3.01	3.65 ± 1.59	5.20 ± 2.02	5.31 ± 1.64		
TXLNB (rs9495425)	*TT/CT*	3.41 ± 0.67	1.11 ± 0.19	2.73 ± 0.62	1.98 ± 0.33	**3e−3**	**e**−8
	CC	5.79 ± 2.99	3.32 ± 1.60	5.44 ± 2.29	4.77 ± 1.79		
ZNF890P (rs62442010)	*CC/AC*	3.38 ± 0.68	1.06 ± 0.19	2.79 ± 0.65	1.97 ± 0.34	**1e−3**	4**e**−9
	AA	6.07 ± 2.61	3.44 ± 1.36	5.33 ± 1.53	4.55 ± 1.35		
AMPH (rs12701634)	*TT/CT*	3.58 ± 0.67	1.06 ± 0.18	2.72 ± 0.61	1.92 ± 0.33	**1e−4**	4**e**−10
	CC	5.49 ± 3.04	3.73 ± 1.48	5.82 ± 2.26	5.12 ± 1.47		
LOXL2 (rs13272093)	*AA/AG*	3.43 ± 0.69	0.99 ± 0.19	2.80 ± 0.65	1.89 ± 0.34	**7e−5**	2.7**e**−10
	GG	5.55 ± 2.86	3.74 ± 1.47	5.52 ± 1.99	5.44 ± 1.28		
C8orf37-AS1 (rs12549485)	*TT/GT*	3.53 ± 0.66	1.06 ± 0.18	2.78 ± 0.62	1.94 ± 0.33	**1e−3**	4**e**−9
	GG	6.08 ± 3.01	3.75 ± 1.55	5.23 ± 1.96	4.93 ± 1.44		
KCNQ3 (rs7818112)	*CC/AC*	3.56 ± 0.68	0.91 ± 0.11	2.74 ± 0.63	1.92 ± 0.33	**5e−3**	2**e**−8
	AA	5.20 ± 2.33	4.22 ± 1.47	4.94 ± 1.63	4.27 ± 1.34		
ST3GAL1 (rs13255060)	*AA/CA*	3.37 ± 0.66	1.02 ± 0.18	2.33 ± 0.48	1.84 ± 0.31	**1e−4**	4**e**−10
	CC	5.88 ± 2.73	3.26 ± 1.40	5.31 ± 2.02	4.69 ± 1.62		
ZBED5 (rs10840501)	*AA/AG*	3.65 ± 0.68	1.10 ± 0.19	2.87 ± 0.62	2.03 ± 0.33	**5e−3**	2**e**−8
	GG	5.96 ± 2.98	3.41 ± 1.57	5.24 ± 2.36	4.54 ± 1.87		
DAOA-AS1 (rs4771495)	*AA/AC*	3.61 ± 0.68	1.11 ± 0.19	2.81 ± 0.62	1.98 ± 0.33	**5e−3**	2**e**−8
	CC	5.78 ± 3.00	3.30 ± 1.61	5.34 ± 2.33	4.71 ± 1.81		
DHRS4L1 (rs10147475)	*AA/AG*	3.56 ± 0.68	1.11 ± 0.19	2.74 ± 0.62	1.90 ± 0.32	**4e−3**	1**e**−8
	GG	5.82 ± 2.98	3.32 ± 1.60	5.75 ± 2.21	4.90 ± 1.75		
CHD2 (rs28458425)	*AA/AT*	3.39 ± 0.65	1.08 ± 0.18	2.75 ± 0.60	1.99 ± 0.33	**8e−3**	3**e**−8
	TT	7.52 ± 3.13	3.53 ± 1.55	5.50 ± 2.39	4.33 ± 1.76		
RGMA (rs7165938)	*AA/AG*	3.50 ± 0.67	1.08 ± 0.18	2.65 ± 0.60	1.91 ± 0.32	**7e−3**	2.7**e**−8
	GG	5.43 ± 3.08	3.61 ± 1.54	5.11 ± 2.41	4.44 ± 1.92		
STXBP4 (rs9303363)	*GG/AG*	3.35 ± 0.69	0.90 ± 0.11	2.53 ± 0.61	1.83 ± 0.32	**0.01**	4**e**−8
	AA	5.53 ± 2.54	3.42 ± 1.29	5.18 ± 2.03	3.98 ± 1.52		
ADCYAP1 (rs304400)	*GG/AG*	3.51 ± 0.66	1.09 ± 0.18	2.75 ± 0.60	1.99 ± 0.33	**7e−3**	2.7**e**−8
	AA	6.28 ± 3.00	3.40 ± 1.58	5.54 ± 2.38	4.34 ± 1.76		
FHOD3 (rs12965274)	*TT/TG*	3.15 ± 0.66	1.07 ± 0.19	2.63 ± 0.63	1.96 ± 0.34	**0.01**	4**e**−8
	GG	9.60 ± 2.72	3.59 ± 1.51	5.11 ± 1.92	4.27 ± 1.55		
RALGAPA2 (rs3827963)	*AA/AG*	3.69 ± 0.70	1.06 ± 0.19	2.87 ± 0.63	2.01 ± 0.34	**0.01**	4**e**−8
	GG	5.90 ± 2.96	3.55 ± 1.56	5.61 ± 2.24	5.01 ± 1.68		

Gene expression was measured using real-time PCR (qPCR). In table, summarizes average gene expression ± standard error (Mean ± SE)

p value significance level; shown in bold statistically significant level ($p < 0.05$)

Table 4 *ABCB1, ABCC1, ABCC2* and *ABCG2* expression level in breast tumor before treatment depending on detected genes polymorphisms in recessive model of studying in validation group

Gene (SNP)	Genotype	The expression level of MDR genes before NAC (mean ± SE)							
		ABCB1	*p* level	*ABCC1*	*p* level	*ABCC2*	*p* level	*ABCG2*	*p* level
DISP1 (rs17535305)	*CC/CG*	***3.51 ± 1.21***	***0.09***	5.81 ± 1.90	0.37	5.6 ± 1.5	0.50	4.41 ± 1.01	0.73
	GG	***0.68 ± 0.27***		6.62 ± 4.21		1.78 ± 0.4		1.96 ± 0.45	
DISP1 (rs61840266)	*AA/AT*	1.36 ± 0.23	0.29	**2.82 ± 1.12**	**8e−3**	5.97 ± 1.7	0.60	***3.57 ± 1.03***	***0.09***
	TT	9.31 ± 4.69		**17.01 ± 5.53**		2.01 ± 0.2		***5.86 ± 1.73***	
SPAG16 (rs35945601)	*TT/CT*	***2.21 ± 0.24***	***0.08***	**3.83 ± 2.02**	**0.01**	4.81 ± 1.7	0.66	3.32 ± 1.12	0.41
	CC	***4.02 ± 1.87***		**8.16 ± 2.78**		5.31 ± 2.2		4.85 ± 1.49	
NAF1 (rs17571991)	*GG/GA*	**1.36 ± 0.26**	**0.05**	**3.48 ± 1.29**	**7e−3**	**3.49 ± 1.0**	**0.02**	**2.83 ± 0.77**	**0.01**
	AA	**9.55 ± 4.62**		**14.84 ± 2.84**		**1.17 ± 4.3**		**8.47 ± 2.41**	
ECHDC1 (rs6569487)	*GG/GA*	**0.27 ± 0.02**	**0.02**	**0.11 ± 0.068**	**0.01**	**1.43 ± 0.6**	**2e−3**	***0.94 ± 0.19***	***0.07***
	AA	**3.35 ± 1.23**		**6.56 ± 1.85**		**0.27 ± 1.5**		***4.47 ± 0.96***	
TXLNB (rs9495425)	*TT/CT*	**1.42 ± 0.29**	**0.03**	**4.09 ± 1.36**	**0.05**	***3.63 ± 0.9***	***0.08***	**3.49 ± 0.95**	**0.02**
	CC	**11.81 ± 1.94**		**15.69 ± 3.42**		***1.57 ± 3.3***		**7.60 ± 2.20**	
ZNF890P (rs62442010)	*CC/AC*	**1.36 ± 0.25**	**0.05**	***4.44 ± 1.73***	***0.06***	**1.89 ± 0.1**	**0.01**	**3.59 ± 0.96**	**0.01**
	AA	**10.60 ± 3.67**		***12.06 ± 4.82***		**8.14 ± 1.4**		**6.38 ± 1.73**	
AMPH (rs12701634)	*TT/CT*	**1.43 ± 0.23**	**0.02**	**3.80 ± 1.21**	**0.03**	**1.82 ± 0.1**	**0.01**	**3.49 ± 0.87**	**0.01**
	CC	**16.59 ± 3.01**		**22.93 ± 6.28**		**8.19 ± 1.4**		**11.37 ± 0.77**	
LOXL2 (rs13272093)	*AA/AG*	1.62 ± 0.32	0.61	5.17 ± 1.69	0.13	4.51 ± 1.3	0.93	3.73 ± 0.94	0.72
	GG	12.70 ± 1.23		17.97 ± 6.97		1.92 ± 0.6		6.88 ± 5.93	
C8orf37-AS1 (rs12549485)	*TT/GT*	3.47 ± 1.33	0.65	6.49 ± 1.95	0.26	4.67 ± 1.4	0.59	3.79 ± 0.84	0.59
	GG	1.11 ± 0.42		3.75 ± 3.43		8.38 ± 4.8		6.28 ± 3.83	
KCNQ3 (rs7818112)	*CC/AC*	**1.26 ± 0.38**	**8e−3**	**2.61 ± 0.44**	**9e−3**	**1.93 ± 0.0.4**	**0.02**	**3.56 ± 1.02**	**0.03**
	AA	**9.51 ± 4.56**		**13.01 ± 1.71**		**16.22 ± 1.70**		**6.02 ± 1.62**	
ST3GAL1 (rs13255060)	*AA/CA*	3.49 ± 1.45	0.59	6.62 ± 2.04	0.64	**3.44 ± 1.13**	**0.04**	**2.93 ± 0.63**	**0.04**
	CC	1.77 ± 0.54		3.23 ± 2.65		**11.09 ± 4.03**		**8.12 ± 3.11**	
ZBED5 (rs10840501)	*AA/AG*	**0.66 ± 0.18**	**0.04**	**3.58 ± 0.18**	**0.01**	**1.17 ± 0.42**	**0.04**	**1.99 ± 1.04**	**0.05**
	GG	**3.67 ± 1.32**		**6.37 ± 1.67**		**6.08 ± 1.68**		**4.57 ± 1.06**	
DAOA-AS1 (rs4771495)	*AA/AC*	3.32 ± 1.23	0.76	6.49 ± 1.93	0.19	2.79 ± 1.34	0.59	***4.46 ± 0.95***	***0.09***
	CC	1.73 ± 1.16		0.96 ± 0.76		2.79 ± 1.76		***1.17 ± 0.62***	
DHRS4L1 (rs10147475)	*AA/AG*	**1.09 ± 0.14**	**0.01**	**3.03 ± 1.25**	**0.02**	***2.36 ± 0.93***	***0.06***	**2.71 ± 0.83**	**0.01**
	GG	**8.66 ± 3.78**		**14.10 ± 4.71**		***9.63 ± 1.97***		**7.52 ± 2.03**	
CHD2 (rs28458425)	*AA/AT*	***1.73 ± 0.45***	***0.09***	***3.30 ± 1.19***	***0.08***	**2.23 ± 0.49**	**5e−4**	***2.69 ± 0.52***	***0.10***
	TT	***3.28 ± 0.61***		***7.13 ± 1.44***		**12.47 ± 3.75**		***8.25 ± 2.82***	
RGMA (rs7165938)	*AA/AG*	**1.04 ± 0.23**	**6e−3**	**2.10 ± 0.92**	**0.001**	**3.68 ± 1.11**	**0.04**	**2.64 ± 0.84**	**6e−3**
	GG	**7.95 ± 3.46**		**14.97 ± 4.48**		**8.99 ± 3.63**		**7.21 ± 2.08**	
STXBP4 (rs9303363)	*GG/AG*	**2.19 ± 0.77**	**0.01**	***4.89 ± 1.51***	***0.10***	***1.84 ± 0.56***	***0.08***	**3.63 ± 0.92**	**0.02**
	AA	**11.01 ± 7.99**		***14.44 ± 9.58***		***5.56 ± 1.53***		**6.95 ± 2.19**	
ADCYAP1 (rs304400)	*GG/AG*	**3.74 ± 1.35**	**6e−3**	**6.98 ± 2.06**	**0.03**	5.88 ± 1.76	0.60	4.51 ± 1.01	0.37
	AA	**0.59 ± 0.14**		**1.87 ± 1.55**		2.42 ± 0.73		2.31 ± 0.80	
FHOD3 (rs12965274)	*TT/TG*	2.58 ± 1.02	0.50	4.38 ± 1.57	0.14	4.87 ± 1.34	0.36	3.31 ± 0.72	0.83
	GG	6.67 ± 4.66		12.03 ± 7.09		6.89 ± 6.19		4.72 ± 2.33	
RALGAPA2 (rs3827963)	*AA/AG*	**2.46 ± 1.18**	**0.01**	**4.07 ± 1.32**	**0.01**	**3.54 ± 1.14**	**0.03**	**2.13 ± 0.40**	**e−3**
	GG	**5.12 ± 2.57**		**11.72 ± 5.50**		**9.85 ± 3.91**		**9.82 ± 2.60**	

Gene expression was measured using real-time PCR (qPCR). In table, summarizes average gene expression ± standard error (Mean ± SE)

p value significance level; shown in bold statistically significant level ($p < 0.05$); bold italic highlighted significance at the clear trend

It should be noted that many genes associated with the expression of the ABC-transporter genes play only an indirect role in forming the drug resistance of tumor cells. For instance, the *ADCYAP1* gene participates in low-level regulation of expression of secretory protein clustering, elevated expression of which is associated with high level of proliferative activity and the development of MDR phenotype, in particular in cervical cancer treated with paclitaxel [31].

The SNPs in *ECHDC1* (*rs6569480*) and *ECHDC1* (*rs7776136*) genes are associated with the response to treatment with tamoxifen [32], the drug is known to be excreted from tumor cells by the ABCB1 and ABCC2 transporters [33, 34], thus suggesting a direct effect of the *ECHDC1* and *ECHDC1* genes on the expression of the ABC-transporters.

Basic regulatory mechanisms of ABC-transporters gene expression can take place at different levels: the influence of various signal cascades in tumor cells and intracellular messengers [35], miRNA, [36, 37], methylation of promotes of the ABC genes [38], and the gene loci deletion [22]. Actually, genetic variation is one of the levels of regulation of chemoresistance-related gene expression.

Conclusion

In the present study, we provided the evidence for the presence of the functional expression cluster of the ABC-transporter genes comprising *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* genes in BC tumors, which likely share common mechanisms of regulation of their expression.

Further studies are required to discover precise mechanisms of the gene cluster regulation, thus providing new approaches and targets to combat the development of the MDR phenotype during chemotherapy.

Based on our results, we established not only genes strongly associated with resistance of tumor cells but also the genes with the unclear role in this process. These data may provide the reference points for further investigations.

Compliance with ethical standards

Conflict of interest All authors declared that they have no potential conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding The study is funded by the Russian Science Foundation No. 17-15-01203 “Metastatic breast tumor clones”.

Informed consent Informed consent was obtained from all individual participants included in the study. The experiments comply with the current laws of the country.

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