Impact of variants in ATP-binding cassette transporters on breast cancer treatment

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There has been substantial interest in the impact of ATP-binding cassette (ABC) transporter variability on breast cancer drug resistance. Here, we provide a systematic review of *ABC* variants in breast cancer therapy. Notably, most studies used small heterogeneous cohorts and their identified associations lack statistical stringency, replication and mechanistic support. We conclude that commonly studied *ABC* polymorphisms are not suitable to accurately predict therapy response or toxicity in breast cancer patients and cannot guide treatment decisions. However, recent research shows that *ABC* transporters harbor a plethora of rare variants with individually small effect sizes, and we argue that a shift in strategy from target variant interrogation to comprehensive profiling might hold promise to drastically improve the predictive power of outcome models.

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Breast cancer constitutes the most prevalent type of malignancy in women and is the leading cause of cancer death accounting for 15% of cancer mortality among women globally [1,2]. Breast cancer is a heterogeneous disease that can be stratified by their molecular and histological features into different subtypes. Histologically, cancers can be distinguished by site into ductal and lobular carcinomas and by stage into pre-invasive and invasive subtypes. The most important molecular determinants are levels of the hormone receptors ER (estrogen receptor) and PR (progesterone receptor), amplification of *ERBB2* (encoding HER2) and mutations in the tumor suppressor genes *BRCA1* or *BRCA2*. For a detailed and updated overview of the molecular events underlying breast cancer ontogeny of the different types, we refer the reader to excellent previous reviews and the references therein [3].

Integration of these histological and molecular features gives rise to clinically distinct and therapeutically actionable subtypes (Figure 1). Luminal-like types are hormone receptor-positive (ER and/or PR) without HER2 activation and are treated by endocrine therapy, such as tamoxifen or aromatase inhibitors, with or without adjuvant chemotherapy depending on the level of proliferation. By contrast, HER2 positive cancers are treated using chemotherapy plus HER2 inhibitors (trastuzumab with or without pertuzumab). Treatment recommendations for triple-negative cancers are chemotherapy with potential addition of platinum-based agents. Furthermore, the use of PARP inhibitors (olaparib or talazoparib) is an additional option for patients with *BRCA* mutations [4,5]. Chemotherapeutic regimens are primarily based on anthracyclines, taxanes and cyclophosphamide [6].

However, while treatment based on molecular features rather than overall tumor burden has drastically improved patient outcomes, drug resistance remains one of the major challenges in breast cancer therapy, particularly for advanced disease. Breast cancer drug resistance is caused by a variety of mechanisms of which increased drug efflux mediated by ATP-binding cassette (ABC) transporters constitutes the most relevant pathway [7–9]. The human *ABC* superfamily of genes encompasses 48 functional transporters distributed across seven subfamilies [10]. Importantly, research over the past decade have implicated a multitude of genetic variations in *ABC* transporter genes in differences in response and toxicity of breast cancer treatment, of which *ABCB1* (encoding MDR1 also known as P-gp), *ABCC1* (encoding MRP1) and *ABCG2* (encoding BCRP) are most extensively studied. While the

Figure 1. Simplified algorithm for breast cancer treatment stratification. Breast cancer management largely depends on the tumor burden and molecular subtypes. Patients with hormone receptor-positive luminal-like types receive adjuvant endocrine therapy after surgery with or without chemotherapy, depending on the risk of recurrence. HER2 positive cancers are treated with chemotherapy and HER2 inhibitors in both neoadjuvant and adjuvant setting. Triple-negative subtypes receive chemotherapy and in case of a lack of complete response after surgery, treatment with PARP inhibitors or immunotherapy are potential options depending on BRCA status and PDL1 expression, respectively. We want to emphasize that the algorithm presented here is simplified for the sake of clarity and we refer to excellent recent reviews for a more detailed stratification of breast cancer management strategies [3,6].

impact of genetic variants in *ABC* transporters on cancer therapy has been excellently reviewed previously, these works were published multiple years ago or did not focus on breast cancer [11–15]. In the following, we thus revisit this topic and provide a comprehensive update and critical review of genetic *ABC* variability as biomarkers for pharmacokinetics, response and toxicity in breast cancer treatment.

ABCB1 (MDR1 or P-gp)

MDR1 is the most extensively studied ABC transporter and mediates the transport of a wide spectrum of chemotherapeutic drugs including anthracyclines, alkaloids, taxanes, bisantrene and other cytotoxic drug substrates [16,17]. MDR1 is highly expressed in intestine, liver and kidney, as well as in the blood–brain barrier. Transporter localization is highly polarized within the plasma membrane, facilitating the directional extrusion of drugs and other substrates. While a total of 990 variants have been identified in *ABCB1* that affect the amino acid sequence of its respective gene product [10], only three exonic and one intronic variant have been implicated in altered toxicity or response to breast cancer therapy (Table 1). The three exonic variants rs2032582, rs1128503 and rs1045642 are localized within a >40 kb haplotype block in strong linkage disequilibrium with $D' > 0.7$ whereas the intronic variant rs3213619 is not linked [18–20].

Rs2032582 encodes a triallelic missense variation of amino acid position 893 of MDR1. The reference genotype G (global MAF = 55%) encodes alanine whereas variants T (MAF = 41.4%) and A (MAF = 3.6%) encode serine and threonine, respectively. Evidence regarding the impact of the different variant states on transporter activity is conflicting [43,44]. In the context of breast cancer, six studies have found significant associations between rs2032582 and toxicity or response. Variant alleles are associated with decreased risk of hematological (leukopenia) and non-hematological (fever, fatigue) adverse events due to docetaxel [21–23] and a reduced risk of paclitaxel resistance [24]. By contrast, rs2032582 variants associated with increased toxicity risk and improved response to anthracyclines [24–26], suggesting substrate-specific differences in variant effects.

The common silent variant rs1045642 (I1145I) has been shown to alter MDR1 function *in vitro* by introduction of a rare codon that alters cotranslational folding [45]. In breast cancer, the rs1045642 variant T allele is consistently

Table 1. *ABCB1* variants associated with pharmacokinetics, toxicity or clinical outcomes in breast cancer treatment.

Variant	RSID	MAF	Drug	Sample size (n)	Variant effect	Effect size	Ref.
c.2677G >T/A (A893S/T)	rs2032582	G: 0.55 T: 0.414 A: 0.036	DTX, EPI and CPA	59	Decreased risk of fever and febrile neutropenia	Fever: OR = 0.15 vs GG Febrile neutropenia: OR = 0.13 vs GG	[21]
			DTX	110	Decreased fatigue	P = 0.03 vs GG	[22]
			PTX/DTX after FAC	132	Decreased risk of leukopenia grade ≥ 2	OR = 0.15, TT vs GG; p = 0.047	[23]
			PTX and ANT	121	Lower risk of drug resistance	PTX: OR = 0.26 vs GG ANT: OR = 0.67 vs GG	[24]
			DOX and CPA	141	Increased risk of leukopenia grade ≥ 3	OR = 3.8 (1.44–9.81); p = 0.007 (A/T vs G)	[25]
c.1236C >T (G412G)	rs1128503	0.545	ANT	487	Increased ANT response	OR = 1.7 (0.79–3.61); p = 0.18 vs GG	[26]
			DOX	62	Increased DOX levels	36.8 vs 21.8 $C_{\max} \cdot \text{dose}^{-1} \cdot \text{BSA}^{-1}$; p = 0.03 (CT + TT vs CC)	[27]
			FAC/FEC	100	Increased FAC/FEC chemotherapy response	OR = 5.2 (1.3–20.2); p = 0.02 (CT vs CC)	[28]
			FAC/FEC	207	Increased grade 2–4 toxicity	OR = 1.9 (1.06–3.39); p = 0.03 (TT vs CT + CC)	[28]
			FAC/FEC	207	Increased dose delay or reduction due to neutropenia	OR = 2.5 (1.15–5.55); p = 0.02 (TT vs CT + CC)	[28]
			DTX	129	Increased response	OR = 2.9 (1.15–7.5); p = 0.03 (TT + CT vs CC)	[29]
			DTX	150	Increased peripheral neuropathy	OR = 1.3 (0.63–2.74) (CT vs CC)	[30]
			PTX	127	Increased peripheral neuropathy grade ≥ 2	OR = 2.4 (1.07–5.4); p = 0.03 (TT vs CC + CT)	[31]
			DOX, CPA and DTX	101	Increased response	OR = 4.6 (1.3–16.1); p = 0.02 (TT vs CC + CT)	[32]
			DOX, CPA, DTX and trastuzumab	86	Increased plasma concentration	AUC 5075 vs 2727 $\text{mg} \cdot \text{h}^{-1} \cdot \text{l}^{-1}$, (CT + TT vs CC)	[33]
c.3435C >T (I1145I)	rs1045642	0.511	ANT	221	Shorter time to progression	PFS 43.25 vs 121.8 months, p = 0.049 (CT + TT vs CC)	[34]
			ANT	153	Worse clinical response	Response rate 33.3% vs 71.2%; p = 0.001 (TT vs CT + CC)	[35]
			ANT	1173	Increased therapeutic response	OR = 2.7 (1.2–6.2); p = 0.02 (CT + TT vs CC)	[26]
			ANT	135	Increased OS and PFS	OS 70.6 vs 49.5 months; p = 0.02 (CT + TT vs CC) PFS 81.2 vs 52.3 months; p = 0.001 (CT + TT vs CC)	[26]
			Adriamycin, CPA and DTX	218	Increased risk of neutropenia	P = 0.015 (TT vs CC)	[36]
			CPA, MTX, FU and TAM	30	Higher risk of recurrence	HR = 5.1 (1.05–24.7); p = 0.043 (CT vs CC)	[37]
			Adjuvant taxane	219	Increased risk of neurotoxicity grade ≥ 2	OR = 2.8 (1.17–6.5); p = 0.017 (TT vs CC + CT)	[38]
			DTX and doxorubicin	216	Increased OS	P = 0.024 (TT vs CC + CT)	[39]
			DTX	110	Increased risk of rash	P < 0.05 (CT vs CC)	[22]

Values in square brackets indicate 95% CI.

ANT: Anthracyclines; AUC: Area under the curve; BSA: Body surface area; CPA: cyclophosphamide; DOX: Doxorubicin; DTX: Docetaxel; EPI: Epirubicin; FAC: 5-Fluorouracil, anthracycline, cyclophosphamide regimen; FEC: 5-Fluorouracil, epirubicin, cyclophosphamide regimen; FU: 5-Fluorouracil; MAF: Minor allele frequency; MTX: Methotrexate; OS: Overall survival; PFS: Progression-free survival; PTX: Paclitaxel; TAM: Tamoxifen.

Table 1. *ABCB1* variants associated with pharmacokinetics, toxicity or clinical outcomes in breast cancer treatment (cont.).

Variant	RSID	MAF	Drug	Sample size (n)	Variant effect	Effect size	Ref.
			PTX	121	Increased OS	HR = 3.5 (1.16–3.51); p = 0.026 (CT vs CC)	[24]
			TAM	71	Longer time to progression	P = 0.012	[40]
			Anastrozole	110	Reduced risk of arthralgia	OR = 0.3 (0.11–0.89); p = 0.029 (TT vs CC + CT)	[41]
c.134–52T >C (intronic)	rs3213619	0.053	PTX	1303	Reduced sensory neuropathy	OR = 0.47 (0.28–0.79); p = 0.004 (C vs T)	[42]

Values in square brackets indicate 95% CI.

ANT: Anthracyclines; AUC: Area under the curve; BSA: Body surface area; CPA: cyclophosphamide; DOX: Doxorubicin; DTX: Docetaxel; EPI: Epirubicin; FAC: 5-Fluorouracil, anthracycline, cyclophosphamide regimen; FEC: 5-Fluorouracil, epirubicin, cyclophosphamide regimen; FU: 5-Fluorouracil; MAF: Minor allele frequency; MTX: Methotrexate; OS: Overall survival; PFS: Progression-free survival; PTX: Paclitaxel; TAM: Tamoxifen.

Table 2. Variants in *ABCC1* and *ABCC2* associated with pharmacokinetics, toxicity or clinical outcomes in breast cancer treatment.

Gene	Variant	RSID	MAF	Drug	Sample size (n)	Variant effect	Effect size	Ref.
<i>ABCC1</i>	c.2012G >T (G671V)	rs45511401	0.04	FEC +/- DOX	1012	Increased risk of febrile neutropenia	OR = 17.6 (2.2–35.8) (TT vs GG)	[46]
	c.825T >C (V275V)	rs246221	0.34	FEC +/- DOX	994	Increased risk of febrile neutropenia	OR = 2.0 (CC vs TT)	[47]
	c.1988 + 219G >T (Intronic)	rs4148350	0.07	FEC +/- DOX	1012	Increased risk of febrile neutropenia	OR = 21.1 (3.1–416.6) (TT vs GG)	[46]
<i>ABCC2</i>	c.4146 + 154A >G (intronic)	rs3740065	0.14	TAM	282	Reduced recurrence-free survival	HR = 10.64 (1.4–79); p = 0.0002 (GG vs AA)	[51]
	c.4544G >A (C1515Y)	rs8187710	0.06	PTX	1303	Increased taxane-related sensory neuropathy	OR = 0.63 (0.42–0.93); p = 0.02 (A vs G)	[42]

Values in square brackets indicate 95% CI.

DOX: Doxorubicin; FEC: 5-Fluorouracil, epirubicin, cyclophosphamide regimen; HR: Hazard ratio; OR: Odds ratio; MAF: Minor allele frequency; PTX: Paclitaxel; TAM: Tamoxifen.

associated with increased toxicity [22,36,38] and improved outcomes [24,39] for patients treated with taxanes. In contrast, the findings for anthracyclines are conflicting and remain inconclusive with reports of increased [26,32] and decreased response and outcomes [34,35] of the variant allele. Similar to rs2032582, effects of rs1045642 on pharmacokinetics and response to MDR1 substrates beyond breast cancer are inconsistent [43,44].

In vitro evidence for the synonymous variant rs1128503 (G412G) suggests that it is functionally neutral by itself [45]. Surprisingly, the available association data in breast cancer unequivocally indicate rs1128503 to be significantly linked with increased exposure (c_{max}) to anthracyclines [27], as well as with increased response and toxicity to anthracyclines [28] and taxanes [29–31]. Given that rs2032582, rs1128503 and rs1045642 are almost completely linked and associations were analyzed by variant and rarely by haplotype, the available data does not allow to precisely assign the observed effects to an individual variant. We conclude based on the available evidence that the three exonic variants rs2032582, rs1128503 and rs1045642 consistently associate with increased exposure, toxicity and efficacy of taxanes, whereas data for anthracyclines is conflicting.

***ABCC1* (MRP1)**

One missense (G671V), one synonymous (V275V) and one intronic variant have been suggested as biomarkers for breast cancer therapy outcomes or toxicity (Table 2). Presence of G671V (rs45511401) correlated with febrile neutropenia in patients treated with fluorouracil, epirubicin and cyclophosphamide (FEC) with odds ratios (ORs) of 1.7–1.8 and 16.4–17.6 for heterozygous and homozygous variant carriers [46,47]. Rs45511401 is ethnogeographically highly variable with highest frequencies in Europeans (5.6%) and lowest frequencies in East Asians (<0.1%) [10]. However, despite the setting of the study being in Europe (Belgium), only 4 homozygous carriers were identified albeit the relatively high study sample size (n = 1012). While this association is corroborated by findings from a

study in non-Hodgkin lymphoma patients that also found increased risk of anthracycline toxicity in variant carriers compared with controls [48], other studies [49,50] did not observe significant effects. Febrile neutropenia risk for breast cancer patients receiving FEC was furthermore reported to be significantly associated with the synonymous variant V275V (rs246221) as well as the intronic variant rs4148350 with ORs of 1.7–2 and 21.1–22.1, respectively [46,47]. In contrast to *ABCB1*, the three variations in *ABCC1* were only weakly linked ($R^2 < 0.2$). Notably, the two studies describing effects of *ABCC1* variations on febrile neutropenia risk [46,47] have been reported by the same group using the same cohort of 1,012 Belgian breast cancer patients (apart from the exclusion of 18 patients receiving chemotherapy prior to FEC in one of the studies) and thus results cannot be seen as independently confirmatory.

We recently analyzed the association of germline variability in ABC transporters with disease-specific survival (DSS) in various cohorts from The Cancer Genome Atlas Program [52]. The results indicated that variant burden in *ABCC1*, but not previously reported ABC candidate variants, was a strong predictor of DSS specifically in those breast cancer patients who received the MRP1 substrates cyclophosphamide ($p = 0.001$) and doxorubicin ($p = 0.009$) but not in hormone receptor-positive patients on endocrine therapy ($p = 0.13$). By contrast *ABCC1* variability was not significantly associated with DSS in clear cell renal carcinoma or hepatocellular carcinoma patients where treatment is not based on MRP1 substrates. These findings support a model in which multiple, often rare variations with individually small effect sizes modulate clinical outcomes in breast cancer patients undergoing chemotherapy.

ABCC2 (MRP2)

Only two SNPs in *ABCC2* have been linked to breast cancer treatment outcomes (Table 2). A study of 1303 early breast cancer patients identified a protective effect of the missense variant rs8187710 (C1515Y) with paclitaxel-induced sensory neuropathy [42]. Paclitaxel is a MRP2 substrate [53] and plausibility of this finding is further supported by result from recombinant *in vitro* systems that show that the variant results in impaired transporter function [54,55].

An intronic variant in *ABCC2* (rs3740065) has furthermore been linked to reduced recurrence-free survival in hormone receptor-positive breast cancer patients on tamoxifen monotherapy with an OR of 10.6 for homozygous variant carriers [51]. However, in contrast to *CYP2D6* genotypes, this variant did not result in alterations of tamoxifen pharmacokinetics. Notably, there is no clear evidence that tamoxifen is a substrate of MRP2 and, to the best of our knowledge, this association has not been replicated in independent studies despite its high MAF of 14% and the alleged high effect size, thus casting considerable doubt on the utility of rs3740065 as a biomarker for tamoxifen treatment outcomes.

ABCG2 (BCRP)

ABCG2 encodes the breast cancer resistance protein (BCRP) that received its name for being originally identified in a breast cancer cell line. Surprisingly however, despite its name, studies that implicate BCRP variability in breast cancer therapy resistance are largely lacking and we only identified a single study that reported an association between *ABCG2* variants and response or therapeutic outcomes in breast cancer patients [56]. In this study, the authors found an association between Q141K (rs2231142) and improved response, as well as a link between V12M (rs2231137) and increased overall survival in hormone receptor-positive breast cancer patients after postoperative anthracycline-based chemotherapy with hazard ratios (HRs) between 0.5 and 0.7 (Table 3). Experimental evidence suggests that V12M is functionally neutral, whereas Q141K results in reduced expression levels [57–60]. Furthermore, Q141K but not V12M have been consistently implicated in differences in the pharmacokinetics of BCRP substrates, such as statins, camptothecins and mitoxantrone [61,62]. However, whereas BCRP provides resistance to a variety of chemotherapeutic agents *in vitro*, BCRP expression does not predict response to chemotherapy, relapse or progression-free survival *in vivo* [63–65]. Substantial *in vitro* evidence moreover suggests that acquired *ABCG2* mutations can increase chemotherapy resistance [66,67]. Again, *in vivo* evidence confirming these findings have to our knowledge not been reported.

Associations with variants in other ABC genes

Only few reports have associated variants in other ABC transporter genes with response or toxicity in breast cancer (Table 3). One report associated *ABCC4* genotypes with toxicity in breast cancer patients receiving cyclophosphamide-containing regimens [68]. The authors report that the intronic variant rs9561778 (MAF = 18%) was associated with both gastrointestinal toxicity ($p = 0.0002$) and leukopenia ($p = 0.014$) in both, an exploratory ($n = 216$) and an independent replication cohort ($n = 187$). Available evidence suggests that cyclophosphamide

Table 3. Variants in other ABC genes associated with pharmacokinetics, toxicity or clinical outcomes in breast cancer treatment.

Gene	Variant	RSID	MAF	Drug	Sample size (n)	Variant effect	Effect size	Ref.
<i>ABCC4</i>	c.3366 + 1243C >T (Intronic)	rs9561778	0.18	CPA	403	Increased risk of leukopenia grade ≥ 3 or gastrointestinal toxicity grade ≥ 2	OR = 2.06 (1.36–3.1); p = 0.04 (T vs C)	[68]
<i>ABCC5</i>	g.+7161T >C (intergenic)	rs1533682	0.38	DOX + CPA	62	Increased DOX clearance	P = 0.04 (CC vs TT)	[69]
<i>ABCC6</i>	c.3803G >A (R1268Q)	rs2238472	0.25	DOX +/- trastuzumab	100	Decreased risk of febrile neutropenia	P = 0.012 (A vs G)	[70]
<i>ABCG1</i>	c.973 + 672G >A (intronic)	rs3788007	0.18	DOX +/- trastuzumab	100	Increased risk of febrile neutropenia	P = 0.0045 (A vs G)	[70]
<i>ABCG2</i>	c.421C >A (Q141K)	rs2231142	0.12	Preoperative ANT	1169	Increased response	OR = 4.7 (0.83–26.4); p = 0.04 (AA vs CC)	[56]
	c.34G >A (V12M)	rs2231137	0.11	ANT	421 postoperative therapy	Increased OS	HR = 0.7; p = 0.044 (AA vs GG)	[56]
				ANT	240 ER+	Increased OS	HR = 0.50; p = 0.023 (AA vs GG)	[56]
				ANT	218 PR+	Increased OS	HR = 0.56; p = 0.042 (AA vs GG)	[56]

Values in square brackets indicate 95% CI.

ANT: Anthracyclines; CPA : Cyclophosphamide; DOX: Doxorubicin; ER: Estrogen receptor; MAF: Minor allele frequency; OS: Overall survival; PR: Progesterone receptor.

is an MRP4 substrate *in vitro* [71] and *in vivo* [72]. However, whether rs9561778 itself has functional effects or is linked to other variants that impact MRP4 function has not been addressed so far.

In addition, in a small study of 62 Asian breast cancer patients the intergenic variant rs1533682 localized 2 kb downstream of *ABCC5* was reported to associate with increased doxorubicin clearance [69]. However, given the lack of further genetic or experimental validations, care is warranted when interpreting this finding. Similarly, Awada and colleagues reported associations for rs2238472 (R1268Q) in *ABCC6* and rs3788007 in *ABCG1* without accounting for multiple testing, strongly suggesting the findings to be statistical artifacts [70].

Conclusion

Robust *in vitro* evidence demonstrates important roles of ABC transporters in chemotherapy resistance in breast cancer cell lines and suggests that both germline and acquired variations can be significant modulators of drug sensitivity. Incentivized by these findings, a multitude of studies have set out to investigate associations between *ABC* variants and various clinical end points, including drug response, toxicity, relapse and survival, with the goal to identify biomarkers that could guide the fine-tuning of therapeutic regimens and assist in prognostic modeling. Here, we systematically reviewed the literature reporting associations between genetic *ABC* transporter variability and treatment outcomes in breast cancer patients and provide a critical synthesis of the available data.

The vast majority of *ABC* associations with treatment outcomes in breast cancer were described for patients undergoing chemotherapy using regimes based on anthracyclines, taxanes and cyclophosphamide and, inversely, the majority of SNPs associated with anthracycline pharmacogenomics were identified in drug transporters [73]. In contrast, no studies have associated *ABC* variants in breast cancer patients with outcomes or toxicity of platinum-based agents, HER2 or PARP inhibitors. Furthermore, only three studies identified *ABC* biomarkers (I1145I in *ABCB1* and the intronic SNP rs3740065 in *ABCC2*) for endocrine therapy using tamoxifen or anastrozole. MDR1 transports the tamoxifen metabolites endoxifen and 4-hydroxytamoxifen [74,75], whereas neither the parent nor its metabolites have been shown to be substrates of MRP2. Based on these findings we conclude that the role of *ABC* transporter polymorphisms for endocrine therapy is limited to few variants in *ABCB1* and there is currently no evidence that *ABC* transporter genotype affects treatment with HER2 or PARP inhibitors.

Changes in transporter activity can be expected to result in alterations of pharmacokinetics and/or in changes of intracellular concentrations. A multitude of studies have evaluated relations between *ABCB1* variants and drug pharmacokinetics. In the context of breast cancer, only two associations with pharmacokinetic changes have been presented in which rs1045642 (I1145I) increases the area under the plasma concentration-time curve of docetaxel [33]

Table 4. Summary of evidence for variant functionality at multiple levels. ‘Efficacy’ and ‘toxicity’ refers to outcomes and risk of adverse events upon treatment with substrates for the respective transporter, respectively. This is a simplified overview and we refer the reader to the discussion of the respective variants in the corresponding sections of the text to understand the quality of the available evidence.

Gene	RSID	Variant	Efficacy	Toxicity	Experimental evidence	Genetic	Ref.
<i>ABCB1</i>	rs2032582	A893S/T	Increased	Conflicting	Conflicting	Decreased IBD risk	[45,86–88]
	rs1128503	G412G	Increased	Increased		Benign	
	rs1045642	I1145I	Conflicting	Conflicting		Benign	
	rs3213619	Intronic	NA	Decreased	NA	NA	
<i>ABCC1</i>	rs45511401	G671V	NA	Increased	Conflicting	Gene not disease-associated	[89,90]
	rs246221	V275V	NA	Increased	NA		
	rs4148350	Intronic	NA	Increased	NA		
<i>ABCC2</i>	rs3740065	Intronic	Decreased	NA	NA	NA	[54,55]
	rs8187710	C1515Y	NA	Increased	Reduced function	Benign	
<i>ABCC4</i>	rs9561778	Intronic	NA	Increased	NA	Gene not disease-associated	
<i>ABCC6</i>	rs2238472	R1268Q	NA	Decreased	NA	Benign	
<i>ABCG1</i>	rs3788007	Intronic	NA	Increased	NA	Gene not disease-associated	
<i>ABCG2</i>	rs2231142	Q141K	Increased	NA	Reduced function	Increased risk of hyperuricemia	[58,59]
	rs2231137	V12M	Increased	NA	Functionally neutral	Decreased risk of hyperuricemia	[59,60]

Disease associations were extracted from ClinVar.
NA: Not available.

and rs1128503 (G412G) increased doxorubicin exposure [27]. Furthermore, no conclusive associations have been identified with other well characterized MDR1 substrates, including cyclosporine [76], various anti-epileptics [77], fexofenadine [78] and digoxin [79]. Similarly, lack of correlation or conflicting results were reported for associations between *ABCG2* genotypes and the tyrosine kinase inhibitor BCRP substrates erlotinib, gefitinib and imatinib [80–82]. These results suggest that the impact of individual *ABC* transporter variants on drug pharmacokinetics is very limited. In contrast, there is more evidence for pharmacogenetic effects on intracellular drug concentrations, as evidenced by genotype-dependent differences in drug efficacy or toxicity.

ABC transporters furthermore have important physiological roles and mutations in multiple genes that encode pharmacologically relevant transporters can also cause genetic disease, including *ABCC2* with Dubin–Johnson syndrome (OMIM 237500), *ABCC6* with pseudoxanthoma elasticum (OMIM 264800) and *ABCG2* with gout (OMIM 138900). Notably, only few of those variants that associate with drug pharmacokinetics, response or toxicity also associate with congenital disease. The most notable example is rs2231142 (Q141K) in *ABCG2*, which correlates with increased (HR = 1.74; $p = 3 \times 10^{-60}$ in Caucasians; HR = 2.1; $p = 10^{-11}$ in Asians) risk of hyperuricemia, thus aligning with the experimentally determined reduction in activity of this transporter variant [83,84]. In addition, rs2231137 (V12M) was found to be mildly protective for hyperuricemia (HR = 0.67; $p = 0.005$) [84]. Besides those *ABCG2* variants, the G allele of rs2032582 (A893) in *ABCB1* was found to associate with IBD by both case–control analysis ($p = 0.002$) and pedigree disequilibrium tests ($p = 0.0002$), providing genetic support for the relatively reduced function of the A893 variant [85]. In the simplest model, it could be expected that reduced transporter activity might result in increased intracellular drug levels, resulting in increased efficacy but also increased risk of toxicity and possibly an association with genetic disease in case of a transporter with physiologically essential functions. However, with the exception of *ABCG2* rs2231142 (Q141K), results are not consistent across experimental, pharmacological and genetic levels of evidence (Table 4).

Importantly, the published studies are highly heterogeneous in quality with regards to sample size, cohort selection and statistical stringency. The majority of studies recruited patients with different breast cancer subtypes, irrespective of hormone receptor and HER2 status, which complicates result interpretation particularly for those studies that considered response as their primary end point. Furthermore, patients were recruited across tumor

stages and grades and these parameters were rarely considered as confounding variables despite their importance for patient outcomes, which hampers the possibility to conduct meaningful meta-analyses. Moreover, most identified biomarkers were only identified in a single study, lacked replication and should thus be interpreted very cautiously. Notable exceptions are variants rs2032582 (A893T/S), rs1128503 (G412G) and rs1045642 (I1145I) in *ABCB1* that have been repeatedly implicated in response and toxicity to anthracyclines and taxanes, albeit sometimes with opposite effects. We see two possible explanations for the lack of replication: the reported result is false-positive or the association is highly sensitive to one or more unidentified factors, such as the molecular or histological signature of the cancer, disease stage, the exact choice of treatment regimen (choice of drugs, doses, timings), demographic or other factors unique for the analyzed cohort, thereby obstructing replication of the finding in question. This lack of replication is particularly concerning for variations with no or little functional or mechanistic support, such as synonymous or intronic variants or missense variants that do not affect transporter function, which combined comprise the majority of identified associations. Of note, while generally assumed to be functionally neutral, such variants can impact transporter function, as evidenced by the synonymous variants G412G and I1145I in *ABCB1* that disrupt the cotranslational folding process via the introduction of rare codons thereby reducing transporter activity [45].

Overall, we conclude that individual *ABC* transporter variants cannot serve as clinically actionable biomarkers for breast cancer therapy. The most promising associations are rs2032582, rs1128503 and rs1045642 in *ABCB1* for the prediction of toxicity and response to taxanes, for which the literature appears consistent. However, the relatively small effect sizes also do not warrant implementation of those variants into clinical decision-support. In contrast, the same variants are poor predictors for clinically relevant end points of anthracycline or endocrine therapy. Variants in other *ABC* transporters lack replication and mechanistic support and are thus at present do not constitute clinically useful biomarkers.

Future perspective

Notably, the vast majority of studies were based on the interrogation of selected candidate variants with mostly high variant frequencies by arrays or targeted PCRs. However, analyses of genomic data from >130,000 individuals revealed that *ABC* transporters harbor more than 62,000 exonic variants as well as many more intronic variations, the vast majority of which (98.5%) are rare with MAFs <1% [10]. This genetic complexity is comparable to other highly polymorphic gene families, such as those encoding CYP enzymes [91,92], SLC [93] or SLCO transporters [94,95]. Analysis of *ABC* variability in breast cancer patients based on next-generation sequencing (NGS) data revealed a multitude of variants with complex haplotype structures and individually small effect sizes whose incorporation drastically improved the predictive power of outcome models [52]. These findings demonstrate the importance of previously neglected rare genetic variants and advocate for a paradigm shift away from the interrogation of few selected SNPs and toward decision-making based on comprehensive sequencing data in the near future [96,97].

Executive summary

- Effects of *ABC* transporter polymorphism on chemotherapy pharmacokinetics, response or toxicity in breast cancer patients is restricted to anthracyclines, taxanes and cyclophosphamide, whereas their relevance for endocrine therapy or HER2/PARP inhibitors are limited.
- Most identified associations lack replication and mechanistic support. This can at least in part be explained by the heterogeneity of the published literature with regards to sample sizes, cohort selection and statistical stringency.
- The only consistent results were obtained for rs2032582, rs1128503 and rs1045642 in *ABCB1* for the prediction of toxicity and response to taxanes.
- Recent research indicated that a large number of variations with individually small effect sizes in *ABCC1* (MRP1) can better explain treatment outcomes in breast cancer patients treated with MRP1 substrates, whereas no association was found with commonly analyzed candidate single nucleotide polymorphisms. These results suggest that variant effects might be combinatorial and advocate for a shift in strategy away from the interrogation of target single nucleotide polymorphisms toward comprehensive and integrative analyses of mutational burden.

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