Review

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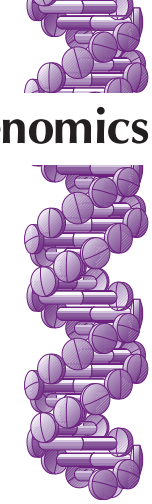
Pharmacogenomics



Pharmacogenetics of anthracyclines

Anthracyclines constitute a fundamental part of the chemotherapy regimens utilized to treat a number of different malignancies both in pediatric and adult patients. These drugs are one of the most efficacious anticancer agents ever invented. On the other hand, anthracyclines are cardiotoxic. Childhood cancer survivors treated with anthracyclines often undergo cardiac complications which are influenced by genetic variations of the patients. The scientific literature comprises numerous investigations in the subject of the pharmacogenetics of anthracyclines. In this review, we provide a comprehensive overview of this research topic. Genetic variants are proposed targets in the personalized treatment in order to individualize dosing and therefore reduce side effects.

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Anthracyclines (ANT), for example, doxorubicin and daunorubicin are widely used chemotherapeutic agents utilized in the treatment of several malignancies including solid tumors of the ovary, breast and gastrointestinal system, and hematologic disorders, for example, leukemia and lymphoma [1,2]. These drugs are very effective and fundamental components of many chemotherapeutic protocols used in pediatric malignancies and contribute to the fact that today the survival is prolonged [3,4]. At the same time serious side effects of anthracyclines cause major clinical problems, like congestive heart failure, pericardial disease and valvular abnormalities [5].

Cardiotoxicity of anthracyclines can have acute, early and late appearance and might emerge in the form of loss of cardiomyocytes or functional damage. Acute cardiotoxicity includes ECG abnormalities, tachycardia and starts within 24 h after the infusion. The subacute form evolves after months as left ventricular systolic dysfunction and pericarditis. The chronic form can appear as dilated cardiomyopathy and congestive heart failure (CHF) 4–20 years after the treatment. The late form is the most common anthracycline treatment-related cardiac adverse effect, mostly occur ≥2 decades after the anthracycline exposure [2,6–7]. Several prevention strategies exist; however, prospective validation of these methods still does not exist [1].

The incidence of heart disease in survivors of childhood cancer is much higher than in the case of their siblings [8–10]. A comprehensive report from 2006 involving 10,397 survivors of childhood cancer (CCSS) has shown that 17.5 years after the diagnosis of cancer, patients, compared with their siblings, had a higher relative risk of congestive heart failure (RR: 15.1, 95% CI: 4.8–47.9, mean age: 26.6 years) [11]. The cumulative incidence of cardiac illness in the same CCSS population in 2013 (median age: 33.7 years) was 5.3% for coronary artery disease, 4.8% for heart failure, 1.5% for valvular disease

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and 1.3% for arrhythmia and it was associated with exposure to cardiotoxic therapies. The same cardiac events were less frequent in the siblings of the patients with 0.9, 0.3, 0.1 and 0.4% cumulative incidences, respectively [12]. An analysis of 1362 childhood cancer survivors showed that they have higher risk of developing symptomatic cardiac events. Especially, there is a very high risk for those receiving concomitant anthracyclines and radiotherapy. After 30 years, one in eight of them will have cardiac problems [13]. Cardiorespiratory fitness of 66.7% of 115 childhood acute lymphoblastic leukemia (ALL) survivors were classified as low compared with the cardiorespiratory fitness of adult healthy participants among whom it was low only in 26.3% ($p < 0.0001$) [14]. Furthermore, cancer survivors can have abnormal myocardial characteristics by echocardiography measurements while being asymptomatic [6,15,16].

The pathogenesis of anthracycline-induced cardiotoxicity is very diverse, several potential and synergistic mechanisms can be in the background in addition to the complexity of the pharmacodynamics and pharmacokinetics of the drug [17]. Oxidative stress induced by anthracyclines is presumed to be the most relevant among these processes and can develop in several ways [2,18]. Radicals derived from oxygen (reactive oxygen species [ROS]) and from nitrogen (reactive nitrogen species [RNS]) are also generated and therefore the antioxidant defense enzymes are of basic importance in the protection of the heart [19]. Cardiac tissue is thought to be more vulnerable to oxidative stress [20,21]. Moreover, other processes might be in the background of anthracycline induced cardiotoxicity, such as inhibition of cardiac-specific gene expression, ion dysregulation, inhibition of DNA polymerase and nucleic acid synthesis, formation of DNA double-strand breaks, binding to TOP2B, secondary alcohol metabolites, binding to cardiolipin in the mitochondria and inhibition of the respiratory chain. Also, doxorubicine is reducted in the presence of free iron and this induces a cycle for free radical generation [2,3,19,20,22–25].

There are several risk factors of anthracycline-induced cardiotoxicity, for example: higher anthracycline cumulative dose, concomitant radiation, younger age at diagnosis, female sex and obesity [20,22,26,27]. Biomarkers may be used to monitor cardiotoxicity. Measuring cTnI (cardiac troponin I) levels is one of the most promising biomarker because it may provide information regarding the cardiac damage before left ventricular dysfunction becomes detectable with echocardiography or via clinical symptoms [28]. Beside these factors, there are high interindividual differences in cardiotoxic events among patients that raise the possibility of a genetic predisposition [27,29,30]. Pharmacogenomics showed that genetic variations might influence drug response and can be used in the clinical practice to tailor therapy [31]. One of the most far advanced examples of genotype-based dosing in the clinical practice is in the case of warfarin. The warfarin (Coumadin) product label approved by the FDA includes recommended dose ranges according to *VKORC1* and *CYP2C9* genotypes [32]. Using genetic variation, it is possible to identify patients who are more susceptible to toxicity or can tolerate larger doses of medication [33,34]. Unfortunately, however, currently there is no pharmacogenomic test with clinical utility available which would predict the cardiotoxic effect of anthracyclines. Complexity of the pharmacodynamics and pharmacokinetics of anthracyclines as well as that of the molecular mechanisms of the side effects of anthracyclines are very high involving lots of genes and targets [2,8,17,19,29]. Concise summary of genes and their function involved in these processes are indicated on Figure 1. We focused especially on genes investigated in pharmacogenetic analyses.

Genetic association studies can be case–control type studies (CCS) or quantitative trait association studies (QTS) [35]. Here below a brief introduction of these study types will be given in the field of pharmacogenetics of anthracyclines. Cases in case–control studies have in general cardiotoxicity, cardiomyopathy or congestive heart failure, whereas controls are without any of these phenotypes. Anthracycline-induced cardiotoxicity is often featured as left ventricular ejection fraction (EF) or fractional shortening (FS) below certain cut off values. Both cases and controls are patients with the same disease (mainly cancer survivors) who received the same medication which included anthracyclines. Genotypes or allele frequencies of the studied SNPs are compared between them. Extensive patient populations are required to obtain comprehensive results to carry out case–control type studies. As an alternative approach, several research groups analyzed EF and FS as a quantitative trait. In quantitative trait association studies left ventricular EF or FS are mainly investigated and mean or median values are compared in genotype groups of the examined SNPs. Figure 2 summarizes the results of the investigations in anthracycline pharmacogenetics. The p-values of case–control and quantitative trait studies are indicated with different symbols.

There are several studies investigating the pharmacogenetics of anthracyclines. In this review, we provide an overview on the pharmacogenetic investigations of anthracycline cardiotoxicity. Variants in the genes of anthracycline pathway can be useful in the individualized anthracycline-based therapy, as these SNPs might contribute to the reduced toxicity while maintaining effectiveness of this drug.

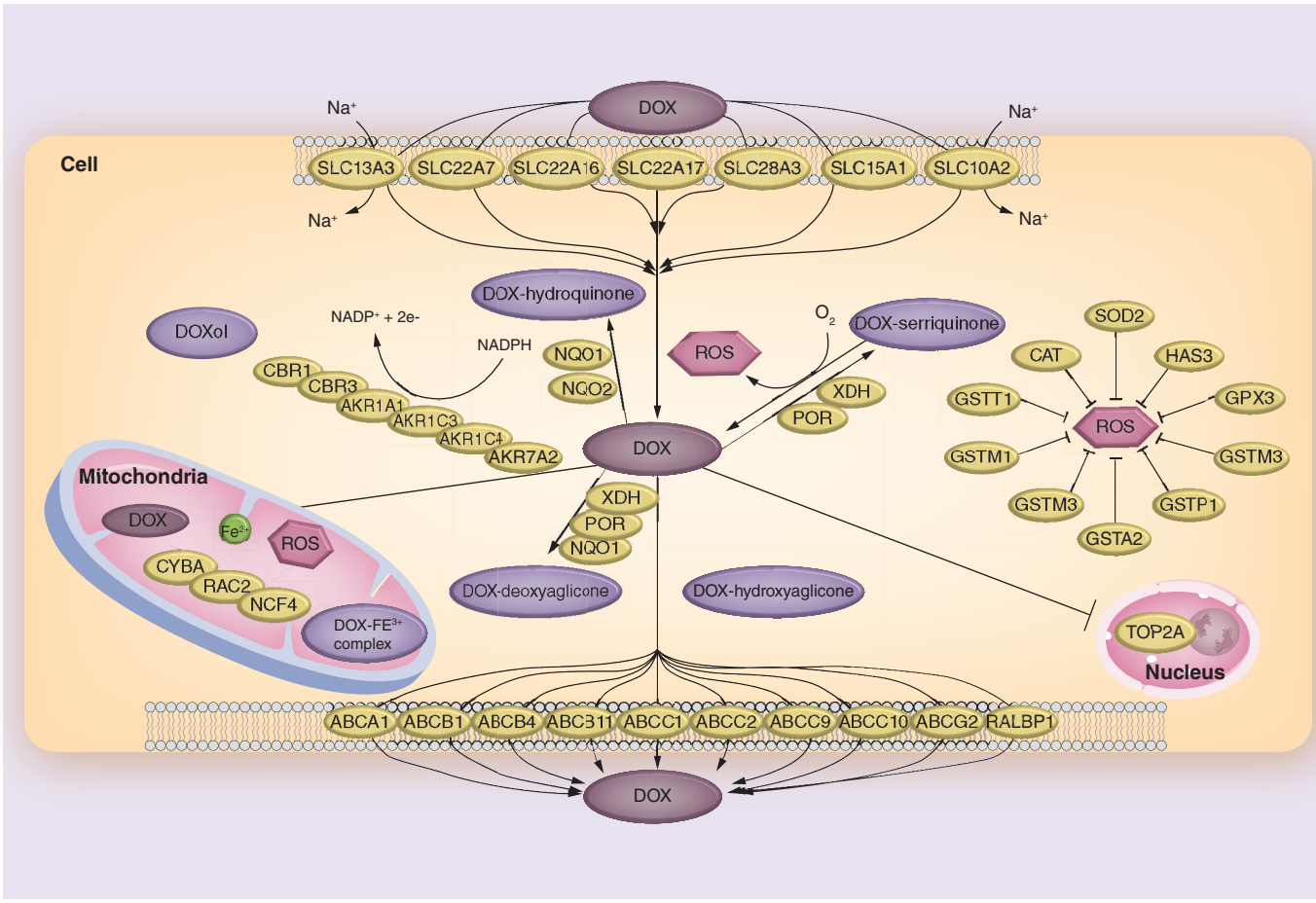

Figure 1. Genes involved in the transport, metabolism and mechanism of side effects of anthracyclines.

Pharmacogenetics of genes in the metabolism of anthracyclines

The articles in the field of pharmacogenetics of anthracyclines are quite diverse. Neither the malignancies of the patient population are homogenous, nor the characterization of the cardiotoxicity. The results of case–control and quantitative trait studies are also very difficult to compare. We summarized the articles in this research area in the online available Supplementary Table 1. Genes in which SNPs were associated with the analyzed cardiac parameters are listed in Supplementary Table 2. Several other genes were also analyzed but showed no association with any studied cardiac parameters, for details see Supplementary Table 3. These are considered here to avoid publication bias. Candidate target genes from the anthracycline pathway and also the results of large-scale studies will be summarized.

Genes relevant to the two-electron reduction of anthracyclines

One of the major metabolic steps that occur with anthracyclines in the cells is the formation of alcohol metabolite by carbonyl reductases and aldo-keto reductases.

CBR3

Case–control studies (CCS)

CBR3 is one of the most investigated genes in anthracycline pharmacogenetics. This enzyme, along with the CBR1 and several other enzymes belonging to the AKR family, catalyze the reduction of anthracyclines to their C13-hydroxymetabolites, doxorubicinol and daunorubicinol (Figure 1) [29,36]. These alcohol metabolites of anthracyclines are thought to be important in the damage of cardiomyocytes [37]. An early pharmacogenetic analysis of children from CCSS population detected no association between anthracycline-induced congestive heart failure (CHF) and SNP V244M (rs1056892A>G) in *CBR3* gene among 30 cases with CHF after childhood cancer and 115 cancer survivors without CHF [38]. Dose dependent association of *CBR3* rs1056892A (V244M) SNP and cardiomyopathy was identified in a population of 170 survivors of childhood cancer with cardiomyopathy when compared with 317 patients without cardiomyopathy. In case of

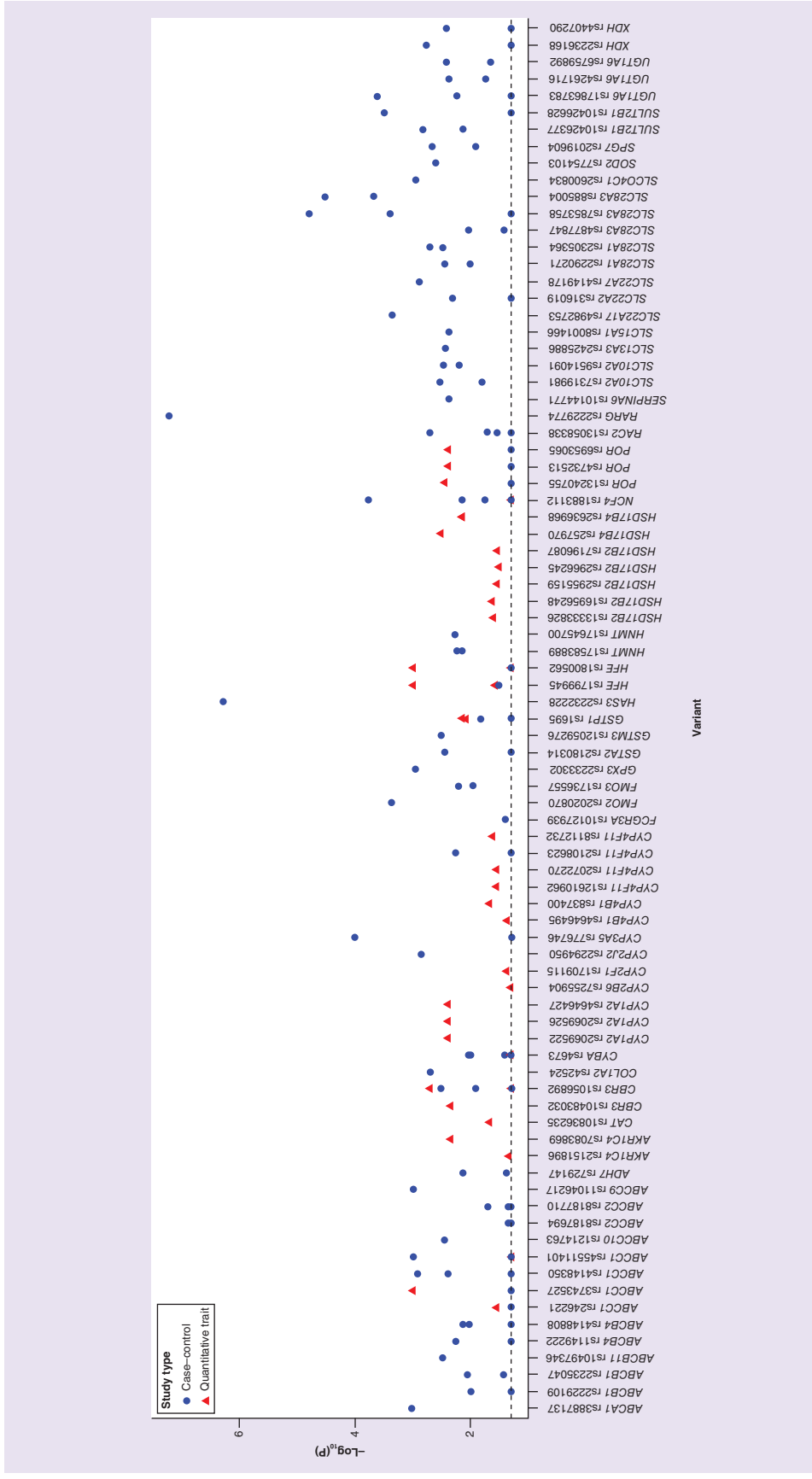

Figure 2. Studied SNPs in anthracycline pharmacogenetics separated by study type. SNPs are displayed on the X-axis; negative logarithm of the p-value is displayed on the y-axis; study type used in the certain article is indicated for all variants.

a subpopulation that received 1–250 mg/m^2 dose of anthracycline, the risk of cardiomyopathy increased in patients with *CBR3* GG genotype (OR: 5.48; 95% CI: 1.81–16.63; p = 0.003). Genotypes were not proven to associate with the risk of cardiomyopathy in the entire population and at high-dose anthracycline treatment [39]. Likewise in survivors of hematopoietic cell transplantation (HCT), *CBR3* rs1056892 did not show association with congestive heart failure (CHF cases n = 77, matched controls without CHF n = 177) [40]. Large studies failed to replicate the association of *CBR3* SNPs and cardiotoxicity in case of a combined cohort of patients (cardiotoxicity cases n = 122, controls n = 398) [41,42]. In addition, a recent report found that *CBR3* rs1056892 associated with cardiotoxicity in survivors of breast cancer (p = 0.012) [43].

Quantitative trait studies (QTS)

Drop in the left ventricular ejection fraction (percentage drop in EF) was used to monitor cardiac function after daunorubicin treatment in 185 acute myeloid leukemia (AML) patients, but none of the studied *CBR3* SNPs rs1056892 (V244M), rs8133052 (C4Y), rs2835285 (V93I) and rs4987121 (M235L) influenced left ventricular EF drop [44]. The same research group performed a more comprehensive study of 465 SNPs involving 81 AML patients receiving daunorubicin or mitoxantrone in combination with cytarabine in their treatment. In this study, more homogenous patient population rs10483032 in *CBR3* associated with drop in left ventricular EF (p = 0.0045) [45]. However, treatment-related cardiac toxicity, expressed as a decrease in EF, was not influenced by *CBR3* SNPs rs8133052, rs1056892 in osteosarcoma patients treated with MAP (methotrexate, doxorubicin and cisplatin based) chemotherapy protocol [46]. A profound decrease in end diastolic volume (EDV) could be observed in patients with *CBR3* rs1056892AA genotype after one year of follow up in a prospective study of 70 patient with cancer (p = 0.002) [47].

CBR1 & CBR4

It seems that variations in other *CBR* genes do not influence the side effects of anthracyclines neither in case–control, nor in quantitative trait type studies. SNPs 1096G>A (rs9024) in the 3′ untranslated region of *CBR1* did not influence the risk of cardiomyopathy or CHF [39,40]. Similarly the lack of association with *CBR1* SNPs rs1143663 (V88I), rs41557318 (P131S) could be observed in another research [44]. Nine SNPs in *CBR1* and six in *CBR4* were analyzed in relation with percentage drop in left ventricular FS, but none of them found to be associate [45]. SNPs in *CBR1* did not show any association with cardiotoxicity [41–43].

AKR family

The family of AKRs was promising potential contributors to the interindividual differences in the side effects of anthracyclines as they are important in the formation of alcohol metabolites of these drugs. Many AKR family genes (*AKR1A1*, *AKR1B1*, *AKR1B10*, *AKR1C1*, *AKR1C2*, *AKR1C3*, *AKR1C4*, *AKR7A2* and *AKR7A3*) were examined in connection with drop (%) in the left ventricular EF in patients with AML. However, there was only one observed association between one SNP (rs7083869) in *AKR1C4* gene and the drop in the left ventricular EF (p = 0.0045) [44,45]. According to a large-scale study risk of cardiotoxicity was not influenced by *AKR* gene SNPs [42].

NQO1

Anthracyclines, as quinones can be subjected to two-electron reduction by NQO1 to from hydroquinone [48,49]. This reaction can be considered as an escape route to by-pass the ROS-generating semiquinone form, however, this picture is not absolutely clear [48].

However, *NQO1* gene polymorphisms did not influence the risk of cardiotoxicity after pediatric cancer in CCSS population, after hematopoietic cell transplantation or in cancer survivors with various disease [38,40,41]. Neither *NQO1* rs1800566 nor rs1131341 influenced ejection fraction in patients with osteosarcoma [46].

Genes in reactive oxygen species generation or elimination

Anthracycline-induced cardiotoxicity can be the result of the formation of ROS in the presence of the drug [19]. These can be generated by semiquinone radical formation catalyzed by several mono-electronic oxidoreductases, for example: XDH or NADH:ubiquinone oxidoreducate subunits which quickly regenerates its parent quinone while generating superoxide anion [19]. Subunits of the membrane-bound enzyme complex NAD(P)H oxidase are also involved in oxidative stress, generating superoxide and other ROS using either NADH or NADPH as an electron donor [2]. Therefore, polymorphisms in genes coding the above-mentioned proteins and also in genes of antioxidant enzymes are important targets in anthracycline pharmacogenetics. Antioxidant enzymes such as superoxide dismutases, glutathione peroxidases, glutathione *S*-transferases and catalase are involved in the defense mechanism by eliminating ROS.

NAD(P)H oxidase functional polymorphism

Widely studied subunits of NAD(P)H oxidase in connection with anthracycline pharmacogenetics are *NCF4*, *CYBA* and *RAC2* [40,50–52]. Genes coding sub-

units of NAD(P)H oxidase were studied among other genes in the first study investigating the pharmacogenetics of anthracycline-induced cardiotoxicity performed by Wojnowski *et al.* [50]. They examined patients with non-Hodgkin lymphoma (NHL). In this research, 206 SNPs were analyzed in 82 genes comparing 87 Caucasian patients with NHL experiencing cardiac problems to 363 NHL patients without any cardiac malfunction. This study found association between chronic anthracycline-induced cardiotoxicity (ACT) and rs1883112 polymorphism in the NAD(P)H oxidase subunit *NFC4* gene. Chronic ACT was defined as the reduction of the ejection fraction <50% or of the fractional shortening <25% after the third cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolon) based chemotherapy. Acute ACT was diagnosed in the cases of arrhythmia, myocarditis, pericarditis and acute heart failure until the end of the third cycle of chemotherapy. The presence of acute ACT was associated with polymorphisms in the NAD(P)H oxidase subunits *CYBA* (rs4673) and *RAC2* (rs13058338) in the same cohort [50].

The genetic variants of NAD(P)H oxidase subunit were studied in the development of cardiac histological lesions in cardiac tissue of deceased patients using a case–control study design, where cases had episodes of CHF. *CYBA* rs4673 protected against patched myocardial necrosis (OR: 0.11; 95% CI: 0.20–0.63, p = 0.039), whereas cardiac interstitial fibrosis was strongly associated with *NCF4* rs1883112 (OR: 5.11; 95% CI: 1.59–16.43; p = 0.018), while no association was detected with *RAC2* rs13058338 [24]. *RAC2* rs13058338 increased the risk of CHF in survivors of haematopoietic cell transplantation (OR: 2.8; 95% CI: 1.4–5.6; p < 0.01); however, *NCF4* rs1883112 and *CYBA* rs4673 did not [40]. In a large case–control study of cancer survivors, SNPs in these three genes did not influence the risk of cardiotoxicity [41]. The same SNPs in *CYBA* and *NFC4* genes did not, but *RAC2* rs13058338 influenced the risk of ACT in diffuse large B-cell lymphoma (DLBCL) population (ACT cases n = 56, matched controls n = 94) when using multivariate logistic regression analysis [52]. A pharmacogenetic investigation of 106 patients with diffuse large B-cell lymphoma (DLBCL) was conducted analyzing 19 SNPs in 15 genes. Rs1883112 in NAD(P)H oxidase subunit *NFC4* was found to be strongly associated with grade 2–4 cardiac toxicity (p = 1.7E-04), while *CYBA* rs4673 and *RAC2* rs13058338 showed only slight association (p = 0.01 and p = 0.019, respectively) [51]. The latest article in the topic found no association with *RAC2*, *NFC4* and *CYBA* SNPs and cardiotoxicity [43]. However, the same SNPs (*NCF4* rs1883112 and *CYBA* rs4673) were not showed association with ejection fraction in osteosarcoma population [46].

HFE & XDH

Iron is supposed to play a crucial role in the anthracycline-induced cardiotoxicity because it is involved in the generation of ROS [2]. Hereditary hemochromatosis is a genetic disease with the symptoms of pathological increase in the iron level of the whole body. The primary mutated gene in its background is the *HFE* gene. It was hypothesized that *HFE* gene mutations might predict cardiotoxicity in patients treated with anthracyclines [53,54]. Two functional SNPs (rs1800562, C282Y and rs1799945, H63D) of the *HFE* gene were examined in relation with cardiac iron deposits in the heart tissue from 97 deceased patients with solid tumors and hematological neoplasms. Haplotypes C/D and Y/H of the two SNPs (C282Y and H63D) in *HFE* gene were associated with higher cardiac iron concentration (p = 0.049 and p = 0.027, respectively) [53]. Additionally examining 184 high-risk childhood ALL patients, a combined genotype of H63D and C282Y showed strong association with several cardiac parameters, for example: fractional shortening, left ventricular mass, end-systolic posterior wall thickness, etc. [54]. In survivors of hematopoietic cell transplantation, multivariate conditional logistic regression revealed that the odds of congestive heart failure was associated with *HFE* rs1799945 SNP (OR: 2.5; 95% CI: 1.0–6.3; p = 0.05) but not with rs1800562 [40].

XDH enzyme might also be involved in the ROS generation. *XDH* gene polymorphisms were associated with anthracycline-induced cardiotoxicity in a recent large-scale analysis [42].

Enzymes scavenging oxygen radicals

Case–control studies

It appears that SNPs in *CAT*, *SOD2* and several glutathione *S*-transferase genes do not influence the side effects of anthracyclines, according to case–control studies. For the first time these genes were studied by the research group of Wojnowski and found no association [50]. Furthermore, neither cardiotoxicity, nor the risk of CHF were influenced by SNPs in *CAT* and *SOD2* genes in survivors of childhood cancer or hematopoietic cell transplantation, respectively [40,41].

Members of the glutathione *S*-transferase family are fundamental enzymes in the scavenge mechanism against oxygen radicals. However, *GSTP1* rs1695 only slightly increased the risk of grade 2–4 cardiac toxicity (OR: 1.83; 95% CI: 1.12–3.01; p = 0.015), while *GSTP1* rs1138272, *GSTA1* rs3957357 and *GSTM1* deletion allele did not according to a study of patients

with diffuse large B-cell lymphoma [51]. None of the SNPs studied in *GSTA1*, *GSTA2*, *GSTA3*, *GSTA4*, *GSTA5*, *GSTM3*, *GSTM4*, *GSTO1*, *GSTO2* and *GSTP1* genes are associated with cardiotoxicity in the work of Visscher *et al.* [41]. Genes of glutathione peroxidases were also studied in the same analysis with no significant result. In this large-scale research 2977 SNPs in 220 genes were studied in 78 cases with cardiotoxicity and 266 controls without cardiotoxicity [41]. The size of the population was further enlarged in a survey in 2015 by the same research group investigating 4578 SNPs in 122 cases with cardiotoxicity and 398 controls without cardiotoxicity. In this analysis the following SNPs in glutathione enzymes were associated with cardiotoxity: rs2233302 in *GPX3*, rs2180314 in *GSTA2* and rs12059276 in *GSTM3*, (p = 0.0011, p = 0.0036, p = 0.0031, respectively) [42].

An especially large-scale association study was performed by Wang *et al.* analyzing 34,912 SNPs in 2100 candidate genes in connection with the risk of cardiomyopathy. In the test for a trend in the gene-environment (anthracycline) interaction between cardiomyopathy and the SNPs, the *HAS3* gene was proven to have a modifying effect (p = 5.3E-7). Furthermore, patients with *HAS3* rs2232228 AA genotype and administered high-dose (>250 mg/m^2) anthracyclines had increased risk for cardiomyopathy (OR: 8.9; 95% CI: 2.1–37.5; p = 0.003). Product of *HAS3* gene is hyaluronan which is the component of extracellular matrix, has an antioxidant activity and has a potential role in cellular recovery after ROS-mediated cardiac injury [55].

Quantitative trait studies

Only one research showed that cardiac damage was influenced by *CAT*. This trait was associated with rs10836235 polymorphism in *CAT* gene (OR: 0.28; 95% CI: 0.09–0.87; p = 0.02), but not with *SOD2* polymorphisms in a Slovenian population of childhood acute lymphoblastic leukemia survivors [56]. SNPs or deletions in *GSTM1* and *GSTT1* showed no association with cardiotoxicity in patients with osteosarcoma nor in childhood acute lymphoblastic leukemia survivors [46,56].

A functional polymorphism, rs1695 (Ile105Val) in *GSTP1* gene was associated with cardiac damage in two studies which investigated the function of the heart as a quantitative trait. Patients with osteosarcoma had increased risk of cardiotoxicity expressed as a decrease in the EF [46]. In a prospective survey of 70 patients with cancer, after 1 year of follow-up, 85% of the *GSTP1* rs1695 G-allele carriers had a decline in their peak filling rate (PFR) value (p = 0.007) [47].

Cytochrome P450 & related enzymes

Numerous members of the superfamily of cytochrome P450 enzymes were thoroughly investigated both in case–control and quantitative trait studies.

Case–control studies

Among the 40 investigated cytochrome P450 genes only rs2108623 in *CYP4F11* was associated with an increased risk of cardiotoxicity (p = 0.0055) in a case–control study of cancer survivors, but SNPs in *POR* gene were not proven to be associate with cardiotoxicity [41]. However, after enlarging the cohort size this association with *CYP4F11* disappeared [57]. Rs776746 in *CYP3A5* in patients with diffuse large B-cell lymphoma increased the risk of grade 2–4 cardiac toxicity (OR: 2.42; 95% CI: 1.53–3.82; p = 1.0E-04), while rs4244285 in *CYP2C19* did not [51]. However, the result with rs776746 in *CYP3A5* was not able to be replicated in breast cancer patients [43].

CYP2J2 was identified as a new potential protective factor concerning anthracycline-induced cardiotoxicity (p = 0.0014) [42] and was not examined previously according to the literature. Cardiomyocyte-specific overexpression of human CYP2J2 provided protection against doxorubicin-induced injury in a mouse model [58].

Quantitative trait studies

Many SNPs in cytochrome P450 were associated with drop (%) in left ventricular EF in the research of Lubieniecka *et al.* in 2013 [45]. Three SNPs (rs2069522, rs2069526, rs4646427) in *CYP1A2* were associated with drop in left ventricular EF (p = 0.004 in all), while several SNPs in other cytochrome P450 enzymes (*CYP2B6*, *CYP2F1*, *CYP4B1*, *CYP4F11*) showed moderate or no association [45]. In the same study, *POR* gene SNPs were also associated with drop in left ventricular EF (rs2868177, rs13240755, rs4732513; p = 0.0025, p = 0.0035, p = 0.004, respectively) [45].

ABC-transporters

Members of the ABC-transporter superfamily (ATP binding cassette transporters) have crucial function in the elimination of anthracyclines from the cell to the extracellular space. These transporters are proteins located in the membrane and utilize the energy derived from the hydrolysis of ATP for their working. There are many articles in the literature supporting their importance in the pharmacokinetics and elimination of doxorubicin. Overexpression of ABCB1 might also confer resistance to doxorubicin [59].

Case–control studies

The first evidence for the relationship between ABC transporters and the side effects of anthracyclines

were published by Wojnowski *et al.* In this investigation of patients with NHL, acute ACT was associated with Gly671Val variant (which is rs45511401) in the ABC transporter *ABCC1* and the Val1188Glu–Cys1515Tyr (rs17222723–rs8187710) haplotype of the *ABCC2* gene. Although other ABC-transporters were also inspected here (*ABCC2*, *ABCC3*, *ABCC4*, *ABCG2*), these showed no association with anthracycline-induced cardiotoxicity [50]. A large candidate gene study of cancer survivor patients was conducted by Visscher *et al.* [41]. Their patient population consisted of survivors of many cancer types. In this large scale report, among many others, 12 ABC-transporter genes were examined (*ABCA1*, *ABCB1*, *ABCB11*, *ABCB4*, *ABCB7*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC4*, *ABCC5*, *ABCC6* and *ABCG2*). Four SNPs in three ABC-transporter genes associated with increased risk of cardiotoxicity. These were rs2235047 in *ABCB1*, rs1149222 and rs4148808 in *ABCB4* and rs4148350 in *ABCC1* gene (p-values are = 0.0087, 0.0054, 0.0093 and 0.0012, respectively) [41]. In order to verify the accuracy of the results the same research group conducted a validation study using two new cohorts which consisted of survivors of several cancer types. These two cohorts were analyzed separately and in combination with the patients in the initial paper [41,57]. The results regarding these SNPs could be replicated for rs2235047 (*ABCB1*), rs4148808 (*ABCB4*) and rs4148350 (*ABCC1*) when the entire combined cohort was considered. Interestingly when they divided the population by gender it was revealed that both of the investigated *ABCB4* SNPs (rs1149222 and rs4148808) increased the risk of ACT in female patients (OR: 2.18; 95% CI: 1.31–3.60; p = 0.0024; OR: 2.53; 95% CI: 1.47–4.36; p = 6.7 x 10^{-4}, respectively) [57].

Previous results regarding *ABCC1* rs45511401 and ACT risk could not be replicated in a more comprehensive report accomplished by the aforementioned research team. Only borderline association was found in the discovery cohort, but no correlation was observed either in the replication, or in the combined cohorts. At the same time some new potential risk factors raised in the following ABC transporter genes: *ABCA1* rs3887137 (p = 9.5 × 10^{-4}), *ABCB11* rs10497346, (p = 0.0033), *ABCC1* rs1214763 (p = 0.0035), *ABCC9* rs11046217, (p = 9.9 × 10^{-4}). Nevertheless, these new potential targets require further validation [42]. Out of the ABC-transporter genes, *ABCB1*, *ABCC2*, *ABCG2*, were studied in relation with grade 2–4 cardiac toxicity in diffuse large B-cell lymphoma (DLBCL) patients and only rs2229109 in *ABCB1* showed slight association with univariate analysis (p = 0.01) [51]. Also in survivors of breast cancer rs1045642 variant of *ABCB1* influenced cardiotoxicity with borderline significance (p = 0.049), but variants in *ABCB1* and *ABCC1* had no effect [43].

The result discovered for *ABCC2* could be replicated in a nested case–control study examining survivors of haematopoietic cell transplantation, risk of congestive heart failure was inflenced by *ABCC2* (rs8187710, OR: 4.3; 95% CI: 1.5–2.5; p < 0.01). In this article, *ABCC1* could not be evaluated due to technical reasons [40]. *ABCC1* and *ABCC2* ABC transporters were studied with nested case–control design of the RICOVER-60 trial. Acute and chronic anthracycline-induced cardiotoxicity cases were compared with matched controls. Risk of ACT was not influenced by *ABCC1* rs45511401, *ABCC2* rs8187710 and *ABCC2* rs8187694 SNPs in diffuse large B-cell lymphoma patients (DLBCL) [52]. The same research group conducted the first anthracycline-induced cardiotoxicity pharmacogenetic study; therefore, they performed a meta-analysis of these two papers. In this final meta-analysis they failed to replicate their associations observed between ACT and SNPs in *ABCC1* and *ABCC2* genes in the first survey [50,52].

Quantitative trait studies

In a retrospective study examining a Hungarian population of 235 pediatric patients with ALL nine SNPs of *ABCC1* were analyzed. Left ventricular fractional shortening was used to characterize the cardiac function of the children at the end of the chemotherapy protocol and at a later follow-up time. *ABCC1* rs3743527TT genotype associated with decreased left ventricular fractional shortening after the chemotherapy (p = 0.001) [33]. Cardiotoxicity was not proven to associate with any of the SNPs of the involved ABC-transporters (*ABCB1*, *ABCG2*, *ABCC1* and *ABCC2*) in 60 patients with osteosarcoma completed MAP chemotherapy [46]. Drop in left ventricular EF was analyzed as the cardiac parameter of survivors of AML. Two ABC-transporters, *ABCB1* and *ABCG2*, were studied with 28 and 16 SNPs, respectively, but none of them influenced the % drop in left ventricular EF [45].

SLC transporters

In humans the solute carrier family consists of almost 400 members. These proteins are located in the membrane and mediate the transport of their substrates into the cells [60]. SLCs take part in the transport of doxorubicin into the cells [42].

Two research groups executed five investigations to explore potential associations between anthracycline-induced cardiotoxicity and SNPs in *SLC* genes. A large study of 220 genes and 1908 analyzed SNPs has been carried out on a discovery cohort of 156 tumor survi-

vor children and two replication cohorts of 188 and 96 patients. Among these genes 28 belonged to the family of solute carriers with several examined SNPs. Two SNPs in *SLC28A3* (rs7853758 and rs885004) were identified as strongly influencing the risk of anthracycline-induced cardiotoxicity (ACT) (OR: 0.31; 95% CI: 0.16–0.60; p = 1.0×10^{-4}; OR: 0.31; 95% CI: 0.15–0.62; p = 2.1×10^{-4}, respectively). Rs4877847 of the same gene associated with OR: 0.60; 95% CI: 0.41–0.89; p = 0.0092. Other SNPs rs7319981 and rs9514091 in *SLC10A2*, rs316019 in *SLC22A2*, rs2290271 and rs2305364 in *SLC28A1* also associated with ACT (p-values from 0.0029 to 0.0049) [41]. Further validations of these SNPs were performed on two validation cohorts and confirmed these findings for all the above mentioned SNPs, except for *SLC22A2* rs316019 [57].

Cardiotoxicity risk was neither influenced by the *SLC28A3* rs7853758 variant nor by the variants of *SLC22A1*, *SLC22A16*, *SLC22A4* and *SLC8A1* in diffuse large B-cell lymphoma patients [52]. *SLC28A3* rs7853758 was not found to associate with cardiotoxicity in breast cancer patients followed up for 6 years [43].

Other genes with uncertain function in ANT pathway

There are some others genes associated with cardiac parameters, but their exact function and role in anthracycline-induced cardiotoxicity pathway is uncertain. These are *ADH7* [41,57]; *COL1A2* [42]; *FCGR3A* [51]; *FMO2* and *FMO3*; *HNMT* [41,57]; *HSD17B2* and *HSD17B4* [45]; *SERPINA6* [42]; *SPG7* [41,57]; *SULT2B1* [41–42,57].

UGT1A6 SNPs (rs6759892, rs4261716, rs17863783) were found to influence the risk of ACT (OR: 1.77; 95% CI: 1.20–2.61; p = 0.0038; OR: 1.76; 95% CI: 1.19–2.59; p = 0.0043; OR: 3.68; 95% CI: 1.45–9.30; p = 0.0059, respectively) [41]. These results were validated on two replication cohorts in a study performed by the same research group. Combined population analysis revealed stronger association of *UGT1A6* rs17863783 with ACT (OR: 4.30; 95% CI: 1.97–9.36; p = 0.00024) [57].

Among these results *SULT2B1* and *UGT1A6* are outstanding, because associated in three reports or with three SNPs, respectively [41,42,57]; although the result with *UGT1A6* was not able to be replicated in the last report in this field [43].

Genome wide association study

A recent genome-wide association study (GWAS) identified a new candidate gene in the risk of ANT-induced cardiotoxicity in survivors of childhood cancer (73 cases with ACT and 383 patients without ACT) [61]. Nonsynonymous variant (rs2229774, Ser427Leu) in *RARG* was associated with increased risk of cardiotoxicity (p = 5.9×10^{-8}; OR: 4.7; 95% CI: 2.7–8.3). The presence of the variant Ser427Leu alters the function of RARG, which therefore does not repress Top2b expression as effectively as wild-type RARG in cellular model. This leads to higher levels of Top2b, which confers increased susceptibility to ANT-induced cardiotoxicity [61].

Conclusion & future perspective

One of the main goals of pharmacogenomics is to develop rational means to optimize drug therapy, with respect to the genotype of the patients, to ensure maximum efficacy with minimal adverse effects. In the last decades, a lot of studies have been carried out with some valuable achievements, but the majority of the results are controversial and not suitable for clinical practice. Presently, for the majority of drugs, there are no reliable pharmacogenetic tests, and also there are very few algorithms for personalized therapies.

Pharmacogenetics on anthracyclines is a narrow area with 21 articles. This implies the difficulties to investigate cardiotoxicity of anthracyclines. The absence of associations is especially controversial. Apart from these, there are some promising potential candidate genes which might have effect on anthracycline induced cardiotoxicity. Variant of *HAS3*, *SLC28A3* and *RARG* genes revealed particularly strong association with cardiotoxicity on rather large patient populations. Variants of *RAC2*, *ABCC1*, *GSTP1*, *NCF4* and *HFE* were found to be associated with cardiotoxicity in more investigations.

Further validation of these findings in prospective independent analyses is recommended. International cooperation is required to be able to gather patient population with appropriate statistical power. The identification of functional polymorphisms would be the most relevant in the clinical practice. Furthermore, the confirmed variants should be tested in clinical trials with long-term follow-up. Nevertheless, due to the late cardiotoxicity, the clinical usage of these trials is expected in decades.

The response of the patients to drugs is influenced by interactions between many genetic and environmental factors. Using systems biologic approaches complex network of interactions can be drawn from these. However, a lot of exact genomic (and epigenomic), clinical and environmental data are needed to establish proper networks, which require large prospective studies. Furthermore, a lot of basic research is required for better understanding the behavior of our genome and the whole organism from molecular to whole body

levels. In addition, better evaluation methods are also needed for extracting as much information from the available data as possible. It is suggested to use new algorithms, for example, Bayesian statistical methodology with which it is possible to investigate whether a variable is directly relevant or its association is mediated. A recently developed and tested, novel statistical method named Bayesian network based on Bayesian multilevel analysis of relevance (BN-BMLA), proved to be superior in the detection of interactions [62–64]. Other fields such as epigenomics or transcriptom analysis could give new insight into the cardiotoxicity of ANTs.

The question is, when it will be a reality that everybody will have genomic data, from which every physician can decide which therapy, or drug will be the most effective and from which the patients will have no adverse drug response.

In the beginning of the 1990s, even leading experts predicted that in a few years these goals would come

Executive summary

Anthracycline-induced cardiotoxicity after anthracycline treatment

- Children and adults surviving cancer treated with anthracyclines (ANT) are at much higher risk to have a cardiac disease than their siblings.
- The pathogenesis of anthracycline-induced cardiotoxicity (ACT) is very complex and diverse with several potential mechanisms.
- Risk factors of ACT are younger age, female sex, dosage of ANT and genetic variants.
- Several researches were performed which studied the potential genetic variants.

Pharmacogenetic insight of anthracyclines

Genes in the two-electron reduction of ANTs

- Among the enzymes in the two electron-reduction of ANT *CBR3* is the most promising candidate.

Genes in reactive oxygen species generation or elimination

- Subunits of NAD(P)H oxidase *CYBA, RAC2, NCF4* are associated with ACT in several populations. Among these genes *NCF4* showed the strongest association.
- Iron is supposed to play a crucial role in the ACT as it is involved in the generation of reactive oxygen species (ROS). Variants of *HFE* gene were connected with cardiac parameters.
- Glutathione *S*-transferases and glutathione peroxidases are essential in the scavenge mechanism against ROS, but only *GPX3, GSTA2, GSTM3* and *GSTP1* SNPs were associated with ACT.
- *HAS3* gene was recently identified in an especially large-scale association study. Its product hyaluronan has a potential role in cellular recovery after ROS-mediated injury.

Cytochrome P450 enzymes & related enzymes

- Cytochrome P450 enzymes have important role in drug metabolism. Among the CYP enzymes *CYP3A5* rs776746 showed the strongest association.

ABC-transporters

- ABC transporters have outstanding function in the elimination of ANT from the cells. It seems that *ABCC1* might be the most important among these, as four SNPs in this gene influenced the side effect of ANT. SNPs of *ABCA1* and *ABCC9* also were prominent among ABC transporters.

SLC transporters

- Solute carriers take part in the transport of doxorubicin into the cell. These widely investigated genes showed associations in many genes. Especially two SNPs in *SLC28A3* (rs7853758 and rs885004) were identified as strongly influencing the risk of ACT.

Genes with uncertain function

- *SULT2B1* were validated in three studies and *UGT1A6* were represented with three SNPs.

Genome-wide association study

- *RARG* were indentified in a recent genome-wide association study.

Conclusion & future perspective

- The main goal of pharmacogenomics is to develop optimized drug therapy with respect to the genotype of the patients, to ensure maximum efficacy with minimal adverse effects.
- Results in pharmacogenomics of ANT are not consistent, further confirmations are recommended; there are very few genomic results that have gone into the practice.
- Prospective surveys in international cooperation are required to be able to study large populations.
- New algorithms can be useful, for example, Bayesian statistical methodology could identify mediated associations and hidden interactions.
- Continuing the development of the last decades it is probable that the number of the usable pharmacogenomic tests or personal therapies will be expanded in the future.

true. But as we have learned the immense complexity of the genome and the whole organism, it turned out that, presently it is not even known whether it will ever be a reality. In 2016, there are very few results of genomic research that would have reached everyday practice. Mainly variations in the protein coding regions with strong effects can give clinically relevant information; the effects of common variants are usually unpredictable and clinically unusable.

Today we are only at the beginning of this process and regarding the huge development of the last decades and with the help of a user-friendly decision–support system, we can be sure that the number of the usable pharmacogenomic tests or personal therapies will be expanded in the future.

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Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/pgs-2016-0036

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