HHS Public Access

Author manuscript

Pharmacol Ther. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Pharmacol Ther. 2016 December ; 168: 113–125. doi:10.1016/j.pharmthera.2016.09.009.

Validating the pharmacogenomics of chemotherapy-induced cardiotoxicity: what is missing?

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Abstract

The cardiotoxicity of certain chemotherapeutic agents is now well established, and has led to the development the field cardio-oncology, increased cardiac screening of cancer patients, and limitation of patients' maximum cumulative chemotherapeutic dose. The effect of chemotherapeutic regimes on the heart largely involves cardiomyocytes death, leading to cardiomyopathy and heart failure, or the induction of arrhythmias. Of these cardiotoxic drugs, those resulting in clinical cardiotoxicity can range from 8–26% for doxorubicin, 7–28% for trastuzumab, or 5–30% for paclitaxel. For tyrosine kinase inhibitors, QT prolongation and arrhythmia, ischemia and hypertension has been reported in 2–35% of patients. Furthermore, newly introduced chemotherapeutic agents are commonly used as part of changed combinational regimens with significantly increased cardiotoxicity incidence. It is widely believed that the mechanism of action of these drugs is often independent of their cardiotoxicity, and the basis for why these drugs specifically effect the heart has yet to be established. The genetic rationale for why certain patients experience cardiotoxicity whilst other patients can tolerate high chemotherapy doses has proven highly illusive. This has led to significant genomic efforts using targeted and genome-wide association studies (GWAS) to divine the pharmacogenomic cause of this predilection. With the advent of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), the putative risk and protective role of single nucleotide polymorphisms (SNPs) can now be validated in a human model. Here we review the state of the art knowledge of the genetic predilection to chemotherapy-induced cardiotoxicity and discuss the future for establishing and validating the role of the genome in this disease.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Keywords

Chemotherapy-induced cardiotoxicity; pharmacogenomics; human induced pluripotent stem cells; cardiomyopathy

1. Introduction

Despite the substantial improvement in cancer care, which has resulted in the increase in 5–year survival rate from 35% in the early 1950's to 70% in 2006–2012, the extensive use of chemotherapeutic agents is concordant with a higher incidence of adverse drug events (ADE). ADEs are one of the leading causes of death worldwide. According to the US Food and Drug Administration (FDA) adverse drug events reporting system (FAERS), about 1 million serious (including death) ADEs were reported in 2014 in the USA alone (fda.gov). Cardiotoxicity is a common ADE for multiple anti-cancer agents, constituting a significant clinical and economic burden, resulting in the establishment of the field of cardio-oncology to elucidate this phenomenon. Chemotherapy-induced cardiotoxicity (CIC) can be defined as subclinical and clinical manifestations including; disturbance in ventricular de/repolarization and QT interval, arrhythmia, bradycardia, tachycardia, decrease in left ventricular ejection fraction (LVEF) and fractional shortening (FS), and irreversible congestive heart failure (CHF), all lead to increased morbidity and mortality. In addition, cardiotoxicity may be classified in to early-onset acute (developed directly or up to 1 year after treatment) or late-onset chronic (detected at 1 to 20 years after starting chemotherapy) making the situation even more complex, as follow up monitoring of patients for life is a substantial clinical burden. The childhood cancer survivor study (CCSS) is a large multi-center, long–term study aimed to follow up survivors diagnosed with cancer in the period between 1960 and 1986. From data collected on ~10,000 cancer patients, the accumulative incidence of severe chronic health conditions, including myocardial infarction and CHF, at 30 years after cancer diagnosis was 73.4%. After adjustment of age, sex, and ethnicity, survivors showed 8.2-fold higher risk of developing severe chronic health conditions (Grade 3 and Grade 4) compared to their siblings who did not receive any cancer treatments (Oeffinger et al., 2006). Hence, identifying risk factors for CIC that make certain patients more susceptible than others, as well as identifying and understanding the underlying mechanism of ADEs is essential to improve clinical outcome of chemotherapy treatment regimens. In this review we will focus on genetically-dependent inter-patient variability in susceptibility to CIC and the extent to which identified genetic polymorphisms are linked to the mechanisms of CIC with an emphasis on doxorubicin pharmacogenomics.

2. Cardiotoxicity of anti-cancer therapeutics

2.1 Anthracyclines

Anthracyclines are anticancer agents initially isolated from natural sources. Daunorubicin and doxorubicin (DOX) are anthracyclines isolated from *Streptomyces peucetius*, a soil-dwelling bacterium and from a mutated strain of the same bacterium, respectively (Arcamone et al., 1969; Di Marco, Cassinelli, & Arcamone, 1981). Other commonly used anthracyclines include epirubicin and idarubicin (Espinosa et al., 2003). Anthracyclines

exert their action primarily through topoisomerase 2-α (TOP2A) inhibition. Topoisomerases are enzymes that cause double stranded DNA breaks that serve to relax DNA supercoiling during DNA replication and transcription. Anthracyclines prevent TOP2A from dissociating from DNA after making a cut, preventing re-ligation. Anthracyclines also directly intercalate with DNA, induce the formation of reactive oxygen species, and modulate histone-DNA binding. Together these effects ultimately lead to programmed cell death (Champoux, 2001)

DOX has been in use for over five decades as backbone of chemotherapy treatment regimens for a wide range of adult and pediatric cancers such as breast cancer, leukemia, and lymphomas. Although DOX treatment has contributed to an increase in the 5-year survival rate in children to more than 80% (Lipshultz et al., 2008), severe dose dependent cardiotoxicity occurs about 50% of treated patients (Swain et al., 2003) and leads to dose limitation or treatment discontinuation. About 26% of patients treated with accumulative DOX dose of 550 mg/m^2 experienced heart failure, and the maximum life time cumulative does is thus limited to 400 to 550 mg/m^2, decreasing the benefits that patients may receive form this potent drug (Swain et al., 2003; Wouters et al., 2005). Notably, up to 65% of pediatric cancer survivors treated with DOX develop measureable impairment in cardiac function, even when treated with DOX doses less than the maximum recommended (van der Pal et al., 2010). As many as 16% of children with these abnormalities will develop subsequent clinical heart failure with a mortality rate as high as 72%. Although DOX has been used for more than 50 years, the mechanism by which it induces cardiotoxicity remains unclear.

2.2 Small molecule tyrosine kinase inhibitors (TKIs)

The protein kinase gene family comprises one of the biggest gene families in the human genome, with more than 538 identified protein kinase encoding genes. Protein kinases play a crucial role in various cellular processes including; metabolism, transcription, cell movement, and intercellular communication. With more than 90 members, tyrosine kinases (TKs) constitute a large sub-family of protein kinases; TKs are enzymes responsible for physiologically reversible polypeptide phosphorylation through the transfer of a phosphate moiety from ATP to tyrosine residues, and thus regulate signaling pathways involved in cancer progression (López-Otín & Hunter, 2010; Manning et al., 2002) Based on this fact, several TK inhibitors (TKIs) have been developed as anti-cancer agents to treat a wide range of cancers including leukemia, breast cancer, renal cell carcinoma, and gastrointestinal stromal tumors. Cardiovascular toxicity has been observed in patients treated with a wide-range of TKIs, and 25 of the 27 currently FDA approved oncology TKIs have some type of cardiovascular toxicity-related warning in their package insert (accessdata.fda.gov).

Imatinib was one of the first small molecules developed to inhibit TKs, targeting the fusion protein breakpoint cluster region-ABL proto-oncogene 1 (BCR-ABL1) tyrosine kinase. Imatinib was approved in 2001 to treat Philadelphia chromosome positive (Ph$^+$) chronic myeloid leukemia (CML), contributing to a better than 90% 5-year survival rate (Druker et al., 2006; Druker et al., 2001). The first cardiovascular adverse effect associated with imatinib therapy was reported by Kerkelä *et al.* They showed that ten individuals who had normal left ventricular function before receiving imatinib, experienced class 3–4 heart

failure approximately 7 months after imatinib therapy (Kerkelä et al., 2006). Studies performed in mouse model showed that one possible mechanism for imatinib-induced cardiotoxicity may be via endoplasmic reticulum stress response-induced pro-death pathway activation including c-Jun N-terminal kinases (JNKs) activation, which leads to subtle alteration in mitochondrial function and cardiomyocyte death. Since the initial report, several studies have demonstrated the implication of imatinib in cardiovascular adverse events (Demetri 2007; Herman et al., 2011; Toubert et al., 2011)

Later on, imatinib was followed by second generation TKIs including dasatinib, nilotinib, and bosutinib. Dasatinib, a second generation BCR-ABL1 TKI was introduced following the dasatinib versus imatinib comparison study in treatment-naive CML patients (DASISION) study demonstrated that dasatinib (100 mg once daily) resulted in faster and deeper molecular responses compared with imatinib (400 mg once daily) however, this was not translated into better overall survival rate (Jabbour et al., 2014). Acquired resistance to TKI is developed due to the formation of polymorphic BCR-ABL1 oncogene, which decreases the binding affinity of TKI. On that basis, Griffin *et al.* successfully developed a second generation BCR-ABL1 TKI, nilotinib which is 30-fold more potent than imatinib. While its role as first line of treatment is still under investigation, it is an excellent therapeutic candidate for patients harboring imatinib-resistant BCR-ABL1 mutants (Weisberg et al., 2005). Importantly, analysis of 2200 electrocardiograms from patients recruited in a dose escalation phase l study of nilotinib showed prolonged QT interval by 5 to 15 milliseconds and thus close monitoring of arrhythmia and QT intervals have been recommended for patients treated with nilotinib (Kantarjian et al., 2006). Prolonged QT intervals could be explained by the inhibitory effect of nilotinib on human Ether-à-go-go-Related Gene (hERG or KCNH2) encoding the alpha subunit of potassium ion channel ($K_v11.1$). $K_v11.1$ is responsible for delayed-rectifier K^+ current in cardiac tissue, and blocking this ion channel by nilotinib thus results in QT wave disturbance (Shopp et al., 2014). Additionally, nilotinib promotes caspase 3/7–induced cardiomyocyte apoptosis, increases ROS production, and alters normal cardiomyocytes morphology generating elongated cardiomyocytes with condensed nuclei (Doherty et al., 2013). Furthermore, vascular adverse events (VAEs) including; rapidly progressive peripheral arterial occlusive disease (PAOD) which is associated with cardiovascular risk factors, myocardial infarction, and sudden death have been reported in CML patients treated with nilotinib (Aichberger et al., 2011; Giles et al., 2013). Although nilotinib and imatinib share common targets, the incidence of undesired vascular events is much lower in patients treated with imatinib when compared to nilotinib. This indicates that the correlation of nilotinib with VAEs is most likely due to off-target rather than on-target effects. Presumably nilotinib has a direct effect on vascular and pre-vascular tissue causing quick development of VAEs after exposure to nilotinib. Nilotinib has a proatherogenic effect on vascular tissue promoting arterial stenosis and vasospasm, in conjunction with increased cholesterol and fasting glucose levels associated with nilotinib, all these conditions may trigger VAEs (Valent et al., 2015). Multiple prospective, retrospective, and meta-analysis studies have reported multiple cardiotoxic events following nilotinib treatment. However incidence rate varies greatly among these studies ranging from 1.3% to 35.7%. This discrepancy could be explained by different cardiovascular endpoints and disparate classification criteria used to define these endpoints from one trial to another.

Bosutinib is an oral second generation TKI which targets BCR-ABL1 along with SRC proto-oncogene (SRC) and is used in imatinib–resistant CML patients. Despite its acceptable tolerability, 10% of patients treated with bosutinib experienced cardiac adverse event, with the major clinical manifestation being hypertension (Brümmendorf et al., 2015). Ponatinib is a third generation TKI with a broad inhibitory profile against; SRC, fibroblast growth factor receptors (FGFRs), platelet–derived growth factor receptors (PDGFRA and PDGFRB), and vascular endothelial growth factor receptor 1–3 (VEGFR1-3), in addition to BCR-ABL1. The incidence of ponatinib-induced cardiotoxicity is directly correlated with the length of follow-up monitoring. The incidence rate of cumulative cardiovascular events increased from 6% after a median follow-up of 12 months to 10% after a median follow-up of 28 months. Similar to bosutinib, ponatinib treatment induced hypertension in 26% of patients more likely due to its VEGFR inhibitory action (Moslehi & Deininger, 2015). The VEGF signaling pathway plays an important role in preserving the activity and structure of vascular endothelium by activating the PI3K-AKT pathway. In that, stimulation of VEGFR2 activates phosphatidylinositol 3–kinase (PI3K) and protein kinase B (AKT1) which propagates the pro-survival signal, endothelial nitric oxide synthase (NOS3) and boost the production of potent vasodilators such as prostacyclin (PGI_2). Accordingly, inhibition of the VEGF signaling cascade will trigger endothelial cell apoptosis, decrease capillary density and capillary dilatory response creating a phenotype known as microvascular rarefaction (Bair, Choueiri, & Moslehi, 2013).

Sunitinib and sorafenib are multi-kinase inhibitors that target several TKs involved in both cancerous cells proliferation and angiogenesis. While sunitinib targets VEGFR1-3, PDGFRA/B, KIT proto-oncogene receptor tyrosine kinase (KIT), FMS-related tyrosine kinase 3 (FLT3), and colony stimulating factor 1 receptor (CSF1R), sorafenib targets intracellular RAF kinases, Raf-1 proto-oncogene, serine/threonine kinase (RAF1), B-Raf proto-oncogene, serine/threonine kinase (BRAF), and mutant BRAF; and the cell surface kinase receptors (VEGFR2/3, PDGFRB, KIT, and FLT3) (Orphanos et al., 2009). Sunitinib and sorafenib are each associated with distinct cardiac adverse events. Sunitinib is associated with a reduction in LVEF and congestive heart failure with incidence rates of 11% and 8%, respectively. Sorafebin treatment results in ischemic heart diseases including myocardial infarction in 3% of treated patients (Chu et al., 2007; Palmer, 2008). Additionally, both TKIs are associated with atrial thromboembolism and hypertension. A meta-analysis including data from 9387 patients reported that patients treated with either sunitinib or sorafenib showed a three-fold higher risk to develop atrial thromboembolism (Choueiri et al., 2010). Finally, in addition to the involvement of endothelin 1 (EDN1) system in sunitinib–, sorafenib– and ponatinib–induced hypertension, all three TKIs share a similar VEGF signaling pathway-linked mechanism of hypertension propagation (Kappers et al., 2010)

2.3 Monoclonal Antibodies

During the last decade, the progress achieved in the field of molecular biology has led to the development of targeted anticancer biologics such as monoclonal antibodies including; rituximab which targets the B lymphocyte antigen membrane spanning 4-domains A1 (MS4A1 or CD20), trastuzumab raised against erb-b2 receptor tyrosine kinase 2 (ERBB2 or HER2), and bevacizumab which targets vascular endothelial growth factor A (VEGFA).

These directed anticancer agents are currently widely used and constitute three of the leading chemotherapy revenues in the USA, in that bevacizumab, and trastuzumab revenues in 2014/2015 in the USA alone were $3 billion, and $2.4 billion, respectively (statista.com). Despite their broad utilization in cancer treatment, FAERS database reported that between 2004 and 2010, trastuzumab had highest number of cardiotoxicity reports followed by bevacizumab (Wittayanukorn et al., 2015).

Trastuzumab is a monoclonal antibody that was approved in 1998 for use in breast cancer with ERBB2 overexpression. A multicenter randomized trial conducted by Piccart-Gebhart *et al.* showed that although one year of trastuzumab treatment improved survival rate by 50% and decreased recurrence by 33%, multiple occurrences of cardiotoxicity events were also reported (Piccart-Gebhart et al., 2005). All patients were prescreened for cardiac exclusion criteria before being recruited in the trial. However, 7.08% of patients displayed decreased LVEF (>10% from baseline to an LVEF of less than 50% at any time) and 1.73% of patient suffered from symptomatic severe CHF. These percentages were recorded after only 12 months of median follow-up and thus higher incidence rates are expected with longer follow-up terms. Guarneri *et al.*, reported that after longer term follow-up (median 32.6 months), 28% of patients experienced cardiac adverse events including decline in LVEF and CHF, (Guarneri et al., 2006). ERBB2 plays an important role in preserving cardiac function in the adult heart (Crone et al., 2002). Neuregulins which are endogenous ligands that activate ERBB2 have been shown to promote survival and growth of cardiac myocytes (Zhao et al., 1998). Furthermore, ERBB2 deficient mice exhibit dilated cardiomyopathy phenotype. Dysregulation of ERBB2 expression by trastuzumab is associated with severe cardiotoxic phenotypes. Taken together, these findings emphasize the crucial role of ERBB2 signaling pathway in the development of cardiotoxicity.

Bevacizumab was approved in 2004 as an angiogenesis inhibitor, and it exerts its action by inhibiting VEGFA tyrosine kinase activity, thus blocking blood supply to tumor cells. As a result of VEGFA inhibition, the production of the natural vasodilator, nitric oxide is reduced stimulating vasoconstriction of blood vessels and increasing the risk of hypertension. A meta-analysis of seven trials comprising 1850 patients treated with bevacizumab demonstrated that bevacizumab is significantly associated with dose-dependent hypertension with relative risks of 3% and 7.5% for low and high dose, respectively (Zhu et al., 2007). The incidence of heart failure and cardiomyopathy after bevacizumab treatment are as low as 2.2% and 3%, however the duration of patients follow-up in this study was only 18 months (Miller et al., 2005). Having considered that hypertension is an independent risk factor for cardiovascular events, cardiotoxicity is therefore highly anticipated with long term follow up. Bevacizumab-induced hypertension a long with VEGFA signaling inhibition have been shown to trigger decompensated heart failure (Chen et al., 2008).

2.4 Alkylating agents

Alkylating agents including nitrogen mustards (cyclophosphamide and ifosfamide) and the platinum-containing molecule, cisplatin, are the oldest class of anticancer agents. They exert their action via binding to negatively charged DNA sites causing DNA strand breaks and DNA strand cross-linking (Espinosa et al., 2003). Cyclophosphamide was introduced in

1958 following early observations that mustard gas reduces peripheral blood lymphocytes and nitrogen-mustard derivatives have cytotoxic properties. Cyclophosphamide is a prodrug which upon activation forms an alkylating molecule that binds to DNA. inter- and intra-strand DNA breaks, resulting in the inhibition of DNA replication and increased cellular apoptosis (Povirk & Shuker, 1994). High doses of cyclophosphamide are associated with cardiotoxicity and a reversible decrease in systolic function. Cyclophosphamide-induced clinical manifestations of cardiotoxicity include, pericardial effusions, myopericarditis and heart failure. Notably 25% of patients treated with cyclophosphamide doses ≥ 1.55 gm/m^2/day exhibited irreversible heart failure. Ifosfamide, a synthetic analog of cyclophosphamide which shares a similar mechanism of action, is also associated with dose dependent acute cardiac toxicity in 17% of patients (Yeh & Bickford, 2009). Cisplatin was the first platinum containing alkylating agent approved to treat several types of cancer. Cisplatin treatment is associated with undesirable vascular events including deep vein thrombosis and pulmonary embolism in 12.9% of patients suffering from urothelial transitional cell carcinoma (Czaykowski et al., 1998). Importantly, cisplatin is associated with late-onset cardiotoxicity. Patients treated with cisplatin develop clinical cardiac events (myocardial infarction and angina pectoris) and subclinical disturbance in systolic LVEF with incidence rates of 6% and 33%, respectively 10 to 20 years after initial treatment with cisplatin (Meinardi et al., 2000)

2.5 Taxanes

Taxanes are another group of chemotherapeutics isolated from natural sources. Paclitaxel and docetaxel are isolated from *Taxus brevifolia* and *Taxus baccata,* respectively (Bissery et al., 1991; Wani et al., 1971) and are used in the treatment of breast, ovarian, and non-small cell lung cancers. Both taxanes exert their action in the cell by binding to microtubule promoting microtubule polymerization and inactivation and eventually inhibiting cell division. The most common cardiac events associated with this class of anticancer agents are arrhythmia and cardiac ischemia. Paclitaxel treatment causes bradycardia in 30% of patients and cardiac ischemia in 5% of treated patients, while, docetaxel is associated with myocardial ischemia occurring with an incidence rate of 1.7%. Co-administration of paclitaxel and doxorubicin has been shown to significantly increase the incidence of CHF to 20%. Presumably, this is due to increasing plasma levels of doxorubicin and thereby boosting intracellular concentration of the DOX toxic metabolite, doxorubicinol in cardiomyocytes (Giordano et al., 2002).

3. Patient-specific toxicity: Pharmacogenomics and personalized medicine

Achieving a tolerable balance between efficacy and toxicity is the most important challenge facing effective chemotherapy treatment. Our knowledge of the pharmacogenomics of chemotherapeutic agents is progressing rapidly. An individual patient’s response to chemotherapy is dependent on the plasma and target site concentration of the anticancer drugs, which are controlled by pharmacokinetics (absorption, distribution, metabolism and excretion, ADME) and pharmacodynamics factors. Inherited polymorphisms in drug metabolizing enzymes and transporters can alter their expression and/or activity influencing pharmacokinetics. Genetic alterations in target enzymes, transporters, ion channels and

receptors may influence drug pharmacodynamics (Evans & McLeod, 2003). Thus a realistic option to improve management and outcome of chemotherapy-induced toxicity is the development of individualized treatment strategies including the use of predictive genetic host factors. Extensive efforts in pharmacogenomics research have been conducted in attempt to uncover the genetic variants associated with chemotherapy clinical outcome. Despite this enormous effort, only few biomarkers are routinely used in clinical practice which reflects the complexity of identifying causal variants. Currently there are more than 150 drugs with FDA approved pharmacogenetic testing information in their drug labels, the majority of which are anticancer agents (Fig. 1) (fda.gov).

Although the terms, “phamacogenetic” and “genetic” testing are used interchangeably, there is a huge difference in their target population and the manner in which each test is used clinical investigation. Pharmacogenetic testing targets subjects experiencing a specific disease. This method is used to provide guidance in selecting the appropriate therapeutic agent; and in some instances, with presence of sufficient clinical data, for individualized dosing selection. On the other hand, genetic testing is utilized when assessing a relative risk of target population to develop certain disease as well as predicting patients’ prognoses.

Similarly, somatic (tumor) and germline (individual) mutations are two types of genetic mutations involved in predicting cancer outcome. Somatic mutations are genetic variations in the tumor tissue which affect tumor microenvironment and determine the cancer profile including prognosis, metastasis and aggressiveness. Studying somatic mutations will be beneficial not only in predicting disease prognosis, but also in developing tumor-specific therapeutics that are capable of targeting particular oncogenic aberration. Germline mutations are genetic variants in a patients’ genome. Inherited mutations in drug transporters and/or drug metabolizing enzymes determine the concentration of drug at the target site and subsequently tuning efficacy and toxicity of cancer therapeutics. Additionally, germline mutations in certain signaling pathways (e.g, genes controlling DNA repair machinery, cell division, and reprogramming) may predispose cancer. Therefore the study of germline aberrations has significant prognostic value of germline aberration. Accordingly, obtaining informative genetic information about both germline and somatic polymorphisms will ideally allow us to draw conclusive decisions about disease prognoses and adequate therapeutics (Hertz & McLeod, 2013).

Pharmacogenomic studies principally adapt a case control study-based design, in which frequencies of genetic variants, mainly single nucleotide polymorphisms (SNP) are detected and compared in cases (subjects with the investigated phenotype) and controls (subjects without the investigated phenotype). Genomic research has accommodated two main approaches: (1) candidate gene studies in which a single gene or a list of well-founded preselected genes are investigated, and (2) genome wide association studies (GWAS) in which genetic variations across the whole genome are analyzed and linked to the investigated phenotype. In terms of the number of SNPs investigated, both genomic studies approaches are quite different. Candidate gene studies investigate anywhere from one SNP to a complete gene sequence while, GWAS analyze a range of several hundred thousand to millions of SNPs.

The momentous advances in the field of next generation sequencing, analysis algorithms, and data storage capacity, coupled with the experimental evidences revealing the role of genetic variation in various diseases, have shifted the paradigm towards whole genome studies to help identify SNPs that protect against or predispose individuals to different clinical conditions and phenotypic traits. The number of GWASs published reports has dramatically increased over the last decade from less than 50 studies in 2006 to about 2000 studies in 2013 (Welter et al., 2014). GWASs are based on the principle of linkage disequilibrium which exists when two or more SNPs at discrete loci are found together more frequently than would likely happen by chance. Accordingly, analyzing only a selected set of tag-SNPs across the genome to act as surrogates for several other linked SNPs, gives complete information about the un-typed SNPs. Linkage disequilibrium-based approach is a very useful as it significantly decreases the number of genotyped SNPs, while providing information about descent number of genetic variants. Nevertheless, this methodology raises the question of whether or not the identified SNP is the causal one. Even though linkage disequilibrium-based genome wide study is an appropriate tool for mapping Mendelian traits that are predisposed due to the segregation of risk alleles within a single gene, it is not as efficient when it comes to polygenic traits like CIC. Multiple genes are implicated in CIC and it thus becomes nearly impossible to identify causal SNPs with just a single association study (Botstein & Risch, 2003). Population stratification constitutes a major limitation for GWAS as heterogeneous subject recruitment significantly affects the output of pharmacogenomic studies. Ethnically diverse populations have different LD profiles caused by distinct recombination rates. Thus, SNPs have significantly different minor allele frequency exist among diverse populations. An exemplary African population has very short LD haplotypes because of cumulative recombination events which make it even more difficult to capture the causal polymorphisms (Reich et al., 2001). Since minor difference in ethnicity between cases and controls could result in false positives even after exclusion of extreme outliers, therefore an odds ratio of at least 2–3 is required for an association to be robust enough to overcome cryptic population stratification. Whereas, odds ratios <1.5 is questionable regardless of the *P*-value (McClellan & King, 2010). Failure to identify large insertions and deletions is considered another GWAS limitation as GWAS primarily focus on single base pair alterations rather than larger genetic mutations. Importantly, the majority of identified GWAS SNPs are located in intergenic or intronic regions and in many instances in genes which are irrelevant to the studied phenotype, where the biological relevance of identified polymorphisms is far from being well described.

4. Pharmacogenomics of doxorubicin

Following the administration of DOX, 50% of the dose is excreted unchanged and the remainder is metabolized intracellularly, where DOX undergoes a two-electron reduction to yield the secondary alcohol doxorubicinol (DOX-ol). DOX and DOX-ol then undergo reductase glycosidation and hydrolase glycosidation to build DOX deoxyaglycone or doxorubicinone from DOX, and DOX-ol hydroxyaglycone or doxorubicinolone (DOX-olone) from DOX-ol, respectively while also forming semiquionone as an intermediate metabolite (Joerger et al., 2005; Licata et al., 2000). Several metabolizing enzymes are involved in this metabolic pathway. Carbonyl reductase 1 (CBR1), carbonyl reductase 1

(CBR3), aldo-keto reductase 1a (AKR1A) and aldo-keto reductase 1C3 (AKR1C3) are responsible for the conversion of DOX into DOX-ol. Mitochondrial NADH dehydrogenases present in the sarcoplasmic reticulum and mitochondria including NDUFS2, NDUFS3, and NDUFS7, as well as cytosolic enzymes such as, NADPH dehydrogenase (NQO1), xanthine oxidase (XDH) and nitric oxide synthases (NOS1, NOS2, and NOS3) that catalyze the reduction of DOX to the DOX-semiquinone metabolite.

Many genes contribute to DIC, and cardiotoxicity phenotype is thus apparently due to combination of four major molecular mechanisms. (1) Serving as electron acceptor, the quinone aromatic ring shared among DOX metabolites promptly takes part in oxidation-reduction reactions, resulting in generation of O_2- and H_2O_2 and the formation of downstream iron-dependent and independent reactive oxygen species (ROS). (2) DOX causes mitochondrial dysregulation via an irreversible mitochondrial transition pore (MTP) or BCL2-associated X protein (BAX) and BCL2 like 1 (BCL2L1) triggered CYCS (cytochrome c) release which ultimately form the apoptosome complex (Minotti et al., 2004). Mitochondria are a key player in the development of cardiotoxicity because of their abundance in adult cardiac cell occupying approximately 30% of cardiomyocytes cell volume. Additionally, mitochondria contribute to about 90% of ATP production in cardiomyocytes, thus making the heart much more vulnerable to DOX insults (Piquereau et al., 2013). (3) DOX inhibits the topoisomerase II-β (TOP2B) re-ligation reaction in cardiomyocytes, and consequently inducing DNA double-strand break-triggered cell apoptosis. (4) DOX activate ryanodine receptor 2 (RYR2) leading to calcium release in the cell. Furthermore, DOX blocks ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (SERCA2 or ATP2A2), preventing calcium re-uptake.

Different genetic and non-genetic factors are known to influence the balance between DOX efficacy and toxicity. Several non-genetic factors have been reported to significantly influence the incidence rates of DIC. Females are more prone to develop DIC compared to males. Patients less than 4 years and more than 65 years old showed a higher incidence of DIC. Higher cumulative DOX doses and chronic disease including hypertension, liver diseases, and cardiac diseases are associated with higher risk of DIC (Octavia et al., 2012).

Experimental and clinical studies have identified several associations between genetic polymorphisms and DOX response or toxicity. Table 1 summarizes the findings of pharmacogenomic studies conducted and genetic variants associated with DOX clinical outcome. The vast majority of these studies are candidate gene approach-based in which only a small number of SNPs were investigated. Only few trials investigated reasonable number of SNPs, located within several genes that have been implicated in DOX response and/or toxicity (Table 1)

To date, only four GWAS regarding DOX clinical outcome have been conducted, one of which focused on DOX-induced febrile neutropenia in cancer patients. In this study, 16,561 SNPs in drug transporter and metabolic genes implicated in neutropenia were genotyped in 155 French breast cancer patients who were tested for association with severe neutropenia (Callens et al. 2015). The other three studies were directed towards DIC and investigated 650,000, 4,578, and 2,977 SNPs, respectively. An early study probing 2,977 SNPs in 220

key drug biotransformation genes (Visscher et al. 2012), and a more recent GWAS (Aminkeng et al. 2015) investigating >650,000 SNPs was carried out in patients receiving DOX in order to identify novel risk alleles for DIC. These GWASs revealed significant risk and protective alleles. However, due to multiple testing issues and limitations in gene coverage, these results definitely do not exclude the existence of additional predictive polymorphisms in well-defined candidate genes.

DOX pharmacogenomic studies have revealed associations within genes that play different roles in DIC. Interestingly 45% of identified SNPs are located in genes encoding transporter proteins, indicating that DOX transportation across cellular membrane is accomplished through several transporters. The rest of the genes are distributed as follows; 27% are located in oxidative stress related genes, 19% are located in DOX metabolizing enzymes and 9% are located in genes involved in DNA repair and replication (Fig. 2)

SNPs implicated in DOX clinical outcome are of significantly different global minor allele frequency (GMAF) ranging from 0.013 (SNP rs2229109) to 0.486 (SNP rs4877847) located in transporter encoding genes, *SLC28A3* and *ABCB1*, respectively (Table 1 and Fig. 3). Furthermore, each individual SNP has diverse minor allele frequency (MAF) among different populations. Having considered that a SNP which is monomorphic in a certain population may be polymorphic in other populations and that the power to detect true genetic associations is in part dependent on tested SNPs MAF (Ardlie et al., 2002), it is crucial to recruit homogenous patient cohorts for both exploration and replication approaches. Additionally, these *data* suggest that population-dependent genetic biomarker screening should be seriously considered.

Although pharmacogenomics research has identified significant association within several genes related to DIC, many other genes shown to be involved in DIC need to be intensively investigated. Examples of such genes include; *ABCC2, ABCG2*, *RALBP1, AKR1A1, CSL1, SOD3, TP53, TOP2B, PPARGC1A (PGC-1α), PPARGC1B (PGC-1β), PPARA, PPARD,* and *CYP2J2. ABCC2* encoding transporter protein MRP2, plays a role in DOX chemoresistance, and knocking down *MRP2* increases cells sensitization towards DOX via increasing DOX intracellular accumulation (Folmer et al., 2007). DOX is a substrate of ABCG2 transporter, and interestingly a mutant variant of *ABCG2* alters substrate specificity and increases DOX resistance in vitro (Stacy et al., 2013). *RALBP1,* gene encoding RalA-binding protein 1 plays an important role in the regulation of intracellular concentration for DOX and its electrophilic cytotoxic metabolite, glutathione-4-hydroxy-t-nonenal (GS-HNE). RALBP1 protects the cells against oxidative stress, and its deletion increased cell sensitivity to DOX (Vatsyayan et al., 2009). *AKR1A1* gene encodes an aldo-keto reductase enzyme, and it is responsible for the conversion of DOX into its alcohol metabolite, DOXol which is linked to the development of cardiotoxicity (Mordente et al., 2009). Genetic polymorphisms in AKR1A1 have been shown to alter its metabolic activity (Bains et al., 2008). *CSL1* encodes cardiolipin synthase 1, which is essential for the synthesis tetraacylphospholipid in mitochondria (Houtkooper & Vaz, 2008). DOX binds irreversibly to cardiolipin, forming a very stable complex at the mitochondrial inner membrane in cardiomyocytes, thus inhibiting many mitochondrial enzymes and leading to mitochondrial dysregulation and eventually cardiotoxicity (Goormaghtigh et al., 1987). Superoxide dismutase (*SOD3)* is an antioxidant

enzyme that protects the cells from oxidative stress generated by DOX. SOD3 is down regulated in patients treated with DOX who experienced DIC compared to patients who did not experience any DIC, indicating its role in DIC precipitation (Burridge et al. 2016). *TOP2B* is another well-founded candidate gene in relation to DIC. DOX binds to *TOP2B* and DNA forming a stable ternary complex and causing double-strand breaks which in turn trigger cell death. Cardiac specific deletion of *TOP2B* in mice has a cardioprotective effect, presumably through maintaining normal expression of transcriptional coactivators; *PGC-1α* and *PGC-1β*. *PGC-1α* and *PGC-1β* bind to nuclear receptors *PPARA* and *PPARD* facilitating their binding to transcription factor that regulate genes involved in downstream mitochondrial biogenesis (Finck & Kelly, 2007). Interestingly, *CYP2J2* over expression activates *PPARA* which subsequently enhance the activity of ROS scavenger enzymes; *CAT,* and *SOD,* and ultimately protecting the cells against DIC (Wray et al., 2009).

Despite many research groups have tried and in part succeeded to identify genetic polymorphisms associated with DOX clinical outcome, these studies were hampered by small sample sizes, inhomogeneous patient cohorts, nonsystematic genetic analysis, and mostly lacked any functional validation. Furthermore, DOX related cardiotoxicity appears to be a polygenic trait, and single SNP-based association tests ignore synergistic and antagonistic effect between different genes polymorphisms. Most pharmacogenomics studies lack any downstream mechanistic studies and thus, the impact of SNPs on the biological system and the relationship between identified SNPs and DIC are poorly understood. Importantly, elucidation of causal mechanisms leading to SNP-associated DOX toxicity and functional changes are important for potential future DOX dosing recommendation. Testing for the causal variants will guarantee that the best possible clinical associations will be detected, however identification of causal variants can be a challenging task. All of these observations taken together, coupled with the fact that multiple neglected candidate genes need to be systematically examined in relation to DIC, emphasize the need for a comprehensive genetic approach to address these issues. It is necessary to validate significant associations in large independent cohorts and conduct proper patient-specific functional studies for validation of SNPs implicated in DIC.

5. Pharmacogenomics of TKIs

Eminent examples of the clinical usefulness of pharmacogenetics in oncology are imatinib, lapatinib and nilotinib. Imatinib specifically inhibits tyrosine kinase activity in patients suffering from myelodysplastic/myeloproliferative diseases (MDS/MPD) associated with platelet-derived growth factor receptor (PDGFR) gene re-arrangements and patients with Philadelphia chromosome positive acute lymphoblastic leukemia. Lapatinib as part of combinatorial therapy has been approved to treat human epidermal growth factor receptor 2 (HER2) protein overexpression positive breast cancer patients. Patients carrying HLA alleles DQA1*02:01 and DRB1*07:01 showed severe lapatinib-induced hepatotoxicity, and consequently testing for these mutations is essential before lapatinib treatment. Patients harboring the UGT1A1*28 allele had a significantly higher risk of developing hyperbilirubinemia as a result of nilotinib treatment. Despite the well-established evidence that TKI treatment causes cardiotoxicity, and the fact that the majority of TKIs have a black box warning for cardiac adverse events, yet there is no identified cardiotoxicity biomarker

currently used in clinical routine investigation, further emphasizing the urgent need for a comprehensive whole genome-based approach for identifying and validating candidate genetic variants in relation to TKI-induced cardiotoxicity.

6. Validation of chemotherapy induced cardiotoxicity associated SNPs

Validating the functional aspects of genetic associations are of great importance in the field of pharmacogenomics. The ultimate goal is not only to detect genetic variants associated with CIC, but also to determine the causality of such gene-disease relationship. Determining the causal SNP/haplotype for DIC will help introduce novel biomarkers for DIC into routine clinical practice. Additionally, identification of the causal genetic polymorphism(s) will be the basis for follow-up studies involving screening for novel cardioprotectants.

Existing methodologies, such as using myocardial biopsy to study the origin of DIC is impractical and invasive; in addition adult cardiomyocytes cannot expand under *in vitro* culturing conditions, making biochemical assays difficult. The substantial physiological and genomics differences between humans and animals constitute a serious limitation for the usage of animal models to study DIC and thus, conclusions based on animal studies cannot be directly translated to humans. All these factors accentuate the usefulness of developing a model which mimics the cardiac host microenvironment to study patient-specific response to doxorubicin.

Patient-specific hiPSC-CMs represent a novel evolving technology which has been successfully applied in modeling cardiovascular and metabolic diseases and screening drugs for efficacy and toxicity. Over the last decade, tremendous improvements have taken place in human somatic cell reprograming, hiPSCs differentiation, and structural and functional phenotypic characterization of the developed hiPSC-CMs; all of which support the usage of hiPSCs-CM in recapitulating patient specific disease phenotypes and pharmacological drug response. Cardiomyocytes generated from patient-specific hiPSCs have been well characterized and have shown to acquire similar characteristics when compared to human cardiac tissue. The human heart share common genomic and transcriptomic profiles with hiPSCs-CMs and human in both continuous culture and following cryopreservation and thawing. hiPSCs-CMs express cardiac markers such as; ion channels implicated in the action potential of human heart (e.g.; *SCN5A*, *KCNJ2*, *CACNA1C*, *KCNQ1*, and KCNH2), cardiac tissue specific markers (*MYH6*, *MYLPF*, *MYBPC3*, *DES*, *TNNT2*, and *TNNI3*), and cardiac transcription factors (*NKX2.5*, *GATA4*, and *GATA6*). In addition, hiPSCs-CMs do not express any pluripotency markers indicating the purity of the generated cardiomyocytes. Furthermore, hiPSCs-CMs exhibit similar electrophysiological, biochemical, contractile, and beating activity when compared with native cardiac myocytes (Babiarz et al., 2012; Ma et al., 2011; Puppala et al., 2013). All taken together, these observations support the superiority of *in vitro* hiPSCs-CMs model in recapitulating human cardiac tissue when compared to animal models, nonhuman primary cells, and immortalized cell lines.

Using a chemically defined media, we have shown the feasibility and reproducibility of generating phenotypically characterized beating cardiomyocytes from hiPSCs with a cardiac differentiation efficiency of 85–95% (Burridge et al., 2011). Importantly, patient–derived

hiPSC-CM have been exploited to study the basal mechanisms and to provide fundamental understanding of the causality of long QT syndrome (LQTS) (Itzhaki et al., 2011; Malan et al., 2016), LEOPARD syndrome (Carvajal-Vergara et al., 2010), Timothy syndrome (Yazawa et al., 2011), arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) (Kim et al., 2013), dilated cardiomyopathy (DCM) (Sun et al., 2012), Barth syndrome (Wang et al., 2014), and diabetic cardiomyopathy (Drawnel et al., 2014). We have recently demonstrated that patient derived hiPSCs-CMs can recapitulate individual patients' predisposition to DIC (Burridge et al., 2016), providing a multi assays-based platform for DIC phenotypic characterization. This platform includes assays to investigate cell viability, mitochondrial and metabolic function, calcium handling, and reactive oxygen species (ROS) production coupled with whole transcriptome analysis. From our findings, we were able to clearly discriminate between patients who are more susceptible to DIC compared to patient of lower susceptibility (Burridge et al., 2016). All these studies support the fact that hiPSCs-CMs could be used to validate genetic variants that confer susceptibility to doxorubicin cardiotoxicity (Fig. 4).

7. Conclusion

The consistent advent of novel targeted chemotherapeutics indeed provides more effective treatment options and leads to great improvements in cancer cure rate. However these gains come with the compromise of increased adverse drug events. Cardiotoxicity is a common established side effect of several anti-cancer agents including; anthracyclines, small molecule TKIs, and monoclonal antibodies. Multiple pharmacogenomic studies adapting both candidate gene and genome wide approaches have tried and in part succeeded in identifying genetic variants associated with chemotherapy-induced cardiotoxicity. The vast majority of these trials are hampered by different factors including the lack of any functional validation. Accordingly, genetic background and mechanistic explanation for chemotherapy-induced cardiotoxicity, as well as intra-individual variability across the population in susceptibility to cardiotoxic events have yet to be determined. Considering all these facts, we believe that a comprehensive whole genome platform based on wide genome genotyping, patient-derived hiPSC–CMs, and utilization of CRISPR technology will help pinpoint robust genotype-phenotype associations and provide functional mechanistic validation for involvement of candidate genes/SNP(s)/haplotypes in CIC (Fig. 5). This methodology will generate a set validated SNPs that are predictive for cardiotoxicity and can be directly used in a clinical cardiotoxicity algorithm that can classify patients who are more susceptible to CIC. Furthermore, this platform would provide cardio-oncologists with an invaluable tool to individualize patient-specific chemotherapies, before beginning treatment rather than experience undesirable cardiotoxicity retrospectively. Taken together, this methodology will help achieve the maximum benefit and minimal side-effects from evolving chemotherapeutics, thus significantly improving cancer treatment.

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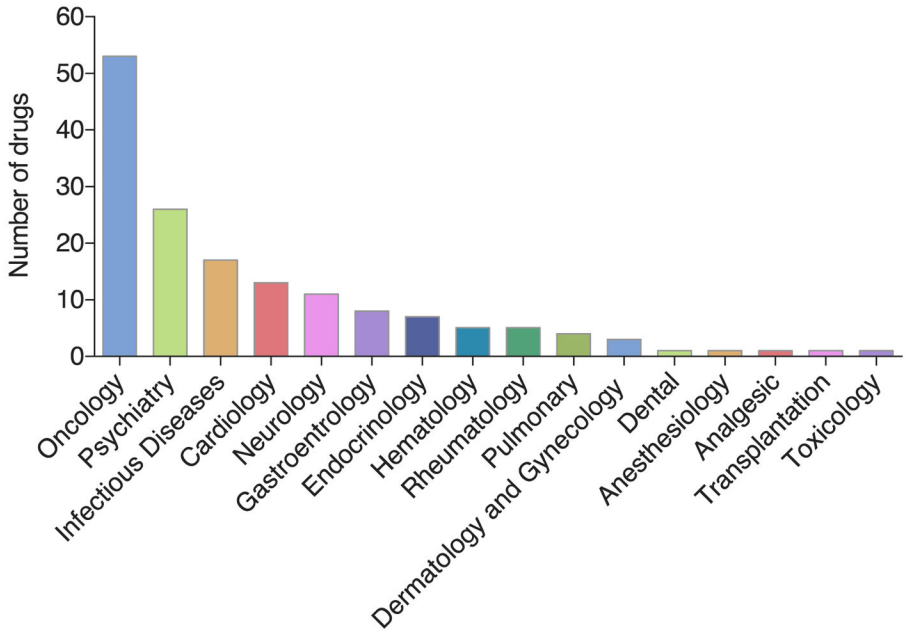

Figure 1. FDA-approved pharmacogenomics biomarker in drug labeling

Bar plot diagram showing number of drugs that contain pharmacogenetic testing information in their package insert, and their distribution across different therapeutic areas (n = 158)

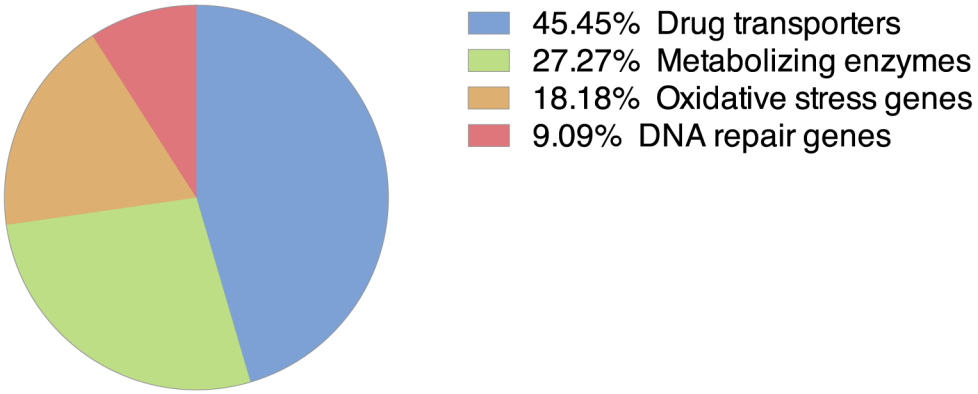

Figure 2. Classification of genes harboring SNPs associated with DOX clinical outcome by class

Pie chart diagram showing the distribution of SNPs associated with DOX clinical outcome across different gene families.

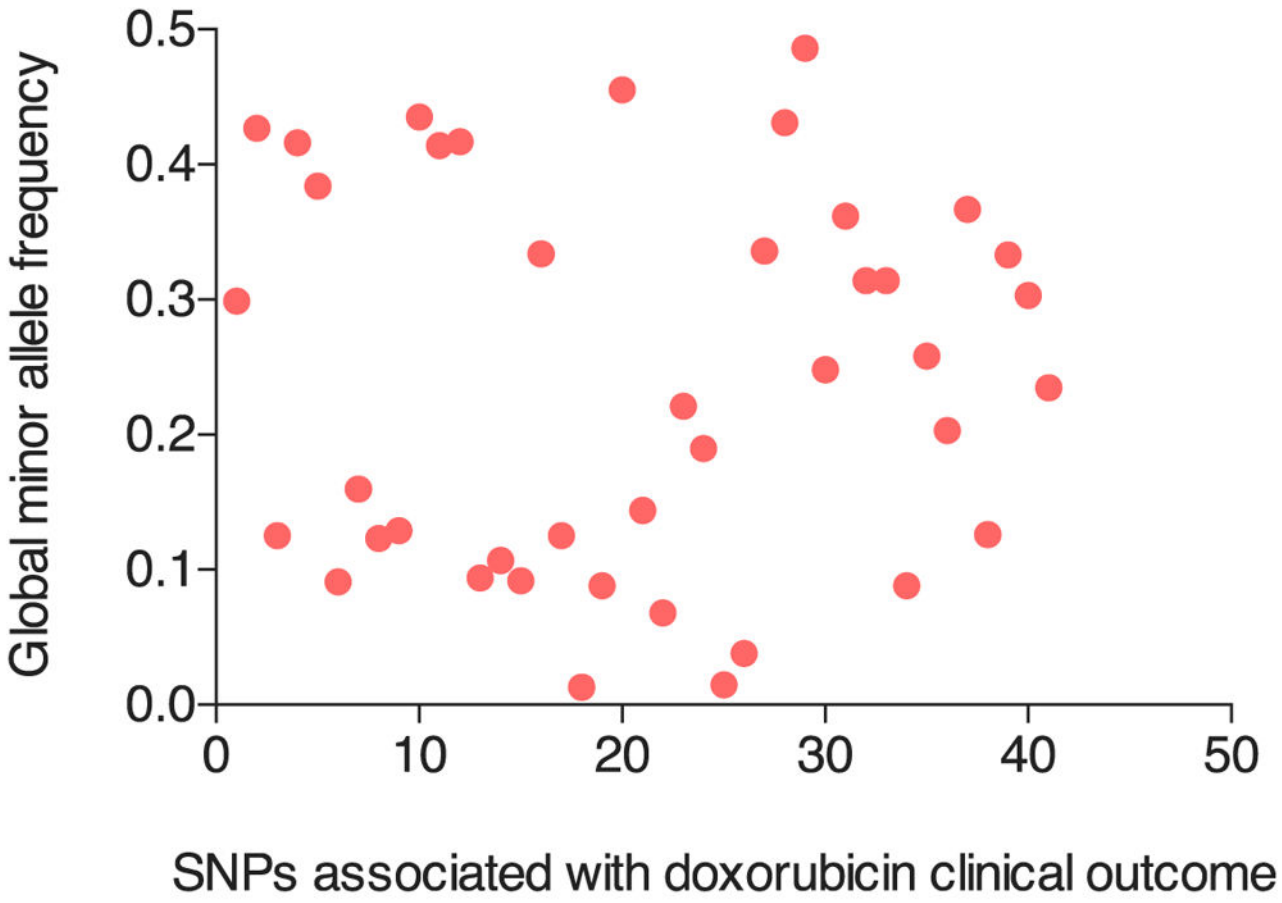

Figure 3. Global minor allele frequency distribution of DOX genetic polymorphisms

Diagram showing global minor allele frequency (GMAF) of SNPs significantly associated with DOX clinical outcome, which demonstrates that individuals SNPs have significantly different allelic frequency in diverse populations. GMAF was adapted according to 1000 genomes project data base. This analysis was done using R/Bioconductor package biomaRt (Durinck et al., 2009).

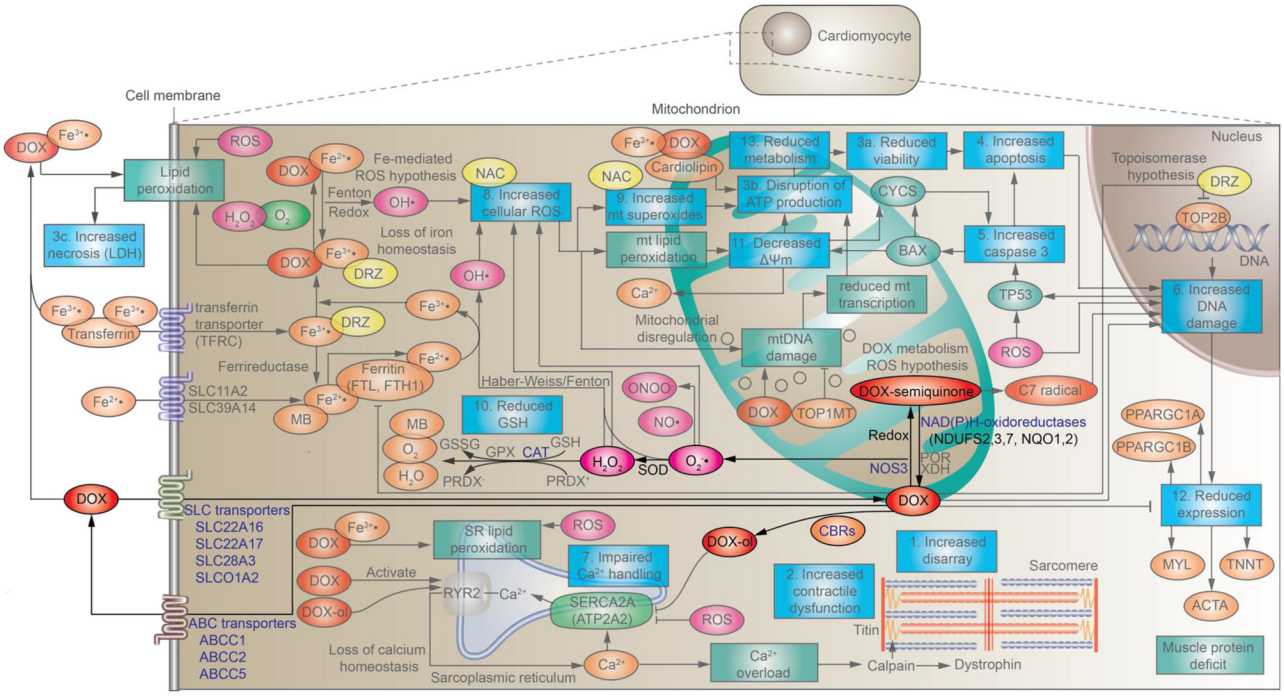

Figure 4. Schematic diagram showing the multiple mechanisms of doxorubicin-induced cardiotoxicity

Genes associated with DOX clinical outcome are written in blue. Blue boxes show assays which identified a differentiation response between patients who had cardiotoxicity (DOXTOX) and patients who did not have toxicity (DOX) (Burridge et al., 2016), highlighting the fact that DOX related cardiotoxicity is a polygenic trait and thus, the comprehensive approach proposed in this project is needed to identify genetic biomarkers for DOX-induced cardiotoxicity. Doxorubicin (DOX), doxorubinol (DOX-ol), doxoerubicin-semiquinone (DOX-semiquinone), C7 centered radical aglycone (C7 radical), nitric oxide synthase 3 (NOS3), NADH dehydrogenases (collectively NAD(P)H oxidoreductases), P450 (cytochrome) oxidoreductase (POR), xanthine oxidase (XDH) superoxide radical (O_2-•), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), nitric oxide (NO•), peroxynitrite (ONOO-), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxide (GSH), glutathione disulfide (GSSG), peroxiredoxin (PRDX), myoglobin (MB), ferrous iron (Fe^{2+}), ferric iron (Fe^{3+}), dexrazoxane (DRZ), N-acetyl-L-cysteine (NAC), topoisomerase (DNA) 1 mitochondrial (TOP1MT), BCL2-associated X protein (BAX), cytochrome C (CYCS) tumor protein p53 (TP53), topoisomerase 2B (TOP2B), ryanodine receptor 2 (RYR2), ATPase, Ca^{2+} transporting, cardiac muscle slow twitch 2 (ATP2A2), myosin light chain (MYL), cardiac troponin T (TNNT), α-actinin (ACTA). Image modified from Burridge et al., 2016, used with permission.

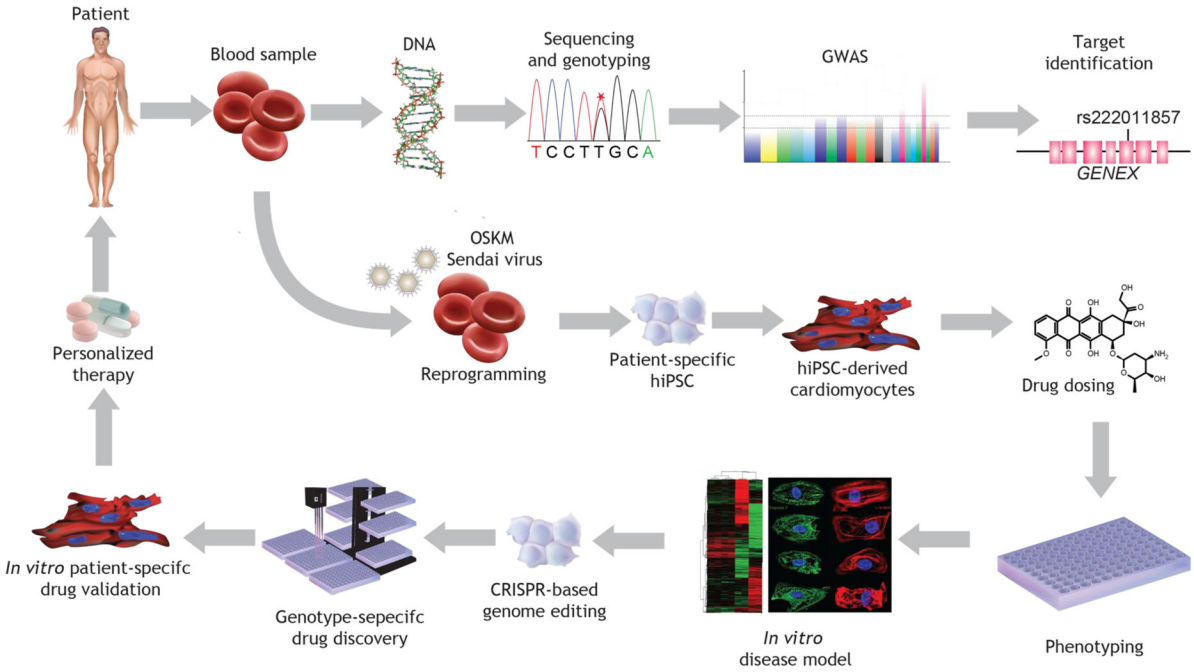

Figure 5.

Schematic of the process for elucidating the role of genetic mutations in chemotherapy-induced cardiotoxicity

Table 1

ative doxorubicin pharmacogenomics studies.

ion of by No. Ps	Study	No. of analyzed SNPs	Gene	Chr	Polymorphism	Location/Residue change	Clinical outcome	No. of patients (age)	Population	Treatment regimen	Cancer
	(Lal, Wong, et al., 2008)	4	*ABCB1*	7	rs1128503	E12/Gly412Gly	Higher Cmax	62	ASI	DOX	Breast
					rs2032582 (tri-allelic)	E21/Ser893Ala/Thr	Lowe CL (T-allele)				
	(Jordheim et al., 2015)	38	*CBR1*	22	rs20572	E3/Ala209=	Severe thrombocytopenia and diarrhea	760	French	R-CHOP	Lymphoma
					rs9024	3′UTR (associated with lower CBR1 hepatic expression and activity (Gonzalez-Covarrubias, Zhang, Kalabus, Relling, & Blanco, 2009)					
			ABCB1	7	rs2229109	E12/Ser400Asn	Severe vomiting and diarrhea				
	(Voon et al., 2013)	9	*AKR1C3*	10	rs1937840	I4	Lower OS & PFS	151	Female Asian	DOX/DOC	Breast
					rs1937841	I4	Protective against neutropenia				
			CBR3	21	rs8133052	E1/Cys4Tyr	Longer OS & lower AUC				
			ABCB1	7	rs2032582 (tri-allelic)	E21/Ser893Ala/Thr	Higher CL (T- allele)				
			SLC22A16	6	rs6907567	E2/Asn104=	Hematological toxicity				
NPs	(Fan et al., 2008)	18	*CBR3*	21	rs8133052	E1/Cys4Tyr	Lower AUC & hematological toxicity	99 (26–68)	Female Asian	DOX/DOC	Breast
	(Bray et al., 2010)	17	*SLC22A16*	6	rs12210538	E5/Met409Thr	Leucopenia & greater incidence of dose delay	230	European	DOX/Cyc	Breast
					rs714368	E2/His49Arg (associated with higher DOX exposure (Lal et al., 2007)	Lower incidence of dose delay				
					rs6907567	E2/Asn104=					
					rs723685	E4/Val252Ala					
			ABCB1	7	rs2032582 (tri-allelic)	E21/Ser893Ala/Thr	Shorter TTP &OS (A-allele)				
	(Krajinovic et al., 2015)	33	*ABCC5*	3	rs7627754	5′UTR	Cardiotoxicity	251 (children)	Caucasian	DOX±DRZ	ALL
			NOS3	7	rs1799983	E7/Glu298Asp	Protective against cardiotoxicity				
	(Lal et al., 2007)	4	*SLC22A16*	6	rs714368	E2/His49Arg	Higher exposure to DOX and DOX- ol	43	Female Asian	DOX	Breast
	(Visscher et al., 2013)	23	*SLC28A3*	9	rs7853758	E14/Leu461Leu	Protective against severe cardiotoxicity	521	Mixed	DOX-based anthracycline	Mixed

ion of by No. Ps	Study	No. of analyzed SNPs	Gene	Chr	Polymorphism	Location/Residue change	Clinical outcome	No. of patients (age)	Population	Treatment regimen	Cancer
			SULT2B1	19	rs10426377	I3					
			UGT1A6	2	rs6759892	E1/Ser7Ala	Cardiotoxicity				
			ABCB4		rs1149222						
					rs4148808						
	(Blanco et al., 2012)	2	*CBR3*	21	rs1056892	E3/Val244Met	Cardiotoxicity	487	Mixed	DOX-based anthracycline	Mixed
	(Rajić et al., 2009)	5	*CAT*	11	rs10836235	I1	Cardiotoxicity	76 (<16)	Caucasian	DOX-based anthracycline	ALL
	(Ikeda et al., 2015)	2	*ABCB1*	7	rs2032582 (tri-allelic)	E21/Ser893Ala/Thr	Neutropenia	141 (>20)	Japanese	DOX/CYC	Breast
	(Tulsyan et al., 2013)	3	*GSTP1*	11	rs1695	Ile105Val	Severe anemia	207	Indian	Anthracycline	Breast
	(Hertz et al., 2016)	27	*ABCB1*	7	rs1045642	Ile1145Ile	Protective against severe cardiotoxicity	166	White	DOX	Breast
			CBR3	21	rs1056892	V244M	Severe cardiotoxicity				
	(Gregers et al., 2015)	4	*ABCB1*	7	rs2229109		High risk of relapse	522 (children)	Nordic Caucasian	DOX/Prednisolone/vincristine	ALL
					rs1045642	Ile1145Ile	Low risk of relapse and severe bone marrow toxicity				
	(Yao et al., 2014)	78	*ABCC1*	16	rs903880	I7	Severe hematological toxicity	882 (≥18)	Mixed (EU83%, AA8%,5 %AS, 4%other)	DOX/CYC	Breast
					rs16967126	I6					
					rs4148350	I15					
SNPs	(Wojnowski et al., 2005)	206	*RAC2*	22	rs13058338	I3	Severe Cardiotoxicity	1697 (18–72)	German	CHOP	NHL
			CYBA	22	rs4673	E4/Tyr72His					
			NCF4	22	rs1883112	5′UTR					
			ABCC1	16	rs45511401	E16/Gly671Val					
			ABCC2	10	rs8187694	Val1188Glu					
					rs8187710	Cys1515Tyr					
	(Hagleitner et al., 2015)	384	*MSH2*	2	rs4638843	I13	Lower 5-year PFS	190	Caucasian	DOX/Cisplatin/MTX	osteosarcoma
			ABCC5	3	rs939338	I5					
			CASP3	4	rs2720376	I4					
0 SNPs	(Callens et al., 2015)	16,561	*SLCO1A2*	12	rs4762699	I2	Severe Febrile neutropenia	155 (18–70)	French women	DOX/Doc	Breast

ion of by No. Ps	Study	No. of analyzed SNPs	Gene	Chr	Polymorphism	Location/Residue change	Clinical outcome	No. of patients (age)	Population	Treatment regimen	Cancer
					rs2857468	I2					
	(Visscher et al., 2012)	2,977	*SLC28A3*	9	rs7853758	E14/Leu461Leu	Protective against severe cardiotoxicity	344	Canadian [EU (77%) and non-EU (23%)]	DOX-based anthracycline	Mixed
			FMO2	1	rs2020870	E2/Asp36Gly					
			SPG7	16	rs2019604	I12					
			SLC10A2	13	rs9514091	I1					
			SLC28A3	9	rs4877847	I1					
			UGT1A6	2	rs6759892	E1/Ser7Ala	Cardiotoxicity				
			ABCB4	7	rs1149222	I10					
			ABCC1	16	rs4148350	I15					
			HNMT	2	rs17583889	I2					
	(Visscher et al., 2015)	4,578	*SLC22A17*	6	rs4982753	3′UTR	Protective against severe cardiotoxicity	562	Mixed	DOX-based anthracycline	Mixed
					rs4149178	I10					
					rs2857468	I2					
SNPs	(Callens et al., 2015)	16,561	*SLCO1A2*	12	rs4762699	I2	Severe Febrile neutropenia	155 (18–70)	French women	DOX/DOC	Breast
					rs2857468	I2					
	(Aminkeng et al., 2015)C	657,694	*RARG*	12	rs2229774	E10/Ser427Leu	Severe Cardiotoxicity	456 (children)	Canadian [EU(82%) and non-EU(18%)]	DOX-based anthracycline	Mixed

American, EU: European, AS: Asian, NHL: Non-Hodgkin's Lymphoma, CHOP: cyclophosphamide, doxorubicin, vincristin, and prednisone, PFS: progression-free survival, OS: overall progression free survival, DOX: doxorubicin, DOC: docetaxel, TTP: time to progression, ALL: Acute lymphoblastic leukemia, DRZ: dexrazoxane