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

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Abstract

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The cardiotoxicity of certain chemotherapeutic agents is now well-established, and has led to the development of the field of 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 cardiomyocyte 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 to 26% for doxorubicin, 7–28% for trastuzumab, or 5–30% for paclitaxel. For tyrosine kinase inhibitors, QT prolongation and arrhythmia, ischemia and hypertension have been reported in 2–35% of patients. Furthermore, newly introduced chemotherapeutic agents are commonly used as part of changed combinational regimens with significantly increased incidence of cardiotoxicity. 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 affect 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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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 1950s to 70% in 2006–2012, the extensive use of chemotherapeutic agents is concordant with a higher incidence of adverse drug events

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(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 or clinical, causing manifestations that include disturbance in ventricular de/polarization and QT interval, arrhythmia, bradycardia, tachycardia, decreases in left ventricular ejection fraction (LVEF) and fractional shortening (FS), and irreversible congestive heart failure (CHF), all of which lead to increased morbidity and mortality. In addition, cardiotoxicity may be classified as 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 lifelong follow-up monitoring of patients is a substantial clinical burden. The childhood cancer survivor study (CCSS) is a large multi-center, long-term effort to follow ~10,000 cancer survivors diagnosed in the period between 1960 and 1986. 30 years after initial diagnosis, the accumulative incidence of severe chronic health conditions, including myocardial infarction and CHF, was 73.4%. After adjustments for age, sex, and ethnicity, survivors showed an 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 improving 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 et al., 1981). Other commonly used anthracyclines include epirubicin and idarubicin (Espinosa et al., 2003). Anthracyclines exert their action primarily through topoisoenzyme 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 the 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 in about 50% of treated patients (Swain et al., 2003) and leads to dose limitation or treatment discontinuation. About 26% of patients treated with an accumulative DOX dose of 850 mg/m² experienced heart failure, and the maximum life time cumulative dose is thus limited to 400 to 550 mg/m², 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 less than the maximum recommended DOX doses (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 (Manning et al., 2002; López-Otin & Hunter, 2010).

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. Cardiotoxicity 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., 2001, 2006). 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 models showed that one possible mechanism for imatinib-induced cardiotoxicity may occur via endoplasmic reticulum stress response-induced pro-death pathway activation including c-Jun N-terminal kinases (JNKs) activation, which leads to subtle alterations in mitochondrial function and cardiomyocyte death. Since the initial report, several studies have implicated imatinib in cardiovascular adverse events (Demetri, 2007; Herman et al., 2011; Toubert et al., 2011).

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), which demonstrated that dasatinib (100 mg once daily) resulted in faster and deeper molecular responses compared with imatinib (400 mg once daily). However, this did not translate into better overall survival rate (Jabbour et al., 2014). Acquired resistance to TKIs is caused by the formation of a polymorphic BCR-ABL1 oncoprote, which decreases the binding affinity of TKIs. 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 a 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 I study of nilotinib showed prolonged QT intervals, ranging from 5 to 15 ms, and thus close monitoring of arrhythmia and QT intervals has 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), which encodes the alpha subunit of potassium ion channel (K,11.1). K,11.1 is responsible for delayed-rectifier K⁺ current in cardiac tissue, and blocking this channel by nilotinib results in QT wave disturbance (Shopp et al., 2005).
Additionally, nilotinib promotes caspase 3/7-induced cardiomyocyte apoptosis, increases ROS production, and alters normal cardiomyocyte morphology, generating elongated cardiomyocytes with condensed nuclei (Doherty et al., 2013). Furthermore, vascular adverse events (VAEs) including rapidly progressive peripheral arterial occlusive disease (PAOD), 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 patients treated with 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 prothrombotic effect on vascular tissue, promoting arterial stenosis and vasospasm. In conjunction with the increased cholesterol and fasting glucose levels associated with nilotinib, these conditions may trigger VAEs (Valent et al., 2015). Multiple prospective, retrospective, and meta-analysis studies have reported multiple cardiovascular 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 a 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 (PDGFRs), 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, most likely due to ponatinib's 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. Stimulation of VEGFR2 activates phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT1), which propagates a pro-survival signal, endothelial nitric oxide synthase (NOS3), and boosts the production of potent vasodilators such as prostacyclin (PGI₂). Accordingly, inhibition of the VEGF signaling cascade triggers endothelial cell apoptosis, decreases capillary density and capillary dilatory response, creating a phenotype known as microvascular rarefaction (Bair et al., 2013).

Sunitinib and sorafenib are multi-kinase inhibitors that target several TKs involved in cancer cell proliferation and angiogenesis. While sunitinib targets VEGFR1–3, PDGFR/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. Sorafenib 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 of developing atrial thromboembolism (Choueiri et al., 2010). Finally, in addition to the involvement of endothelin 1 (EDN1) 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 (MSA41 or CD20); trastuzumab, an antibody 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. 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 (Wittayankorn et al., 2015).

Trastuzumab is a monoclonal antibody that was approved in 1998 for use in breast cancer patients 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 patients 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 a dilated cardiomyopathy phenotype. Dysregulation of ERBB2 expression by trastuzumab is associated with severe cardiotoxic phenotypes. Taken together, these findings emphasize the crucial role of an ERBB2 signaling pathway in the development of cardiotoxicity.

Bevacizumab was approved in 2004 as an angiogenesis inhibitor, and it exerts its action by inhibiting VEGF tyrosine kinase activity, thus blocking the blood supply to tumor cells. As a result of VEGF 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 doses, respectively (Zhu et al., 2007). The incidences of heart failure and cardiomyopathy after bevacizumab treatment are as low as 2.2% and 3%, however the duration of patient follow-ups in this study was only 18 months (Miller et al., 2005). Considering that hypertension is an independent risk factor for cardiovascular events, cardiotoxicity is therefore highly anticipated with long-term follow up. Bevacizumab-induced hypertension, along with VEGF 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 and causes 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 g/m²/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, (Wani et al., 1971; Bissery et al., 1991) 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 microtubules, promoting microtubule polymerization and inactivation, 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 at an incidence 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, thereby boosting the 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 an attempt to uncover the genetic variants associated with chemotherapy clinical outcome. Despite this enormous effort, 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, “pharmacogenetic” and “genetic” testing are used interchangeably, there is a significant difference in their target population and the manner in which each test is used in 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 the presence of sufficient clinical data, for individualized dosing selection. On the other hand, genetic testing is utilized when assessing a relative risk of a target population to develop a certain disease, as well as when 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 aberrations. 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, subsequently tuning the 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 cause a predisposition to cancer. Therefore, the study of germline aberrations has significant prognostic value. 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 (SNPs), 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 study 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 GWAS 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). GWAS are based on the principle of linkage disequilibrium (LD), 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. The linkage disequilibrium-based approach is very useful as it significantly decreases the number of genotyped SNPs while providing information about the descent number of genetic variants. Nevertheless, this methodology raises the question of whether or not the identified SNP is the causal one. Even though the 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 having significantly different minor allele frequencies 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). Because minor differences in ethnicity between cases and controls can result in false positives even after exclusion of extreme outliers, an odds ratio of at least 2–3 is required for an association to be robust enough to overcome cryptic population stratification. Odds ratios <1.5 are 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 hydroxylase glycosidation, yielding DOX deoxyaglycone or doxorubicinolone from DOX, and DOX-ol hydroxyaglycone or doxorubicinolone (DOX-olone) from DOX-ol, respectively, while also forming semiquinone as an intermediate metabolite (Licata et al., 2000; Joerger et al., 2005). Several metabolizing enzymes are involved in this metabolic pathway. Carbonyl reductase 1 (CBR1), carbonyl reductase 3 (CBR3), aldo-keto reductase 1A1 (AKR1A1) 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 NDUF52, NDUF53, and NDUF57, as well as cytosolic enzymes such as NAPDH dehydrogenase (NQO1), xanthine oxidase (XDH) and nicotinic acid synthases (NOS1, NOS2, and NOS3) catalyze the reduction of DOX to the DOX-semiquinone metabolite.

Many genes contribute to DIC, and the cardiotoxicity phenotype is thus apparently due to a 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 O2•− and H2O2 and the formation of downstream iron-dependent and independent reactive oxygen species (ROS). (2) DOX causes mitochondrial disrygulation 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 cardiomyocyte 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-α (TOP2B) re-ligation reaction in cardiomyocytes, consequently inducing DNA double-strand break-triggered cell apoptosis. (4) DOX activates ryanodine receptor 2 (RYR2), leading to calcium release in the cell. Furthermore, DOX blocks ATPase sarcoplasmic/endoplasmic reticulum Ca2+ 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 conditions 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. Few trials have investigated a reasonable number of SNPs located within genes that have been implicated in DOX response and/or toxicity (Table 1).

To date, only four GWAS investigating 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, 4578, and 2977 SNPs, respectively. An early study probing 2977 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 GWAS revealed significant risk and protective alleles. However, due to multiple testing issues and limitations in gene coverage, these results 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
<table>
<thead>
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<th>Classification of the studies by No. of SNPs</th>
<th>Study</th>
<th>No. of analyzed SNPs</th>
<th>Gene</th>
<th>Chr</th>
<th>Polymorphism</th>
<th>Location/residue change</th>
<th>Clinical outcome</th>
<th>No. of patients (age)</th>
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<tr>
<td>0–50 SNPs</td>
<td>(Lal et al., 2008)</td>
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<td>ABCB1</td>
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<td>CBR1</td>
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<td>rs9024</td>
<td>E13/Ala209 = 3'UTR (associated with lower CBR1 hepatic expression and activity (Gonzalez-Covarrubias et al., 2009)</td>
<td>Severe thrombocytopenia and diarrhea</td>
<td>760</td>
<td>French</td>
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<td>6</td>
<td>rs8133052, rs9070567</td>
<td>E2/Asn104 =</td>
<td>Hematological toxicity, Lower AUC &amp; hematological toxicity, Leucopenia &amp; greater incidence of dose delay, Lower incidence of dose delay</td>
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<td>Female Asian</td>
<td>DOX/DOC</td>
<td>Breast</td>
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<td>33</td>
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<td>251 (children)</td>
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<td>4</td>
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<td>6</td>
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<td>Protective against severe cardiotoxicity</td>
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<td>Protective against severe cardiotoxicity</td>
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<td>Ile105Val</td>
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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 a SNP’s MAF (Ardlie et al., 2002), it is crucial to recruit homogeneous 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 shown to be involved in DIC need to be intensely investigated. Examples of such genes include: ABC2, ABCG2, RALBP1, AKR1A1, CS1, SOD3, TP53, TOP2B, PPARGC1A (PGC-1α), PPARGC1B (PGC-1β), PPARA, PPARD, and CYP2J2. ABC2, encoding transporter protein MR2P, plays a role in DOX chemoresistance, and knocking down MR2P expression increases the cell’s sensitization towards DOX via increasing DOX intracellular accumulation (Folmer et al., 2007). DOX is a substrate of the ABCG2 transporter, and interestingly, a mutant variant of ABCG2 alters substrate specificity and increases DOX resistance in vitro (Stacy et al., 2013). RALBP1, the gene encoding RalA-binding protein 1, plays an important role in the regulation of intracellular concentration of DOX and its electrophilic cytotoxic metabolite, glutathione-4-hydroxy-t-nonenal (GS-HNE) (Chaiswing et al., 2005). RALBP1 protects the cell against oxidative stress, and its deletion increases cell sensitivity to DOX (Vatsayan et al., 2009). The 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 tetraacyclolphospholipid 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 cell 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 DNA 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 PPARδ, facilitating their binding to transcription factors that regulate genes involved in downstream mitochondrial biogenesis (Finck & Kelly, 2007). Interestingly, CYP2J2 over expression activates PPARA which subsequently enhances the activity of the ROS scavenger enzymes CAT and SOD, ultimately protecting the cells against DIC (Wray et al., 2009).

Despite the efforts and partial successes of many research groups 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 effects between different genes polymorphisms. Most pharmacogenomics studies lack any downstream mechanistic validation and thus, the impact of SNPs on the biological system and the relationships 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 recommendations. 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

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**Fig. 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.

**Fig. 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 individual 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).
significant associations in large independent cohorts and conduct proper patient-specific functional studies for validation of SNPs implicated in DIC.

5. Pharmacogenomics of tyrosine kinase inhibitors

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 combinational 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 have 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, there is no identified cardiotoxicity biomarker currently used in clinical routine investigation, further emphasizing the urgent need for a comprehensive whole genome-based approach to identify and validate candidate genetic variants related to TKI-induced cardiotoxicity.

6. Validation of chemotherapy induced cardiotoxicity associated single nucleotide polymorphisms

Validating the functional aspects of genetic associations is 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 relationships. 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 biopsies 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 genomic 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 responses 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 reprogramming, hiPSC differentiation, and structural and functional phenotypic characterization of the developed hiPSC-CMs, all of which support the usage of hiPSC-CM in recapitulating

Fig. 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). Edema (Doxrubicin: DOX), doxorubicin (DOX), doxorubicin (DOX), doxorubicin-oxidoreductase (DOX-oxidoreductase), C7 centered radical aglycone (C7 radical), nitric oxide synthase 3 (NOS3), NADH dehydrogenase (collectively NAD(P)H oxidoreductase), NOS5 (cytochrome) oxidoreductase (POR), xanthine oxidase (XDH) superoxide radical (O2−), hydrogen peroxide (H2O2), 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 (Fe2+), ferric iron (Fe3+), deseroxoxane (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, Ca2+ 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.
patient specific disease phenotypes and pharmacological drug response. Cardiomyocytes generated from patient-specific hiPSCs have been well characterized and display characteristics similar to human cardiac tissue. The human heart shares common genomic and transcriptomic profiles with hiPSC-CMs in both continuous culture and following cryopreservation and thawing. hiPSC-CMs express cardiac markers such as: ion channels implicated in the action potential of the human heart (e.g., SCN5A, KCNJ2, CACNA1C, KCNQ1, and KCNH2), cardiac tissue specific markers (MYH6, MYLFP, MYBPC3, DES, TNNT2, and TNNI3), and cardiac transcription factors (N2K2.5, GATA4, and GATA6). In addition, hiPSC-CMs do not express any pluripotency markers, indicating the purity of the generated cardiomyocytes. Furthermore, hiPSC-CMs exhibit similar electrophysiological, biochemical, contractile, and beating activity when compared with native cardiac myocytes (Ma et al., 2011; Babiarz et al., 2012; Puppala et al., 2013). Taken together, these observations support the superiority of an in vitro hiPSC-CM 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-CMs 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 hiPSC-CMs can recapitulate individual patients’ predisposition to DIC (Burridge et al., 2016), providing a multi-assay-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 patients with lower susceptibility (Burridge et al., 2016). All these studies support the fact that hiPSC-CMs can 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 have been 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 the involvement of candidate genes/SNP(s)/haplotypes in CIC (Fig. 5). This methodology will generate a set of 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. This methodology will

![Fig. 5. Schematic of the process for elucidating the role of genetic mutations in chemotherapy-induced cardiotoxicity.](image-url)
help achieve maximal benefit and minimal side-effects from evolving chemotherapeutics, thus significantly improving cancer treatment.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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