Editorial

Pharmacogenomics

The future role of pharmacogenomics in anticancer agent-induced cardiovascular toxicity

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"A mandatory follow-up on any GWAS should be to experimentally validate positive SNPs and to provide a mechanistic explanation for significant genotype–phenotype correlations"

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Toxicity to the heart and vascular system is a major concern with nearly all anticancer agents. Toxicity can range from drug-induced hypertension, atherosclerosis (peripheral artery disease) and venous thromboembolism, to QT prolongation, arrhythmias, myocardial infarction/ischemia, left ventricular dysfunction and heart failure, with obvious long-term consequences [1]. Incidence of toxicity is low in the case of venous thromboembolism and myocardial infarction (<10%), rising to moderate with left ventricular dysfunction (1–28%) and common with hypertension (4–61%) [2]. Toxicity also occurs across all classes of drugs including anthracyclines, monoclonal antibodies, tyrosine kinase inhibitors, platinum derivatives, uracil derivatives and proteosome inhibitors [1]. For many of these phenotypes, the drug mechanisms responsible are unknown and even whether this is an on- or off-target effect is not established.

Factors that might predict a patient's disposition to anticancer agent-induced cardiovascular cardiotoxicity are far from clear, although there is a demonstrated role for cumulative dose and infusion rate for some drugs. Many of these toxicities also occur in children, increasing the likelihood a genomic basis of predisposition by reducing the influence of factors such as BMI, diet, lifestyle, smoking, exposures, comorbidities and polypharmacy. Combined, this has piqued interest in a pharmacogenomic approach to both genetically predict which patients will experience toxicity and personalize patient care, as well as provide insights into the drug toxicity mechanisms of action by highlighting genes involved, potentially informing protective drug discovery.

Pharmacogenomic research has utilized increasingly sophisticated approaches to pinpoint genetic variants associated with a particular phenotype from candidate gene association studies (CGAS) and chip-based genome-wide association studies (GWAS) using tag SNPs, to candidate gene resequencing, expression qualitative trait loci (eQTL) mapping, exome sequencing and whole-genome sequencing (WGS). The immense evolution in sequencing techniques, data storage capacity and analysis pipelines has led to a dramatic decrease in the cost of WGS per individual from about $100 million USD in 2000 to as little as $600 USD [3] in 2017. The time required to sequence one human genome is now as little as 2 days, with commercial vendors offering DNA to WGS processed data turn-around in as little as 20–30 days [4]. These advances have shifted the trend in GWAS toward adapting WGS for variant discovery, providing data on ~3 billion base pairs, rather than genotyping 500,000–2.5 million SNPs across the genome as with chip-based GWAS.

Currently, there are several ongoing large-scale human genomics projects adapting WGS being held in many countries across the world primality targeted toward cancer and rare diseases, such as the Cancer Genome Atlas, the Cancer Genome Project, and the 100,000 Genomes Project. The capacity of these projects in terms of number of individuals varies ranging between a few thousand up to two million individuals [5]. The goal of the vast majority of these projects is to identify genetic variants that are responsible for interindividual variability in drug response, and

Future Medicine

accordingly tailor cancer treatments toward specific population, subpopulation or even single individuals providing the best therapeutic efficacy with minimal side effects.

To date only a small number of cardiovascular toxic anticancer agents have been studied mainly by CGASs and a small number of GWASs. The premier among them is the anthracycline doxorubicin (Adriamycin), for which >40 CGASs have been performed [1] and three GWASs [6–8]. Noticeably, out of more than 25 SNPs found to be associated with anthracyline-induced cardiotoxicity, only nine could be replicated as risk alleles in a replication cohort [1,9]. Of these nine SNPs, the correlation with cardiotoxicity was found insignificant in several other pharmacogenomics studies. The cardiotoxicity of the humanized monoclonal antibody trastuzumab (Herceptin) has also been studied by six CGAS [1]. Two SNPs located in the trastuzumab main target receptor *ERBB2* have been identified as linked with trastuzumab-induced cardiotoxicity; however, as with doxorubicin-induced cardiotoxicity-related SNPs, the effect of these SNPs was also not consistently replicated [10–12]. It is clear that a lack concurrance of between various CGAS and GWAS studies is a major issue, and raises considerable concerns of the validity of much of the existing data in the field.

Several factors contribute to this lack of consistency including; heterogeneity of studied populations; difference in studied primary end points; different modality used to determine these end points; implementation of different genetic approaches from single SNP genotyping passing through genotyping microarrays reaching WGS; the usage of diverse genotyping microarrays across pharmacogenomics studies; different covariates included in the genotype-phenotype correlation analysis; and using distinctive statistical approaches for multiple testing correction to set a proper significance level. Having considered all these facts, it is clear that the field needs an adequate genomic approach that provides a comprehensive coverage across the entire genome; and a validation *in vitro* cell model that are able to mimic SNPs physiological micro-environment and could be used to experimentally replicate SNP associations, providing further mechanistic evidence about how associated genetic variants are implicated in cardiac toxicity.

With only few exceptions, published GWASs depend solely on genotyping chips that utilize tag SNP approaches capturing only hundreds of thousands of SNPs across the entire genome. Although these GWASs are able to identify interesting associated SNPs with some investigated phenotypes, the causality of these variants is still far from proven. In order to increase the density of SNPs across genome and to reveal the causal SNP, GWASs use 'genotype imputation' which is a multipoint statistics-based genetic approach that could predict the un-typed SNPs by comparing the genotyped SNPs to a reference haplotype panel of relevant population. Imputation accuracy can be very easily affected by several variables including but not limited to; number of individuals in the reference panel; length of haplotype stretches which are shorter in populations with more diverse and complex genetic structure such as Africans; number of SNPs on the genotyping chip; and even the algorithm used to impute un-genotyped SNPs [13].

Applying WGS-based approach in pharmacogenomics research provides a wide variety of genetic information compared with aforementioned ones. GWAS adapting WGS was able to identify more than 22 million SNPs [14] compared with only approximately 5 million SNPs revealed by using genotyping microarray (500,000 SNPs) followed by imputation [15], enhancing downstream fine mapping and causative SNPs analysis. Importantly, SNP–SNP and gene–gene interactions especially in polygenic traits play a crucial role in predisposing a particular phenotype. The comprehensive genome coverage provided by WGS will help perform haplotype assessment in relation to the phenotype of interest rather than performing single-locus genotype–phenotype correlation analysis, allowing us to investigate these types of genetic interactions. Additionally, WGS provides huge genomic knowledge which otherwise would have been missed upon using other approaches including; copy number variations, insertions and deletions. Taken together, we believe that WGS should be the standard approach for GWASs as it indeed increases the probability to pinpoint the causal SNP/haplotype for studied phenotypes.

A mandatory follow-up on any GWAS should be to experimentally validate positive SNPs and to provide a mechanistic explanation for significant genotype–phenotype correlations, because only genetic testing for causal SNP/haplotype in routine clinical practice assures the best correlation with the phenotype of interest. To be able to assess GWAS positive genetic hits associated with cardiotoxicity, we will need an *in vitro* cell model that: could be used to a mimic and recapitulate human heart; carry the exact same genetic signature of patients harboring the associated SNP; could be used to study many different cardiovascular toxicity phenotypes; and could be scaled up to fit high-throughput characterization and quantification of cardiovascular toxicity.

The value of human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) as an efficient cell model originates from their ability to fulfill the aforementioned requirements [16]. Starting with ~500 µl of blood

samples from a participant in a GWAS cohort, hundreds of thousands of human somatic cells can be isolated and reprogrammed to hiPSCs in a process that has become highly reproducible and cost-effective [17]. hiPSCs are then differentiated into cardiomyocytes that carry the same exact genetic signature of relevant patient. Over the last decade, great progress in optimization of cardiac differentiation protocols has increased the purity of phenotypically characterized generated hiPSC-CMs from 8 to ~95% [18]. hiPSC-CMs have shown to recapitulate human heart in many aspects including gene expression profile, contractility, beating and electric activity; and have been used to model various cardiovascular diseases, and to recapitulate patient-specific drug-induced cardiac toxicity [19–21].

Utilization of the proper subpopulation of cardiomyocytes that is relevant to the mechanisms of action, on-, and off-target toxicity of tested drugs is essential for drug efficacy and toxicity screening assays. Applying minor modification to differentiation protocols such as adding neuregulin inhibitors [22] and retinoic acid regulators [23,24], and the usage of reporter gene constructs have proven successful in generating a relatively pure subtypes of cardiomyocytes including ventricular, atrial and nodal cardiomyocytes [25].

Huge efforts have been exerted to achieve cardiomyocytes mass production, and to improve high-throughput compatible dissociation and re-platting techniques. Using monolayer differentiation protocol, we are able to generate up to 30–50 million cardiomyocytes from only a single 15 cm culturing plate which are then re-plated into 96- or 384-well format. The availability of next-generation toxicity screening platforms enhance our ability to use hiPSC-CMs as an *in vitro* cell model to investigate diverse cardiotoxicity phenotypes and inter-individual patient-specific drug response. High-throughput screening platforms include among others; automated patch clamp for assessing electrophysiological parameters; luminescent-based assays for assessing viability, caspase activity, apoptosis, and reactive oxygen species production; kinetic high content imaging for assessing calcium handling; atomic force microscopy for assessing single cell contractility; and flow cytometry for assessing mitochondrial membrane potential and superoxide production.

We believe that adapting WGS-based variant calling pipeline in GWASs, when coupled to patient derived hiPSC-CMs for experimental validation of protective and risk alleles, constitutes an efficient platform that helps complete the missing part of the puzzle shifting the paradigm toward more comprehensive pharmacogenomics research, accelerating the inclusion of pharmacogenomics in routine clinical practice.

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