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EDITORIAL COMMENT

Harnessing Genomics to Predict and Prevent Anthracycline-Associated Cardiotoxicity*

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Anthracyclines, highly potent chemotherapy drugs used in approximately 60% of pediatric patients with solid and hematological malignancies, have contributed to an improvement in the overall 5-year pediatric cancer survival rate from 58% in the 1970s to 85% today (cancer.gov). Soon after the introduction of anthracyclines into pediatric cancer treatment protocols, the potential for cardiotoxicity was recognized (1), with the risk being dose-dependent, particularly when doses exceed 250 mg/m^2 (2). Acute cardiotoxicity can manifest as a reduction in left ventricular ejection fraction (LVEF), arrhythmia, or symptomatic heart failure, potentially severe enough to warrant heart transplantation (3). Studies of long-term survivors of childhood cancer have reported a risk of anthracycline-associated cardiotoxicity to be 15-fold higher compared with sibling controls (4), with the cumulative incidence of heart failure to be 7% to 10% at 30 years postexposure (2). Although the association between cardiotoxic anthracycline exposure and cardiomyopathy risk is well-described in childhood cancer survivors, cumulative anthracycline dose alone does not adequately explain individual risk, with contributions of other risk factors, including younger age at diagnosis of primary cancer, female sex, chest radiation, and presence of cardiovascular risk factors, such as diabetes, hypertension, and obesity (5).

Several studies have suggested a genetic contribution to anthracycline-associated cardiotoxicity susceptibility. Initial efforts focused on investigations of candidate genes, resulting in identification of more than 40 single nucleotide polymorphisms (SNPs) associated with cardiotoxicity, although only a few have been replicated in independent samples (6). This limited success is likely due to inconsistency in ascertainment and criteria for defining cardiotoxicity, as well as different patient characteristics, reduced study power associated with small sample sizes, and failure to account for multiple testing and population stratification. To date, 2 genome-wide association studies (GWAS) for anthracycline-associated cardiotoxicity among childhood cancer survivors have been conducted, but neither has identified genome-wide significant associations. However, they detected variants with p values that approached genome-wide significance, including a nonsynonymous coding SNP, rs2229774, in *RARG* that was replicated in both European and non-European samples, and an SNP (rs1786814) in *CELF4* that was also independently replicated. Although these studies have provided important information about the pathophysiology of anthracycline-associated cardiotoxicity, they examined only common variants (occurring with more than 5% allele frequency in populations), which may limit our understanding of the full spectrum of genetic variation influencing anthracycline-associated cardiotoxicity.

In this issue of *JACC: CardioOncology*, Chaix et al. (7) investigated the role of rare and low-frequency variants in anthracycline-associated cardiotoxicity among childhood cancer survivors. Whole-exome

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sequencing was performed in 289 anthracycline-exposed survivors from the Preventing Cardiac Sequelae in Pediatric Cancer Survivors study who were unselected for race/ethnicity. Using a nested case-control design and extreme phenotypes, the analysis compared 183 who received cumulative anthracycline dose of ≤250 mg/m^2 and experienced cardiotoxicity (defined as LVEF ≤50% or 10% LVEF decline to ≤55% from a prior echocardiogram, or low LVEF ≤55%) with 106 who did not develop cardiac dysfunction (LVEF >55%) despite their exposure to >250 mg/m^2 of anthracycline. The authors tested the joint effects of rare and low-frequency variants within each gene on the risk of anthracycline-associated cardiotoxicity using the Burden Test, Sequence Kernel Association Test (SKAT) and SKAT-Optimized. Although no gene achieved exome-wide statistical significance after accounting for multiple testing, 28 genes showed nominal significance ($p < 0.001$) in at least 2 of the 3 gene-based tests and 3 additional biologically relevant genes exhibited $p < 0.001$ in at least 1 test. It should be noted that these 3 gene-based tests are not completely independent of each other and their results are correlated to some degree except in extreme scenarios. The authors observed a significantly lower burden of variants (odds ratio: 0.09; $p = 3.98 \times 10^{-15}$) in these 31 genes among survivors with (42.6%) compared with those without (89.6%) cardiac dysfunction, which is expected from a post hoc analysis of nominally significant set of genes. Among Caucasian survivors alone, the variant burden was persistently lower (odds ratio: 0.33; $p = 0.019$), although the burden in survivors with cardiac dysfunction increased substantially (72%) from that observed in an analysis including survivors of all ethnicities. This suggests the estimate of variant burden among all survivors was likely influenced by population stratification and thus underscores the need and importance of a genetically homogeneous population in gene discovery efforts.

Of the 31 genes, 5 (*ZNF827*, *ELAC2*, *SEC62, USP42,* and *PIK3R2*), based on biological plausibility, were considered for functional analysis using 2 published human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) lines generated from 2 healthy male donors. Among these, *ZNF827*, *ELAC2,* and *PIK3R2* were significantly upregulated after 24-h treatment with doxorubicin compared with dimethyl sulfoxide. Considering availability of targeted inhibitors, *ZNF827* and *PIK3R2* were further prioritized for their potential roles in cardioprotection against anthracycline-associated cardiotoxicity. The authors found that TGX-221 (PI3KR2 inhibitor) and metformin (ZNF827 inhibitor) were effective at blocking doxorubicin-induced decrease in cardiomyocyte viability. Both metformin and TGX-221 were superior or comparable in their cardioprotective effect compared with the nontargeted inhibitor, dexrazoxane, indicating pharmacologic disruption of pathways associated with *ZNF827* and *PIK3R2* provided cardioprotection against doxorubicin. The lower variant burden in *PIK3R2* with respect to anthracycline-associated cardiotoxicity was replicated in an independent sample of survivors (odds ratio: 0.196; $p = 0.038$). Although these results indirectly support the observed cardioprotective effect of variants in these pathways, additional studies using patient-derived hiPSC-CMs with and without the variant(s) of interest are needed to confirm these observations.

The few studies that assessed potential utility of genetic variants in cardiotoxicity risk stratification found the area under the receiver operating characteristic curve (AUC) improved from 0.68 to 0.69 to 0.79 to 0.87 when genetic variants were added to the risk-prediction model including clinical risk factors alone (8). Consistent with these findings, Chaix et al. (7) also showed improvement in the AUC of the risk-prediction model from 0.59 to 0.72 when genetic risk factors were added to the clinical model. Although these results are encouraging, none of the prediction models that included genetic variants were validated in independent external samples, and selecting associated variants followed by evaluating their contribution to prediction in the same dataset does not prove their utility. Further studies are needed to independently assess their potential utility in clinical risk stratification.

Considering the limited yield of candidate-gene studies, an agnostic approach such as GWAS may be more suitable to discover novel genetic determinants of anthracycline-associated cardiotoxicity. Genotyping arrays allow assessment only of common genetic variation, and therefore, future GWASs for anthracycline-associated cardiotoxicity would benefit from whole genome sequencing, which is the only single platform that can fully catalog all genomic variation. Limited sample size is a challenge for genome-wide interrogations for studies on adverse drug effects such as those of anthracyclines because of the need for well-phenotyped homogeneous populations. Both national and international collaborative research efforts are required to increase the sample size and achieve the needed study power and statistical rigor to detect and replicate genetic variants robustly associated with anthracycline-associated cardiotoxicity.

Most genetic studies assessing anthracycline exposure and cardiac dysfunction have been performed in European American survivors, although African American (AA) survivors have a significantly higher prevalence of cardiac dysfunction (9). Because of increased genetic diversity in AAs, they may harbor population-specific genetic variants that cannot be found by studies in populations of European ancestry alone. Moreover, without studies focused on AA survivors, we risk excluding them from advances in personalized genomic medicine and clinical care, potentially increasing racial disparity. A recent study demonstrated that patient-derived hiPSC-CMs are efficient predictors of anthracycline-associated cardiotoxicity (10). The hiPSC-CM model represents the clear genetic background of an individual removing the effect of environmental factors, such as age, cardiotoxicity, and drugs, and is highly amenable to genetic modification with CRISPR/Cas9. Thus, the patient-specific hiPSC-CMs can be used to investigate molecular mechanisms underlying identified genetic associations that may elucidate novel mechanistic insights and targets for cardioprotection. Together, genomic studies offer the potential to advance understanding of the pathophysiology of cardiac dysfunction, which could inform new approaches to predict, prevent, and treat this treatment-related cardiotoxicity.

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AUTHOR DISCLOSURES

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