JACC: CARDIOONCOLOGY
PUBLISHED BY ELSEVIER ON BEHALF OF THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION.

VOL. 5, NO. 3, 2023

VIEWPOINT

Genetic Testing in Evaluating Risk of Anthracycline Cardiomyopathy

Are We There Yet?

Jeannette R. Wong-Siegel, MD, MPH,[a] Yuri Kim, MD, PhD,[b] Nathan O. Stitziel, MD, PhD,[c,d,e] Ali Javaheri, MD, PhD[b]

Childhood cancer survivors are at increased risk of cardiac dysfunction following anthracycline therapy, and those who subsequently develop heart failure (HF) are at increased risk of mortality. Genetic variation contributes to the pathogenesis of anthracycline-induced cardiac dysfunction,[1] and may be a larger contributor to HF risk in the pediatric population, where there are typically fewer modifiable cardiovascular disease risk factors. In this Viewpoint, we present our recent clinical experience with a pediatric patient who developed HF in the context of anthracycline therapy as an illustrative example of the potential role of genetic testing in stratifying risk for developing anthracycline-induced cardiomyopathy (AIC).

A 6-year-old, previously healthy girl who initially presented with left knee pain was diagnosed with high-grade osteosarcoma. She had no significant family history of malignancy or cardiovascular disease. After receiving a cumulative doxorubicin dose of 367 mg/m^2 over 6 months, her left ventricular ejection fraction (LVEF) declined from 52% to 29%, further worsening to 13% despite cessation of anthracycline therapy. She continued to have a severely decreased LVEF, requiring routine paracentesis and continued medical therapy. Unfortunately, while awaiting adequate remission time before consideration for heart transplantation, she developed metastatic recurrence not amenable to further therapy. The acuity, severity, and persistence of her cardiac dysfunction, despite only receiving 73% of the protocol's anthracycline dose, led to our hypothesis that a genetic cause might be contributing to her presentation.

We performed whole exome sequencing (WES) on the patient and her biological parents, both of whom were of nonconsanguineous European ancestry and had no history of cardiomyopathy or anthracycline exposure. Genomic DNA samples were quantified using the Qubit Fluorometer 3.0 (Thermo Fisher Scientific) or the VarioSkan Flash (Thermo Fisher Scientific) with 25 ng used for sample assessment. Following fragmentation using the Covaris LE220 to achieve 200- to 250-base pair (bp) fragment sizes, libraries were constructed using the KAPA Hyper Prep Kid (KAPA Biosystems, Cat #7962363001) in conjunction with automated Perkin Elmer SciCloneG3 NGS (96-well configuration). Libraries were then pooled and subsequently hybridized with the xGen Exome Research Panel v1.0 reagent (IDT Technologies) that spans a 39-Mb target region (19,396 genes) of the human genome. Library fragments were sequenced on an Illumina NovaSeq-6000 using 150-bp paired-end reads. Mean coverage depth for the proband, mother, and father was 92×, 127×, and 127×, respectfully.

We first evaluated for likely pathogenic variants in genes that had been previously associated with inherited cardiomyopathies and any variants previously reported as contributors to AIC. We also evaluated other genes for rare (defined as having a minor allele frequency <1% in the Genome Aggregation Database) loss-of-function variants and missense variants predicted to be pathogenic by the rare exome variant ensemble learner (REVEL), combined annotation dependent depletion (CADD), or ANNOtate

From the [a]Washington University School of Medicine, Department of Pediatrics, St. Louis, Missouri, USA; [b]Brigham and Women's Hospital, Cardiovascular Division, Boston, Massachusetts, USA; [c]Washington University School of Medicine, Department of Medicine, St. Louis, Missouri, USA; [d]McDonnell Genome Institute, Washington University School of Medicine, St. Louis, Missouri, USA; and the [e]Washington University School of Medicine, Department of Genetics, St. Louis, Missouri, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2666-0873 https://doi.org/10.1016/j.jaccao.2023.02.003

VARiation (ANNOVAR) algorithms. We considered both de novo and transmitted variants since 1 of her parents might have carried an allele predisposing to AIC that did not manifest without drug exposure.

Although we did not identify any likely pathogenic variants in genes known to cause inherited cardiomyopathies, WES revealed 3 variants within genes previously reported to be associated with AIC. First, the patient had a paternally inherited, rare nonframeshift deletion in *PRDM2* (p.E1261del), which encodes the PR/SET Domain 2 zinc finger protein. Considered a tumor suppressor gene, *PRDM2* is critical for BRCA1-dependent repair of DNA double-strand breaks[2] and impairment of BRCA1 in cardiomyocytes exacerbates anthracycline cardiotoxicity.[2]

Second, WES identified a more common maternally inherited missense variant in *ABCC1* (p.G671V), which was predicted to be likely pathogenic. *ABCC1* encodes the multidrug resistance protein 1 (MRP1) protein, a member of the ATP-binding cassette (ABC) transporter family. MRP1 is a cellular efflux pump and confers resistance against many chemotherapeutics (including anthracycline) by reducing intracellular drug accumulation. Residue 671 is located in a highly conserved region involved in retention of anthracycline and has previously demonstrated a significant association with AIC in a cohort of non-Hodgkin lymphoma patients.[3]

Finally, we discovered a rare, de novo heterozygous mutation with predicted moderate pathogenicity in *CCDC51* (p.R4H), a nuclear gene that encodes the mitochondrial potassium channel protein MITOK. Located in the mitochondrial inner membrane, MITOK forms a protein complex with ABCB8/MITOSUR that mediates ATP-dependent potassium currents. *Abcb8* knockout mice exhibit increased anthracycline-induced reactive oxygen species production and mitochondrial damage,[4] suggesting a plausible connection by which this de novo mutation may increase risk of AIC.

Although there are currently no guideline-based recommendations supporting genetic testing to assess risk of cardiac dysfunction before initiation of chemotherapy, several prior studies have evaluated potential underlying genetic mechanisms associated with AIC—from single candidate single nucleotide polymorphism to less biased gene discovery strategies, such as the whole genome or exome sequencing. The identified genetic associations can be classified based on reported mechanisms related to AIC[1] that include alterations in anthracycline transport and metabolism, generation of reactive oxygen species, DNA damage response, mitochondrial dysfunction, and sarcomere disruption that contribute to myocardial damage and cardiac dysfunction. In our case, WES identified variants within genes previously implicated in AIC pathogenesis, such as p.G671V in *ABCC1* and p.E1261del in *PRDM2*, as well as a potential rare genetic modifier p.R4H in *CCDC51* that may influence cellular response following anthracycline exposure. Although we did not evaluate common genetic variants, given a growing appreciation that polygenic risk associated with common genetic variation across the genome can modify the penetrance and phenotypic expressivity of disease-associated Mendelian mutations,[5] it is possible that combining common and rare genetic variant assessment in a comprehensive evaluation will prove useful in the future.

Mutations that cause inherited cardiomyopathy are also observed in patients who developed AIC and increase risk of developing cardiac dysfunction after anthracycline exposure.[6] For example, among 213 patients with cancer therapy-induced cardiomyopathy who underwent targeted sequencing of 9 cardiomyopathy-associated genes, those with therapy-associated cardiomyopathy had significantly more titin-truncating variants (TTNtvs) detected compared with the general population.[6] Whereas most of the TTNtvs were noted in adult cancer patients, a single nucleotide polymorphism in *CELF4* reported among childhood cancer survivors resulted in alternative splicing of TNNT2, which was significantly associated with cardiomyopathy.[7]

In the United States, guidelines have been recently published for genetic testing in inherited cardiovascular diseases, with testing reserved for patients with a confirmed or suspected diagnosis based on rigorous, disease-appropriate phenotyping, or those who are at high-risk secondary to a previously identified pathogenic variant in a close family member.[8] Genetic risk prediction models for AIC demonstrated a modest ability to discriminate risk and will require external validation.[9] Thus, given the limited evidence to date, we do not currently recommend upfront genetic testing for a patient newly diagnosed with a malignancy before initiation of therapies with cardiotoxic potential. However, in our view, if a decline in LVEF is detected after initiation of chemotherapy, clinical genetic testing using an inherited cardiomyopathy gene panel could be pursued.

With further data, we could envision that genetic testing might be utilized to inform clinical strategies to prevent AIC. For example, one might ask whether identification of increased genetic risk of cardiomyopathy may influence the use of dexrazoxane, which reduces oxygen free radical formation when administered before anthracycline therapy. Despite

JACC: CARDIOONCOLOGY, VOL. 5, NO. 3, 2023
JUNE 2023:406–408

concerns of increased risk for secondary malignant neoplasms, dexrazoxane may be considered for those patients receiving higher doses of anthracycline.[10] Perhaps those identified with a genetic predisposition for AIC might consider dexrazoxane at lower cumulative anthracycline doses. Earlier initiation of medical therapy once any cardiac dysfunction is noted may be considered, but the exact treatment regimen for AIC remains controversial as typical HF medications have not demonstrated consistent benefit. For our patient, she was placed on several HF medications with no appreciable recovery of cardiac function, and her genetic testing results, unfortunately, did not provide any information to change her clinical course.

In summary, growing evidence supports the contribution of genetic variation to AIC. We currently do not advocate for the routine use of genetic testing before initiation of chemotherapy as there are insufficient data to support its utility to prevent AIC at this time. Further genetic characterization of larger cohorts with chemotherapy-associated cardiomyopathy is critical in order to elucidate the risk related to specific genetic variants in both pediatric and adult populations. This work is needed to help define screening and testing protocols, risk stratification strategies for patients before initiation of chemotherapy, and appropriate treatment regimen.

ACKNOWLEDGMENTS The authors thank Drs Deepa Mokshagundam, Aecha Ybarra, Daniel Willis, and Megha Malhotra for their clinical guidance.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This study was funded by The Interesting Patient Study (TIPS) and the Division of Physician Scientists (DPS) at Washington University School of Medicine. TIPS and DPS also provided mentorship support. The Genome Technology Access Center (GTAC) at the McDonnell Genome Institute at Washington University School of Medicine provided help with genomics services. GTAC is partially supported by NCI Cancer Center Support Grant #P30 CA91842 to the Siteman Cancer Center and by ICTS/CTSA Grant# UL1TR002345 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. This publication is solely the responsibility of the authors and does not necessarily represent the official view of NCRR or NIH. Dr Stitziel has received investigator-initiated research funding from Regeneron Pharmaceuticals unrelated to the content of this study. Dr Javaheri serves on the scientific advisory board for Mobius Scientific, has received research funding from AstraZeneca, Bitterroot, Inc., and has patents related to the use of apolipoprotein for the treatment of heart failure and eye diseases. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Ali Javaheri, Washington University School of Medicine, CSRB 8818, St. Louis, Missouri 63105, USA. E-mail: ali.javaheri@wustl.edu. Twitter: @Alicardsdoc.

REFERENCES

1. Bhatia S. Genetics of anthracycline cardiomyopathy in cancer survivors. *J Am Coll Cardiol CardioOnc*. 2020;2(4):539–552. https://doi.org/10.1016/j.jaccao.2020.09.006

2. Shukla PC, Singh KK, Quan A, et al. BRCA1 is an essential regulator of heart function and survival following myocardial infarction. *Nat Commun*. 2011;2(1):593. https://doi.org/10.1038/ncomms1601

3. Wojnowski L, Kulle B, Schirmer M, et al. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. *Circulation*. 2005;112(24):3754–3762. https://doi.org/10.1161/CIRCULATIONAHA.105.576850

4. Ichikawa Y, Ghanefar M, Bayeva M, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest*. 2014;124(2):617–630. https://doi.org/10.1172/JCI72931

5. Fahed AC, Wang M, Homburger JR, et al. Polygenic background modifies penetrance of monogenic variants for tier 1 genomic conditions. *Nat Commun*. 2020;11(1):3635. https://doi.org/10.1038/s41467-020-17374-3

6. Kim Y, Seidman JG, Seidman CE. Genetics of cancer therapy-associated cardiotoxicity. *J Mol Cell Cardiol*. 2022;167:85–91. https://doi.org/10.1016/j.yjmcc.2022.03.010

7. Wang X, Sun CL, Quiñones-Lombraña A, et al. CELF4 variant and anthracycline-related cardiomyopathy: a children's oncology group genome-wide association study. *J Clin Oncol*. 2016;34(8):863–867. https://doi.org/10.1200/JCO.2015.63.4550

8. Musunuru K, Hershberger RE, Day SM, et al. Genetic testing for inherited cardiovascular diseases: a scientific statement from the American Heart Association. *Circ Genom Precis Med*. 2020;13(4):e000067. https://doi.org/10.1161/HCG.0000000000000067

9. Visscher H, Ross C, Rassekh S, et al. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. *J Clin Oncol Off J Am Soc Clin Oncol*. 2012;30(13):1422–1428. https://doi.org/10.1200/JCO.2010.34.3467

10. de Baat EC, Mulder RL, Armenian S, et al. Dexrazoxane for preventing or reducing cardiotoxicity in adults and children with cancer receiving anthracyclines. *Cochrane Database Syst Rev*. 2022;(9):CD014638. https://doi.org/10.1002/14651858.CD014638.pub2

KEY WORDS chemotherapy, genomics, heart failure, pediatric cancer, prediction