

STATE-OF-THE-ART REVIEW

# Genetics of Anthracycline Cardiomyopathy in Cancer Survivors

## JACC: CardioOncology State-of-the-Art Review



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### ABSTRACT

Anthracyclines are an integral part of chemotherapy regimens used to treat a variety of childhood-onset and adult-onset cancers. However, the development of cardiac dysfunction and heart failure often compromises the clinical utility of anthracyclines. The risk of cardiac dysfunction increases with anthracycline dose. This anthracycline-cardiac dysfunction association is modified by several demographic and clinical factors, such as age at anthracycline exposure (<4 years and  $\geq 65$  years); female sex; chest radiation; presence of cardiovascular risk factors (diabetes, hypertension); and concurrent use of cyclophosphamide, paclitaxel, and trastuzumab. However, the clinical variables alone yield modest predictive power in detecting cardiac dysfunction. Recently, attention has focused on the molecular basis of anthracycline-related cardiac dysfunction, providing an initial understanding of the mechanism of anthracycline-related cardiomyopathy. This review describes the current state of knowledge with respect to the pathogenesis of anthracycline-related cardiomyopathy and identifies the critical next steps to mitigate this problem. (J Am Coll Cardiol CardioOnc 2020;2:539-52) © 2020 The Author. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

**A**nthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin) form the backbone of chemotherapy regimens used to treat acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma, Ewing sarcoma, osteosarcoma, neuroblastoma, and breast cancer. Up to 60% of patients with cancer receive anthracycline-based regimens (1-6). In fact, the World Health Organization lists doxorubicin as an essential cytotoxic medicine (7). The mechanisms by which anthracyclines exert antitumor effects include: 1) inhibition of deoxyribonucleic acid (DNA) synthesis and gene expression; 2) DNA binding, alkylation, and cross-linking; 3) interference with DNA strand separation; and 4) free radical formation and lipid peroxidation.

The clinical utility of anthracyclines is compromised by cardiac dysfunction, which is often

identified on imaging studies, that may progress to heart failure. Compared with the general population, childhood cancer survivors are at a 5- to 15-fold higher risk of cardiac dysfunction (4-9). In adults with cancer, the prevalence of cardiac dysfunction approaches 10% (10). Once diagnosed with heart failure, historical data suggest the 5-year survival may be <50% (11). The risk of cardiac dysfunction increases with anthracycline dose (Figure 1) (10,12-14). Other risk factors include extremes of age at exposure to anthracyclines (<4 years;  $\geq 65$  years); female sex; chest radiation; presence of cardiovascular risk factors (diabetes, hypertension); and concurrent use of cyclophosphamide, paclitaxel, and trastuzumab (15-17). However, despite an established dose-dependent association, there is interpatient variability in the risk of cardiac dysfunction for any given

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**ABBREVIATIONS  
AND ACRONYMS**

<b>AUC</b>	= area under the curve
<b>CBR</b>	= carbonyl reductase
<b>CI</b>	= confidence interval
<b>CM</b>	= cardiomyocyte
<b>DNA</b>	= deoxyribonucleic acid
<b>GST</b>	= glutathione S-transferase
<b>hiPSC-CM</b>	= human-induced pluripotent stem cell cardiomyocyte
<b>iPSC-CM</b>	= induced pluripotent stem cell cardiomyocyte
<b>OR</b>	= odds ratio
<b>RNA</b>	= ribonucleic acid
<b>SNP</b>	= single nucleotide polymorphism
<b>Top2b</b>	= topoisomerase-II $\beta$

anthracycline dose; clinical variables alone yield modest predictive power in detecting cardiac dysfunction (18,19). Recently, attention has focused on single nucleotide polymorphisms (SNPs) associated with cardiac dysfunction (18,20-28). The poor prognosis coupled with the interindividual variability in risk has resulted in an increasing interest in developing prediction tools to identify patients at highest risk of this complication (24,29-31). Such a risk prediction tool could inform personalized decisions regarding anthracycline-based treatment as well as post-treatment surveillance.

**PATHOGENESIS OF ANTHRACYCLINE-RELATED CARDIOMYOPATHY**

Myocardial injury begins with single-cell myocytolysis. It progresses to patchy myocardial necrosis, leading to interstitial fibrosis, and finally to multifocal myocardial fibrosis (32). This leads to disruption of myocardial structure and eventually, if unmitigated, presents clinically as overt heart failure. The pathogenesis of anthracycline-related cardiomyopathy is an area of

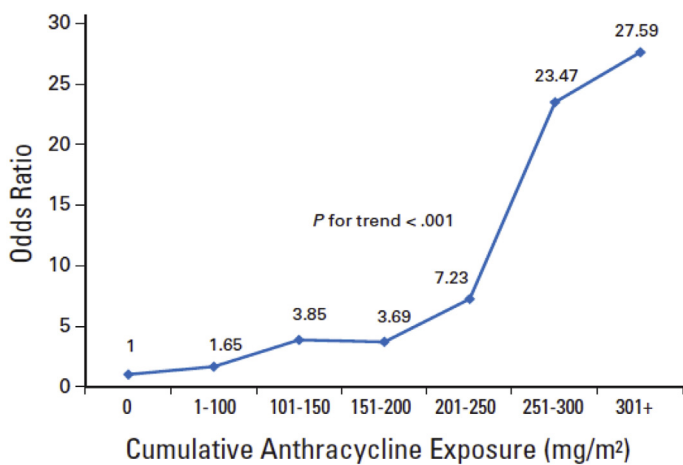
**HIGHLIGHTS**

- Anthracycline chemotherapy results in an increased risk of cardiac dysfunction.
- Most recent studies have suggested that there is a genetic basis for anthracycline-related cardiac dysfunction.
- Integration of genetics with the clinical characteristics may be used to enhance the ability to predict the risk for anthracycline-related cardiomyopathy.

active investigation. The tetracyclic ring structure of anthracyclines with the quinone and hydroquinone moieties on adjacent rings permits electron gains and losses. Anthracyclines bind to DNA through intercalation between specific bases and block DNA and ribonucleic acid (RNA) synthesis, causing DNA strand scission and interference with cell replication. Anthracyclines inhibit topoisomerase II and generate semiquinone and free radicals through an iron-dependent, enzyme-mediated reductive process. Anthracyclines bind to cellular membranes to alter fluidity and ion transport (33). Presence of quinone groups cause anthracyclines to produce free radicals that react with oxygen to produce superoxide anion radicals, causing cardiotoxicity (33). Decreased adenosine triphosphate production, direct damage to mitochondria, mitochondria-dependent apoptosis of cardiomyocytes, and lipid peroxidation of the cardiac myocyte membrane are additional mechanisms by which anthracyclines exert cardiotoxicity (34,35). The anthracycline-induced cardiotoxicity is also driven by TOP2B-mediated DNA damage (36). Doxorubicin targets topoisomerase-II $\beta$  (Top2b) and forms iron (III)-doxorubicin complex (37). Cardiomyocyte-specific deletion of Top2b can reduce defective mitochondrial biogenesis and reactive oxygen species formation (36). In particular, the heart is uniquely vulnerable to anthracyclines owing to the high density of mitochondria in cardiomyocytes, which make up 35% of the total cell volume (38). Other cellular changes in anthracycline-exposed cardiomyocytes include depleted cardiac stem cells, impaired DNA synthesis, impaired cell signaling that triggers cell death, altered gene expression, inhibited calcium release from the sarcoplasmic reticulum, impaired formation of the protein titin in sarcomeres, and impaired mitochondrial creatine kinase activity and function (39-42).

**GENETIC VARIANTS.** As summarized in Table 1, existing evidence supports the hypothesis that genetic variation contributes to chemotherapy-related

**FIGURE 1** Dose-Response Relationship Between Cumulative Anthracycline Exposure and Risk of Cardiomyopathy



The dose-response relationship between cumulative anthracycline exposure and risk of cardiomyopathy is shown. A total of 170 survivors with cardiomyopathy (cases) were compared with 317 survivors with no cardiomyopathy (control subjects; matched on cancer diagnosis, year of diagnosis, length of follow-up, and race/ethnicity) using conditional logistic regression techniques. A dose-dependent association was observed between cumulative anthracycline exposure and cardiomyopathy risk (0 mg/m<sup>2</sup>: reference; 1 to 100 mg/m<sup>2</sup>: odds ratio [OR]: 1.65; 101 to 150 mg/m<sup>2</sup>: OR: 3.85; 151 to 200 mg/m<sup>2</sup>: OR: 3.69; 201 to 250 mg/m<sup>2</sup>: OR: 7.23; 251 to 300 mg/m<sup>2</sup>: OR: 23.47; >300 mg/m<sup>2</sup>: OR: 27.59;  $p_{\text{trend}} < 0.001$ ). Reprinted with permission from Blanco et al. (28).

**TABLE 1 A Summary of Studies Examining the Role of Genetic Susceptibility to Anthracycline-Related Cardiomyopathy**

First Author (Year) (Ref. #)	Study Design	Age at Anthracycline Exposure (yrs)	Type of Cancer	Definition of Cardiotoxicity	Results	Replication
<b>Pediatric Cancer Populations</b>						
Semsei et al. (2012) (45)	Cohort n = 235	5.7 ± 3.8	ALL	Change in LV FS	ABCC1 rs3743527TT genotype and rs3743527TT-rs246221TC/TT genotype combination (OR = NA)	No replication performed
Visscher et al. (2012) (24)	Cohort Discovery: n = 156; 38 cases Replication: 1) n = 188; 40 cases 2) n = 96; 43 cases	Discovery Cases: 5.5 (0-17) Control subjects: 3.9 (0-16.5) Replication 1) Cases: 6.2 (0.4-17.6) Control subjects: 3.7 (0.05-16.9) Replication 2) Cases: 9.0 (0.5-16.8) Control subjects: 10.6 (2.1-17.7)	ALL, AML, other leukemia, HL, NHL osteosarcoma, rhabdomyosarcoma, Ewing sarcoma, other sarcoma, Wilms tumor, hepatoblastoma, neuroblastoma, carcinoma	1. FS ≤ 26% 2. Signs and symptoms indicating need for cardiac compromise intervention based on CTCAEv3	SLC28A3 rs7853758 (OR: 0.20; p = 7.1 × 10 <sup>-6</sup> ) FMO2 rs2020870 OR: 0.09; p = 2.1 × 10 <sup>-4</sup> SPG7 rs2019604 (OR: 0.33; p = 0.003) SLC10A2 rs9514091 OR: 0.41; p = 0.007 SLC28A3 rs4877847 OR: 0.54; p = 0.009 UGT1A6 rs6759892 OR: 2.93; p = 2.2 × 10 <sup>-5</sup> ABCB4 rs1149222 OR: 2.31; p = 0.002 ABCC1 rs4148350 OR: 3.77; p = 0.005 HNMT rs17583889 OR: 2.21; p = 0.009	Replication in a second cohort of 188 children from across Canada and further replication of the top SNP in a third cohort of 96 patients from the Netherlands
Visscher et al. (2013) (25)	Replication: 1) n = 128 2) n = 90	Replication 1) Cases: 9.1 (0.5-16.8) Control subjects: 11.2 (1.8-17.7) Replication 2) Cases: 12.6 (0.9-17.0) Control subjects: 4.9 (0.5-16.0)	ALL, AML, HL, NHL osteosarcoma, rhabdomyosarcoma, Ewing sarcoma, other sarcoma, Wilms tumor, hepatoblastoma, neuroblastoma, carcinoma, germ cell tumor	1. FS ≤ 26% 2. Signs/symptoms of cardiac compromise indicating need for intervention	UGT1A6 rs17863783 (p = 1.6 × 10 <sup>-5</sup> ) and SLC8A3 rs885004 (p = 3.0 × 10 <sup>-5</sup> ) were replicated in this analysis	
Visscher et al. (2015) (23)	Cohort Discovery: n = 335; 78 cases Replication: n = 185; 44 cases	Cases: 7.4 (0.04, 17.6) Control subjects: 4.9 (0.1-17.7)	Leukemia, lymphoma, sarcoma, and others	1. FS <26% 2. Echo and/or symptoms of cardiac compromise indicating need for intervention	Significant associations in SLC22A17 rs4982753 (p = 0.0078) and SLC22A7 rs4149178 (p = 0.003), with replication in the second cohort (p = 0.007 and 0.047, respectively)	Significant associations identified in SLC22A17 and SLC22A7 were replicated in the replication cohort (p = 0.0071 and p = 0.047, respectively)
Blanco et al. (2012) (28)	Case control Cases: n = 170 Control subjects: n = 317	Cases: 7.3 (0-20.7) Control subjects: 7.6 (0-21.1)	HL, NHL, bone tumors, soft tissue sarcoma, ALL, AML, other	1. Signs/symptoms of cardiac compromise based on AHA criteria 2. Echo evidence of LV dysfunction (LVEF ≤40%; FS ≤28%)	CBR3 V244M homozygous G genotypes (CBR3:GG), exposure to low- to moderate-dose anthracyclines increased cardiomyopathy risk when compared with individuals with CBR3:GA/AA genotypes unexposed to anthracyclines (OR: 5.48; p = 0.003), as well as exposed to low- to moderate-dose anthracyclines (OR: 3.30; p = 0.006)	No replication performed
Aminkeng et al. (2015) (22)	Cohort Discovery: n = 280; 32 cases Replication European patients: n = 96; 22 cases Non-European patients: n = 80; 19 cases	Discovery: cases: 9.0 (2.5-14); controls: 4 (2-7.5) Replication (Dutch population): cases: 7.5 (5-12); controls: 11 (6-14)	ALL, AML, HL, NHL, osteosarcoma, rhabdomyosarcoma, Ewing sarcoma, hepatoblastoma, neuroblastoma, Wilms tumor	Cases were defined as exhibiting FS ≥24% or signs and symptoms of cardiac compromise indicating need for intervention based on CTCAEv3, whereas control subjects had FS ≥30% and no symptoms of cardiac compromise for at least 5 yrs after treatment	Nonsynonymous variant (rs2229774, p.Ser427Leu) in RARG was associated with anthracycline-related cardiomyopathy (p = 5.90 × 10 <sup>-8</sup> , (OR: 4.7; 95% CI: 2.7-8.3)	Replication in similarly treated cohorts of 96 European and 80 non-European patients

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TABLE 1 Continued

First Author (Year) (Ref. #)	Study Design	Age at Anthracycline Exposure (yrs)	Type of Cancer	Definition of Cardiotoxicity	Results	Replication
Krajinovic et al. (2016) (46)	Cohort Discovery: n = 251 Replication: n = 44	Mean age of the Discovery cohort: 6.16 (1-18) Mean age of the Replication cohort: 5.27 (1-17)	ALL	Reduction in FS and EF	Individuals with the <i>ABCC5</i> TT-1629 genotype had an average of 8% to 12% reduction of EF and FS (EF: $p < 0.0001$ , FS: $p = 0.001$ ). A protective effect of the <i>NOS3</i> TT894 genotype on EF was seen in high-risk patients ( $p = 0.02$ ), especially in those who did not receive dexrazoxane ( $p = 0.002$ ).	Analysis of an additional cohort of 44 ALL patients replicated the <i>ABCC5</i> association but not the <i>NOS3</i> association
Wang et al. (2016) (21)	Discovery Case-control Cases: n = 112 Control subjects: n = 219 Replication Case only: n = 54	Discovery: cases: median 7.5 (0-20); controls 7.9 (0-21) Replication: cases 5.6 (1.1-17.3); controls 7.7 (0-20.6)	Discovery HL, NHL, Sarcoma, AML, ALL, and others Replication HL, NHL, Sarcoma, AML, ALL, and others	1. Signs/ symptoms of cardiac compromise based on AHA criteria 2. Absence of symptoms/ signs with echo evidence of LV dysfunction (EF $\leq 40\%$ and/or FS $\leq 28\%$ )	Anthracyclines $>300$ mg/m <sup>2</sup> , <i>CELF4</i> rs1786814 GG genotype conferred a 10.2-fold ( $p < 0.001$ ) increased risk of cardiomyopathy compared with those who had GA/AA genotypes and anthracycline exposure $\leq 300$ mg/m <sup>2</sup>	Gene-environment interaction was successfully replicated in an independent set of patients with anthracycline-related cardiomyopathy
Singh et al. (2020) (47)	Case-control Cases: n = 75 Control subjects: n = 92	Cases: 7.8 (3.8-11.5) Control subjects: 9.6 (3.3-14.8)	ALL, AML, HL, NHL, bone tumors, kidney tumor, sarcoma, neuroblastoma	LVEF $<40\%$ and/or FS $<28\%$	A significant association was observed between the risk of cardiomyopathy and the <i>GSTM1</i> null genotype (OR: 2.7; 95% CI: 1.3, 5.9; $p = 0.007$ )	No replication performed
<b>Pediatric and Adult Cancer Populations</b>						
Armenian et al. (2013) (30)	Case-control Cases: n = 77 Control subjects: n = 178	Cases: 49.2 (16-68.8) Control subjects: 51.0 (6.4-72.6)	Leukemia, myeloma, lymphoma status post-hematopoietic cell transplantation	Sign/symptoms of cardiac compromise indicating need for intervention based on AHA criteria	<i>ABCC2</i> rs8187710 (OR: 4.3; $p < 0.01$ ) <i>RAC2</i> rs13058338 (OR: 2.48; $p < 0.01$ ) <i>HFE</i> rs1799945 (OR: 2.5; $p = 0.05$ )	No replication performed
Lipshultz et al. (2013) (57)	Cohort n = 184	15.2 (3.1-31.4)	ALL	1. cTnT $> 0.01$ ng/ml 2. NT-proBNP $> 150$ pg/ml ( $<1$ yr) 3. NT-proBNP $>100$ pg/ml ( $\geq 1$ yr)	<i>C282Y HFE</i> carriers associated with myocardial injury	No replication performed
Wang et al. (2014) (20)	Discovery Case-control: n = 287; 93 cases Replication Case only: n = 76	Discovery Cases: 19.4 (0.4-41.7) Control subjects: 18.5 (3.5-49.2) Replication Cases: 48 (13-68)	Discovery HL, NHL bone tumors, soft tissue sarcoma, ALL, AML, other Replication HL, NHL, ALL, AML, other	AHA criteria for cardiac compromise: 1. Symptoms/signs of cardiac compromise 2. Echo evidence of LV dysfunction (LVEF $\leq 40\%$ and/or FS $\leq 28\%$ )	rs2232228, in <i>HAS3</i> exerted a modifying effect on anthracycline dose-dependent cardiomyopathy risk ( $p = 5.3 \times 10^{-7}$ )	Gene-environment interaction successfully replicated in an independent set of 76 patients with anthracycline-related cardiomyopathy
Leger et al. (2016) (56)	Nested case cohort Cases: n = 79 Comparison cohort: n = 267	Adult and pediatric patients	BMT recipients for acute leukemia/MDS, chronic leukemia, lymphoma, multiple myeloma, solid tumors, nonmalignant disorder	Administrative data sources (National Death Index; state hospital discharge and death registry records) and self-report	Identified association with previously reported genetic associations among early onset cardiomyopathy cases, including rs1786814 ( <i>CELF4</i> ), rs2232228 ( <i>HAS3</i> ), and rs17863783 ( <i>UGT1A6</i> )	No replication performed
<b>Adult-Onset Cancer Populations</b>						
Reichwagen et al. (2015) (48)	Case-control n = 520	68 (61, 80)	NHL	Grade $>0$ based on CTCAEv2	Accumulation of <i>RAC2</i> subunit genotypes TA/AA among cases was statistically significant on adjustment for sex, age, and doxorubicin dose in a multivariate logistic regression analysis (OR: 2.3; $p = 0.03$ )	No replication performed

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**TABLE 1 Continued**

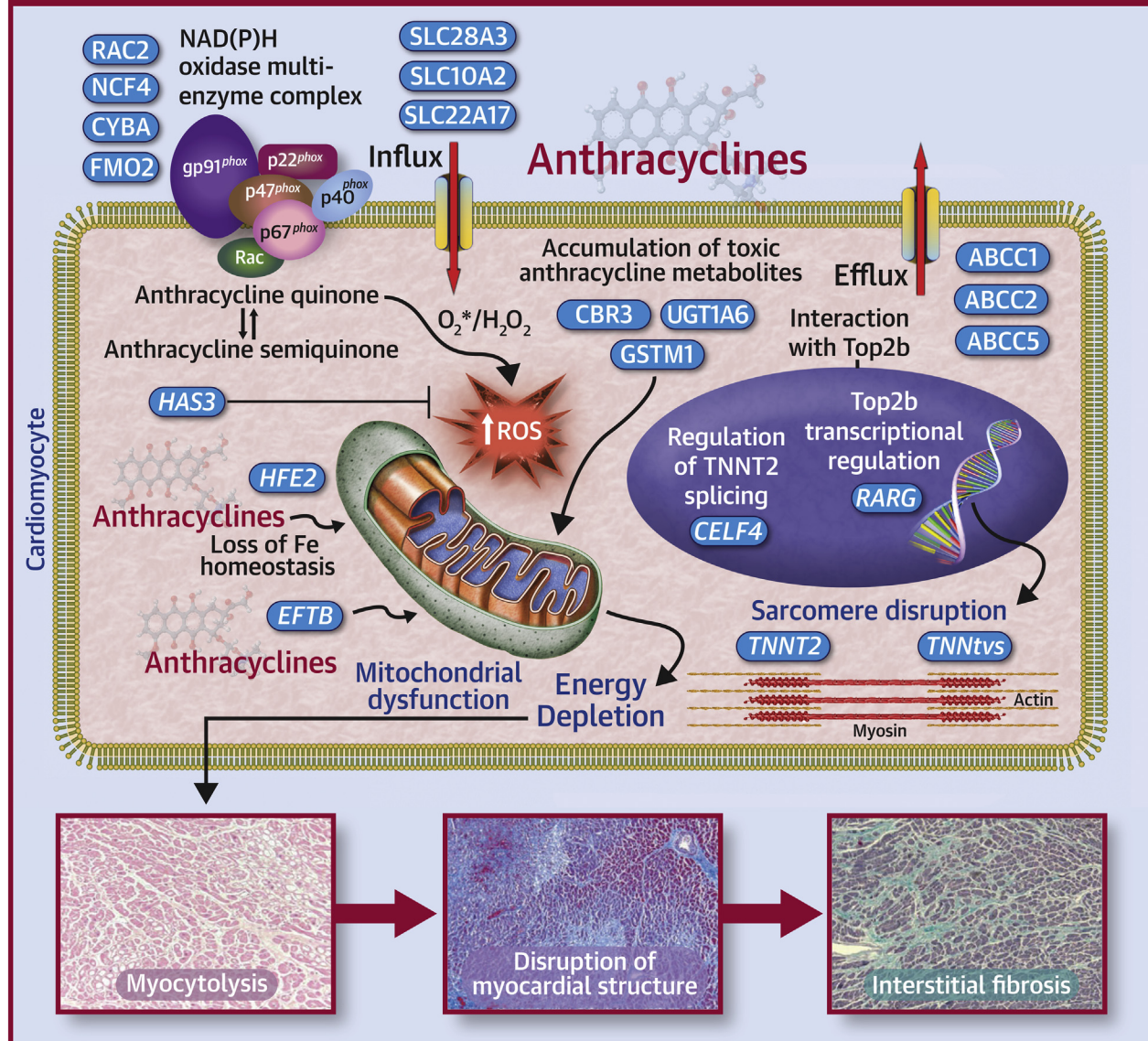
First Author (Year) (Ref. #)	Study Design	Age at Anthracycline Exposure (yrs)	Type of Cancer	Definition of Cardiotoxicity	Results	Replication
Wojnowski et al. (2005) (26)	Case-control Cases: n = 87 Control subjects: n = 363	Cases: 62.0 ± 10.9 Control subjects: 61.3 ± 11.0	NHL	1. Arrhythmia in the absence of arrhythmia before treatment 2. Myocarditis 3. Acute heart failure 4. LVEF <50% or FS <25%	<i>ABCC1</i> rs45511401 (OR: 2.39; 95% CI: 1.2-4.9) <i>ABCC2</i> rs8187710 (OR: 1.98; 95% CI: 1.01-3.90) <i>ABCC2</i> rs8187694 (OR: 1.98; 95% CI: 1.0-3.9) <i>CYBA</i> rs4673 (OR: 1.93; 95% CI: 1.2-3.2) <i>RAC2</i> rs13058338 (OR: 1.68; 95% CI: 1.1-2.7)	No replication performed
Vulsteke et al. (2015) (49)	Cohort n = 877; 153 cases	Mean: 50.3	Breast cancer	Asymptomatic decrease of LVEF >10% and cardiac failure grade 3-5 (CTCAEv4.0)	Heterozygous carriers of the rs246221 T allele in <i>ABCC1</i> relative to homozygous carriers of the T allele were significantly associated with LVEF decline of >10% (OR: 1.3; 95% CI: 1.1-1.4; p < 0.001, and OR: 1.6; 95% CI: 1.1-2.3; p = 0.02)	No replication performed
Hertz et al. (2016) (50)	Cohort n = 166; 19 cases	50 (24-80)	Breast cancer	Asymptomatic (LVEF <55%)	<i>ABCB1</i> (3435C>T, p = 0.049) and <i>CBR3</i> (V244M, p = 0.01) (uncorrected)	No replication performed
Rossi et al. (2009) (51)	Cohort n = 658; 106 cases	66 (56-75)	DLBCL	Grade 2-4 cardiac toxicity	<i>NCF4</i> rs1883112 was an independent predictor against cardiac toxicity (OR: 0.37; p = 0.02)	No replication performed
Ruiz-Pinto et al. (2018) (52)	Cohort Discovery: n = 71; 18 cases Replication: n = 83; 31 cases	Discovery Cases: 49 (27-73) Control subjects: 59.5 (36-72) Replication Cases: 5.1 (1.4-16.9) Control subjects: 10.4 (1.2-21.1)	Discovery: breast cancer Replication: childhood cancer	1. Cardiac failure grade 3-5 using CTCAEv4.0 (grade 3: severe symptoms at rest or with minimal activity or exertion, intervention indicated; grade 4: life-threatening consequences, urgent intervention indicated; grade 5: death) 2. Asymptomatic decrease of LVEF >10%	<i>ETFB</i> , related to anthracycline-mediated mitochondrial dysfunction	Successfully replicated in an independent cohort of 83 anthracycline-treated pediatric cancer patients
Garcia-Pavia et al. (2019) (53)	Case-cohort Cases: n = 213 Comparison: n = 2,498	Cohort A: adults with diverse cancer with cardiomyopathy: 48.7 ± 17.1 Cohort B: breast cancer patients with cardiomyopathy: 49.6 ± 10.8 Cohort C: children with AML with cardiomyopathy: 10.8 ± 5.6	Cases: adults with diverse cancers (n = 99); breast cancer (n = 73); children with AML (n = 41) Comparison cohort: Cancer Genome Atlas participants (n = 2,053); healthy volunteers (n = 445)	LVEF to <50 (cohort B) or <53% (cohorts A and C) and ≥10% reduction from baseline by echo or <50% and ≥10% reduction from baseline by radionuclide ventriculography, in the absence of established coronary artery disease, cardiomyopathy, primary valvular disease, or uncontrolled hypertension	Titin-truncating variants	No replication performed
Wells et al. (2017) (54)	Cohort Discovery: n = 385 Replication: n = 181	Discovery: 52 (40-61) Replication: 53 (45-61)	Diverse cancers	Maximal change in LVEF from pre-chemotherapy measurement	rs7542939—a susceptibility locus near <i>PRDM2</i> . Variation in genes belonging to pathways related to DNA repair, metabolism, and cardiac remodeling may influence changes in LV function after anthracycline exposure.	rs7542939 successfully replicated
Schneider et al. (2017) (55)	Cohort Discovery: n = 1,055; 68 cases Replication 1) n = 930; 47 cases 2) n = 322; 24 cases	Adults (details not available)	Breast cancer	Centrally reviewed, cardiologist-adjudicated HF	rs28714259—within the binding site for glucocorticoid receptor protein; important roles in the structural and functional maturation of fetal heart	rs28714259 was successfully validated in replication cohorts

Values are mean ± SD or median (interquartile range) unless otherwise indicated.

AHA = American Heart Association; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; BMT = bone marrow transplant; CI = confidence interval; CTCAEv3: Clinical Terminology Criteria for Adverse Events version 3; cTnT, cardiac troponin T; DLBCL = diffuse large B-cell lymphoma; DNA = deoxyribonucleic acid; EF = ejection fraction; FS = fractional shortening; HF = heart failure; HL = Hodgkin lymphoma; LV = left ventricular; LVEF = left ventricular ejection fraction; MDS = myelodysplastic syndrome; NA = not available; NHL = non-Hodgkin lymphoma; NT-proBNP = N-terminal pro-B-type natriuretic peptide; OR = odds ratio; SNP = single nucleotide polymorphism.

**CENTRAL ILLUSTRATION** Proposed Pathogenesis of Anthracycline-Related Cardiomyopathy

## Pathophysiologic Mechanisms of Anthracycline-Related Cardiomyopathy



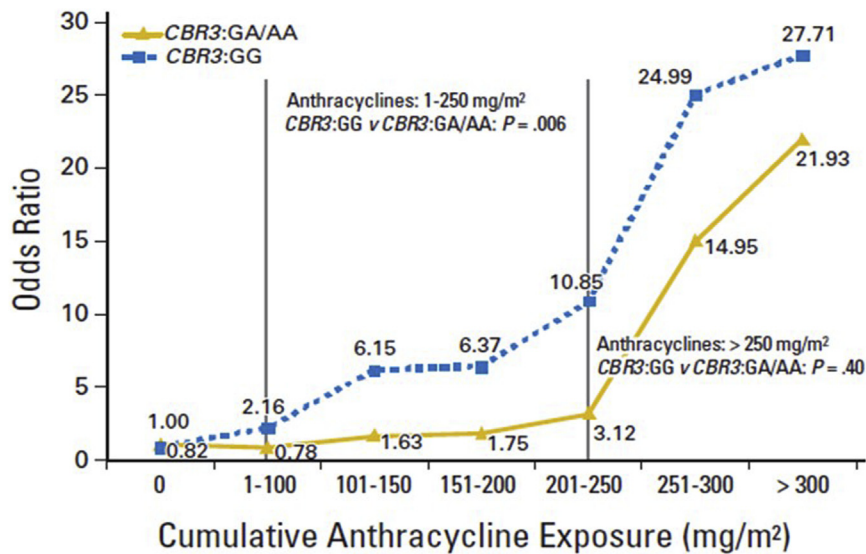
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The identified genetic associations are placed in the context of a proposed pathogenesis of anthracycline-related cardiomyopathy. The proposed mechanisms include transport of anthracyclines across the cardiomyocyte cell membrane, generation of reactive oxygen species (ROS), deoxyribonucleic damage response and repair, mitochondrial dysfunction, generation of cardiotoxic anthracycline metabolites, and sarcomere disruption. Top2b = topoisomerase-II $\beta$

cardiac dysfunction (43,44). The existing evidence has been used to construct the proposed pathophysiologic mechanism of anthracycline-related cardiomyopathy in the **Central Illustration**. Whereas some studies utilized childhood cancer (21-25,27,28,45-47)

or adult-onset cancer populations (26,48-55), others included patients across the age spectrum (20,30,56,57). Several studies examined homogeneous cancer populations (leukemia, osteosarcoma, breast cancer, lymphoma), whereas others examined

**FIGURE 2** Dose-Response Relationship Between Cumulative Anthracycline Exposure and Risk of Cardiomyopathy Stratified by Patient *CBR3* Genotype Status

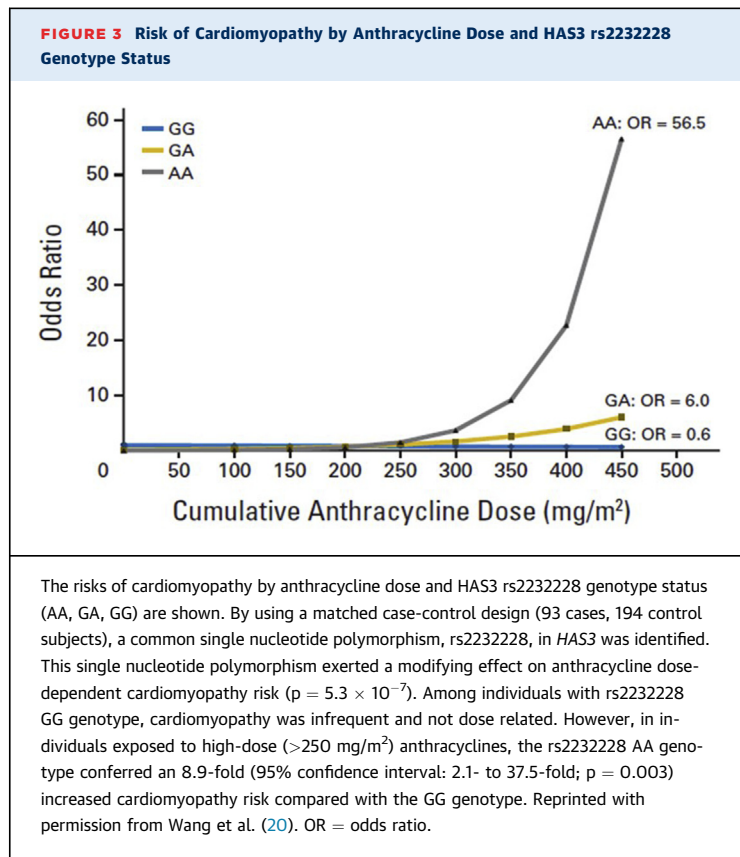


The dose-response relationship between cumulative anthracycline exposure and risk of cardiomyopathy stratified by patients' *CBR3* genotype status (*CBR3*:GG and *CBR3*:GA/AA) is shown. A total of 170 survivors with cardiomyopathy (cases) were compared with 317 survivors with no cardiomyopathy (control subjects; matched on cancer diagnosis, year of diagnosis, length of follow-up, and race/ethnicity) using conditional logistic regression techniques. Among individuals carrying the variant A allele (*CBR1*:GA/AA and/or *CBR3*:GA/AA), exposure to low- to moderate-dose anthracyclines (1 to 250 mg/m<sup>2</sup>) did not increase the risk of cardiomyopathy. Among individuals with *CBR3* V244M homozygous G genotypes (*CBR3*:GG), exposure to low- to moderate-dose anthracyclines increased cardiomyopathy risk when compared with individuals with *CBR3*:GA/AA genotypes unexposed to anthracyclines (odds ratio: 5.48;  $p = 0.003$ ), as well as exposed to low- to moderate-dose anthracyclines (odds ratio: 3.30;  $p = 0.006$ ). High-dose anthracyclines (>250 mg/m<sup>2</sup>) were associated with increased cardiomyopathy risk, irrespective of *CBR* genotype status. Reprinted with permission from Blanco et al. (28).

a mix of several cancers. Doxorubicin, daunorubicin, and epirubicin were the most common anthracyclines examined. A few studies reported cumulative anthracycline dose in doxorubicin-equivalent doses (58). Objective assessments (based on echocardiographic findings, Clinical Terminology Criteria for Adverse Events criteria) were used to define the phenotype for all studies, except one that used self-reports (56). The quest for causal genes has involved the use of a variety of strategies, including examination of single SNPs to customized arrays and agnostic genome-wide approaches. Both cohort and case-control study designs were used; the median number of cases per study was small: 54 (range 12 to 213). Twelve of the 26 studies (46%) reviewed here, did not attempt a replication of their salient findings. However, some of these studies were either examining a single SNP with targeted activity and established mechanism of cardiotoxicity (28,57) or had extended their findings by demonstrating differential gene expression (47) or by validating in preclinical mouse models (26). Furthermore, some of these studies replicated findings from previous studies (30,48-51).

**Drug biotransformation genes. GSTM1.** Glutathione S-transferases (GSTs) facilitate elimination of anthracyclines. GSTs could play a role in oxidative damage-induced cardiomyopathy as free radical scavengers. A previous report indicates an association between the *GSTM1* null genotype and iron-overload-related cardiomyopathy in patients with thalassemia (59,60). Singh et al. (47) sought to identify an association between the *GSTM1* null genotype and anthracycline-related cardiomyopathy in childhood cancer survivors. They examined the *GSTM1* gene deletion in 75 survivors with clinically validated cardiomyopathy and 92 survivors without cardiomyopathy. They observed a significant association between the risk of cardiomyopathy and the *GSTM1* null genotype (odds ratio [OR]: 2.7; 95% confidence interval [CI]: 1.3 to 5.9;  $p = 0.007$ ).

Singh et al. (47) measured *GSTM1* expression levels in peripheral blood from 20 cases and 20 control subjects and also examined human-induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) from childhood cancer survivors (3 with cardiomyopathy, 3 without) for *GSTM1* gene expression levels. There was



a significant down-regulation of *GSTM1* expression in the peripheral blood RNA from cases compared with from control subjects (average relative expression:  $0.67 \pm 0.57$  vs.  $1.33 \pm 1.33$ , respectively;  $p = 0.049$ ). Along similar lines, hiPSC-CMs from patients who had cardiomyopathy-revealed reduced *GSTM1* expression ( $p = 0.007$ ).

**CBR3.** Carbonyl reductases (CBRs) catalyze reduction of anthracyclines to cardiotoxic alcohol metabolites. Polymorphisms in *CBR3* influence synthesis of these metabolites. Blanco et al. (28) examined the role of SNPs in *CBR3* (*CBR3* V244M) in modifying the dose-dependent risk of anthracycline-related cardiomyopathy in childhood cancer survivors. A total of 170 survivors with cardiomyopathy were compared with 317 survivors with no cardiomyopathy. Among individuals with *CBR3* V244M homozygous G genotypes (*CBR3*:GG), exposure to low- to moderate-dose anthracyclines (1 to 250 mg/m<sup>2</sup>) increased cardiomyopathy risk when compared with individuals with *CBR3*:GA/AA genotypes unexposed to anthracyclines (OR: 5.48;  $p = 0.003$ ), as well as in those exposed to low- to moderate-dose anthracyclines (OR: 3.30;  $p = 0.006$ ). High-dose anthracyclines (>250 mg/m<sup>2</sup>) were associated with increased cardiomyopathy risk,

irrespective of *CBR3* genotype status. This study demonstrates increased anthracycline-related cardiomyopathy risk at doses as low as 101 to 150 mg/m<sup>2</sup> (Figure 2). An independent study of patients with breast cancer treated with anthracyclines with or without trastuzumab replicated the association between *CBR3* and decline in left ventricular ejection fraction (61).

*CBR3* is present in the liver at low basal levels but is highly inducible by the transcription factor Nrf2. *Cbr3* messenger RNA and *CBR3* protein are highly expressed in the livers of *Gclm*<sup>-/-</sup> mice (a mouse model of glutathione deficiency) relative to wild-type mice. Schaupp et al. (62) investigated the ability of *CBR3* to metabolize doxorubicin. Incubation of doxorubicin and purified recombinant murine *CBR3* were analyzed for doxorubicinol formation using high-performance liquid chromatography, showing that doxorubicin is a substrate of recombinant murine *CBR3* (62). Moreover, hepatocytes from *Gclm*<sup>-/-</sup> mice produced more doxorubicinol than *Gclm*<sup>+/+</sup> hepatocytes did. In addition, differentiated rat myoblasts (C2C12 cells) cocultured with primary *Gclm*<sup>-/-</sup> murine hepatocytes were more sensitive to doxorubicin-induced cytostasis and/or cytotoxicity than incubations with *Gclm*<sup>+/+</sup> hepatocytes were. These results indicate a potentially important role for *CBR3* in doxorubicin-induced cardiotoxicity.

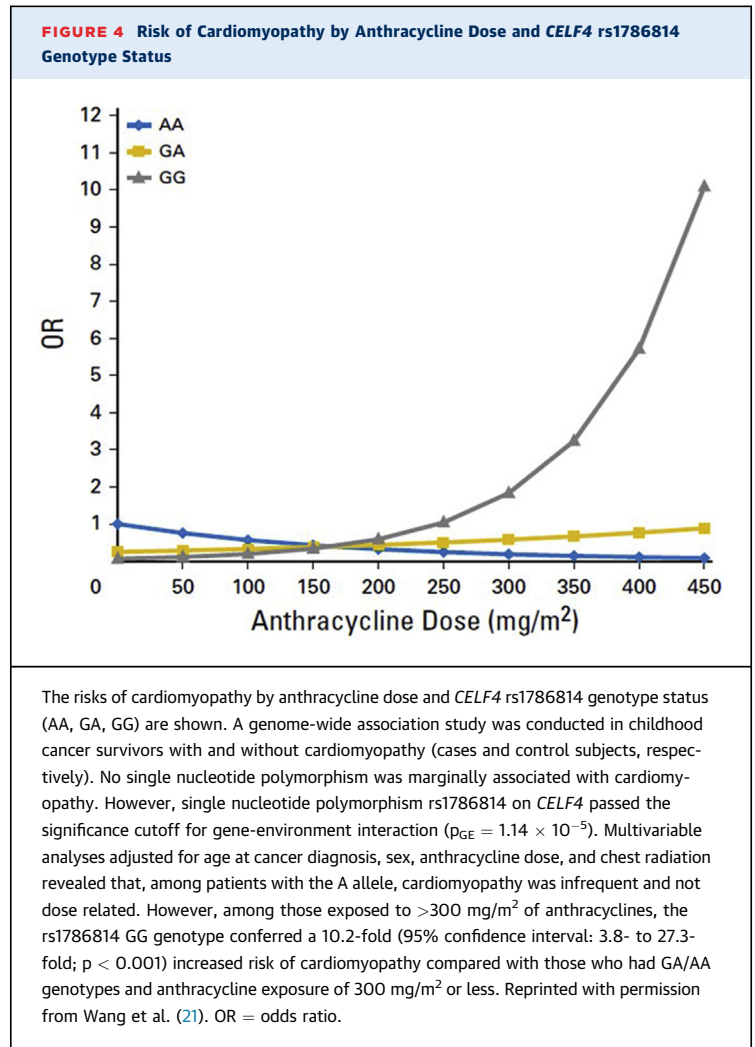
**SLC28A3, SLC22A17, SLC22A17, UGT1A6.** Visscher et al. (24) studied 2,977 SNPs in 220 key drug biotransformation genes in a discovery cohort of 156 anthracycline-treated children from British Columbia, with replication in a second cohort of 188 children and further replication of the top SNP in a third cohort of 96 patients. They identified a significant association of a synonymous coding variant rs7853758 (L461L) within the *SLC28A3* gene with anthracycline-related cardiomyopathy (OR: 0.35;  $p = 1.8 \times 10^{-5}$  for all cohorts combined). Additional associations ( $p < 0.01$ ) with risk and protective variants in other genes including *SLC28A1* and several adenosine triphosphate-binding cassette transporters (*ABCB1*, *ABCB4*, and *ABCC1*) were present. They then aimed to replicate these findings by testing the association between 23 genetic variants and cardiomyopathy in an independent cohort of 218 patients. They confirmed the association of rs17863783 in *UGT1A6* and anthracycline-related cardiomyopathy in the replication cohort (OR: 7.98;  $p = 0.006$ ). Additional evidence for association of rs7853758 (OR: 0.46;  $p = 0.058$ ,) and rs885004 (OR: 0.40;  $p = 0.058$ ) in *SLC28A3* was found (combined  $p = 1.6 \times 10^{-5}$  and  $p = 3.0 \times 10^{-5}$ , respectively) (25). They extended the genotyping panel to include over 4,500 SNPs in more



than 300 genes relevant in pharmacokinetics and dynamics, including anthracycline transport, metabolism, and toxicity (23). They identified two novel SNPs— rs4982753 (*SLC22A17*) and rs4149178 (*SLC22A7*)— in the discovery cohort; these findings were successfully replicated.

**Antioxidant mechanisms. HAS3.** Co-occurrence of cardiovascular risk factors (diabetes and hypertension) exacerbate the dose-dependent association between anthracyclines and cardiomyopathy (15,63). Institute for Translational Medicine and Therapeutics/Broad/Candidate-gene Association Resource cardiovascular SNP array profiles common SNPs in 2,100 genes considered relevant to de novo cardiovascular disease (20), including cardiovascular risk factors that exacerbate the risk of anthracycline-related cardiomyopathy. Wang et al. (20) investigated host susceptibility to anthracycline-related cardiomyopathy using the Institute for Translational Medicine and Therapeutics/Broad/Candidate-gene Association Resource cardiovascular SNP array. Using a matched case-control design (93 cases; 194 control subjects), they identified a common SNP, rs2232228, in *HAS3* that exerted a modifying effect on anthracycline dose-dependent cardiomyopathy risk ( $p = 5.3 \times 10^{-7}$ ) (Figure 3). Among individuals with rs2232228 GG genotype, cardiomyopathy was infrequent and not dose-related. However, in individuals exposed to high-dose (>250 mg/m<sup>2</sup>) anthracyclines, the rs2232228 AA genotype conferred an 8.9-fold (95% CI: 2.1 to 37.5;  $p = 0.003$ ) higher cardiomyopathy risk than the GG genotype did. Relative *HAS3* messenger RNA levels measured in healthy hearts tended to be lower among individuals with AA compared with those with GA genotypes ( $p = 0.09$ ). Hyaluronan produced by *HAS3* is a ubiquitous component of the extracellular matrix and plays a role in tissue remodeling. In addition, hyaluronan reduces reactive oxygen species-induced cardiac injury. In this setting, cardiomyopathy risk could be due to inadequate remodeling and/or inadequate protection of the heart from reactive oxygen species-mediated injury on high anthracycline exposure.

**TOP2B-mediated DNA damage. RARG.** Aminkeng et al. (22) performed a genome-wide association study in 280 patients of European ancestry (32 cases; 248 control subjects) treated for childhood cancer with independent replication in cohorts of 96 European patients (22 cases; 74 control subjects) and 80 non-European patients (19 cases; 61 control subjects). Cases exhibited fractional shortening of <24% or signs/symptoms of cardiac compromise requiring



intervention; controls had no symptoms and fractional shortening  $\geq 30\%$ . A nonsynonymous variant (rs2229774) in *RARG* was associated with anthracycline-related cardiomyopathy (OR: 4.7; 95% CI: 2.7 to 8.3;  $p = 5.9 \times 10^{-8}$ ). This variant alters *RARG* function leading to derepression of the key genetic determinant, *Top2b*. As indicated earlier, *Top2b* plays a role in the development of cardiomyopathy in a murine model (36), whereas in a rat cardiomyoblast (H9c2) cell line, cardioprotectant dexrazoxane results in decreased *Top2b* levels (64). *RARG* is expressed in the heart (Nuclear Receptor Signaling Atlas) and is induced in murine cardiac cells following cardiac injury (65).

**Alternative splicing of *TNNT2*. CELFB4.** Wang et al. (21) conducted a genome-wide association study in childhood cancer survivors with and without cardiomyopathy (cases and control subjects, respectively), and independently replicated the SNPs that surpassed a pre-specified threshold for statistical

significance. They used healthy hearts to seek the mechanistic significance of the validated SNP(s). SNP rs1786814 on *CELF4* passed the significance cutoff for gene-environment interaction ( $p_{GE} = 1.14 \times 10^{-5}$ ). Among patients with A allele, cardiomyopathy was infrequent and not dose-related. However, among those exposed to  $>300 \text{ mg/m}^2$  of anthracyclines, the rs1786814 GG genotype conferred a 10.2-fold (95% CI: 3.8 to 27.3;  $p < 0.001$ ) increased risk of cardiomyopathy compared with those with GA/AA genotypes and anthracycline exposure of  $\leq 300 \text{ mg/m}^2$  (Figure 4). Cytosine-uridine-guanine repeat binding protein and embryonic lethal abnormal vision-type RNA-binding protein-3-like factor proteins (encoded by *CELF4*) control developmentally regulated splicing of *TNNT2*, the gene that encodes for cardiac troponin T, a biomarker of myocardial injury. Coexistence of more than 1 cardiac troponin T variant results in a temporally split myofilament response to calcium, which causes decreased contractility. Analysis of *TNNT2* splicing variants in healthy human hearts suggested an association between the rs1786814 GG genotype and coexistence of more than 1 *TNNT2* splicing variant (90.5% GG vs. 41.7% GA/AA;  $p = 0.005$ ). Thus, the modifying effect on the dose-dependent association between anthracyclines and cardiomyopathy possibly occurs through a pathway that involves the expression of abnormally spliced *TNNT2* variants.

**METABOLOMICS.** Disturbances in cardiac metabolism underlie most cardiovascular diseases. Metabolomics, one of the newer omics technologies, has emerged as a powerful tool for defining changes in both global and cardiac-specific metabolism that occur across a spectrum of cardiovascular disease states (66). Findings from metabolomics studies have contributed to better understanding of the metabolic changes that occur in heart failure and have identified new cardiovascular disease biomarkers. Armenian et al. (67) performed plasma metabolomic analyses (8 pathways; 354 metabolites) in 150 asymptomatic anthracycline-exposed childhood cancer survivors. Median time from cancer diagnosis to study participation was 12.4 years (interquartile range: 2.6 to 37.9 years); median anthracycline dose was  $350 \text{ mg/m}^2$  (interquartile range: 25 to  $642 \text{ mg/m}^2$ ). Thirty-five participants (23%) had cardiac dysfunction defined as left ventricular end-systolic wall stress  $>2$  SD by echocardiogram. Plasma levels of 15 compounds in 3 metabolic pathways (carbohydrate, amino acid, and lipid metabolism) were significantly different between individuals with cardiac dysfunction and those with normal systolic function. After adjusting for multiple comparisons, individuals with cardiac

dysfunction had significantly lower plasma carnitine levels (relative ratio: 0.89;  $p < 0.01$ ) compared with those in individuals with normal systolic function. If confirmed in future studies, findings such as this could suggest a role for carnitine supplementation for primary prevention (before/during anthracycline administration) or secondary prevention in long-term survivors.

**iPSC-DERIVED CMs.** An innovative approach to study cardiomyopathy is to harness iPSCs and derived differentiated cells as in vitro model systems, shown to serve as powerful model systems for understanding cell type-specific genetic regulation of transcription (68-72). The response of iPSC-derived CMs has been extensively characterized by BurrIDGE et al. (73,74). iPSC-CMs derived from 4 individuals who developed cardiomyopathy after doxorubicin treatment (DOXTOX group) and 4 who did not (DOX group) showed clear differences in viability (via apoptosis), metabolism, DNA damage, oxidative stress, and mitochondrial function when exposed to doxorubicin. These observations suggest that iPSC-CMs recapitulate in vivo interindividual differences in doxorubicin sensitivity. Knowles et al. (75) used a panel of iPSC-CMs from 45 individuals exposed to 5 different drug concentrations to understand the genetic basis of the interindividual differences in doxorubicin sensitivity. They found several genetic variants that modulate transcriptomic response, including some that act on alternative splicing. The transcriptomic response correlated with cardiac troponin levels in culture. They also showed that the mapped genetic variants were enriched in the top genetic variants in a genome-wide association study of anthracycline-related cardiomyopathy.

Christidi et al. (76) used iPSC-CMs to investigate the functional role of the RARG variant previously shown to be associated with anthracycline-related cardiomyopathy (22). iPSC-CMs from individuals who experienced anthracycline-related cardiomyopathy (cases) showed significantly greater sensitivity to doxorubicin than did iPSC-CMs from doxorubicin-treated individuals who did not develop cardiomyopathy (control subjects) in cell viability and optical mapping experiments. Using CRISPR/Cas9 technology (CRISPR Therapeutics, Cambridge, Massachusetts), isogenic cell lines differing only at the RARG locus were generated. Genetic correction of RARG-S427L to wild type resulted in reduced doxorubicin-induced double-stranded DNA breaks, reactive oxygen species production, and cell death. Conversely, introduction of RARG-S427L increased susceptibility to doxorubicin. Finally, genetic

disruption of *RARG* resulted in protection from cell death due to doxorubicin treatment. These findings suggest that the presence of *RARG-S427L* increases sensitivity to anthracycline-related cardiomyopathy.

Holmgren et al. (77) investigated doxorubicin-induced cardiotoxicity in hiPSC-CMs using proteomics. In addition, they combined different sources of omics data (protein, messenger RNA, and microRNA) from the same experimental setup to identify differential expression in data of various origin and types. In this experimental model system, they exposed hiPSC-CMs to doxorubicin for up to 2 days, followed by a washout period of 12 days. Besides an effect on the cell morphology and cardiomyocyte functionality (treated cells exhibited reduced contractility and inhibited cell proliferation), the data showed a strong effect of doxorubicin on all molecular levels investigated. They identified differential expression patterns that showed a linkage between the proteome, transcriptome, and the regulatory microRNA network. Pathway over-representation analysis of the differentially expressed proteins at each time point revealed a clear effect on cardiomyocyte-related signaling pathways, including mitochondrial apoptotic signaling. Effects were also seen on the abundance of proteins that constitute the myofibrils, such as myosin light chains; myYH-homologous genes/proteins; LP18.3, Psb32, and MOLO-1; and troponins; as well as proteins involved in the mitochondrial function, such the cyclooxygenase family, MT-CO2, NADH:ubiquinone oxidoreductase supernumerary subunits family, adenosine triphosphate family, and UQCRI0.

Taken together, these studies provide evidence for the use of iPSC-CMs as a suitable platform to identify and characterize the genetic basis and molecular mechanism of anthracycline-related cardiomyopathy. However, there are some limitations that need to be taken into account: these include the immature and indeterminate phenotype of the cardiomyocytes; and the lack of other cardiac cell types, such as endothelial cells and fibroblasts. Nonetheless, by using this model, it should be possible to validate the relevance of genetic variants through SNP arrays.

### PREDICTION OF ANTHRACYCLINE-RELATED CARDIOMYOPATHY

There have been several attempts at developing risk prediction models for identifying patients at highest risk for anthracycline-related cardiomyopathy. Clinical risk prediction models have yielded modest predictive power for identifying patients at risk for anthracycline-related cardiomyopathy (31,78),

resulting in studies that combined genetic variants with clinical and demographic variables to improve the ability to discriminate anthracycline-exposed survivors by their risk of developing cardiomyopathy. Visscher et al. (25) combined multiple variants from drug biotransformation genes together with clinical risk factors into a prediction model and classified patients into 3 risk groups. In the high-risk group, the model was able to predict 75% of the patients who actually developed anthracycline-related cardiomyopathy. Equally as importantly, in the low-risk group, the model was able to predict 96% of the patients who did not to develop cardiomyopathy. They extended the genotyping panel including over 4,500 SNPs in more than 300 genes pre-selected for relevance in pharmacokinetics and dynamics. An improved prediction model constructed using replicated genetic variants as well as clinical factors discriminated significantly better between cases and control subjects than did clinical factors alone in both original (area under the curve [AUC]: 0.77 vs. 0.68;  $p = 0.003$ ) and replication (AUC: 0.77 vs. 0.69;  $p = 0.06$ ) cohorts. Armenian et al. (30) included SNPs on genes coding for the NAD(P)H-oxidase subunit *RAC2* (rs13058338), *HFE* (rs1799945), and the doxorubicin efflux transporter *ABCC2* (rs8187710) to create a combined (clinical + genetic) heart failure predictive model. The combined model performed better (AUC: 0.79) than the genetic (AUC: 0.67) or the clinical (AUC: 0.69) models alone. However, none of these models were externally validated, presenting a need for robust external validation in independent patient populations. These validated models could provide a framework on which to base future screening strategies and interventions targeted to those at highest risk of developing cardiomyopathy. Equally important is the identification of those at lowest risk, to allow the maximally tolerated dose of anthracyclines as well as to be spared the need for screening for early detection of cardiomyopathy. Thus, there is a possibility for setting an individualized threshold of maximum cumulative anthracycline dose where patients with a favorable risk profile might benefit from an increased cumulative dose with larger antitumor effect. In contrast, those with an unfavorable profile would benefit from alternative therapeutic options and more frequent cardiac monitoring, facilitating early detection of cardiac compromise.

**SUMMARY.** This review summarizes the current knowledge regarding the etiology and pathogenesis of anthracycline-related cardiomyopathy. Even though a lot of work has been accomplished, there are significant gaps in knowledge. For example, with an

exception of few, most studies lack robust patient numbers and functional validation. Most of the studies have not examined gene-environment (gene-anthracycline dose) interactions, or are not powered to do so; thus, genes such as *HAS3*, *CELF4*, and *CBR3* (all demonstrating strong gene-environment interactions) have been replicated by very few. Most of the studies on the adult-onset cancer patients are restricted to breast cancer of lymphoma survivors; there is a need to expand the portfolio of research to a diverse group of cancer survivors. There is also a need to expand the sample size, to allow the use of an agnostic approach as we integrate genomics, transcriptomics, proteomics, and metabolomics. The ultimate goal is 3-fold: 1) identify those at highest risk such that both primary and secondary measures can be instituted to prevent this complication; 2) use the

findings from integrated omics analyses to serve as biomarkers of those at highest risk; and 3) use the leads from these studies to identify therapeutic targets.

## AUTHOR DISCLOSURES

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