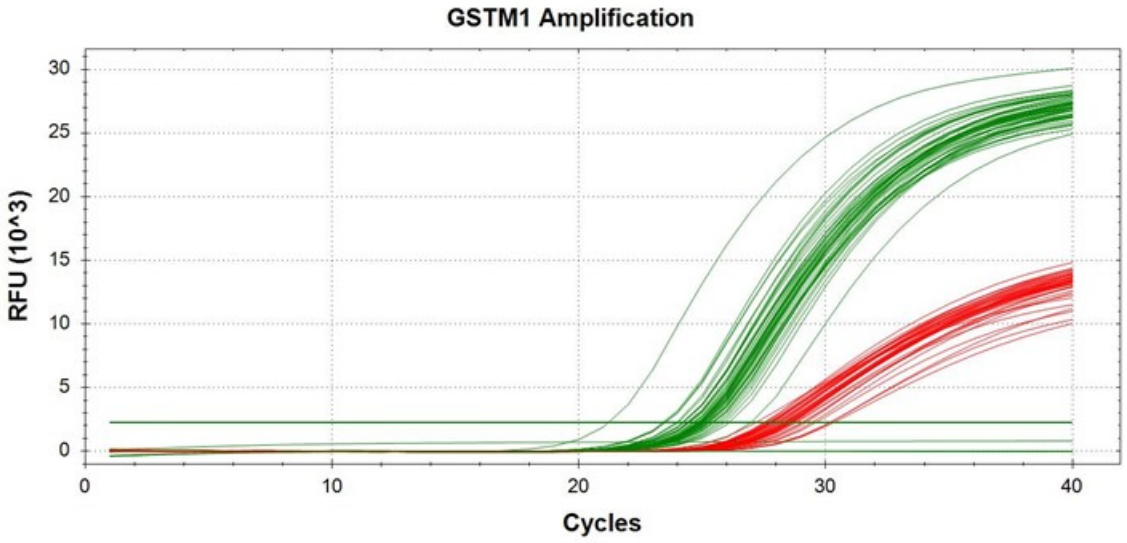
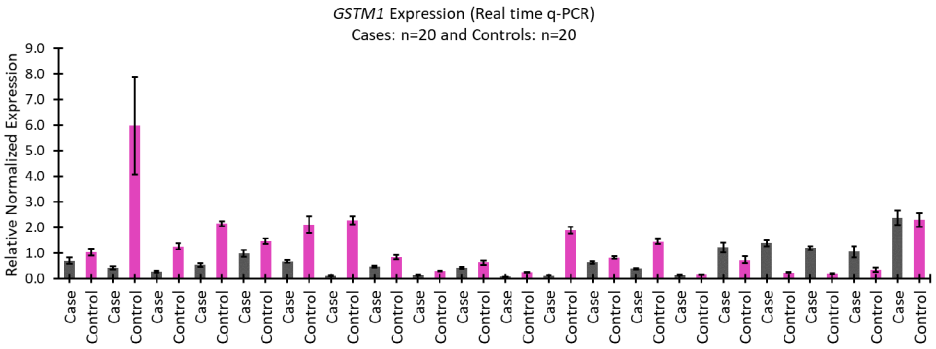

GSTM1 Amplification
RFU (10^3)
30
25
20
15
10
5
0
0
10
20
30
40
Cycles


GSTM1 Expression (Real time q-PCR)
Cases: n=20 and Controls: n=20
Relative Normalized Expression
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Case
Control

Supporting Figure 1. The chart shows the amplification profiles for *GSTM1* (FAM probe). Green curves show homozygous (2N) or heterozygous (1N) positive genotype and red curves show homozygous null genotype.

Supporting Figure 2. Relative gene expression of significantly deregulated *GSTM1* (Taqman custom design primers and probes spanning Exons 7-8) in anthracycline-induced cardiomyopathy normalized to β-actin gene (*ACTB*). Matched Cases (grey) and Controls (pink) were analyzed in triplicates by qPCR assay. Error bars indicate the standard deviation from the mean in each sample.

SUPPORTING METHODS

GSTM1 genotyping: The multiplex PCR assay does not distinguish heterozygous or homozygous wild type genotypes (2N and 1N) and therefore only the presence (at least 1 allele present, homozygote or heterozygote) or the absence (complete deletion of both alleles, null) was reported **(see Supporting Fig. 1)**. Reagents were obtained from Thermo Fisher Scientific (Grand Island, NY). Thermal cycling was carried out in 96-well plates using the CFX96 Touch™ Real-Time PCR Detection System (BioRad). Genomic DNA (Cat. Nos. NA12236, NA17657 and NA18540) from Coriell biorepository were used as positive and negative control in PCR-based genotyping reactions.[1]

Microarray: Each RNA sample was amplified using Illumina TotalPrep RNA Amplification kit (Thermal Fisher Scientific) with biotin-UTP (Enzo Life Sciences, Inc., Farmingdale, NY, USA) labeling. The TotalPrep Illumina RNA Amplification kit uses T7-oligo(dT) primer to generate single-stranded cDNA followed by a second-strand synthesis to generate double-stranded cDNA and purified through spin column, using T7 RNA polymerase to synthesize biotin-labeled cRNA, and re-purified. The cRNA was then quantified using the ND-1000 Spectrophotometer (Thermo Fisher Scientific). A total of 1.5 µg cRNA from each sample was hybridized on each array using standard Illumina protocols with streptavidin-Cy3 (Amersham Biosciences Corp., Piscataway, NJ, USA) for detection. Slides were scanned on Illumina HiScan scanner and analyzed using GenomeStudio (Illumina, Inc.). Raw files of Illumina HumanHT-12 v4.0 expression array were extracted and normalized (Quantile) in GenomeStudio Module. We analyzed all probes from Illumina HT-12v4 expression array for *GSTM1* gene, with one probe (ILMN_2391861) showing signal intensities above housekeeping genes.

RT-qPCR: cDNA synthesis was performed using SuperScript™ IV VILO™ Master Mix with ezDNase™ enzyme following the manufacturer's instructions (Thermo Fisher Scientific) using Taqman assays and TaqMan® Fast Advanced Master Mix on a CFX96 Real-Time PCR system (Bio-Rad) in triplicate. The normalized expression analysis (ΔΔCT) was performed using the CFX Maestro software and the relative normalized

expression levels were plotted to compare cases and controls.

RNA sequencing in cardiomyocytes: Library preparation was done using a TruSeq RNA v2 kit (Illumina) and sequencing with NextSeq 500 instrument (Illumina) by Northwestern's NuSeq core facility, generating ~40 million single-end 75 bp reads for each sample. Reads were mapped to the GRCh38 reference human genome using Subread software. [2]

References

1. Pratt VM, Everts RE, Aggarwal P, et al. Characterization of 137 Genomic DNA Reference Materials for 28 Pharmacogenetic Genes: A GeT-RM Collaborative Project. J Mol Diagn. 2016;18: 109-123.

2. Liao Y, Smyth GK, Shi W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 2013;41: e108.

Supporting Table 1. Participating Children's Oncology Group Institutions

A.B. Chandler Medical Ctr - University of Kentucky
A.I. duPont Hospital for Children
Advocate Hope Children's Hospital
All Children's Hospital
Allan Blair Cancer Centre
Baptist Children's Hospital
British Columbia's Children's Hospital
Brooklyn Hospital Center
C.S. Mott Children's Hospital
Cancer Research Center of Hawaii
CancerCare Manitoba
Cedars-Sinai Medical Center
Children's Healthcare of Atlanta, Emory University
Childrens Hospital & Clinics Minneapolis & St Paul
Childrens Hospital London Health Sciences
Childrens Hospital Los Angeles
Childrens Hospital Medical Center-Akron, Ohio
Childrens Hospital Oakland
Children's Hospital of Eastern Ontario
Children's Hospital of Michigan
Childrens Hospital of Philadelphia
Children's Hospital of the Greenville Hospital System
Childrens Hospital-King's Daughters
Children's Medical Center Dayton
Children's Memorial Medical Center at Chicago
Children's National Medical Center - D.C.
Children's of New Orleans/LSUMC CCOP
Cincinnati Children's Hospital Medical Center
City of Hope National Medical Center
Connecticut Children's Medical Center
Cook Children's Medical Center
Dana-Farber Cancer Institute and Children's Hosp
Driscoll Children's Hospital
East Tennessee Childrens Hospital
East Tennessee State University
Eastern Maine Medical Center
Emanuel Hospital-Health Center
Hackensack University Medical Center
Helen DeVos Children's Hospital
Hospital Sainte-Justine
Hospital for Sick Children
Hurley Medical Center
Indiana University - Riley Childrens Hospital
Inova Fairfax Hospital
IWK Health Centre
Kaiser Permanente Medical Group, Inc., Northern CA

Kalamazoo Center for Medical Studies
Kingston General Hosp/Kingston Regional Cancer
Kosair Childrens Hospital
M.D. Anderson Cancer Center
Maimonides Medical Center
Mayo Clinic and Foundation
McGill University Health Center - Montreal Children's Hosp
McMaster University
Medical College of Georgia Childrens Medical Ctr
Memorial Sloan Kettering Cancer Center
Methodist Children's Hospital of South Texas
Miami Children's Hospital
Michigan State University
Midwest Children's Cancer Center
Nationwide Children's Hospital
Nemours Children's Clinic-Jacksonville
Nevada Cancer Research Foundation - CCOP
New York Medical College
Newark Beth Israel Medical Center
Primary Childrens Medical Center
Princess Margaret Hospital for Children
Rady Children's Hosp San Diego
Rainbow Babies and Childrens Hospital
Royal Children's Hospital, Brisbane
Royal Children's Hospital, University of Melbourne
Sacred Heart Children's Hospital
Sacred Heart Hospital
Saint Barnabas Medical Center
Saint Peter's University Hospital
Saskatoon Cancer Center
Scott & White Memorial Hospital
Seattle Children's
South Carolina Cancer Center
St John Hospital and Medical Center
St. Joseph's Hospital and Medical Center
St. Jude Children's Research Hospital Memphis
St. Vincent Children's Hospital - Indiana
St. Vincent Hospital - Wisconsin
Stanford University Medical Center
State University of New York at Stony Brook
Stollery Children's Hospital
SUNY Upstate Medical University
Swiss Pediatric Oncology Group Geneva
Tampa Children's Hospital
Texas Children's Cancer Center at Baylor College of Medicine
Texas Tech UHSC - Amarillo
The Children's Hospital - Denver, CO

The Children's Hospital of Southwest Florida Lee Memorial Health System
The Childrens Mercy Hospital
The University of Chicago Comer Children's Hosp
Tulane University Medical Center
UCLA David Geffen School of Medicine
University of Alabama
University of Florida
University of Iowa Hospitals & Clinics
University of Kansas Medical Center
University of Minnesota Cancer Center
University of Mississippi Medical Center Children's Hospital
University of Missouri - Columbia
University of New Mexico School of Medicine
University of North Carolina at Chapel Hill
University of Oklahoma Health Sciences Center
University of Pittsburgh
University of Texas Health Science Center at San Antonio
University of Vermont College of Medicine
University of Wisconsin - Childrens Hosp Madison
UT Southwestern Medical Center
Vanderbilt Children's Hospital
Virginia Commonwealth Univ Health System-MCV
Wake Forest University School of Medicine
Washington University Medical Center
West Virginia University HSC - Charleston
Winthrop University Hospital
Women's and Children's Hospital, Adelaide
Yale University School of Medicine

Supporting Table 2. Echocardiographic evidence of case *vs* control determination

Clinical Characteristics	N	Mean EF (%)	Mean SF (%)
Cases	75	39.42	22.23
Cases with EF and SF	37	42.25	21.87
Cases with only EF	13	29.15	-
Cases with only SF	15	-	23.33
Controls	92	65.97	36.72
Controls with EF and SF	26	67.38	36.27
Controls with only EF	13	63.15	-
Controls with only SF	17	-	37.42
MD note indicating no symptom	36	-	-

Supporting Table 3. Univariable analysis of *GSTM1* gene variants with Anthracycline- induced cardiomyopathy

Variables	**Cases (N=75)**	**Controls (N=92)**	**p-value***
***GSTM1* (N, %)**			
null	45 (60.00)	35 (38.04)	**0.005**
positive	30 (40.00)	57 (61.96)	

* Estimated using chi-square for categorical variables

Supporting Table 4. Risk Factors associated with Anthracycline-related Cardiomyopathy

Risk Factor	OR (95% CI)	p-value*
Age at primary cancer diagnosis (years)		
Per year increase in age	0.95 (0.88-1.02)	0.1
Anthracycline Dose (mg/m^2) [¥]		
Per mg increase in dose	1.0 (1-1.01)	**0.04**
Chest Radiation		
No	1.00	
Yes	1.5 (0.6-3.6)	0.4
Sex		
Male	1.00	
Female	1.6 (0.8-3.3)	0.2
Genotype		
GSTM1 positive	1.00	
GSTM1 null	**2.5 (1.2-5.2)**	**0.01**

* Estimated from conditional logistic regression model that included age at cancer diagnosis, sex, chest radiation and anthracycline dose (continuous)

[¥] Doses not available for one case and one control

Abbreviations: CI, confidence interval; OR, odds ratio

Supporting Table 5. Risk Factors associated with Anthracycline-related Cardiomyopathy stratified by cumulative anthracycline exposure

Anthracycline Dose (mg/m²) [¥]	<250 (N=65)		≥250 (N=100)	
Risk Factor	**OR (95% CI)**	**p-value***	**OR (95% CI)**	**p-value***
Age at primary cancer diagnosis (years)				
Per year increase in age	0.96 (0.88-1.05)	0.4	0.95 (0.88-1.02)	0.2
Chest Radiation				
No	1.00		1.00	
Yes	2.6 (0.7-10.25)	0.2	1.02 (0.4-2.43)	0.9
Sex				
Male	1.00		1.00	
Female	1.8 (0.6-5.4)	0.3	1.9 (0.8-4.4)	0.1
Genotype				
GSTM1 positive	1.00		1.00	
GSTM1 null	2.3 (0.7-6.7)	0.1	**2.6 (1.15-6.1)**	**0.02**

* Estimated from logistic regression model that included age at cancer diagnosis, sex and chest radiation

[¥] Doses not available for one case and one control

Abbreviations: CI, confidence interval; OR, odds ratio