BACKGROUND: Cancer therapy–induced cardiomyopathy (CCM) is associated with cumulative drug exposures and preexisting cardiovascular disorders. These parameters incompletely account for substantial interindividual susceptibility to CCM. We hypothesized that rare variants in cardiomyopathy genes contribute to CCM.

METHODS: We studied 213 patients with CCM from 3 cohorts: retrospectively recruited adults with diverse cancers (n=99), prospectively phenotyped adults with breast cancer (n=73), and prospectively phenotyped children with acute myeloid leukemia (n=41). Cardiomyopathy genes, including 9 prespecified genes, were sequenced. The prevalence of rare variants was compared between CCM cohorts and The Cancer Genome Atlas participants (n=2053), healthy volunteers (n=445), and an ancestry-matched reference population. Clinical characteristics and outcomes were assessed and stratified by genotypes. A prevalent CCM genotype was modeled in anthracycline-treated mice.

RESULTS: CCM was diagnosed 0.4 to 9 years after chemotherapy; 90% of these patients received anthracyclines. Adult patients with CCM had cardiovascular risk factors similar to the US population. Among 9 prioritized genes, patients with CCM had more rare protein-altering variants than comparative cohorts (P≤1.98e–04). Titin-truncating variants (TTNtv) predominated, occurring in 7.5% of patients with CCM versus 1.1% of The Cancer Genome Atlas participants (P=7.36e–08), 0.7% of healthy volunteers (P=3.42e–06), and 0.6% of the reference population (P=5.87e–14). Adult patients who had CCM with TTNtv experienced more heart failure and atrial fibrillation (P=0.003) and impaired myocardial recovery (P=0.03) than those without. Consistent with human data, anthracycline-treated TTNtv mice and isolated TTNtv cardiomyocytes showed sustained contractile dysfunction unlike wild-type (P=0.0004 and P<0.002, respectively).

CONCLUSIONS: Unrecognized rare variants in cardiomyopathy-associated genes, particularly TTNtv, increased the risk for CCM in children and adults, and adverse cardiac events in adults. Genotype, along with cumulative chemotherapy dosage and traditional cardiovascular risk factors, improves the identification of patients who have cancer at highest risk for CCM.

Clinical Perspective

What Is New?

• This is the first study to consider the association between rare genetic variants in a large set of cardiomyopathy genes and the occurrence of cancer therapy–induced cardiomyopathy (CCM).

• We demonstrated an increased prevalence of rare variants in cardiomyopathy genes, in particular, truncating variants in the TTN gene, in adult and pediatric patients who have cancer with CCM.

• We confirmed human genetic data with experimental analyses, showing that anthracyclines induced protracted left ventricular dysfunction in mice with titin-truncating variants, but not in wild-type mice.

What Are the Clinical Implications?

• Our findings show that variants in cardiomyopathy genes contribute to CCM susceptibility among adult and pediatric patients with cancer.

• The identification of genetic risk factors opens new opportunities to define patients at high risk for CCM and associated adverse outcomes.

• Future investigations to define patients who have cancer with high risk for CCM through genetic testing can assess the efficacy of prophylactic cardioprotective drugs and treatment regimens to reduce CCM while providing effective cancer therapy.

Several candidate gene and genome-wide association studies have identified common genetic variants that are associated with CCM through candidate gene analyses and genome-wide association studies.2,8–12 Although a recent systematic literature review concluded that the overall evidence supporting variant associations with CCM was limited, genetic data were robust for one intergenic variant (rs28714259) and variants in proximity to 4 other genes.13 Rare variants in genes that cause familial cardiomyopathies14 have also been identified in several small case series and isolated patients with CCM.13,15–17

To better understand the clinical and genetic determinants in CCM, we studied 3 CCM cohorts comprising adult and pediatric patients with diverse malignancies, of whom 90% received anthracyclines. We then corroborated our human findings through cardiac phenotyping of anthracycline-treated mice. From these analyses, we demonstrate the direct and prevalent involvement of variants in genes associated with dilated cardiomyopathy and, in particular, titin-truncating variants (TTNtv) in CCM.

METHODS

The data that support the findings of this study are available within the article, the online supplementary files, and publicly available databases. Additional requests, from qualified researchers trained in human subject confidentiality protocols, for anonymized data may be sent to the corresponding authors.

CCM Cohorts, Healthy Volunteer, and Population Controls

Research protocols were reviewed and approved by the institutional ethics board at each participating site. Adult patients with CCM (cohorts A and B), parents of minor patients with CCM (cohort C), and healthy volunteers provided written informed consent. Cohort A includes non-Finnish European patients with CCM retrospectively collected from 6 European heart failure or cardiac transplantation clinics in Spain and the United Kingdom. Cohort B includes prospectively enrolled patients with breast cancer, participating in cardiotoxicity studies of cancer treatments (clinicaltrials.gov NCT01173341). Cohort C includes pediatric patients with newly diagnosed acute myeloid leukemia, enrolled in a clinical therapy trial therapy (AAML031; clinicaltrials.gov NCT01371981). Cohorts B and C are US patients with non-Finnish European, African, or Asian ancestry, who had prespecified clinical assessments with cardiac imaging (echocardiograms or multigated acquisition scans) before, during, and after chemotherapy. Table 1 provides additional demographic profiles on these cohorts.

CCM was diagnosed irrespective of symptoms based on LVEF to <50 (cohort B) or <53% (cohorts A and C)5,18,19 and ≥10% reduction from baseline by echocardiography 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.

Considerable advances in cancer therapies have led to major improvements in long-term survival for many malignancies, but also to unintended side effects, including cardiotoxicity.1,2 Cancer therapy–induced cardiomyopathy (CCM), identified as reduced left ventricular ejection fraction (LVEF) with or without signs and symptoms of overt heart failure,3 can occur during, shortly after, or many years beyond cancer treatments and affects the long-term prognosis of patients.1,4,5 Anthracyclines, which are commonly used to treat both solid tumors and hematologic malignancies in children and adults,2 cause cardiotoxicity in up to 10% of patients with cumulative dosages of 250 mg/m² but in 65% of patients receiving cumulative dosages >550 mg/m².6 Combining anthracyclines with other therapies, such as trastuzumab (an antibody targeting HER-2), can provoke greater cardiotoxicity with depressed LVEF occurring in ≥18% to 34% of treated individuals, and severe, symptomatic heart failure in 2% to 4%.3,7 Additional clinical parameters are recognized to contribute to CCM, including female sex, extremes of age, and preexisting cardiac risk factors.2 Even when accounting for these factors, predicting individual susceptibility to CCM remains challenging.
Additional clinical information including follow-up duration and adverse outcomes was obtained from medical and clinical trial records and patient reports. Where prechemotherapy cardiac imaging was absent, patients were included when LVEF was ≤45% and no alternative cause for cardiac dysfunction other than chemotherapy was identified. LVEF recovery was defined by a final LVEF ≥50% with ≥5% LVEF increase or restoration of LVEF to the baseline value.18

Healthy volunteers of European ancestry (n=445) were prospectively recruited participants into the U.K. Digital Heart Project
Gene Variants in Patients With CCM

We previously identified 9 genes with an excess of rare missense and in-frame insertions/deletion or truncating variants among patients with cardiomyopathy.29 Within cardiac transplant clinics. Two US cohorts were identified through prospective longitudinal cardiac evaluations obtained throughout cancer therapy: Cohort B comprised 73 patients (mean age at treatment=49.6±10.8 years) with European, African, or Asian ancestry, enrolled from breast cancer clinics as part of a prospective study of who developed CCM during treatment; Cohort C comprised 41 pediatric patients with newly diagnosed acute myelogenous leukemia (mean age at treatment=10.8±5.6 years) of diverse ancestries. Although individual treatments varied, 90% of all patients with CCM received anthracycline and 33% of adults received trastuzumab. After normalizing anthracycline doses27 the cumulative equivalent dose was <400 mg/m² in 93.9% of patients in cohort A, 100% of patients in cohort B, and 2.3% of patients in cohort C.

We assessed clinical risk factors for CCM in these cohorts. Seventy-six percent of all patients were CCM females, predominantly treated for breast cancer. In cohorts A and B the prevalence of cigarette smoking, hypertension, and diabetes mellitus was comparable (P=not significant) to that of the general US population,28 but hypercholesterolemia in patients with cancer was less common (P=3.0e–09). Three patients in cohort A, without prechemotherapy imaging studies, had family histories of cardiomyopathy of unknown cause. Patients in cohort C were considerably younger (mean age=10.8±5.6 years), without cardiovascular risk factors, and all had normal LVEF at study entry.

The median time after the initiation of cancer treatment to CCM diagnosis in cohort A was 3.0 (range=1–9) years, but 0.3 to 0.7 years for cohorts B and C because of cardiac surveillance during treatment in these 2 cohorts. At CCM diagnosis, the mean LVEF decrease was 23.4±9.2% in cohort A, 13.5±3.3% in cohort B, and 19.7±6.0% in cohort C. Across all cohorts, treatment with high cumulative anthracycline dose (>400 mg/m²) was not associated with poorer left ventricular (LV) dysfunction at CCM diagnosis (mean LVEF=42.0±9.6%). Patients (cohorts A and B) who received trastuzumab without anthracycline had similar cardiovascular risk factors and no significant differences in either baseline or postchemotherapeutic LVEF (mean LVEF decrease=13.9±3.6%) in comparison with patients receiving anthracyclines with or without other agents (mean LVEF decrease=16.7±7.5%). Cardiac recovery occurred in approximately half of patients with CCM from each cohort, but 9% of patients in cohort A underwent cardiac transplantation. Cardiac deaths occurred in 3% of patients in cohort A and in 5% of patients in cohort C.

RESULTS

Patients With CCM

We studied 3 CCM cohorts (Table 1). Cohort A includes 99 patients of European ancestry with hematologic, breast, or other solid-tumor cancer (mean age at treatment=48.7±17.1 years), recruited from heart failure and
To Table II in the online-only Data Supplement), healthy

Pbreast or lung TCGA participants (combined,

with CCM (n=16; 7.5%) in comparison with unselected

ly increased. TTNtvs were highly enriched in all patients

TTN only variants in

volunteers (P=3.90e–05), and reference populations

tering variants across all 9 genes was significantly higher

motherapeutic agent. The prevalence of rare protein-al-

expect that most TCGA participants received this che-

TCGA24 (Table 2). Because anthracyclines are highly ef-

volunteers, and all breast and lung cancer participants in

2019;140:31–41. DOI: 10.1161/CIRCULATIONAHA.118.037934 July 2, 2019 35

are shown in bold. CCM indicates cancer therapy-induced cardiomyopathy; and DCM, dilated cardiomyopathy.

The number of subjects with variants in each gene is noted in the table, and percentages are noted in parentheses (%). Types of variants analyzed for each
gene: all protein-altering variants (BAG3, LMNA, TAPC, TNN1, TNN2), missense variants and in-frame deletion or insertion only (MYH7), frameshift variant, stop-
gained, splice-donor, and splice-acceptor variants only (DSP, SCNA, TTN). P-values were calculated via Fisher exact test for all CCM vs breast and lung cancer TCGA
participants (TCGA) and healthy volunteers (HVOL) and binomial test for all CCM vs gnomAD.23 Bonferroni corrections are provided in parentheses. Significant values
are shown in bold. CCM indicates cancer therapy-induced cardiomyopathy; and DCM, dilated cardiomyopathy.

*TCGA denotes all breast and lung cancer participants (n=2053) in The Cancer Genome Atlas.24

‖HVOL denotes 445 healthy volunteers without cardiovascular disease based on detailed evaluations.

§NFE denotes non-Finnish Europeans ancestries. The subanalyses of NFE patients with CCM are compared with NFE gnomAD reference population n=113,482.

†gnomAD denotes reference population with African American, non-Finnish European, and East Asian ancestries.

| Gene | Cohort A (n=99) | Cohort B (n=73) | Cohort C (n=41) | All CCM (n=213) | TCGA* Breast/ Lung (n=2053) | HVOL† (n=445) | P Values Comparisons of
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BAG3</td>
<td>1 (1.0%)</td>
<td>2 (2.7%)</td>
<td>0 (0.0%)</td>
<td>3 (1.4%)</td>
<td>18 (0.9%)</td>
<td>4 (0.9%)</td>
<td>0.44 (1)</td>
</tr>
<tr>
<td>DSP</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>4 (0.2%)</td>
<td>0 (0.0%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>LMNA</td>
<td>0 (0.0%)</td>
<td>1 (1.4%)</td>
<td>1 (0.5%)</td>
<td>0 (0.0%)</td>
<td>16 (0.8%)</td>
<td>2 (0.4%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>MYH7</td>
<td>3 (3.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>3 (1.4%)</td>
<td>35 (1.7%)</td>
<td>5 (1.1%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>SCNA</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>3 (0.2%)</td>
<td>1 (0.2%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>TAPC</td>
<td>1 (1.0%)</td>
<td>0 (0.0%)</td>
<td>1 (2.4%)</td>
<td>0 (0.0%)</td>
<td>2 (0.9%)</td>
<td>0 (0.0%)</td>
<td>0.05 (0.45)</td>
</tr>
<tr>
<td>TNN1</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (0.1%)</td>
<td>0 (0.0%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>TNN2</td>
<td>0 (0.0%)</td>
<td>1 (1.4%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (0.5%)</td>
<td>7 (3.0%)</td>
<td>0.55 (1)</td>
</tr>
<tr>
<td>TTN</td>
<td>10 (10.0%)</td>
<td>4 (5.5%)</td>
<td>2 (4.9%)</td>
<td>16 (7.5%)</td>
<td>22 (1.1%)</td>
<td>3 (0.7%)</td>
<td>7.36e–08 (6.62e–07)</td>
</tr>
<tr>
<td>8 genes (no TTN)</td>
<td>5 (5.1%)</td>
<td>4 (5.5%)</td>
<td>1 (2.4%)</td>
<td>10 (4.7%)</td>
<td>86 (4.2%)</td>
<td>12 (2.7%)</td>
<td>0.72</td>
</tr>
<tr>
<td>9 genes</td>
<td>15 (15.1%)</td>
<td>8 (11%)</td>
<td>3 (7.3%)</td>
<td>26 (12.2%)</td>
<td>108 (5.3%)</td>
<td>15 (3.4%)</td>
<td>1.98e–04</td>
</tr>
</tbody>
</table>

The number of subjects with variants in each gene is noted in the table, and percentages are noted in parentheses (%). Types of variants analyzed for each
gene: all protein-altering variants (BAG3, LMNA, TAPC, TNN1, TNN2), missense variants and in-frame deletion or insertion only (MYH7), frameshift variant, stop-
gained, splice-donor, and splice-acceptor variants only (DSP, SCNA, TTN). P-values were calculated via Fisher exact test for all CCM vs breast and lung cancer TCGA
participants (TCGA) and healthy volunteers (HVOL) and binomial test for all CCM vs gnomAD.23 Bonferroni corrections are provided in parentheses. Significant values
are shown in bold. CCM indicates cancer therapy-induced cardiomyopathy; and DCM, dilated cardiomyopathy.

*TCGA denotes all breast and lung cancer participants (n=2053) in The Cancer Genome Atlas.24

†HVOL denotes 445 healthy volunteers without cardiovascular disease based on detailed evaluations.

§NFE denotes non-Finnish Europeans ancestries. The subanalyses of NFE patients with CCM are compared with NFE gnomAD reference population n=113,482.

†gnomAD denotes reference population with African American, non-Finnish European, and East Asian ancestries.

these prespecified genes, we examined rare variants (de-

fined as minor allele frequency <1.0e–4) among ancestry-
matched reference populations;25 CCM cohorts, healthy

volunteers, and all breast and lung cancer participants in

TCGA;24 (Table 2). Because anthracyclines are highly ef-

efective and widely used to treat these malignancies,6 we

expect that most TCGA participants received this che-

motherapeutic agent. The prevalence of rare protein-al-

tering variants across all 9 genes was significantly higher

in a combined CCM cohort than in unselected lung and

breast cancer TCGA participants (P=1.98e–04), healthy

volunteers (P=3.90e–05), and reference populations

(P=1.78e–06). Although patients with CCM had rare vari-

ants in several established dilated cardiomyopathy (DCM)
genes (BAG3, LMNA, MYH7, TAPC, TNN1, and TTN),

only variants in TTN, which encodes titin, were signifi-

cantly increased. TTNtvs were highly enriched in all patients

with CCM (n=16; 7.5%) in comparison with unselected

breast or lung TCGA participants (combined, P=7.36e–08

and Table I in the online-only Data Supplement), healthy

volunteers (P=3.42e–06), and the reference population

(P=5.87e–14). Subanalyses of patients with CCM and the

reference population stratified by ancestry (Table II in the

online-only Data Supplement), although limited by small

numbers, confirmed the observed enrichment of TTNtvs

in all patients with CCM. TTNtvs that are significantly

increased in patients with DCM,30 reside in exons that

are highly expressed in LV tissues, especially those that

encode the A-band and distal I-band.31 TTNtvs identified

in patients with CCM shared these characteristics (Table 3).

We extended these analyses to include 40 other genes

that have been implicated in cardiomyopathies.29 Vari-

ants in these genes account for a very small fraction of

unselected patients with cardiomyopathy. There was no

significant difference in the prevalence of all rare protein-

altering variations (minor allele frequency <1.0e–4; Tables

III and IV in the online-only Data Supplement) or variants

predicted as damaging (Tables V and VI in the online-only

Data Supplement) in patients with CCM in each cohort or

the combined CCM cohort, in comparison with healthy

volunteers or in the reference population. For individual

genes, the prevalence of rare variants was nominally in-

creased only in FKR (encoding fukutin-related protein); re-

cessive FKR mutations cause several forms of muscu-

lar dystrophies with cardiac involvement.32

Clinical Outcomes in Adult Patients Who Have CCM With or Without TTNtvs

Patients with CCM in cohorts A and B were predomin-

antly women (81%), with breast cancer (73%), with

traditional cardiovascular risk factors, who received
anthracyclines (86.6%) or trastuzumab (33%), and with follow-up between 8.4 months and 18 years (Table 1). We defined the clinical courses among patients who have CCM with TTNtv and compared risk factors for CCM and outcomes among patients with and without TTNtv (Table 4 and Tables VII through IX in the online-only Data Supplement). At diagnosis of CCM, the mean LVEF of patients with (34.9±7.4) and without TTNtv (36.8±9.5; P=not significant) were comparable; however, patients with TTNtv had more heart failure hospitalizations and atrial fibrillation (P=0.003 for each) than those without TTNtv. Recovery occurred in both groups, although the final mean LVEF was more depressed in patients with TTNtv (39.6±14.2 versus 48.9±10.8; P=0.03).

Modeling CCM in TTNtv Mice

Given the multiple variables that can influence cardiotoxicity in human patients, we assessed whether TTNtv increased susceptibility to anthracycline-induced cardiomyopathy in an experimental model. Doxorubicin was administered (3 doses of 5 mg/kg at weekly intervals; cumulative=45 mg/m²) to genetically identical mice, with the exception of the absence (wild-type) or presence (Ttntv/+) of a heterozygous A-band titin truncation in one gene copy. Untreated Ttntv/+ mice have normal LV function (not significantly different from wild-type mice) and anthracycline administration comparably depressed LV function in both genotypes at week 4 after treatments (Figure). However, at week 8, LV function recovered to baseline in wild-type mice but remained depressed in mice with TTNtv.

Table 3. Summary of TTNtv Identified From All 3 Cohorts With CCM

<table>
<thead>
<tr>
<th>Variant</th>
<th>Impact</th>
<th>TTN Band</th>
<th>Affected Exon</th>
<th>PSI of Affected Exon</th>
<th>Cancer Type</th>
<th>Chemotherapy</th>
<th>LVEF at CCM (Change)</th>
<th>Follow-Up, y</th>
<th>Adverse Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr2:179399704 c.101638G&gt;T</td>
<td>Nonsense (p.Glu33880X)</td>
<td>M</td>
<td>359</td>
<td>1</td>
<td>Breast</td>
<td>Epirubicin</td>
<td>30% (NA)</td>
<td>14</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179400742 c.100731dupA</td>
<td>Frameshift (p.Ser33578IlefsTer15)</td>
<td>A</td>
<td>358</td>
<td>0.99</td>
<td>Breast</td>
<td>Doxorubicin + Trastuzumab</td>
<td>37% (24%)</td>
<td>4</td>
<td>VT, HF</td>
</tr>
<tr>
<td>chr2:179410112 c.95722+2delT</td>
<td>Splicing</td>
<td>A</td>
<td>345</td>
<td>0.99</td>
<td>Endometrial</td>
<td>Doxorubicin</td>
<td>20% (NA)</td>
<td>5</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179414849 c.91715dupA</td>
<td>Frameshift (p.Asn30572fs)</td>
<td>A</td>
<td>338</td>
<td>0.98</td>
<td>Breast</td>
<td>Epirubicin</td>
<td>42% (NA)</td>
<td>5</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179422284 c.60512-2A&gt;C</td>
<td>Splicing</td>
<td>A</td>
<td>330</td>
<td>0.96</td>
<td>Breast</td>
<td>Doxorubicin + Trastuzumab</td>
<td>43% (17%)</td>
<td>2.2</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179425091 c.85768C&gt;T</td>
<td>Nonsense (p.Arg28590X)</td>
<td>A</td>
<td>327</td>
<td>0.95</td>
<td>Breast</td>
<td>Doxorubicin</td>
<td>35% (NA)</td>
<td>2</td>
<td>HF, VT, transplantation</td>
</tr>
<tr>
<td>chr2:179428124 c.82734dupA</td>
<td>Frameshift (p.Val27579SerfsTer15)</td>
<td>A</td>
<td>327</td>
<td>0.95</td>
<td>Non-Hodgkin lymphoma</td>
<td>Doxorubicin</td>
<td>29% (NA)</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179432234 c.78625G&gt;T</td>
<td>Nonsense (p.Glu26209X)</td>
<td>A</td>
<td>327</td>
<td>0.95</td>
<td>Non-Hodgkin lymphoma</td>
<td>Doxorubicin</td>
<td>34% (NA)</td>
<td>3</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179435679 c.75179delA</td>
<td>Frameshift (p.Asp22081MetfsTer31)</td>
<td>A</td>
<td>316</td>
<td>0.86</td>
<td>Bone sarcoma</td>
<td>Doxorubicin</td>
<td>25% (NA)</td>
<td>6</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179441250 c.69715+6T&gt;C</td>
<td>Splicing</td>
<td>A</td>
<td>326</td>
<td>0.95</td>
<td>Breast</td>
<td>Trastuzumab</td>
<td>38% (12%)</td>
<td>0.4</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179446855 c.66240delA</td>
<td>Frameshift (p.Asp2081MetfsTer31)</td>
<td>A</td>
<td>316</td>
<td>0.86</td>
<td>Bone sarcoma</td>
<td>Doxorubicin</td>
<td>25% (NA)</td>
<td>6</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179448325 c.63096delT</td>
<td>Frameshift (p.His21032fs)</td>
<td>A</td>
<td>305</td>
<td>0.97</td>
<td>AML</td>
<td>Daunorubicin+ Mitoxantrone</td>
<td>36% (22%)</td>
<td>2.8</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179453355 c.63096delT</td>
<td>Frameshift (p.Asp21032fs)</td>
<td>A</td>
<td>263</td>
<td>0.89</td>
<td>AML</td>
<td>Daunorubicin+ Mitoxantrone</td>
<td>50% (18%)</td>
<td>0.8</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179478777 c.49345+2T&gt;C</td>
<td>Splicing</td>
<td>A</td>
<td>102</td>
<td>0.88</td>
<td>Breast</td>
<td>Doxorubicin</td>
<td>35% (15%)</td>
<td>1.6</td>
<td>None</td>
</tr>
<tr>
<td>chr2:179571683 c.29042-2A&gt;C</td>
<td>Splicing</td>
<td>I</td>
<td>49</td>
<td>0.95</td>
<td>Breast</td>
<td>Doxorubicin</td>
<td>31% (30%)</td>
<td>6</td>
<td>HF</td>
</tr>
<tr>
<td>chr2:179604819 c.13141G&gt;T</td>
<td>Nonsense (p.Glu4381X)</td>
<td>I</td>
<td>41</td>
<td>1</td>
<td>Breast</td>
<td>Epirubicin</td>
<td>45% (NA)</td>
<td>6</td>
<td>HF</td>
</tr>
</tbody>
</table>

Variants are defined based on the meta-transcript (LRG_391_t1 / ENST00000589042) that incorporates all exons in described TTN isoforms (including fetal and noncardiac isoforms) with the exception of exons that are unique to the novex transcripts.

AML indicates acute myeloid leukemia; CCM, cancer therapy–induced cardiomyopathy; HF, heart failure hospitalization; LVEF, left ventricular ejection fraction; NA, not available; PSI, proportions spliced in; TTNtv, titin-truncating variant; and VT, ventricular tachycardia.
Table 4. Comparisons of Risk Factors and Outcomes in Adult Patient Who Has CCM With and Without TTNtv

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>TTNtv (n=14)</th>
<th>Non-TTNtv (n=158)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>2 (14.3)</td>
<td>31 (19.6)</td>
<td>1</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current or past smokers</td>
<td>3 (21.4)</td>
<td>55 (34.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (28.6)</td>
<td>54 (34.2)</td>
<td>0.78</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>6 (42.9)</td>
<td>38 (24.1)</td>
<td>0.20</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (14.3)</td>
<td>25 (15.8)</td>
<td>1</td>
</tr>
<tr>
<td>Oncological treatments, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin ± other</td>
<td>13 (92.9)</td>
<td>136 (86.1)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

At CCM diagnosis

| LVEF at CCM diagnosis, %                        | 34.9±7.4     | 36.8±9.5          | 0.38    |
| NYHA functional class III–IV, n (%)            | 6 (42.9)     | 39 (24.7)         | 0.2     |

Follow-up

| Last LVEF, %                                   | 39.6±14.2    | 48.9±10.8         | 0.03†   |
| LVEF recovery, n (%)                           | 6 (42.9)     | 77 (48.7)         | 0.78    |
| On neurohormonal blockers, t n (%)            | 10 (71.4)    | 121 (76.6)        | 0.74    |
| Atrial fibrillation, n (%)                    | 5 (35.7)     | 10 (6.3)          | 0.003†  |
| Heart failure-related hospitalization, n (%)  | 9 (64.3)     | 39 (24.7)         | 0.003†  |
| Cardiac transplantation, n (%)                | 2 (14.3)     | 7 (4.4)           | 0.16    |
| Aborted sudden cardiac death, n (%)           | 1 (7.1)      | 6 (3.8)           | 0.45    |
| Cardiac death, n (%)                          | 0 (0.0)      | 3 (1.9)           | 1       |
| Death from all cause, n (%)                   | 0 (0.0)      | 5 (3.2)           | 1       |

CCM indicates cancer therapy–induced cardiomyopathy; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; and TTNtv, titin-truncating variant.

*P values were calculated using the Fisher exact test for categorical variables and the Welch t test for continuous variables.
†Number of patients who were taking at least one of β-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, or mineralocorticoid receptor antagonists. No data were available to review for 1 of the patients with TTNtv.
‡Significant values.

CCM: anthracycline-treated Ttntv/+ mice and isolated cardiomyocytes had protracted LV and cellular dysfunction in comparison with wild-type.

Clinical outcomes among patients with CCM showed considerable variability, but cardiac function improved in 45% to 68% of adult and pediatric patients. Recovery occurred in 83 adults and 28 children, and was not significantly correlated (P≥0.5) with preexisting cardiovascular risk factors in adults, TTNtv, high (>400 mg/m²) anthracycline dose, or trastuzumab therapy. However, adult patients who have CCM with TTNtv had more heart failure hospitalizations and atrial fibrillation, as occurs in patients with DCM caused by TTNtv, and cardiac function was worse in patients than in patients without these variants.

In addition to TTNtv, our analyses identified rare protein-altering variants in 5 genes previously studied in patients with DCM. Mutations in BAG3, LMNA, MYH7, and TNNT2 are established autosomal dominant causes of DCM. TCAP mutations are occasionally identified in patients with DCM, but more commonly cause a recessive form of limb-girdle muscular dystrophy. Despite the low prevalence of variants in these genes across all CCM cohorts (4.7%), their critical roles in myocyte biology imply that variants identified here may contribute to an individual's risk for CCM.

The increased burden of rare variants, including TTNtv, indicate that genetics is an important component in CCM susceptibility and adverse outcomes. We demonstrate that genetics is associated with CCM susceptibility across different cancer types and treatment regimens, in particular, those including anthracycline and trastuzumab (Table 1). Genetic variants in previously identified cardiomyopathy genes were increased among adult cancer survivors with overt CCM and severe clinical courses, and among prospectively studied adult and pediatric patients with mild CCM identified during ongoing cancer treatment. It is notable that heart failure, cardiac transplantation, aborted sudden death, and cardiac death occurred years after completion of chemotherapy regimens in some patients with CCM (Table 4), an observation that underscores the controls. Although the majority of the patients with CCM have European ancestry, the frequency of cardiomyopathy variants in other patients with CCM who have other ancestries was not significantly different (Table II in the online-only Data Supplement). TTNtv were identified in 16 of 213 CCM cases (7.5%), a considerably higher prevalence than in unselected breast and lung cancer TCGA participants (1.1%, P=7.36e–08) or healthy volunteers (0.7%, P=3.42e–06) and enriched in comparison with ancestry-matched reference populations (P=5.87e–14). Because cardiac status is not recorded for TCGA participants, these data provide conservative estimates of the burden of TTNtv in CCM. Further support that TTNtv contribute to CCM is derived from a mouse model of CCM: anthracycline-treated Ttnvtv/+ mice and isolated cardiomyocytes showed no significant increase in fibrosis or apoptosis in comparison with wild-type.

**DISCUSSION**

We demonstrate an increased prevalence of DCM-associated gene variants, predominantly TTNtv, in adult patients who have cancer and pediatric patients who have acute myelogenous leukemia with CCM relative to depressed through week 12 in Ttnvtv/+ mice (P=0.0004 versus wild-type). Functional analyses in isolated cardiomyocytes confirmed that LV dysfunction reflected cell autonomous effects of anthracyclines (Figure B). Histological analysis of cardiac tissues from anthracycline-treated wild-type or Ttnvtv/+ mice were comparable and showed no significant increase in fibrosis or apoptosis in comparison with untreated mice.
need for continued cardiac surveillance in patients with CCM.

These data establish a genetic relationship between DCM and CCM. Cardiomyopathy variants were found in 12.2% of patients with CCM (Table 2), whereas these occur in ≈40% of patients with familial and sporadic DCM.19,29,30 Whether broader genomic analyses may uncover additional genetic contributors to CCM is worthy of study. TTNtv are significantly prominent in DCM, occurring in 15% of ambulatory and 25% of end-stage patients,29,31,34,38 but are rarely identified in CCM. TTNtv are ≈15% in the A-band (Table 3). TTNtv also occur in patients with CCM, like those in patients with DCM, disrupted exons that are constitutively expressed in the heart and are overrepresented in the A-band (Table 3). TTNtv also occur in ≈15% of patients with peripartum cardiomyopathy21 and in ≈10% of individuals with alcoholic cardiomyopathy.42 Findings that imply additional cardiovascular stress can unmask the deleterious cardiac effects of TTNtv. Consistent with this supposition, in vitro analyses of human isogenic cardiomyocytes (derived from induced pluripotent stem cells) demonstrate that titin provides an essential mechanical connection that propagates diastolic traction stresses from β-cardiac myosin during sarcomere formation. Cardiomyocytes with TTNtv have diminished reassembly of sarcomeres after stress in comparison with cells without TTNtv.43 We suggest that chemotherapy, like pregnancy and excessive alcohol, is an important provocation that is poorly tolerated by TTNtv, a conclusion that is supported both by these human data and by analyses of anthracycline-treated TTNtv mice.

We recognize several limitations in this study. Given the demographic profiles of the cohorts studied here, further analyses of patients with diverse ancestries are needed. Cohort A was retrospectively recruited to WT and Ttnm/+ mice (n=15 per genotype) in 3 successive weekly doses (cumulative dose=45 mg/m²). Serial echocardiograms showed persistent significantly depressed systolic function (mean fractional shortening ±SD) in Ttnm/+ in comparison with WT mice (P=0.0004). B, Isolated cardiomyocytes (n=52 per group) were studied 12 weeks after initial doxorubicin injection. Cardiomyocytes from doxorubicin-treated Ttnm/+ mice had significantly depressed contractility (P=0.002) in comparison with cardiomyocytes from doxorubicin-treated WT mice or untreated mice. NS indicates not significant.

Figure. Persistent cardiac dysfunction in Ttnm/+ mice after anthracycline treatment. A, Untreated Ttnm/+ mice have left ventricular function comparable with wild-type (WT) mice. Intrapertoneal doxorubicin (5mg/kg) was administered (arrows) to WT and Ttnm/+ mice (n=15 per genotype) in 3 successive weekly doses (cumulative dose=45 mg/m²). Serial echocardiograms showed persistent significantly depressed systolic function (mean fractional shortening ±SD) in Ttnm/+ in comparison with WT mice (P=0.0004). B, Isolated cardiomyocytes (n=52 per group) were studied 12 weeks after initial doxorubicin injection. Cardiomyocytes from doxorubicin-treated Ttnm/+ mice had significantly depressed contractility (P=0.002) in comparison with cardiomyocytes from doxorubicin-treated WT mice or untreated mice. NS indicates not significant.

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can optimize cancer and cardiovascular treatments to reduce CCM while providing effective cancer therapy.

ARTICLE INFORMATION

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Disclosures

Drs C. E. and J. G. Seidman are founders and owners shares in Myokardia Inc, a startup company that is developing therapeutics that target the sarcosome. James S. Ware receives grant support and honoraria from Myokardia. Myokardia had no involvement in this study. The authors report no conflicts.

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