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PRINCIPAL INVESTIGATOR: Zoltan Arany, MD PHD

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14. ABSTRACT

Peripartum cardiomyopathy (PPCM) is marked by loss of cardiac contractile function in women late in pregnancy or soon after delivery. PPCM affects approximately 1:1000 births worldwide, and frequently leads to chronic heart failure, cardiac transplantation, or death. Our proposal is focused on understanding how mutations in TTN predispose to PPCM.

Aim 1: Human genetics: we have collated a cohort of >600 clinically annotated women with PPCM, and have performed deep sequencing of the TTN gene, followed by detailed phenotype/genotype correlations, including evaluation of correlation between TTN mutations and severity of disease on presentation, and recovery from PPCM. We will be submitting a manuscript describing this work in the next few weeks. We have also analyzed the UPenn and Geisinger Biobank data sets of >70,000 exomes for TTN truncating variants, and concluded that surprisingly large number of people carry these variants without having PPCM or other dilated cardiomyopathy. This work was published in Circulation in April of last year.

Aim 2: Mouse genetics: we have generated mice genetically modified to encode mutations in *ttn* that precisely match those found in three women with PPCM. Baseline phenotyping revealed little baseline dysfunction. Initial data with cardiotoxic stressors such as chemotoxic agents has revealed surprisingly little effect, compared to control animals. On the other hand, preliminary data suggests that repeated pregnancies (n=five) do worsen function in these animals, compared to controls. We are actively pursuing these results.

Aim 3: Cellular studies: we have generated iPSCs bearing the same TTN mutations as in mice above, and are actively evaluating the biomechanical and metabolic consequences. We have also harvested adult human cardiomyocytes from post-transplant hearts of patients bearing TTN truncating variants (n=4 so far) and compared their biomechanical phenotype compared to patients with cardiomyopathy without TTN variants, and to nonfailing donor subjects. We have found surprisingly that TTNtv-bearing cells contract nearly as well as cells from nonfailing controls, and clearly better than cells from other failing hearts. This work is being submitted for publication.

15. SUBJECT TERMS

Peripartum Cardiomyopathy, Human genetics, Women's cardiovascular disease

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INTRODUCTION

Peripartum Cardiomyopathy (PPCM) is a disease characterized by systolic heart failure presenting in the last month of pregnancy or the 1st five months postpartum. It affects anywhere from 1:300 to 1:3000 births, with certain geographic “hot spots” like Haiti.¹⁻⁴ In about half of the cases, heart failure persists, and often leads to cardiac transplantation or death. PPCM accounts for >50% of cases of maternal cardiogenic shock in the US⁵, and has become the leading cause of maternal peripartum death.^{4,6,7} The disease affects all socioeconomic and racial sectors, but is more common and bears significantly worse prognosis in women of African descent^{8,9}, a group that is also more highly represented in the US Armed Forces. Mortality rates are as high as 25% at 5 years in African American women in the US.⁴ The consequences of PPCM are thus often devastating for otherwise healthy young women and their newborn infants. The precise cause of PPCM remains elusive. There is limited ongoing research into the mechanisms underlying PPCM, and almost none in the US. Advocacy for PPCM has been poor, limiting public awareness and funding support. Recent work from our lab and others in Germany, however, has uncovered the novel notion that PPCM is a vascular disease.¹⁰⁻¹² And most recently, we have made the additional breakthrough observation that many women with PPCM bear mutations in the large sarcomeric protein titin (the gene *TTN*).¹³ These observations have raised considerable excitement about potential novel ways to diagnose, prognose, and treat PPCM. The paradigm-shifting finding that women with PPCM frequently bear mutations in *TTN* has led to key unanswered questions:

Clinically: Does the presence of *TTN* mutations in women with PPCM predict severity of disease and/or clinical outcome? Based on *post-hoc* analyses of our initial study, we **hypothesize that the presence of *TTN* mutations correlates with diminished cardiac recovery and higher risk of recurrence with subsequent pregnancy.**

Mechanistically: How do mutations in *TTN* affect cardiomyocyte function and interact with pregnancy to cause PPCM? **We hypothesize that *TTN* mutations reduce the metabolic efficiency of contraction/relaxation, leading to contractile defects that become pathogenic in the metabolically vulnerable context of late pregnancy.**

Guided by these hypotheses, we proposed to take a comprehensive functional genomics approach to investigate in depth the contribution of *TTN* mutations to PPCM, including clinical, pre-clinical, and cellular studies:

Aim 1: Test in humans if the presence of *TTN* mutations predicts clinical outcomes.

Aim 2: Test in mouse models if and how *TTN* truncating variants predispose to PPCM.

Aim 3: Test in cell culture if and how *TTN* variants cause contractile and metabolic insufficiency.

KEYWORDS

Peripartum cardiomyopathy, heart failure, titin, TTN, pregnancy, women, vascular

KEY RESEARCH ACCOMPLISHMENTS

Aim 1: During the current period, we have continued to expand our multi-center PPCM Collaborative Network, and have assembled now more than 600 cases of PPCM, including blood samples and comprehensive clinical annotations. We have performed extended sequencing of these samples, in which genomic DNA is sheared, and individually bar-coded libraries are generated, amplified, and enriched by bead-based hybridization capture with custom capture reagent (Sure Select Target Enrichment, Agilent Technologies) containing ~50 genes of interest. Eluted DNA is amplified and pooled for paired-end NextGen massive parallel sequencing (NextSeq, Illumina). We have identified a number of key observations. First, we have identified a ~10% prevalence of *TTN* truncating variants in this cohort, ~20x excess over a population control, and in keeping with our prior results. In addition, we have identified a number of additional loss-of-function mutations in genes other than *TTN*, including *FLNC* and *VCL*, which are statistically overrepresented in our cohort, and thus likely pathogenic. Finally, extensive genotype/phenotype correlations focusing on the *TTN*tv variants has revealed that women bearing *TTN*tv present with significantly lower left ventricle ejection fractions (LVEF) than do women without *TTN*tv. On the other hand, recovery rates do not appear to differ between genotype groups. We also find no racial differences, or differences in time from delivery to clinical presentation. This work is under preparation for submission for publication in the next few weeks.

In parallel work, we leveraged our large cohort of women with PPCM at the University of Pennsylvania to query if preeclampsia, and/or delay of diagnosis after delivery, impacted clinical outcomes. We found that preeclampsia had little bearing on recovery rates, but that delay of diagnosis had a strong impact, explaining a significant proportion of racial disparities in outcomes. This work was published in Hypertension in January of this year (see Appendix).

Aim 2: We have used CRISPR/Cas9 to generate 3 knock-in mice bearing *TTN* mutations identical to those identified in women with PPCM. Baseline phenotyping has revealed little baseline dysfunction, as measured by echocardiography and classic morphometric measurements. We proceeded to challenge the mice with various stressors, including chemotoxic agents, because we had found, in work with Drs. Jon and Cricket Seidman, that women with breast cancer and with *TTN* truncating variants were also predisposed to develop chemotherapy-induced cardiomyopathy. Interestingly, the knock-in mice bearing *TTN* mutations were overly sensitive to treatment with doxorubicin, demonstrating synergy between these genetic and environmental factors in vivo, but the mice were not sensitized to bortezomib, a proteasome inhibitor often used in the treatment of breast cancer. Part of this work was published in Circulation in July of last year¹⁵ (see Appendix). In addition, in preliminary data, we find that repeated pregnancies (five) cause more cardiac dysfunction in mice bearing *TTN* truncating variants than in control mice, consistent with a model of PPCM. We are actively further characterizing these observations, and preparing to test the role of sFLT1 in the process.

Aim 3: We have generated iPSCs bearing the same *TTN* mutations as in mice above, and have evaluated the biomechanical and contractile consequences. We worked with Drs. Ken Margulies and Ben Prosser to harvest adult human cardiomyocytes from post-transplant hearts of patients bearing *TTN* truncating variants (n=4) and compared their biomechanical phenotype compared to patients with cardiomyopathy without *TTN* variants, and to nonfailing donor subjects. This exciting work yielded the surprising observation that cells bearing *TTN* truncating variants derived from failing hearts performed in isolated contractile assays nearly as well as cells from nonfailing hearts, and significantly better than cells from failing hearts that did not bear *TTN* variants. This surprising observation suggests both a fundamental underlying difference between *TTN* variant positive and negative failing hearts, and that in hearts with *TTN* variants non-cell autonomous factors (e.g. preload or afterload, or extracellular milieu) play a

critical role in determining heart failure. We have submitted a manuscript that includes these results for publication to Science Translational Medicine.

REPORTABLE OUTCOMES

Importance of Early Diagnosis in Peripartum Cardiomyopathy.

Lewey Jennifer, Levine Lisa D, Elovitz Michal A, Irizarry Olga C, **Arany Zoltan**

Hypertension. 75(1): 91-97, Jan 2020.

Genomics-First Evaluation of Heart Disease Associated With **Titin**-Truncating Variants.

Haggerty CM, Damrauer SM, Levin MG, Birtwell D, Carey DJ, Golden AM, Hartzel DN, Hu Y, Judy R, Kelly MA, Kember RL, Lester Kirchner H, Leader JB, Liang L, McDermott-Roe C, Babu A, Morley M, Nealy Z, Person TN, Pulenthiran A, Small A, Smelser DT, Stahl RC, Sturm AC, Williams H, Baras A, Margulies KB, Cappola TP, Dewey FE, Verma A, Zhang X, Correa A, Hall ME, Wilson JG, Ritchie MD, Rader DJ, Murray MF, Fornwalt BK, **Arany Z**.

Circulation. 2019 Jul 2;140(1):42-54. doi: 10.1161/CIRCULATIONAHA.119.039573. Epub 2019 Jun 20. PMID: 31216868

Genetic Variants Associated With Cancer Therapy-Induced Cardiomyopathy.

Garcia-Pavia P, Kim Y, Restrepo-Cordoba MA, Lunde IG, Wakimoto H, Smith AM, Toepfer CN, Getz K, Gorham J, Patel P, Ito K, Willcox JA, **Arany Z**, Li J, Owens AT, Govind R, Nuñez B, Mazaika E, Bayes-Genis A, Walsh R, Finkelman B, Lupon J, Whiffin N, Serrano I, Midwinter W, Wilk A, Bardaji A, Ingold N, Buchan R, Tayal U, Pascual-Figal DA, de Marvao A, Ahmad M, Garcia-Pinilla JM, Pantazis A, Dominguez F, John Baksi A, O'Regan DP, Rosen SD, Prasad SK, Lara-Pezzi E, Provencio M, Lyon AR, Alonso-Pulpon L, Cook SA, DePalma SR, Barton PJR, Aplenc R, Seidman JG, Ky B, Ware JS, Seidman CE.

Circulation. 2019 Jul 2;140(1):31-41. doi: 10.1161/CIRCULATIONAHA.118.037934. Epub 2019 Apr 16. PMID: 30987448

CONCLUSION

In summary, our project continues to advance rapidly and successfully on all three aims. The work is revealing important phenotype/genotype relationships, and has already contributed to three major publications in *Circulation* and *Hypertension*, with two additional manuscripts either submitted or soon-to-be submitted. Success in our studies will provide deep mechanistic insight into PPCM, a poorly understood disease, and will open novel therapeutic opportunities for a disease that currently has no specific treatment.

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15. Garcia-Pavia, P., *et al.* Genetic Variants Associated With Cancer Therapy-Induced Cardiomyopathy. *Circulation* **140**, 31-41 (2019).

Importance of Early Diagnosis in Peripartum Cardiomyopathy

Jennifer Lewey, Lisa D. Levine, Michal A. Elovitz, Olga C. Irizarry, Zoltan Arany

See Editorial, pp 33–34

Abstract—Peripartum cardiomyopathy (PPCM) can lead to long-term systolic dysfunction, especially among black women. Hypertensive disorders of pregnancy (HDP) are the strongest risk factor for PPCM, but controversy remains on whether HDP predict a favorable outcome. Women with HDP are also often diagnosed with PPCM earlier than those without HDP. Our objective is to determine recovery of systolic function in patients with PPCM stratified by HDP, timing of diagnosis, and race. We conducted a retrospective cohort study of 220 patients (55% black) diagnosed with PPCM. Patients with PPCM and HDP were diagnosed earlier postpartum than patients without HDP ($P=0.013$), an effect that was most pronounced in nonblack patients. Rates of left ventricular ejection fraction (LVEF) recovery were similar among PPCM patients with and without HDP (68.4% versus 62.6%, $P=0.425$). In contrast, patients with PPCM diagnosed after 1-month postpartum had lower rates of LVEF recovery than patients diagnosed <1-month postpartum (53.7% versus 69.9%, $P=0.035$). LVEF at time of diagnosis is a strong predictor of LVEF recovery, and patients with PPCM diagnosed after 1-month postpartum had lower baseline LVEF compared to patients presenting earlier ($P=0.041$). The presence of HDP does not correlate with LVEF recovery in our racially diverse PPCM cohort. In contrast, early diagnosis portends a favorable outcome. Early diagnosis is associated with higher LVEF at presentation, likely explaining the improved outcomes in these women. These findings underscore the need for early monitoring and diagnosis, especially in at-risk and underserved populations. (*Hypertension*. 2020;75:91–97. DOI: 10.1161/HYPERTENSIONAHA.119.13291.) • [Online Data Supplement](#)

Key Words: cardiomyopathies ■ healthcare disparities ■ preeclampsia ■ pregnancy ■ women

Peripartum cardiomyopathy (PPCM) is an often severe form of cardiomyopathy diagnosed towards the end of pregnancy or in the months after delivery that affects at least 1 in 2000 deliveries in the United States.^{1,2} Recovery to normal systolic function occurs in 61% to 72% of patients in contemporary cohorts with ≈13% of patients having persistent severe dysfunction or major adverse cardiovascular events such as left ventricular (LV) assist device, heart transplant, or death.^{1,3,4}

Preeclampsia and gestational hypertension are hypertensive disorders of pregnancy (HDP) that are more common in black women and strongly predispose to the development of PPCM.^{1,5} At least 22% of patients with PPCM have preeclampsia and a third have any hypertension, including preeclampsia, gestational hypertension, or chronic hypertension.⁶ Preeclampsia and PPCM likely share pathophysiological mechanisms, including the upregulation of antiangiogenic and vasculotoxic hormones secreted by the placenta.^{1,7,8}

In patients in whom HDPs contribute to the development of PPCM, resolution of postpartum hypertension, and normalization of angiogenic imbalance may theoretically

accelerate resolution of PPCM. Consistent with this notion, PPCM cohorts in Germany and Japan have demonstrated a higher likelihood of LV recovery among patients with HDP compared to patients without HDP.^{9,10} In contrast, however, several studies in the United States have demonstrated no such relationship^{3,11} or conflicting outcomes.¹²

Timing of diagnosis may confound the relationship between HDP and PPCM since women with HDP are often treated with antihypertensives postpartum and require close follow-up in the days to weeks after delivery. Early postpartum follow-up may facilitate the early diagnosis and treatment of PPCM in women at risk. The prospective PPCM cohort in the Investigations of Pregnancy-Associated Cardiomyopathy registry suggested that earlier diagnosis is a predictor of recovery of systolic function, but interaction with HDP was not probed, and this relationship has not been explored in other cohorts.¹¹

Finally, we and others have shown that important racial disparities exist in PPCM outcomes in the United States and worldwide.^{4,11,13–16} In our cohort of 220 women with PPCM, black women were almost half as likely to recover and took

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From the Division of Cardiology (J.L., Z.A.) and Maternal and Child Health Research Center, Department of Obstetrics and Gynecology (L.D.L., M.A.E., O.C.I.), University of Pennsylvania Perelman School of Medicine, Philadelphia.

The online-only Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.119.13291>.

Correspondence to Jennifer Lewey, Perelman Center for Advanced Medicine, 3400 Civic Center Blvd, 2-E Pavilion, Philadelphia, PA 19104. Email jennifer.lewey@pennmedicine.upenn.edu

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twice as long to do so. Interestingly, we also noted that black women were diagnosed significantly later than nonblack women, typically a month or more after delivery.⁴ Together, these observations raise the question of how race, HDP, and timing of diagnosis interplay, if at all, to impact prognosis in PPCM. Our objective was, therefore, to understand how HDP and timing of postpartum diagnosis impact PPCM outcomes in one of the largest and racially diverse PPCM cohorts in the United States.

Methods

We conducted a retrospective cohort study of women diagnosed with PPCM between 1986 until 2016 treated within the University of Pennsylvania Health System (UPHS). This study was approved by the University of Pennsylvania Institutional Review Board. To minimize the possibility of unintentionally sharing information that can be used to reidentify patients, a subset of the data that support the findings of this study are available from the corresponding author on reasonable request.

Cohort

We queried the UPHS clinical data warehouse, Penn Data Store, to identify all patients with a potential diagnosis of PPCM, based on *International Classification of Diseases, Ninth Revision* diagnosis code of 674.5 or the presence of an echocardiogram within 6 months of delivery. We manually reviewed medical records, including echocardiography reports, consultation notes, and discharge summaries of all potentially eligible women. We included patients 18 years or older if they were diagnosed with PPCM or heart failure towards the end of pregnancy or in the several months following delivery and had no other identifiable cause for their heart failure.¹⁷ Inclusion criteria for PPCM diagnosis included (1) systolic dysfunction as determined by left ventricular ejection fraction (LVEF) <45% or fractional shortening <30% on echocardiogram at time of diagnosis; (2) documentation of systolic dysfunction in clinical notes at time of diagnosis; or (3) ongoing systolic dysfunction documented by echocardiogram or clinical notes among women transferred to UPHS after diagnosis. Exclusion criteria included a history of congenital heart disease, significant valvular disease predating PPCM diagnosis, and a history of radiation or cardiotoxic chemotherapy.

Covariates

We extracted relevant demographic and clinical information from Penn Data Store including diagnosis details, past obstetric and medical history, and clinical outcomes. Race was self-reported as documented in Penn Data Store. We dichotomized race as black versus nonblack as described previously⁴ and based upon reports that black women with PPCM have worse outcomes as described above. We included gestational hypertension, preeclampsia, and eclampsia as HDP as diagnosed on retrospective chart review. Timing of PPCM diagnosis was recorded as a categorical variable in the original cohort and was dichotomized into early (<1 month after delivery) versus late (≥1 month after delivery).

Outcomes

Our primary outcome was full recovery of LV function, defined as LVEF >50%, consistent with other contemporary cohorts.^{3,4,11} Secondary outcomes included time to recovery (days) for patients with documented dates of diagnosis and follow-up echocardiograms and adverse cardiovascular events, including LV assist device placement, heart transplantation, or death. Patients without follow-up echocardiogram data were censored. Patients were followed for the duration of time that they received care at UPHS, through 2016. As LVEF at time of diagnosis is one of the strongest predictors of subsequent LVEF recovery, we compared differences in baseline LVEF at time of diagnosis stratified by HDP and timing of PPCM diagnosis among the cohort of patients with available baseline echocardiographic reports.

Statistical Analysis

We presented continuous variables as mean with SD and median with interquartile range. We assessed continuous variables using the Student *t* test and Wilcoxon rank-sum tests as appropriate. Categorical variables are presented as counts and percentages and were compared utilizing χ^2 tests. We used Kaplan-Meier survival analysis for recovery time and group differences were compared using the Wilcoxon rank-sum test. We used logistic regression to analyze predictors of LVEF recovery. A *P*<0.05 was considered significant. We performed the analysis using SAS version 9.4 software (SAS Institute Inc, Cary, NC).

Results

We identified 220 PPCM patients (121 black patients, 55%) between 1986 and 2016. We were able to ascertain HDP status in 180 patients (81.8%) and timing of diagnosis in 193 patients (87.7%). The median duration of follow-up was 42.6 months (interquartile range 9.5–89.2 months).

HDP Baseline Characteristics

Baseline characteristics of patients with and without HDP stratified by race are presented in Table 1. Patients with HDP are younger, more likely to be nulliparous, and have twin gestation (known risk factors for HDP). These differences were observed in both black and nonblack patients, although statistical significance was not reached in the stratified analysis. Chronic hypertension was similar between patients with and without HDP (9.1% versus 8.2%, *P*=0.841) but was present almost exclusively in black patients. Both black and nonblack patients with HDP were more than twice as likely to be diagnosed in the first week postpartum compared to patients without HDP, as demonstrated in Figure. This effect was particularly notable for nonblack patients with HDP, among whom 72.7% presented in the first postpartum week. In comparison, only 27.9% of black patients with HDP were diagnosed in the first week after delivery (*P*=0.0001). There were no nonblack patients with HDP diagnosed during pregnancy.

In the 130 patients with complete echocardiographic data at baseline, LVEF at time of diagnosis was similar between patients with and without HDP in the entire cohort (median LVEF 28.8% versus 30%, *P*=0.636) with similar rates of EF<30% documented in the 2 groups (Table 1, Figure S1 in the [online-only Data Supplement](#)). Among black patients with HDP, 63.6% had a baseline LVEF<30% whereas only 33.3% of nonblack patients with HDP had an LVEF<30%.

Time to Diagnosis Baseline Characteristics

Patients diagnosed early (during pregnancy or within one month of delivery) trended towards being older and having higher rates of HDP, were more likely to have twin gestation and to undergo Cesarean delivery than women diagnosed later in the postpartum period (Table 2). In contrast to comparisons based on HDP above, patients with early postpartum diagnosis had a higher median LVEF compared to patients diagnosed 1 month or later postpartum (30% versus 22.5%, *P*=0.041) and more patients in the late diagnosis group had LVEF<30% (63.2% versus 44.8%, *P*=0.054), see Table 2 and Figure S1.

Table 1. Baseline Characteristics Stratified by HDP and Race

Characteristic	Entire Cohort (N=180)			Blacks (N=96)			Nonblacks (N=84)		
	No HDP	HDP	P Value	No HDP	HDP	P Value	No HDP	HDP	P Value
Mean age, y (SD)	30.4 (5.8)	28.0 (7.2)	0.012	29.2 (5.5)	26.0 (7.1)	0.016	31.7 (5.8)	30.6 (6.5)	0.442
Age >30 y, n (%) n=180	54 (54.0)	28 (35.0)	0.011	20 (40.0)	11 (23.9)	0.092	34 (68.0)	17 (50.0)	0.097
Timing of diagnosis, n (%) n=170									
During pregnancy	11 (11.7)	4 (5.3)	0.013	4 (8.7)	4 (9.3)	0.359	7 (14.6)	0 (0)	0.006
<1 wk PP	22 (23.4)	36 (47.4)		6 (13.0)	12 (27.9)		16 (33.3)	24 (72.7)	
≥1 wk and <1 mo PP	30 (31.9)	18 (23.7)		14 (30.4)	12 (27.9)		16 (33.3)	6 (18.2)	
1–5 mo PP	23 (24.5)	16 (21.1)		16 (34.8)	13 (30.2)		7 (14.6)	3 (9.1)	
>5 mo PP	8 (8.5)	2 (2.6)		6 (13.0)	2 (4.7)		2 (4.2)	0 (0)	
Early diagnosis PP, n (%) n=170	63 (67)	58 (76.3)	0.183	28 (65.1)	24 (52.2)	0.216	39 (81.3)	30 (90.9)	0.229
Chronic hypertension, n (%) n=172	9 (9.1)	6 (8.2)	0.841	8 (16.0)	6 (14.3)	0.820	1 (2.0)	0 (0)	0.424
Nulliparous, n (%) n=180	45 (45.0)	48 (60.0)	0.045	20 (40.0)	25 (54.4)	0.159	25 (50.0)	23 (67.7)	0.109
Twin pregnancy, n (%) n=180	8 (8.0)	14 (17.5)	0.053	2 (4.0)	4 (8.7)	0.342	6 (12.0)	10 (29.4)	0.046
Cesarean delivery, n (%) n=177	50 (51.2)	50 (63.3)	0.102	24 (49.0)	25 (55.6)	0.524	26 (53.1)	25 (73.5)	0.060
Median EF at diagnosis, % (IQR) n=130	30 (15–40)	28.8 (21.3–38.8)	0.636	30 (15–37.5)	25 (15–37.5)	0.867	32.5 (15–40)	32.5 (25–40)	0.429
EF at diagnosis <30%, n (%) n=130	34 (48.6)	30 (50.0)	0.871	16 (48.5)	21 (63.6)	0.215	18 (48.7)	9 (33.3)	0.221

EF indicates ejection fraction; HDP, hypertensive disorder of pregnancy; IQR, intraquartile range; and PP, postpartum.

PPCM Outcomes

Patients with HDP had similar rates of recovery of LV function compared with patients without HDP (68.4% versus 62.6%, $P=0.425$; Table 3). Black patients demonstrated similar rates of recovery despite HDP status, whereas non-black patients with HDP trended towards having higher rates of recovery (78.8% versus 66%, $P=0.209$). Among patients who recovered ($n=114$), 63.1% had documentation on time to recovery within 2 years of diagnosis. In this smaller subset, the variability of time to recovery was high and no significant difference existed between those with and without HDP. Rates of adverse outcomes (persistent dysfunction resulting in transplant, LV assist device, or death) were low and similar in patients with and without HDP (4% versus 7.1%, $P=0.378$).

Patients with early diagnosis (Table 3) had significantly higher rates of LV recovery (69.9% versus 53.7%, $P=0.035$). This trend was consistent in the analysis stratified by race. Patients with early diagnosis had a shorter time to recovery compared to patients with late diagnosis (4 versus 7 months, $P=0.047$). This result was driven by differences in time to recovery among black patients (4.3 versus 15.7 months, $P=0.013$). Rates of adverse outcomes were similar in patients diagnosed early and late (9.8% versus 14.8%, $P=0.323$). In univariate logistic regression analysis, nonblack race and early diagnosis were associated with a 2-fold increase in odds of LV recovery, whereas baseline LVEF $\geq$ 30% was associated with an almost 4-fold increase in odds of recovery (Table 4).

We performed several sensitivity analyses to confirm the robustness of our findings. First, we repeat our analysis among women with documented preeclampsia and found similar trends. Second, given that our cohort spans 30 years,

during which time the standard of heart failure care has evolved, we repeated the analysis stratified by date of diagnosis (diagnosis date before 2006 or 2006 and later). Rates of recovery were similar in both cohorts when stratified by HDP and timing of diagnosis.

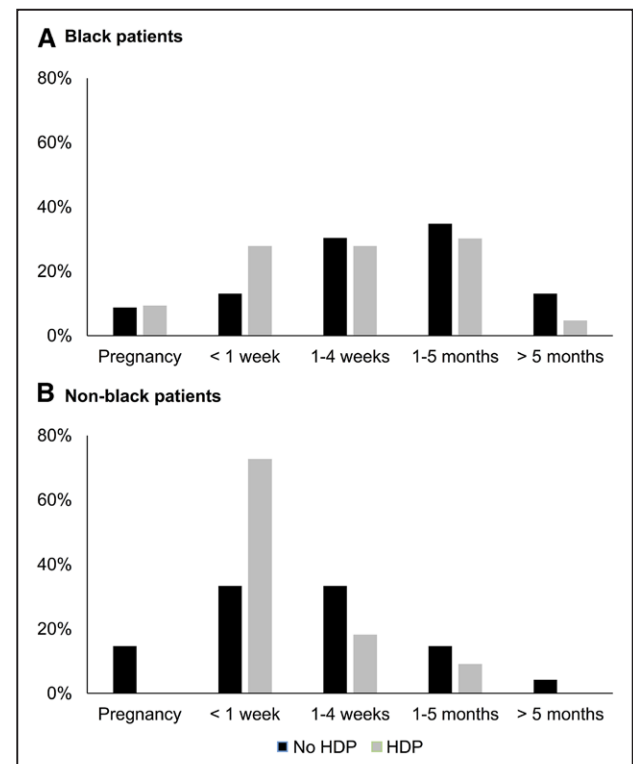


Figure. Timing of diagnosis by hypertensive disorder of pregnancy (HDP) and race. Timing refers to the postpartum period.

Table 2. Baseline Characteristics Stratified by Time to Diagnosis and Race

Characteristic	Entire Cohort (N=193)			Blacks (N=104)			Nonblacks (N=89)		
	Early	Late	P Value	Early	Late	P Value	Early	Late	P Value
Mean age, y (SD)	30.1 (6.8)	28.2 (5.8)	0.073	28.3 (6.8)	27.5 (6.3)	0.554	31.6 (6.5)	30.5 (3.0)	0.535
Age >30 y, n (%)	69 (50)	19 (34.6)	0.052	22 (35.5)	12 (28.6)	0.461	47 (61.8)	7 (53.8)	0.586
Timing of diagnosis, n (%)									
During pregnancy	17 (12.3)	0	<0.0001	10 (16.1)	0	<.0001	7 (9.2)	0	<0.0001
<1 wk PP	66 (47.8)	0		22 (35.5)	0		44 (57.9)	0	
≥1 wk and <1 mo PP	55 (39.9)	0		30 (48.4)	0		25 (32.9)	0	
1–5 mo PP	0	44 (80.0)		0	33 (78.6)		0	11 (84.6)	
>5 mo PP	0	11 (20.0)		0	9 (21.4)		0	2 (15.4)	
Hypertensive disorder of pregnancy n=170	58 (47.9)	18 (36.7)	0.183	28 (53.9)	15 (40.5)	0.216	30 (48.5)	3 (25.0)	0.229
Chronic hypertension, n (%) n=164	11 (9.4)	3 (6.4)	0.532	10 (19.6)	3 (8.6)	0.16	1 (1.5)	0	0.668
Nulliparous, n (%) n=193	72 (52.2)	25 (45.5)	0.399	28 (45.2)	17 (40.5)	0.636	44 (57.9)	8 (61.5)	0.805
Twin pregnancy, n (%) n=192	22 (15.9)	3 (5.6)	0.054	5 (8.1)	2 (4.9)	0.529	17 (22.4)	1 (7.7)	0.224
Cesarean delivery, n (%) n=188	82 (60.3)	21 (40.4)	0.014	30 (49.2)	16 (41.0)	0.425	52 (69.3)	5 (38.5)	0.032
Median EF at diagnosis, % (IQR) n=143	30 (20–40)	22.5 (12.5–37.5)	0.041	27.5 (18.8–38.8)	21.3 (12.5–32.5)	0.139	32.5 (20–40)	30 (15–41.3)	0.866
EF at diagnosis <30%, n (%) n=143	47 (44.8)	24 (63.2)	0.052	24 (54.6)	20 (66.7)	0.297	23 (37.7)	4 (50)	0.503

EF indicates ejection fraction; IQR, intraquartile range; and PP, postpartum.

Discussion

We have made several clinically important observations in our analysis. First, women with HDP are diagnosed with PPCM earlier in the postpartum period. This effect is largely driven by the very early presentation of nonblack patients. Second, early diagnosis (during or within 1 month of delivery) is strongly associated with recovery of LV function whereas HDP is not. Finally,

early postpartum diagnosis is independently associated with higher baseline LV function, which may represent the mechanism by which early diagnosis mediates improved recovery.

HDP and PPCM Outcomes

Prior studies exploring the prognostic impact of preeclampsia or any hypertensive disorder on PPCM outcomes have had

Table 3. Recovery Outcomes Stratified by Postpartum Time to Diagnosis, HDP, and Race

Outcome	HDP (n=175)			Early diagnosis (n=187)		
	Yes	No	P Value	Yes	No	P Value
Recovery of EF ≥50%, n (%)						
Entire cohort	52 (68.4)	62 (62.6)	0.425	93 (69.9)	29 (53.7)	0.035
Blacks	26 (60.5)	29 (59.2)	0.901	37 (63.8)	21 (51.2)	0.211
Nonblacks	26 (78.8)	33 (66.0)	0.209	56 (74.7)	8 (61.5)	0.327
Time to recovery, mo (IQR)						
Entire cohort (n=72/114)	3.5 (2.1–11.4)	4.5 (1.3–9.4)	0.918	4.0 (1.7–9.6)	7.0 (5.4–17.1)	0.047
Blacks (n=23/55)	2.9 (1.1–14.3)	7.3 (4.5–10.2)	0.379	4.3 (1.3–9.3)	15.7 (8.6–17.5)	0.013
Nonblacks (n=49/59)	3.8 (2.1–9.8)	4.0 (1.3–9.1)	0.599	4.0 (1.7–9.7)	5.4 (1.7–7.0)	0.886
Transplant, LVAD, or death, n (%)						
Entire cohort	3 (4.0)	7 (7.1)	0.378	13 (9.8)	8 (14.8)	0.323
Blacks	2 (4.7)	3 (6.1)	0.756	7 (12.1)	6 (14.6)	0.71
Nonblacks	1 (3.0)	4 (8.0)	0.352	6 (8.0)	2 (15.4)	0.393

HDP indicates hypertensive disorder of pregnancy; IQR, interquartile range; and LVAD, left ventricular assist device.

Table 4. Characteristics Associated With Recovery of LV Function

Characteristic	OR (95% CI)	P Value
Age >30 y	1.06 (0.60–1.85)	0.851
Nonblack race	1.90 (1.07–3.37)	0.029
Early diagnosis	2.0 (1.05–3.84)	0.036
Hypertensive disorder of pregnancy	1.29 (0.69–2.43)	0.426
Chronic hypertension	0.75 (0.25–2.22)	0.604
Nulliparous	1.76 (1.0–3.1)	0.05
Twin pregnancy	1.79 (0.72–4.46)	0.21
Cesarean delivery	1.01 (0.57–1.81)	0.965
Baseline EF $\geq$ 30%	3.91 (1.88–8.15)	0.0003

EF indicates ejection fraction; LV, left ventricle; and OR, odds ratio.

mixed results (Table S1). In a German cohort of 115 patients, HDP were highly prevalent and strongly associated with partial or full recovery of LV function compared to patients without HDP (49% versus 7%, respectively, $P=0.009$).⁹ Similarly in a Japanese cohort of 102 patients, HDP were associated with shorter hospitalization at time of diagnosis and improved LV recovery.¹⁰ Studies evaluating black patients in Soweto, South Africa have demonstrated improved PPCM outcomes with higher blood pressure at time of diagnosis but notably these studies excluded women with high blood pressure.^{14,15}

Race may mediate the relationship between preeclampsia and PPCM outcomes and explain why different outcomes are observed in cohorts based on geography and racial composition. In contrast to the German and Japanese cohorts, HDP have not been associated with favorable outcomes in the US-based cohorts (Table S1). The prospective Investigations of Pregnancy-Associated Cardiomyopathy cohort of 100 patients (30% black) demonstrated high rates of HDP but no association of HDP with recovery of LV function.¹¹ Goland et al³ reported no difference in LV recovery at up to 48 months of follow-up among 187 patients (24% black) with and without HDP. A small, predominately black cohort in St Louis (39 patients, 77% black) found that women with preeclampsia had lower 1-year rates of event-free survival, although among survivors with complete echocardiographic follow-up, women with preeclampsia had significantly higher rates of LV recovery (80% versus 25%).¹²

Our study here is one of the largest cohorts to date that addresses the question of how HDP impact outcomes and the first to evaluate the impact of race. We find that HDP are not associated with improved LV recovery. Moreover, this association was maintained in the analysis stratified by race. However, we observed a number of trends that should be further explored in future analyses with larger populations. Nonblack women with HDP were more likely to present in the first week after delivery compared to black women with HDP. Nonblack women with HDP also trended towards having higher baseline LV function, an important predictor of LV recovery, compared to women without HDP. Overall rates of LV recovery were similar between women with and without HDP, but recovery rates did trend towards being higher in those with HDP compared to those without among the 76 nonblack women (79% versus 66%, $P=0.285$), underscoring

the notion that different outcomes observed in international cohorts among PPCM women with HDP may be related to differences in racial composition of those cohorts. It is possible that a potentially protective effect of preeclampsia in nonblack populations is not present in black patients because of competing factors that adversely impact outcomes, perhaps related to socioeconomic or biological factors.

Time to Diagnosis and PPCM Outcomes

We have previously shown in this cohort that black women have lower rates of LV recovery and longer time to recovery compared to nonblack women. Here, we demonstrated that delayed time to diagnosis postpartum is associated with lower rates of LV recovery. Moreover, this observation was maintained in both black and nonblack women and was independent of HDP status. Thus, our study strongly suggests that timing of diagnosis is a key factor in overall outcome. Few studies have explored the relationship between timing of diagnosis and outcomes. In the Investigations of Pregnancy-Associated Cardiomyopathy cohort, black women were enrolled on average at 42 days postpartum, compared to 26 days for nonblack women. Longer time to postpartum diagnosis was significantly associated with worse outcomes in univariate analysis.¹¹ In the St Louis cohort, Lindley et al¹² demonstrated that none of the women diagnosed during pregnancy experienced LV recovery but did not evaluate timing of postpartum presentation.

Delayed diagnosis may explain part of the striking differences between outcomes in black versus nonblack women with PPCM. Socioeconomic factors can lead to the delayed recognition of heart failure symptoms by patients and physicians, leading to delays in the diagnosis and early treatment of PPCM. Adherence to evidence-based medications is lower in vulnerable patient populations and is associated with worse cardiovascular outcomes.¹⁸ The initiation of evidence-based therapies such as angiotensin-converting enzyme inhibitors or β -blockers in nonischemic cardiomyopathy is associated with LV reverse remodeling and even short-term changes in LVEF with medication are associated with prolonged survival.^{19,20} We previously demonstrated that prescription rates of angiotensin-converting enzymes inhibitors and β -blockers were similar between black and nonblack patients in our cohort at time of diagnosis.⁴ We would expect that women with a delayed diagnosis would also have delayed initiation of these evidence-based therapies, leading to worse LV function at baseline and, therefore, lower likelihood of recovery. None of the patients in our cohort were treated with bromocriptine.

Limitations

Our study also has limitations. Retrospective cohort data spanning 30 years may have incomplete data capture as patients were treated according to clinician discretion and not all patients had continuous follow-up within the UPHS system. Differences in follow-up frequency between patient groups could contribute to differences in time to recovery. Although our cohort is among the largest in the United States, our sample size limits our ability to interrogate subgroups for all clinically relevant variables that may impact

recovery. Referral bias may be present as sicker patients are more likely to be referred to a major academic medical center. Preeclampsia diagnoses may be underreported and misclassified; for this reason, we used HDP diagnoses more broadly with the assumption that some cases of gestational hypertension may represent preeclampsia. We included patients with a diagnosis of PPCM and LV systolic dysfunction based on echocardiogram reports and documentation in clinical records but had to exclude some patients due to inability to obtain full reports of all baseline and follow-up echocardiograms. Finally, although we have documentation of medication prescriptions at the time of diagnosis, we do not have more detailed data regarding the duration of treatment, dose titration, or adherence to medications, which may also impact recovery. Despite our study representing the largest clinical PPCM cohort reported in the United States, we are limited by sample size to generalize potentially important trends observed in the stratified analysis by race. Moving forward, efforts to combine clinical cohorts and create a prospective, longitudinal, and adjudicated PPCM registry should be prioritized to better understand how patient characteristics impact prognosis.

Conclusions

In a racially diverse cohort of patients with PPCM, early postpartum presentation is strongly associated with recovery of LV function whereas HDP are not. Later diagnosis is associated with lower EF at presentation, likely explaining the overall worse outcomes observed in black women. These findings underscore the need for early monitoring and diagnosis, especially in at-risk underserved populations.

Perspectives

The association between HDP and recovery of LV function in patients with PPCM is not clear. In this racially diverse cohort of women with PPCM, HDP were not associated with improved rates of LV recovery. Receiving a diagnosis of PPCM during pregnancy or within 1 month after delivery was associated with improved LV recovery compared to patients diagnosed later in the postpartum period. Early diagnosis was associated with higher LVEF, which likely mediates the relationship between late diagnosis and lower recovery rates. Black women are more likely to be diagnosed later in the postpartum period and have lower rates of recovery. Understanding and addressing factors that lead to later diagnosis in this population may lead to improved clinical outcomes.

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Disclosures

None.

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Novelty and Significance

What Is New?

- In a racially diverse cohort of women with peripartum cardiomyopathy (PPCM) living in the United States, hypertensive disorders of pregnancy were not associated with improved recovery rates.
- Early diagnosis (within 1-month postpartum) was significantly associated with improved recovery compared to late diagnosis. This trend was consistent among black and nonblack patients.
- Patients with late diagnosis had a lower ejection fraction at time of presentation, which likely mediates the relationship between late diagnosis and lower recovery rates.

What Is Relevant?

- Earlier diagnosis of PPCM in black women may prevent the worse clinical outcomes currently observed in this population. Cardiac symptoms in women with PPCM risk factors should lead to prompt evaluation.

- Controversy exists in the literature about the association of HDP and PPCM recovery. Our study contributes to the growing literature in the United States demonstrating that women with PPCM do not experience improved recovery rates, differential effects based on race and geography may exist.

Summary

Recovery rates of PPCM in the United States are high but a significant proportion of women still develop severe cardiomyopathy or adverse outcomes. Black women are diagnosed significantly later than white women and have worse rates of recovery. Improving the early monitoring and diagnosis of PPCM in at-risk women may improve clinical outcomes in traditionally underserved populations.



Genetic Variants Associated With Cancer Therapy–Induced Cardiomyopathy

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 ($P \leq 1.98 \times 10^{-4}$). Titin-truncating variants (TTNtrvs) predominated, occurring in 7.5% of patients with CCM versus 1.1% of The Cancer Genome Atlas participants ($P=7.36 \times 10^{-8}$), 0.7% of healthy volunteers ($P=3.42 \times 10^{-6}$), and 0.6% of the reference population ($P=5.87 \times 10^{-14}$). Adult patients who had CCM with TTNtrvs 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 TTNtrv mice and isolated TTNtrv 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 TTNtrvs, 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 TRIAL REGISTRATION: URL: <https://www.clinicaltrials.gov>. Unique identifiers: NCT01173341; AAML1031; NCT01371981.

Pablo Garcia-Pavia, MD, PhD*
Yuri Kim, MD, PhD*
Maria Alejandra Restrepo-Cordoba, MD*
et al

*Drs Garcia-Pavia, Kim, and Restrepo-Cordoba contributed equally.

†Drs Barton, Aplenc, J. G. Seidman, Ky, Ware, and C. E. Seidman are co-senior authors (see page 39).

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Key Words: cardiomyopathies
■ drug therapy ■ genetics ■ medical oncology ■ titin

Sources of Funding, see page 39

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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.

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.

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 systemic 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 (TTN_{trvs}) 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 (AAML1031; 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)^{3,18,19} 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.

Table 1. Clinical Characteristics at Baseline and Follow-Up in Patients With CCM in Study Cohorts

Characteristics	Cohort A (n=99)	Cohort B (n=73)	Cohort C (n=41)
Baseline			
Age at chemotherapy onset, y	48.7±17.1	49.6±10.8	10.8±5.6
Male sex, n (%)	33 (33)§	0 (0)§	17 (41)§
Family history of cardiomyopathy, n (%)	3 (3)	0 (0)	NA
Ethnicity, n (%)			
White	99 (100)§	46 (63)§	25 (61)§
Black	0 (0)§	23 (32)§	9 (22)§
Asian	0 (0)§	4 (5)§	3 (7)§
Others	0 (0)§	0 (0)§	4 (10)§
Comorbidities, n (%)			
Current or past smokers	33 (33)	25 (34)	0 (0)
Hypertension	29 (29)	29 (39)	0 (0)
Hypercholesterolemia	27 (27)	17 (23)	0 (0)
Diabetes mellitus	20 (20)	7 (10)	0 (0)
Oncological disease, n (%)			
Breast cancer	51 (52)§	73 (100)§	0 (0)§
Hematological malignancy	38 (38)§	0 (0)§	41 (100)§
Other solid tumor	10 (10)§	0 (0)§	0 (0)§
Exposure to anthracycline, n (%)	91 (91)	58 (79)	41 (100)
Cumulative anthracycline dose, * mg/m ²	300.7±153.2	240±0	488.3±23.4
Exposure to anti-HER2, n (%)	20 (20)§	36 (49)§	0 (0)§
Radiation therapy to chest, n (%)	32 (32)§	47 (64)§	0 (0)§
LVEF before chemotherapy,† %	62.1±5.4§	55.0±4.7§	64.5±5.8§
At CCM diagnosis			
Time from chemotherapy to diagnosis, y	3 (1–9)§	0.7 (0.4–1.2)§	0.3 (0.2–0.4)§
LVEF at CCM diagnosis, %	33.0±10.0§	41.5±5.5§	44.8±6.7§
NYHA functional class III–IV, n (%)	44 (44)§	1 (1.3)§	NA§
Follow-up			
Duration of follow-up, y	4 (1–7)§	1.7 (0.7–3.3)§	2.3 (1.1–3.2)§
Final LVEF, %	47.3±13.2	49.3±8.2	54.8±13.0
LVEF recovery,‡ n (%)	50 (50)	33 (45)	28 (68)
Cardiac transplantation, n (%)	9 (9)	0 (0)	0 (0)
Cardiac death, n (%)	3 (3)	0 (0)	2 (5)

Data are expressed as numbers (%) or mean±SD, except for time from chemotherapy to cardiotoxicity diagnosis and duration of follow-up, which are expressed as median (Q1–Q3). Comorbidities are those present at diagnosis of CCM. Patient characteristics which are significantly different (adjusted *P*-value <0.05 using Fisher exact test for categorical variables and Welch *t* test for continuous variables) between adult cohorts A and B are shown in bold. CCM indicates cancer therapy-induced cardiomyopathy; HER-2, human epidermal growth factor receptor-2; LVEF, left ventricular ejection fraction; and NYHA, New York Heart Association.

*Cumulative anthracycline dose for 7 patients in cohort A is unknown; these patients were excluded from calculation. Fifty-eight patients in cohort B who underwent anthracycline treatment received 240 mg/m². Planned cumulative anthracycline dose for cohort C (492 mg/m² for 40 patients and 342 mg/m² for 1 patient) is noted in the table, but actual dose may have varied, because 17 patients underwent dose modification of unknown quantity during their treatment period.

†LVEF before chemotherapy was available to review in 31, 73, and 41 subjects in cohorts A, B, and C, respectively.

‡LVEF recovery was defined as an absolute increase in LVEF ≥5% with final LVEF ≥50% in the adult cohorts and ≥53% in the pediatric cohort.

§Significant values.

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

(<https://digital-heart.org/>)²⁰ with no cardiovascular disease or risk factors by self-report and normal cardiac magnetic resonance imaging.

Next-Generation Sequencing and Variant Analysis

Genomic DNA extracted from peripheral blood samples was used to produce DNA-sequencing libraries that were captured and sequenced on Illumina TruSight Cardio Sequencing kit and a custom Agilent array (DCMv5) as described.^{20,21} Variants were identified using the Genome Analysis Tool Kit (GATK) HaplotypeCaller tool following GATK Best Practices.²² Rare variants (minor allele frequency $<1.0 \times 10^{-4}$, assessed in ancestry-matched subjects in the Genome Aggregation Database [gnomAD]²³) were annotated by SnpEFF and GRCh37.68 (also see the [online-only Data Supplement](#)). The cumulative burden of rare variants in cardiomyopathy genes was compared in CCM cohorts with all patients who had breast ($n=1042$) and lung ($n=1011$) cancer participating in The Cancer Genome Project (TCGA),²⁴ healthy volunteers, and gnomAD²³ subjects with non-Finnish European, African, and Asian ancestries (combined and in ancestry-specific analyses).

Anthracycline Treatment of Mice

Protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Harvard Medical School (Boston, MA). Wild-type and heterozygous C57BL/6N mice with a titin A-band truncation ($Ttn^{tv/+}$)^{25,26} received 3 doses of intraperitoneal doxorubicin (5 mg/kg) at weekly intervals (≈ 45 mg/m²). Cardiac function was assessed in vivo at baseline (age=10–14 weeks) and weekly using a digital ultrasound system (Vevo 2100 Imaging System and M5550D transducer; FujiFilm VisualSonics) by an experienced observer blinded to mouse genotype and treatment. Cardiomyocytes from treated and untreated wild-type and $Ttn^{tv/+}$ mice were isolated and sarcomere contractility was measured (see the [online-only Data Supplement Methods](#)).

Statistical Analyses

Cohort and subgroup analyses, and comparisons with TCGA genomic data,²⁴ healthy volunteers, and reference populations²³ were performed by Fisher exact test (2-tailed), binomial test, or Pearson χ^2 test of association for categorical values. Welch t test and Kruskal-Wallis rank sum tests were used to assess numerical data. Analyses were conducted using either the Stata SE package (version 14, StataCorp) or the R statistical package (version 3.4.0; <http://www.R-project.org/>).

Additional method details are provided in the [online-only Data Supplement](#).

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 $\pm$ 17.1 years), recruited from heart failure and

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 $\pm$ 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 $\pm$ 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 were 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 ($P=3.0 \times 10^{-9}$). 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 $\pm$ 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 $\pm$ 9.2% in cohort A, 13.5 $\pm$ 3.3% in cohort B, and 19.7 $\pm$ 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 $\pm$ 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 $\pm$ 3.6%) in comparison with patients receiving anthracyclines with or without other agents (mean LVEF decrease=16.7 $\pm$ 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.

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

Table 2. Burden Analysis of 9 DCM Genes in CCM Cohorts

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			
							All CCM Versus			NFE§ CCM (n=170) Versus NFE gnomAD
							TCGA	HVOL	gnomAD‡	
BAG3	1 (1.0%)	2 (2.7%)	0 (0.0%)	3 (1.4%)	18 (0.9%)	4 (0.9%)	0.44 (1)	0.69 (1)	0.44 (1)	0.37 (1)
DSP	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.2 %)	0 (0.0%)	1 (1)	1 (1)	1 (1)	1 (1)
LMNA	0 (0.0%)	1 (1.4%)	0 (0.0%)	1 (0.5%)	16 (0.8%)	2 (0.4%)	1 (1)	1 (1)	1 (1)	1 (1)
MYH7	3 (3.0%)	0 (0.0%)	0 (0.0%)	3 (1.4%)	35 (1.7%)	5 (1.1%)	1 (1)	0.72 (1)	1 (1)	0.75 (1)
SCN5A	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.2%)	1 (0.2%)	1 (1)	1 (1)	1 (1)	1 (1)
TCAP	1 (1.0%)	0 (0.0%)	1 (2.4%)	2 (0.9%)	2 (0.1%)	0 (0.0%)	0.05 (0.45)	0.10 (0.90)	0.07 (0.62)	1 (1)
TNNC1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)	1 (1)	1 (1)	1 (1)	1 (1)
TNNT2	0 (0.0%)	1 (1.4%)	0 (0.0%)	1 (0.5%)	7 (0.3%)	0 (0.0%)	0.55 (1)	0.32 (1)	0.50 (1)	0.34 (1)
TTN	10 (10.0%)	4 (5.5%)	2 (4.9%)	16 (7.5%)	22 (1.1%)	3 (0.7%)	7.36e–08 (6.62e–07)	3.42e–06 (3.08e–05)	5.87e–14 (5.28e–13)	2.03e–10 (1.82e–09)
8 genes (no TTN)	5 (5.1%)	4 (5.5%)	1 (2.4%)	10 (4.7%)	86 (4.2%)	12 (2.7%)	0.72	0.25	0.01	0.21
9 genes	15 (15.1%)	8 (11%)	3 (7.3%)	26 (12.2%)	108 (5.3%)	15 (3.4%)	1.98e–04	3.90e–05	1.78e–06	6.98e–06

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, TCAP, TNNC1, TNNT2), missense variants and in-frame deletion or insertion only (MYH7), frameshift variant, stop-gained, splice-donor, and splice-acceptor variants only (DSP, SCN5A, 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.
‡gnomAD denotes reference population with African American, non-Finnish European, and East Asian ancestries.
§NFE denotes non-Finnish Europeans ancestries. The subanalyses of NFE patients with CCM are compared with NFE gnomAD reference population n≥113482.
||Significant values.
See Table II in the online-only Data Supplement for subanalyses of patients with CCM and reference populations stratified by ancestry.

these prespecified genes, we examined rare variants (defined as minor allele frequency <1.0e–4) among ancestry-matched reference populations,²³ CCM cohorts, healthy volunteers, and all breast and lung cancer participants in TCGA²⁴ (Table 2). Because anthracyclines are highly effective and widely used to treat these malignancies,⁶ we expect that most TCGA participants received this chemotherapeutic agent. The prevalence of rare protein-altering variants across all 9 genes was significantly higher in a combined CCM cohort than in unselected lung and breast cancer TCGA participants ($P=1.98\text{e}–04$), healthy volunteers ($P=3.90\text{e}–05$), and reference populations ($P=1.78\text{e}–06$). Although patients with CCM had rare variants in several established dilated cardiomyopathy (DCM) genes (*BAG3*, *LMNA*, *MYH7*, *TCAP*, *TNNT2*, and *TTN*), only variants in *TTN*, which encodes titin, were significantly increased. TTNts were highly enriched in all patients with CCM (n=16; 7.5%) in comparison with unselected breast or lung TCGA participants (combined, $P=7.36\text{e}–08$ and Table I in the online-only Data Supplement), healthy volunteers ($P=3.42\text{e}–06$), and the reference population ($P=5.87\text{e}–14$). 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 TTNts in all patients with CCM. TTNts that are significantly

increased in patients with DCM^{20,30} reside in exons that are highly expressed in LV tissues, especially those that encode the A-band and distal I-band.³¹ TTNts 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 Variants 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 variants (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 increased only in *FKRP* (encoding fukutin-related protein); recessive *FKRP* mutations cause several forms of muscular dystrophies with cardiac involvement.³²

Clinical Outcomes in Adult Patients Who Have CCM With or Without TTNts
Patients with CCM in cohorts A and B were predominantly women (81%), with breast cancer (73%), with traditional cardiovascular risk factors, who received

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

Variant	Impact	TTN Band	Affected Exon	PSI of Affected Exon	Cancer Type	Chemotherapy	LVEF at CCM (Change)	Follow-Up, y	Adverse Outcome
chr2:179399704 c.101638G>T	Nonsense (p.Glu33880X)	M	359	1	Breast	Epirubicin	30% (NA)	14	HF
chr2:179400742 c.100731dupA	Frameshift (p.Ser33578IlefsTer15)	A	358	0.99	Breast	Doxorubicin + Trastuzumab	37% (24%)	4	VT, HF
chr2:179410112 c.95722+2delT	Splicing	A	345	0.99	Endometrial	Doxorubicin	20% (NA)	5	HF
chr2:179414849 c.91715dupA	Frameshift (p.Asn30572fs)	A	338	0.98	Breast	Epirubicin	42% (NA)	5	HF
chr2:179422284 c.60512-2A>C	Splicing	A	330	0.96	Breast	Doxorubicin + Trastuzumab	43% (17%)	2.2	None
chr2:179425091 c.85768C>T	Nonsense (p.Arg28590X)	A	327	0.95	Breast	Doxorubicin	35% (NA)	2	HF, VT, transplantation
chr2:179428124 c.82734dupA	Frameshift (p.Val27579SerfsTer15)	A	327	0.95	Non- Hodgkin lymphoma	Doxorubicin	29% (NA)	2	None
chr2:179432234 c.78625G>T	Nonsense (p.Glu26209X)	A	327	0.95	Non- Hodgkin lymphoma	Doxorubicin	34% (NA)	3	HF
chr2:179435679 c.75179delA	Frameshift (p.Asn25060fs)	A	327	0.95	Breast	Doxorubicin + Trastuzumab	44% (12%)	3.4	None
chr2:179441250 c.69715+6T>C	Splicing	A	326	0.95	Breast	Trastuzumab	38% (12%)	0.4	None
chr2:179446855 c.66240delA	Frameshift (p.Asp22081MetfsTer31)	A	316	0.86	Bone sarcoma	Doxorubicin	25% (NA)	6	HF
chr2:179453355 c.63096delT	Frameshift (p.His21032fs)	A	305	0.97	AML	Daunorubicin+ Mitoxantrone	36% (22%)	2.8	None
chr2:179478777 c.49345+2T>C	Splicing	A	263	0.89	AML	Daunorubicin+ Mitoxantrone	50% (18%)	0.8	None
chr2:179571683 c.29042-2A>C	Splicing	I	102	0.88	Breast	Doxorubicin	35% (15%)	1.6	None
chr2:179604819 c.13141G>T	Nonsense (p.Glu4381X)	I	49	0.95	Breast	Doxorubicin	31% (30%)	6	HF
chr2:179631116 c.9693_9694delCT	Frameshift (p.Tyr3232CysfsTer5)	I	41	1	Breast	Epirubicin	45% (NA)	6	HF

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.

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 (Ttn^{tw/+}) of a heterozygous A-band titin truncation in one gene copy.^{25,26} Untreated Ttn^{tw/+} 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

Table 4. Comparisons of Risk Factors and Outcomes in Adult Patient Who Has CCM With and Without TTNtv

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

depressed through week 12 in *Ttn*^{tv/+} 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 *Ttn*^{tv/+} mice were comparable and showed no significant increase in fibrosis or apoptosis in comparison with untreated mice.

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

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%, $P=7.36\text{e-}08$) or healthy volunteers (0.7%, $P=3.42\text{e-}06$) and enriched in comparison with ancestry-matched reference populations ($P=5.87\text{e-}14$). 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 *Ttn*^{tv/+} 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 ($P\geq 0.5$) 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,^{33,34} and cardiac function was worse in patients with 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.²⁹ Mutations in *BAG3*, *LMNA*, *MYH7*, and *TNNT2* are established autosomal dominant causes of DCM.^{14,35} *TCAP* mutations are occasionally identified in patients with DCM,³⁶ but more commonly cause a recessive form of limb-girdle muscular dystrophy.³⁷ 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

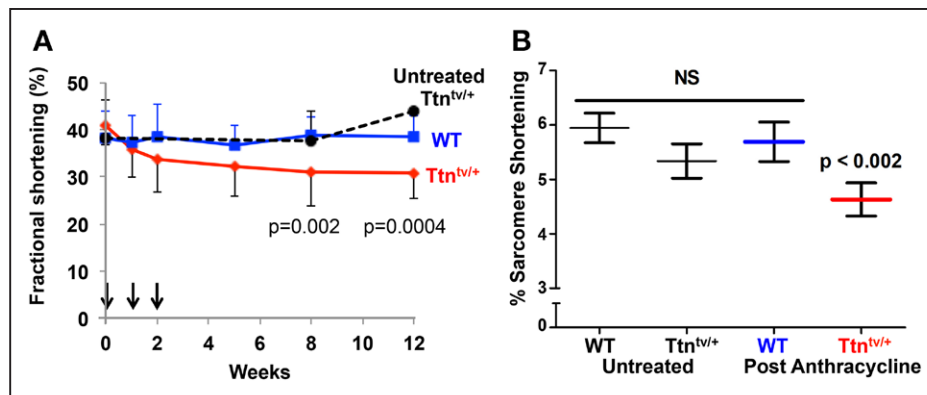


Figure. Persistent cardiac dysfunction in *Ttn^{tv/+}* mice after anthracycline treatment.

A, Untreated *Ttn^{tv/+}* mice have left ventricular function comparable with wild-type (WT) mice.^{25,26} Intraperitoneal doxorubicin (5mg/kg) was administered (arrows) to WT and *Ttn^{tv/+}* 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 $\pm$ SD) in *Ttn^{tv/+}* in comparison with WT mice ($P=0.0004$). **B**, Isolated cardiomyocytes ($n\geq 52$ per group) were studied 12 weeks after initial doxorubicin injection. Cardiomyocytes from doxorubicin-treated *Ttn^{tv/+}* mice had significantly depressed contractility ($P<0.002$) in comparison with cardiomyocytes from doxorubicin-treated WT mice or untreated mice. NS indicates not significant.

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 $\approx 40\%$ of patients with familial and sporadic DCM.^{31,38–40} Whether broader genomic analyses may uncover additional genetic contributors to CCM is worthy of study. TTNts are significantly prominent in DCM, occurring in 15% of ambulatory and 25% of end-stage patients,^{30,31,34,38} but are rarely identified in childhood-onset DCM,⁴¹ whereas here we identified TTNts in 8.1% of adults and 5.0% of children with CCM. TTNts found 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). TTNts also occur in $\approx 15\%$ of patients with peripartum cardiomyopathy²¹ and in $\approx 10\%$ of individuals with alcoholic cardiomyopathy,⁴² findings that imply additional cardiovascular stress can unmask the deleterious cardiac effects of TTNts. Consistent with this supposition, in vitro analyses of human isogenic cardiomyocytes (derived from induced pluripotent stems cells) demonstrate that titin provides an essential mechanical connection that propagates diastolic traction stresses from β -cardiac myosin during sarcomere formation. Cardiomyocytes with TTNts have diminished reassembly of sarcomeres after stress in comparison with cells without TTNts.⁴³ We suggest that chemotherapy, like pregnancy and excessive alcohol, is an important provocation that is poorly tolerated by TTNts, 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

after diagnosis of CCM, and these patients had more severe phenotypes and longer durations of follow-up than the prospectively identified patients in cohorts B and C. Because breast cancer was the most common diagnosis in adult patients with CCM and all pediatric cases had acute myelogenous leukemia, these findings may not be relevant to other cancers and other treatment regimens. All patients had individual chemotherapy dosages and additional treatments based on clinical practice and treatment protocols. These and other variables may influence susceptibility to CCM. This study compared the frequency of TTNts among patients with CCM to the frequency of TTNts in a large cohort of patients with cancer participating in TCGA, some fraction of whom likely developed CCM. A limitation of our study is that we do not know which TCGA subjects developed CCM, potentially affecting the accuracy of the TTNtv frequency estimate in the CCM-free cancer cohort. A more ideal comparison group would have been patients treated with chemotherapy who did not develop cardiomyopathy. While recognizing these issues, we suggest that enrichment of protein-altering variants and TTNts across all cohorts strongly supports the conclusion that genetics, like high-dose anthracycline and combination therapy, and cardiovascular risk factors contribute to CCM.

Current strategies to diagnose CCM focus on imaging and circulating biomarkers^{1,2,18,44–46} and treatment guidelines are limited, often recommending interruption or discontinuation of chemotherapy that can negatively impact the survival of patients with cancer. The identification of genetic risk factors opens new opportunities to identify patients with cancer at high risk for CCM and to assess the efficacy of prophylactic cardioprotective drugs and treatment regimens.^{47–49} Future investigations will determine if early recognition of patients who have cancer with high CCM risk through genetic testing

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

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Authors

Pablo Garcia-Pavia MD, PhD*; Yuri Kim, MD, PhD*; Maria Alejandra Restrepo-Cordoba*; Ida G. Lunde, PhD; Hiroko Wakimoto, MD, PhD; Amanda M. Smith, BA; Christopher N. Toepfer, PhD; Kelly Getz, PhD; Joshua Gorham, MSc; Parth Patel, MD; Kaoru Ito, MD, PhD; Jonathan A. Willcox, PhD; Zoltan Arany, MD, PhD; Jian Li, PhD; Anjali T. Owens, MD; Risha Govind, MSc; Beatriz Nuñez, MD; Erica Mazaika, MSc; Antoni Bayes-Genis, MD; Roddy Walsh, PhD; Brian Finkelmann, MD, PhD; Josep Lupon, MD, PhD; Nicola Whiffin, PhD; Isabel Serrano, MD; William Midwinter, BSc; Alicja Wilk, BSc; Alfredo Bardaji, MD, PhD; Nathan Ingold; Rachel Buchan, MSc; Upasana Tayal, PhD, MRCP; Domingo A. Pascual-Figal, MD, PhD; Antonio de Marvao, PhD, MRCP; Mian Ahmad, PhD; Jose Manuel Garcia-Pinilla, MD, PhD; Antonis Pantazis, MD; Fernando Dominguez, MD, PhD; A. John Baksi, PhD, MRCP; Declan P. O'Regan, PhD; Stuart D. Rosen, MD; Sanjay K. Prasad, MD; Enrique Lara-Pezzi, PhD; Mariano Provencio, MD, PhD; Alexander R. Lyon, PhD; Luis Alonso-Pulpon, MD, PhD; Stuart A. Cook, PhD; Steven R. DePalma, PhD; Paul J.R. Barton, PhD†; Richard Aplenc, MD, PhD†; Jonathan G. Seidman, PhD†; Bonnie Ky, MD, MSCE†; James S. Ware, PhD, MRCP†; Christine E. Seidman, MD†

Correspondence

Pablo Garcia-Pavia, MD, PhD, Department of Cardiology, Hospital Universitario Puerta de Hierro, Manuel de Falla, 2; 28222 Madrid, Spain; or Christine E. Seidman, MD, Departments of Medicine & Genetics, NRB Room 256, Harvard Medical School, 77 Ave Louis Pasteur, Boston, MA 02115. Email pablogpavia@yahoo.es or cseidman@genetics.med.harvard.edu

Affiliations

Hospital Universitario Puerta de Hierro, Madrid, Spain (P.G.-P., M.A.R.-C., F.D., L.A.-P.). Centro de Investigación Biomédica en Red Enfermedades en Cardiovascular Diseases (CIBERCV), Madrid, Spain (P.G.-P., M.A.R.-C., A.B.-G., J. Lupon, D.A.P.-F., J.M.G.-P., F.D., E.L.-P., L.A.-P.). University Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain (P.G.-P.). Harvard Medical School, Boston, MA (Y.K., I.G.L., H.W., C.N.T., J.G., P.P., K.I., J.A.W., S.R.D., J.G.S., C.E.S.). Massachusetts General Hospital, Boston (Y.K.). Oslo University Hospital and University of Oslo, Norway (I.G.L.). Perelman School of Medicine and University of Pennsylvania Health System, Philadelphia (A.M.S., K.G., Z.A., J. Li, A.T.O., B.F., R.A., B.K.). University of Oxford (C.N.T.). National Heart & Lung Institute, Imperial College London, UK (R.G., E.M., R.W., N.W., W.M., A.W., N.I., R.B., U.T., A.d.M., M.A., A.P., A.J.B., S.D.R., S.K.P., E.L.-P., A.R.L., S.A.C., P.J.R.B., J.S.W.). Royal Brompton & Harefield NHS Foundation Trust, London, UK (R.G., E.M., R.W., N.W., W.M., A.W., N.I., R.B., U.T., A.d.M., M.A., A.P., A.J.B., S.D.R., S.K.P., A.R.L., P.J.R.B., J.S.W.). Hospital Universitario Puerta de Hierro, Universidad Autónoma de Madrid, Spain (B.N., M.P.). Hospital Universitario Germans Trias i Pujol, Badalona, Spain (A.B.-G., J. Lupon). MRC London Institute of Medical Sciences, Imperial College UK (N.W., D.P.O., S.A.C., J.S.W., C.E.S., A.d.M.). Hospital Universitario de Tarragona Joan XXIII. IISPV, Rovira Virgili University, Spain (I.S., A.B.). Hospital Universitario Virgen de la Arrixaca, University of Murcia, Spain (D.A.P.-F.). Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain (E.L.-P.). National Heart Centre Singapore and Duke-National University of Singapore (S.A.C.). Howard Hughes Medical Institute, Chevy Chase, MD (S.R.D., C.E.S.). Brigham and Women's Hospital, Boston MA (P.P., C.E.S.). Hospital Universitario Virgen de la Victoria, IBIMA, Malaga, Spain (J.M.G.-P.).

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Disclosures

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

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ORIGINAL RESEARCH ARTICLE

Genomics-First Evaluation of Heart Disease Associated With Titin-Truncating Variants

BACKGROUND: Truncating variants in the Titin gene (TTNtvs) are common in individuals with idiopathic dilated cardiomyopathy (DCM). However, a comprehensive genomics-first evaluation of the impact of TTNtvs in different clinical contexts, and the evaluation of modifiers such as genetic ancestry, has not been performed.

METHODS: We reviewed whole exome sequence data for >71 000 individuals (61 040 from the Geisinger MyCode Community Health Initiative (2007 to present) and 10 273 from the PennMedicine BioBank (2013 to present) to identify anyone with TTNtvs. We further selected individuals with TTNtvs in exons highly expressed in the heart (proportion spliced in [PSI] >0.9). Using linked electronic health records, we evaluated associations of TTNtvs with diagnoses and quantitative echocardiographic measures, including subanalyses for individuals with and without DCM diagnoses. We also reviewed data from the Jackson Heart Study to validate specific analyses for individuals of African ancestry.

RESULTS: Identified with a TTNtv in a highly expressed exon (hiPSI) were 1.2% individuals in PennMedicine BioBank and 0.6% at Geisinger. The presence of a hiPSI TTNtv was associated with increased odds of DCM in individuals of European ancestry (odds ratio [95% CI]: 18.7 [9.1–39.4] {PennMedicine BioBank} and 10.8 [7.0–16.0] {Geisinger}). hiPSI TTNtvs were not associated with DCM in individuals of African ancestry, despite a high DCM prevalence (odds ratio, 1.8 [0.2–13.7]; $P=0.57$). Among 244 individuals of European ancestry with DCM in PennMedicine BioBank, hiPSI TTNtv carriers had lower left ventricular ejection fraction ($\beta=-12\%$, $P=3\times 10^{-7}$), and increased left ventricular diameter ($\beta=0.65$ cm, $P=9\times 10^{-3}$). In the Geisinger cohort, hiPSI TTNtv carriers without a cardiomyopathy diagnosis had more atrial fibrillation (odds ratio, 2.4 [1.6–3.6]) and heart failure (odds ratio, 3.8 [2.4–6.0]), and lower left ventricular ejection fraction ($\beta=-3.4\%$, $P=1\times 10^{-7}$).

CONCLUSIONS: Individuals of European ancestry with hiPSI TTNtv have an abnormal cardiac phenotype characterized by lower left ventricular ejection fraction, irrespective of the clinical manifestation of cardiomyopathy. Associations with arrhythmias, including atrial fibrillation, were observed even when controlling for cardiomyopathy diagnosis. In contrast, no association between hiPSI TTNtvs and DCM was discerned among individuals of African ancestry. Given these findings, clinical identification of hiPSI TTNtv carriers may alter clinical management strategies.

Christopher M. Haggerty,
PhD*
Scott M. Damrauer, MD*
et al

*Drs Haggerty and Damrauer contributed equally.

†Drs Fornwalt and Arany are co-senior authors (see page 52).

Full author list is available on page 52

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Clinical Perspective

What Is New?

- Genetic variants causing premature truncation of the sarcomere protein, Titin, are highly prevalent in numerous forms of nonischemic cardiomyopathy, but little is known about the consequences of these Titin variants ascertained independently of cardiomyopathy (ie, genomics-first).
- We report that individuals of European ancestry identified through a genomics-first approach have much greater odds of developing dilated cardiomyopathy, and have lower left ventricular function than their peers whether a clinical diagnosis of dilated cardiomyopathy is present or not.
- We also find that the association of Titin variants and dilated cardiomyopathy is much weaker in individuals of African ancestry.

What Are the Clinical Implications?

- Truncating genetic variants in Titin have a measurable effect in large clinical populations, with respect to both strong associations with cardiomyopathy and arrhythmia and quantitative differences in cardiac structure and function, although these associations appear strongest in individuals of European ancestry.
- Identification of such variants, either through targeted genetic testing in the setting of cardiomyopathy or screening for secondary findings, may provide clinical value for individualized risk stratification and preventive cardiology.

Titin is a large sarcomeric protein that is vital for cardiac contraction and relaxation.¹ Genetic sequencing of large cohorts of patients ascertained based on the diagnosis of familial or idiopathic dilated cardiomyopathy (DCM) has identified heterozygous variants in *TTN* that are predicted to lead to protein truncation (*TTN*-truncating variants, or *TTN*ts) in ~15% to 20% of these cases.^{2–7} Subsequent work has demonstrated that this association is driven by *TTN*ts in exons that are constitutively expressed (proportion spliced in [PSI]>0.9, or hiPSI) in the primary cardiac isoforms of *TTN* (N2BA and N2B).³ However, the prognostic significance of identifying a *TTN*ts, in particular, as an incidental finding, is uncertain because of substantial challenges in variant interpretation and the high frequency of *TTN*ts in the general population.^{2,8}

A critical step toward enhancing the clinical utility of identifying a *TTN*ts is to define the phenotypic impact of *TTN*ts independent of DCM-based ascertainment.⁹ This is important because DCM may represent an extreme on a spectrum of *TTN*ts-related disease that includes milder, but still clinically relevant forms.¹⁰ For example, a recent study found that a small group (n=15)

of healthy volunteers incidentally identified with *TTN*ts (*PSI*>0.15) had mild increases in left ventricular (LV) volumes by cardiac MRI, suggesting possible deleterious effects of *TTN*ts in the absence of clinically recognized DCM.¹¹ In addition, little is known about the associations of *TTN*ts with disease in non-European populations, despite some suggestion for differential effects in individuals of African ancestry.³

Linking large-scale genomic sequence data to electronic health records (EHRs) provides a powerful opportunity to leverage a genomics-first approach to answer such questions, including the possible identification of novel genotype-phenotype associations, and deep characterization of heterogeneous disease based on individual genotype. Therefore, we comprehensively evaluated phenotypes associated with *TTN*ts in 2 large biobanks with whole exome sequence data linked to EHRs. These 2 biobanks represent informative extremes of clinical contexts: the Geisinger MyCode project¹² represents a general clinical population with high engagement and continuity; in contrast, the PennMedicine Biobank (PMBB) represents a tertiary care center with a high referral rate, and hence a higher disease prevalence. We hypothesized that individuals with *TTN*ts would demonstrate a distinct clinical phenotype characterized by associations of clinical diagnoses and differences in ventricular size and function in comparison with individuals without *TTN*ts.

METHODS

The data, analytic methods, and study materials from the University of Pennsylvania will be made available to other researchers for purposes of reproducing the results with approval of the University of Pennsylvania institutional review committee. The data and study materials from Geisinger will not be made available. The study was approved by the University of Pennsylvania institutional review committee and Geisinger institutional review committee, and all subjects gave informed consent.

Cohorts

The MyCode Community Health Initiative at Geisinger is a precision health project in which participants, with opt-in informed consent, provide biospecimens for broad research use, including permission to link associated data to EHRs.¹² Exome sequencing was performed through the DiscovEHR collaboration between Geisinger and the Regeneron Genetics Center.^{13–17} This study included the first 92 455 participants who underwent sequencing; this cohort is of predominantly European ancestry.

PMBB recruits patients from throughout the University of Pennsylvania Health System for genomic and precision medicine research. Participants actively consent to allow the linkage of biospecimens to their longitudinal EHR. Currently, >50 000 participants are enrolled in the PMBB; ~23% of these individuals self-identify as having African ancestry. This study included a subset of 10 289 individuals who have undergone

whole exome sequencing, performed through a collaboration with the Regeneron Genetics Center.

The Jackson Heart Study is a population-based observational study evaluating the etiology of cardiovascular, renal, and respiratory diseases among African Americans residing in the 3 counties (Hinds, Madison, and Rankin) that make up the Jackson, Mississippi metropolitan area. Data and biological materials have been collected from 5306 participants, aged 35 to 84 years. The study population is characterized by a high prevalence of diabetes mellitus, hypertension, obesity, and related disorders.

Genetic Sequencing, Variant Calling, and Genotype Assignment

Sample preparation and exome sequencing were completed per the standard Regeneron Genetics Center methodology, as described by Dewey et al¹⁴ and detailed in the [online-only Data Supplement Methods](#). Sequence quality was sufficient to provide >20 times read depth in >90% of samples for 96% of the bases in *TTN* for Geisinger and 82% in PMBB (83.6% in individuals of African ancestry and 82.1% in individuals of European ancestry).

After cohort sequencing, samples showing disagreement between genetically determined and reported sex, low-quality sequence data, genetically identified sample duplicates, and individuals with closer than third-degree relatedness were excluded. After exclusions, 61 040 samples from Geisinger and 10 289 samples from PMBB remained. Samples with quality scores at a given site, normalized by depth <3 (<5 for indels), site read depth <7 (<10 for indels), and alternate allele balance <25% (or <5 alternate reads) were removed. Sufficient positive predictive value for these thresholds was verified by Sanger sequence confirmation in selected samples.

Variant Annotation and Selection

We selected all *TTN* variants with minor allele frequency <0.001 and further selected all predicted loss of function, truncating variants (ie, stop gain, frameshift, splice site) using the Ensembl Variant Effect Predictor or ANNOVAR.^{18,19} Splice site variants included those affecting canonical donor and acceptor splice sites (ie, 2 bases flanking either side of each exon). PSL>0.9 (see below) was considered high.³

Phenotype Evaluation

For the Geisinger cohort, we collated available demographic (birthdate, age at last encounter, sex, race, vital status), diagnostic (*International Classification of Diseases* codes from clinical encounters), and quantitative data from the most recent echocardiogram with a physician-reported left ventricular ejection fraction (LVEF) from the Geisinger Phenomic-Initiative database as of April 19, 2018.

Within PMBB genetic ancestry was determined using principal components analysis based on HapMap3 reference populations.¹³ For individuals of European or African ancestry, quantitative ancestry estimates were performed using ADMIXTURE.²⁰ PMBB phenotype data were directly queried from Penn Data Store, which agglomerates EHR data from across the health system, as of January 18, 2018.

DCM was defined as ≥ 2 encounters with the I42.0 (Dilated cardiomyopathy) *International Classification of Diseases, 10th Revision* code; or ≥ 2 instances of nonspecific *International Classification of Diseases, Ninth Revision, Clinical Modification* or *International Classification of Diseases, 10th Revision* codes 425.4 (Other primary cardiomyopathies), 425.8 (Cardiomyopathy in other diseases classified elsewhere), 425.9 (Secondary cardiomyopathy, unspecified), I42.8 (Other cardiomyopathies), or I42.9 (Cardiomyopathy, unspecified) and mention of dilated or DCM in the underlying diagnosis code or on the left ventricular findings from echocardiography (Geisinger) or the phrases dilated cardiomyopathy or DCM in the free text encounter notes (PMBB). Individuals with only one occurrence of either specific or nonspecific codes, or free text, were excluded from analysis considering DCM as an outcome. Transthoracic echocardiographic parameters were extracted from structured reports (outpatient only for PMBB).

Because phenotype data on DCM were not directly collected in the Jackson Heart Study, DCM was determined based on cardiac morphology derived from echocardiography and cardiac MRI. Nonischemic DCM was defined based on age, sex, and body surface area standardized reductions in LVEF and increases in LV end-diastolic dimensions in individuals without a previous history of myocardial infarction or coronary revascularization.³

Statistics

Statistical analyses were performed using R (version 3.5).²¹ Individuals with hiPSI TTNtv were compared with the rest of their respective sequenced cohorts (excluding individuals with low PSI TTNtv) by using phenome-wide association (PheWAS), adjusted for age, sex, and principal components of ancestry (first 4 components for Geisinger, first 10 for PMBB).^{22,23} Logistic regression was used to specifically evaluate the association of TTNtv with DCM, adjusting for age, age,² sex, and principal components of ancestry. Because of the high percentage of individuals of African ancestry in PMBB, analyses were performed separately by genetic ancestry and combined with inverse variance weighted meta-analysis. Available echocardiography measurements were compared on a case/control basis, independently for the Geisinger, PMBB, and Jackson Heart Study cohorts, using the Welch *t* test or Wilcoxon test, as appropriate. Comparisons in patient subsets exclusively with (PMBB) and without (Geisinger) DCM were also made using robust linear regression²⁴, adjusting for age, sex, and ancestry. Bootstrapping with the adjusted percentile method (1000 resamples, with replacement) was used to calculate 95% CIs of the regression coefficients.²⁵ Controls were selected for echocardiography analyses in the Geisinger cohort from the sequenced population lacking a TTNtv and matched (5 controls per TTNtv individual) by sex and date of birth to the TTNtv cases.²⁶ In PMBB, the remaining sequenced population without a TTNtv was used for controls for all analyses.

RESULTS

Prevalence and Distribution of TTNtv

Four hundred ninety-two truncating variants were identified across *TTN* in the Geisinger cohort ([Table 1](#) in

the online-only Data Supplement). Of these, 208 were frameshifting insertions/deletions, 194 were stop gains, and 90 were essential splice site variants. One hundred eighty-nine TTNts were identified in PMBB, including 80 frameshifts, 66 stops, and 43 splice variants (Table II in the online-only Data Supplement). Figure 1 shows the distribution of variants within the *TTN* gene, and compares them with the Exome Aggregation Consortium (ExAC) reference population.²⁷ The variant distribution in the Geisinger cohort was similar to ExAC, whereas the distribution in PMBB strongly biased the A band and constitutively expressed exons (Figure 1, $P<0.005$). For Geisinger, 245 TTNts (49.8%) were located in high PSI (hiPSI) exons, whereas 126 variants in PMBB (75.1%) were hiPSI (Table III in the online-only Data Supplement).

Individuals and Demographic Details

A total of 501 individuals, representing 0.7% of the combined cohorts, were identified with a hiPSI TTNv. Demographics are shown in Table 1 for the full cohorts, and shown by ancestry in Table IV in the online-only Data Supplement. The fraction of individuals of African ancestry was 2.8% and 21% in the Geisinger and PMBB cohorts, respectively. The prevalence of hiPSI TTNts in PMBB patients of European descent was 1.5%, significantly higher than in ExAC subjects of European descent (0.5%; $P<1\times10^{-6}$). In contrast, the prevalence of hiPSI TTNts in PMBB patients of African descent was

0.9%, not significantly different from that in ExAC subjects of African descent (0.7%; $P=0.14$).

Association With DCM

In the Geisinger cohort, 1.0% of all individuals carried a diagnosis of DCM, in contrast to 5.6% of PMBB, reflecting the large difference in disease prevalence between the cohorts. The distribution of TTNts associated with DCM diagnosis in both cohorts is indicated in red in Figure 1.

In the Geisinger cohort, 7.5% of individuals with a hiPSI TTNv had a clinical diagnosis of DCM. The age- and sex-adjusted odds ratio for DCM in individuals with hiPSI TTNv was 10.8 (95% CI, 7.0–16.0; $P=2\times10^{-29}$). Of all sequenced individuals with a DCM diagnosis, 4.2% had a hiPSI TTNv. The age- and sex-adjusted odds ratio for DCM in individuals with hiPSI TTNv was 10.8 (95% CI, 7.0–16.0; $P=2\times10^{-29}$).

In PMBB, 30% of individuals with a hiPSI TTNv had DCM, representing 7% of individuals with DCM. Surprisingly, hiPSI TTNts were more prevalent in patients with DCM of European ancestry than in patients with DCM of African ancestry (40 [10%] of 397 patients versus 3 [1.4%] of 216 patients; $P=2\times10^{-5}$). The latter prevalence did not differ from the prevalence of hiPSI TTNv in individuals of African ancestry in ExAC (0.5%, $P=0.15$). Furthermore, among individuals of European ancestry with TTNv, the age- and sex-adjusted odds ratio for DCM was

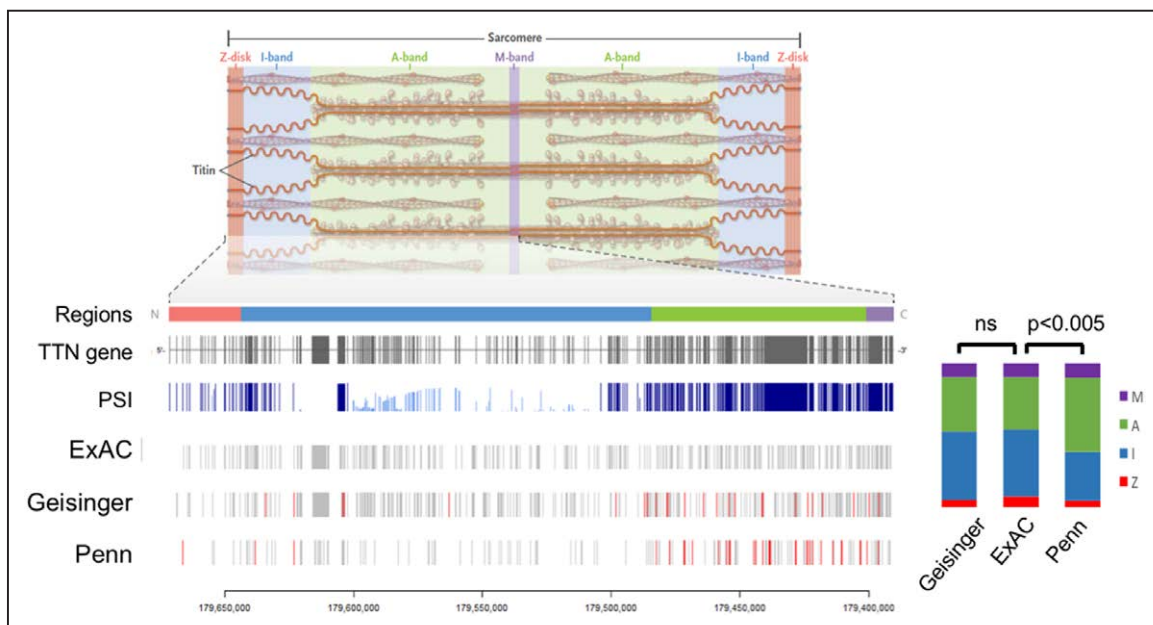


Figure 1. Location within the *TTN* gene of identified variants in the Geisinger and Penn cohorts.

Titin, a protein encoded by *TTN*, is an integral part of the cardiac sarcomere, spanning from Z-disk to M-line. Regions of the *TTN* gene and protein are designated according to their spatial domain in the sarcomere: Z-disk, I-band, A-band, M-band. The exons of the *TTN* gene and their associated proportion spliced in (PSI) are shown, and the distributions of truncating variants in those exons from the Exome Aggregation Consortium (ExAC) reference population and the Geisinger and Penn cohorts, as well. Variants found in patients with dilated cardiomyopathy are designated in red. The relative distribution of variants by *TTN* region is represented on the right stacked bar plots, showing that A-band variants are overrepresented in the Penn cohort in comparison with ExAC, whereas the Geisinger and ExAC distributions are comparable. ns indicates not significant.

Table 1. Cohort Demographics

Demographics	Geisinger			PMBB		
	Non-hiPSI Cohort	hiPSI	P Value	Non-hiPSI Cohort	hiPSI	P Value
Participants	60 681	359		10 147	142	
Male, n (%)	24 538 (40)	139 (39)	0.52	5997 (59)	95 (67)	0.07
EUR ancestry, n (%)	57 371 (95)	340 (95)	>0.99	8024 (79)	122 (86)	0.05
Age at last encounter	60 (45–71)	57 (43–70)	0.015	68 (59–77)	66 (57–74)	0.10
Diabetes mellitus, n (%)	11 528 (19)	55 (15)	0.08	3286 (32)	53 (37)	0.24
Hypertension, n (%)	32 214 (53)	172 (48)	0.06	7505 (74)	102 (72)	0.90
CAD, n (%)	11 005 (18)	93 (26)	3×10^{-4}	4449 (44)	55 (39)	0.79
Myocardial infarction, n (%)	924 (2)	10 (3)	0.08	2103 (21)	26 (18)	0.53
Heart failure, n (%)	5515 (9)	86 (24)	2×10^{-17}	3947 (39)	93 (66)	3×10^{-10}
Nonischemic DCM, n (%)	595 (1)	27 (8)	4×10^{-17}	570 (5.6)	43 (30)	2×10^{-21}
Heart transplant, n (%)	20 (<1)	1 (<1)	0.12	339 (3.3)	20 (14)	1×10^{-7}

Data are presented as number (%) or median (interquartile range). CAD indicates coronary artery disease; DCM, dilated cardiomyopathy; EUR, European; hiPSI, having variants within exons with high proportion spliced in to Titin transcripts; and PMBB, PennMedicine Biobank.

18.7 (95% CI, 9.1–39.4; $P=4 \times 10^{-15}$), whereas the odds ratio among individuals of African ancestry was only 1.8 (95% CI, 0.2–13.7; $P=0.57$) and was not statistically significant.

To further probe the interaction between ancestry and the association of TTNtv with DCM, we modeled the DCM outcome in the overall PMBB cohort while including both the principal components of genetic ancestry, and the percentage of African genetic ancestry from ADMIXTURE analysis, as well (Figure 1 in the online-only Data Supplement). With this approach, the odds ratio (OR) for the association of hiPSI TTNtv variants with DCM was 17 (95% CI, 9–31; $P=2.5 \times 10^{-21}$) in individuals with no African ancestry. Each 10% increase in percentage of African ancestry decreased the magnitude of this association by 22% (OR for interaction between hiPSI and 10% African ancestry on ADMIXTURE=0.78; 95% CI, 0.63–0.95; $P=0.02$; Figure 2 in the online-only Data Supplement). The median percentage of African ancestry in African Americans in the PMBB cohort is 85; based on this, we calculate the effect of carrying a hiPSI TTNtv on the risk of DCM in the PMBB African American individuals as only 2.2-fold increased. In contrast, the risk of DCM based on ancestry alone from our admixture models is higher, with African

Americans in PMBB being estimated to have a 3.4-fold increased risk of DCM than those without any African ancestry (OR, 1.13 per 10% African ancestry; 95% CI, 1.10–1.15; $P=0.02$).

To validate the observed lack of association between TTNtv and DCM in patients of African ancestry, we turned to the Jackson Heart Study, which conducted whole exome sequencing and extensive phenotypic evaluations of 2974 individuals, all of African descent. Among these individuals, we identified 29 (1%) with echocardiographic parameters specific for DCM (Table V in the online-only Data Supplement). Zero of these individuals were among the 18 identified with hiPSI TTNtv ($P=0.02$, assuming expected prevalence of 12%). There was no association between TTNtv carrier status and any echocardiographic or cardiac MRI parameter or incident heart failure in the participants in the Jackson Heart Study (Table VI in the online-only Data Supplement).

One possible explanation for a weaker/absent association of TTNtv with DCM in individuals of African ancestry may be an alternate exon-splicing pattern. In other words, the exons in *TTN* that are constitutively expressed (hiPSI) may vary by ancestry, such that the TTNtv in individuals of African ancestry in our analysis may be occurring in exons with low or intermediate PSI. To

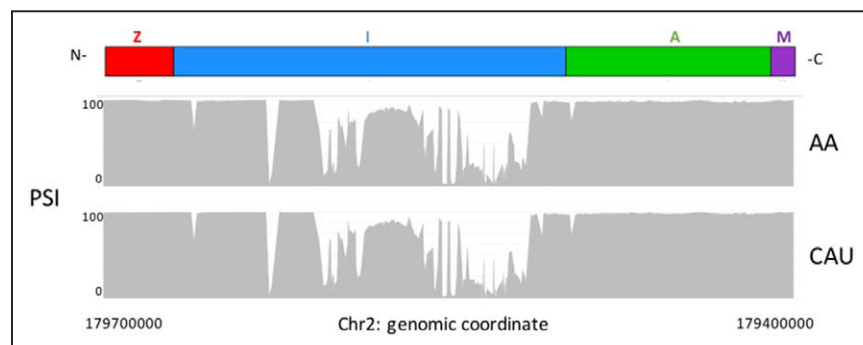


Figure 2. Proportion spliced in (PSI) exons in adult hearts from Caucasian (CAU) and African Americans (AA), explanted after cardiac transplantation for dilated cardiomyopathy.

No significant differences were noted between groups.

test this possibility, we performed RNA-sequencing of *TTN* mRNAs in 166 posttransplant hearts (89 of Caucasian descent, 77 of African descent), and 155 nonfailing discarded donor hearts (122 of Caucasian descent, 33 of African descent). As shown in Figure 2, PSI patterns were nearly identical in all 4 groups. Alternative splicing patterns thus do not differ between these races.

Disease Association

We performed a gene burden PheWAS in the Geisinger and PMBB cohorts to identify associations of broader disease diagnoses with hiPSI *TTN*tv (Figure 3A and 3B). In both cohorts, several diagnoses were significantly more common in the group with hiPSI *TTN*tv. These included cardiomyopathies (OR, 7–9), heart failure (OR, 4–8), arrhythmias (OR up to 13), and need for cardiac pacemaker or defibrillator (OR, 7–12; see Table VII in the online-only Data Supplement for OR and 95% CIs for individual phenotype codes by cohort). No associations with diagnoses were observed for low or intermediate PSI (<0.9) *TTN*tv (Figure III in the online-only Data Supplement), supporting the conclusion that tissue

expression level (PSI) is a critical factor in the pathogenesis of *TTN*tv-related disease.

Because many of these phenotypes are likely the downstream consequences of DCM, we repeated the PheWAS adjusting for the lead cardiomyopathy diagnoses. No associations were noted in the PMBB cohort after this adjustment. In the Geisinger cohort, however, several associations remained significant, including atrial fibrillation (OR, 2.4), other dysrhythmias (OR, 2–4), and heart failure (OR, 3–4; Figure 3C and 3D, Table VII in the online-only Data Supplement). Thus, in the Geisinger cohort, hiPSI *TTN*ts associate strongly with atrial fibrillation and arrhythmia even after adjustment for DCM, and the implications of hiPSI *TTN*tv (such as heart failure) exceed the extent of clinically recognized DCM.

Quantitative Phenotype

Measures of cardiac structure and function for individuals with a clinically obtained echocardiogram ($n=200$ [56%] for Geisinger, $n=67$ [40%] for PMBB) are provided in Table VIII in the online-only Data Supplement. Individuals with hiPSI *TTN*tv in both cohorts had lower

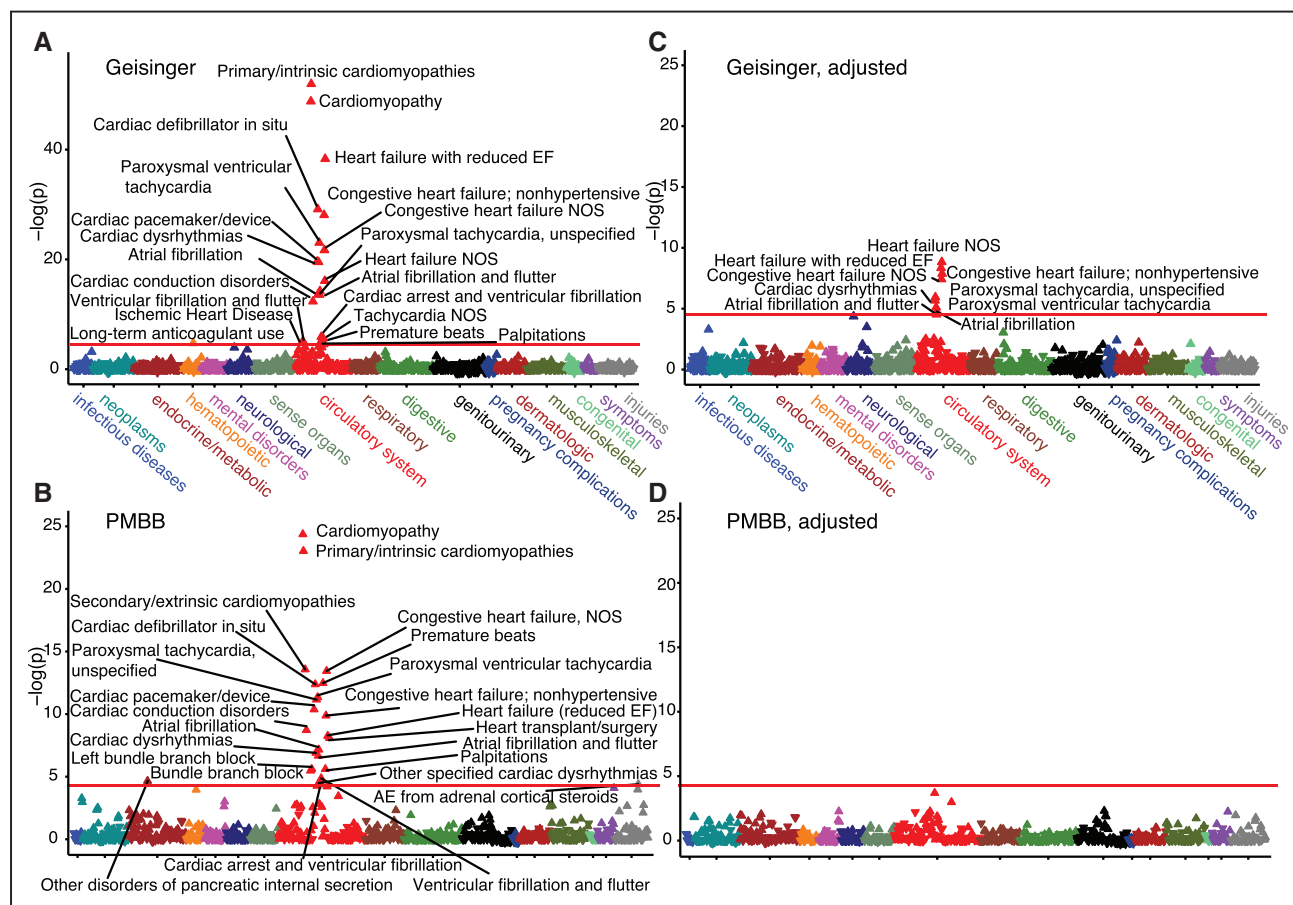


Figure 3. Manhattan plots representing the Phenome-wide Association findings.

Results from the Geisinger (A) and PennMedicine Biobank (PMBB; B) cohorts demonstrating numerous significant cardiovascular phenotypes associated with *TTN* truncating variants (TTNtv). After controlling for cardiomyopathy, atrial fibrillation remained significantly associated with TTNtv in the Geisinger cohort (C), whereas no significant associations were observed in PMBB (D). EF indicates ejection fraction; and NOS, not otherwise specified.

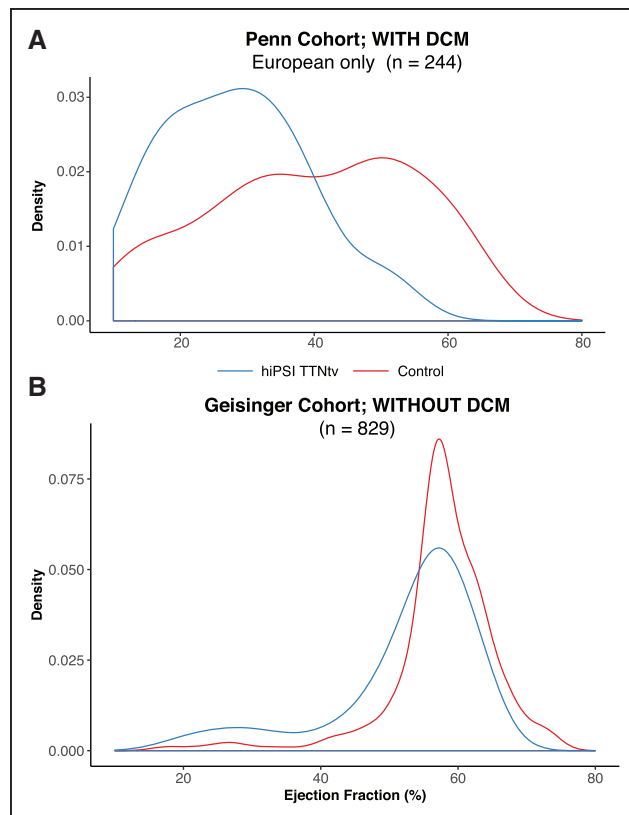


Figure 4. Density plots comparing left ventricular ejection fraction (LVEF) between hiPSI TTNtv (blue) and controls (red) among patients with (Penn, A) and without (Geisinger, B) dilated cardiomyopathy (DCM) diagnoses.

In both cases, LVEF was significantly lower in the group with TTNtv. hiPSI indicates having variants within exons with high proportion spliced in to Titin transcripts; Penn, PennMedicine BioBank; and TTNtv, Titin-truncating variants.

LVEF, decreased septal thickness and increased diastolic and systolic LV internal diameters (Table VIII in the online-only Data Supplement, Figure IV in the online-only Data Supplement). We also observed that LVEF was statistically different in individuals with hiPSI variants (>0.9) in comparison with individuals with intermediate (0.16 – 0.9) and low (0 – 0.15) PSI variants (Figure V in the online-only Data Supplement), further supporting the importance of PSI in determining disease association. Conversely, we did not observe variations in LVEF according to position (band) within the *TTN* gene (Figure VI in the online-only Data Supplement) for variants in hiPSI exons.

To examine the effect of hiPSI TTNtv within the subset of individuals with DCM, we focused on the PMBB cohort, where the number of individuals with DCM and hiPSI TTNtv was high ($n=43$). We restricted the analysis to patients who have DCM with available LVEF measurements, and of European ancestry, in light of the low number of hiPSI TTNtv-positive patients of African ancestry. Among individuals with DCM, we observed significantly lower LVEF and higher LV systolic diameter in patients with hiPSI TTNtv, in comparison with those without (Figure 4, Table 2). These differences persisted

after adjustments for age, sex, and ancestry (Table 2), and held whether evaluating median, minimum, or most recently measured LVEF (Figure VII in the online-only Data Supplement).

To examine the effect of hiPSI TTNtv among individuals without DCM, we focused on the Geisinger cohort, where the number of such individuals is high ($n=132$). For this analysis, DCM was defined broadly as any encounter coded with “dilated cardiomyopathy” (I42), “other (primary) cardiomyopathies” (425.4, I42.8), “cardiomyopathy in other diseases classified elsewhere” (425.8), “cardiomyopathy, unspecified” (I42.9), or “secondary cardiomyopathy, unspecified” (425.9). The lack of DCM was further confirmed through blinded physician chart review. After these exclusions, differences in LVEF, but not LV diastolic diameter, persisted adjusted for age, sex, and ancestry (Figure 4, Table 2). We further excluded any individuals with a heart failure diagnosis, and the association with lower LVEF persisted ($\beta=-2.3$ [95% CI, -3.3 to -1.2 ; $P=4\times 10^{-5}$).

DISCUSSION

In this genomics-first study, we combined *TTN* exome sequencing with extensive clinical phenotyping derived from EHRs to determine genotype-phenotype relationships in individuals bearing TTNtv. We leveraged 2 cohorts, a general clinical population (Geisinger) and patients from a tertiary referral center (PMBB). In both contexts, we found clear phenotypic differences in individuals with hiPSI TTNtv identified from a genomics-first approach. In a general clinical population (Geisinger), our findings demonstrate that: (1) hiPSI TTNtvs convey significantly increased odds for DCM and are also associated with other related cardiac phenotypes, such as systolic heart failure and arrhythmias, some of which may be independent of DCM; and (2) individuals with a hiPSI TTNtv and a clinically indicated echocardiogram have decreased LV function, even in the absence of a clinical diagnosis of nonischemic DCM. In a referral population (PMBB), our findings show that: (1) hiPSI TTNtvs are strongly associated with cardiac phenotypes, largely driven by DCM; (2) individuals with DCM and hiPSI TTNtv have markedly increased LV size and decreased LV function in comparison with patients with DCM who lack a TTNtv; and (3) the association between hiPSI TTNtvs and DCM in individuals of African ancestry is much weaker than in individuals of European ancestry.

Yield of DCM From Genomics-First TTNtv

Overall, 7.5% of Geisinger individuals and 30% of PMBB individuals with hiPSI TTNtv had DCM, corresponding with ORs of 10.8 and 18.7, respectively

Table 2. Echocardiogram Measures in hiPSI TTNtv+ Individuals and hiPSI TTNtv− Controls Without (Geisinger) or With (PMBB) DCM Diagnosis

Echocardiogram Measures	hiPSI TTNtv ⁺	hiPSI TTNtv [−]	Effect Estimate (95% CI)*	P Value
Geisinger (without DCM)				
LVEF, % (last)				
Median	57	57	−3.4 (−4.7 to −2.2)	1×10 ^{−7}
IQR	52–58	57–62		
n	123	706		
LVIDd, cm				
Median	4.6	4.6	0.04 (−0.09 to 0.16)	0.56
IQR	4.1–5.1	4.1–5.0		
n	107	604		
LVIDs, cm				
Median	3.2	3.0	0.24 (0.10 to 0.40)	1×10 ^{−3}
IQR	2.7–3.7	2.6–3.4		
n	95	501		
LVPWd, cm				
Median	1.0	1.0	−0.02 (−0.05 to 0.02)	0.44
IQR	0.9–1.2	0.9–1.2		
n	106	603		
IVSd, cm				
Median	1.1	1.1	−0.02 (−0.06 to 0.02)	0.33
IQR	0.9–1.2	0.9–1.2		
n	106	603		
MV E/A				
Median	1.0	1.0	−0.02 (−0.10 to 0.07)	0.70
IQR	0.8–1.2	0.8–1.2		
n	78	486		
LA dim†				
Median	3.7	3.7	0.05 (−0.08 to 0.19)	0.44
IQR	3.2–4.2	3.2–4.1		
n	90	547		
PMBB (with DCM; excluding transplant)				
LVEF, % (last)				
Median	29	40	−12.1 (−16.2 to −7.0)	3×10 ^{−07}
IQR	15	22.2		
n	26	218		
LVIDd, cm				
Median	5.67	5.42	0.37 (−0.01 to 0.77)	0.07
IQR	1.22	1.17		
n	25	193		
LVIDs, cm				
Median	4.82	4.12	0.65 (0.13 to 1.12)	0.009
IQR	1.25	1.32		
n	25	192		

(Continued)

Table 2. Continued

Echocardiogram Measures	hiPSI TTNtv ⁺	hiPSI TTNtv ⁻	Effect Estimate (95% CI)*	P Value
LVPWd, cm				
Median	0.92	1.02	−0.09 (−0.17 to 0.01)	0.07
IQR	0.22	0.24		
n	26	203		
IVSd, cm				
Median	0.92	1.04	−0.11 (−0.19 to −0.04)	0.004
IQR	0.21	0.28		
n	26	203		
MV E/A				
Median	91	0.92	0.05 (−0.18 to 0.30)	0.67
IQR	0.4	0.56		
n	16	165		
LA dim†				
Median	24.1	22.1	−0.09 (−3.75 to 4.72)	0.97
IQR	7.2	10.5		
n	21	162		

DCM indicates dilated cardiomyopathy; E/A, E-wave velocity/A-wave velocity; hiPSI, having variants within exons with high proportion spliced in to Titin transcripts; IQR, interquartile range; IVSd, interventricular septum thickness, diastole; LA, left atrium; LVEF, left ventricular ejection fraction; LVID (d/s), left ventricular internal diameter (diastole/systole); LVPWd, left ventricular posterior wall thickness, diastole; MV, mitral valve; PMBB, PennMedicine Biobank; and TTNtv, Titin truncating variants.

*Effect estimates computed from linear regression, adjusted for age, sex, and ancestry (principal components 1–4 [Geisinger] or 1–10 [PMBB]). 95% CIs defined with bootstrapping with 1000 samples.

†LA dimension (dim) represents parasternal diameter for Geisinger and 4-chamber area for PMBB.

(Europeans only for PMBB). The DCM/TTNtv association has been well documented through genetic sequencing of patients with DCM,^{2–4} but not previously documented through a genomics-first approach. The prevalence and OR differences likely reflect patient ascertainment into the respective biobanks. For Geisinger, participants are recruited across a large regional health system, comprising both primary care and specialty clinics, and are thus more representative of the population of central Pennsylvania. For PennMedicine, a tertiary care facility, a large percentage of participants have been referred for specialty care, consistent with the higher observed proportion of advanced disease. Hence, these cohorts represent opposite ends of the clinical spectrum for ascertaining patients with DCM, but our findings demonstrate that TTNtv have a meaningful and measurable impact in both settings.

Other Clinical Phenotype Associations

In addition to DCM, we found numerous other significant cardiac phenotype associations of TTNtv (in hiPSI exons) in individuals identified by using this genomics-first approach. These included diagnoses of cardiomyopathies; other common clinical manifestations associated with cardiomyopathies, such as heart failure, and ventricular and atrial dysrhythmias; and common interventions for cardiac arrhythmias and dysfunction, such

as defibrillator or pacemaker placement. Moreover, the fact that no phenotype is associated with low PSI TTNtv in our PheWAS reinforces the presumed benign nature of variants in low and intermediate PSI exons.

To evaluate whether TTNtv are associated with other diseases independent of DCM, we repeated the phenotype association while conditioning for cardiomyopathy diagnoses. Conditioning on cardiomyopathy in the PMBB cohort removed all other associations, suggesting they were all secondary to cardiomyopathy in this cohort with more advanced disease. However, several associations remained significant after adjustment in the Geisinger cohort, suggesting independence from DCM. These include atrial fibrillation, which is consistent with recent work showing increased early arrhythmic risk in patients who have DCM with TTNtv,²⁸ and genome-wide associations of loci in or near *TTN* and atrial fibrillation, as well.^{29–31} In addition, several systolic heart failure diagnoses remained significant. These findings are unlikely to be independent of DCM, but do suggest a clinical underrecognition of *TTN*-associated cardiomyopathy.

Quantitative Imaging Phenotype

Few data exist to address the question of whether an incidental finding of a hiPSI TTNtv is associated with structural cardiac dysfunction. The best evidence to date

comes from cardiac MRI data in 15 healthy individuals with intermediate or high PSI variants ($\text{PSI} > 0.15$) showing increased ventricular volumes.¹¹ Using our larger cohort of 132 individuals with a hiPSI TTNtv and a clinically indicated echocardiogram, but without a diagnosis of nonischemic DCM, we found that hiPSI TTNtv strongly correlated with lower LVEF, but no difference in diastolic diameter, suggesting a deficit in contractility. Although other heart conditions, such as ischemic disease, were present in our clinically based cohort, the extent to which they are related to or interacting with the TTNtv is unclear. Thus, clinical follow-up of incidental findings of hiPSI TTNtv, such as routine echocardiography, may be warranted to potentially identify underlying heart disease. Prospective studies will be needed to determine if subsequent early intervention before the development of overt DCM and heart failure, such as neurohormonal blockade, may prevent the development of symptoms in individuals with hiPSI TTNtv.

For patients with DCM, we find that hiPSI TTNtv were strongly associated with cardiac enlargement and as much as a 10% absolute reduction in LVEF, suggesting that hiPSI TTNtv DCM may represent a more severe form of disease. These findings contrast with recent findings in the Netherlands of a mild form of DCM associated with hiPSI TTNtv.³² In addition, a recent longitudinal study in the United Kingdom found that TTNtv were not associated with worse cardiac parameters in a DCM cohort.³³ This contrast between studies may reflect different patient populations, despite our adjustments for age, sex, and ancestry. For example, the mean age of patients with TTNtv in the Dutch cohort was 49 years, in comparison to 66 years in our cohort. The contrast between studies may also reflect patient ascertainment, acuity of presentation, or comorbidity profile. This heterogeneity between cohorts underscores the challenge of generalizing observations from one clinical population to another.

Ancestry-Stratified Differences of Prevalence of TTNtv in DCM

Among patients with DCM in the PMBB ($n=613$), the overall prevalence of hiPSI TTNtv (7%) was less than has been reported in ascertained DCM cohorts.^{2,33} Ancestry-specific carrier rates, however, likely explain this difference. Among patients with DCM of European ancestry the carrier rate of hiPSI TTNtv was 10%, which is akin to previously reported values.^{33,34} In striking contrast, the prevalence of TTNtv in individuals of African ancestry with DCM was 1.4%, which is not significantly different from that reported in a general population of African Americans (ExAC). In addition to this, hiPSI TTNtv were not associated with DCM in individuals of African ancestry in the PMBB cohort, nor with echocardiographic or cardiac MRI evidence of DCM in

subjects in the Jackson Heart Study. It is possible that we underestimated the true burden of DCM across Jackson Heart Study because some participants with echocardiographic or MRI findings close to the normal range might have been treated and had improvement in ejection fraction. However, based on the results in the PMBB cohort, we expect this be less likely to occur in patients with DCM carrying hiPSI TTNtv than those without variants. Unfortunately, previous DCM cohort studies have not reported on hiPSI TTNtv carrier status by genetic ancestry,^{2,5} although the presence of TTNtv in women with peripartum cardiomyopathy, a DCM-like disease, does seem to be equivalently high in women of European and African ancestry.⁷

A number of reasons may explain the lack of observed association between TTNtv and DCM in patients of African descent. It has been speculated that the development of DCM in the setting of hiPSI TTNtv requires a second hit (ie, extrinsic stress), such as volume overload or chemotherapy.^{7,11,35} Alternatively, environmental factors may be responsible for the lack of association, including access to health care and social determinants of health, both of which are worse in African American populations.^{36,37} In addition, patients of African descent have higher rates of DCM in general, such as perhaps higher rates of hypertension-associated DCM, and this high prevalence may dilute the measurable risk conferred by TTNtv. Also, it is possible that referral rates for advanced heart failure differ by race. Finally, it is possible that the genetic background in individuals of African ancestry limits the impact of TTNtv-induced risk of developing DCM. However, one possible such explanation, alternative splicing allowing more TTNtv to fall in low PSI exons, does not appear to be the case. It will be of future interest to evaluate the TTNtv/DCM relationship in cohorts of other genetic ancestries and with different approaches to ascertainment.

Limitations

The *TTN* gene is the largest and one of the most complex coding regions of the genome, and still presents technical challenges to next-generation sequencing. These technical limits may introduce confounding factors to our analysis, such as a higher than normal false discovery rate. We took steps to mitigate this potential, such as Sanger confirmation for a subset of samples to identify appropriate quality cutoffs, but these steps may not have removed all false-positive cases. However, because the likely effect of false-positive calls is to introduce noise in the signal, these potential errors are unlikely to explain our findings and may in fact lead to an underestimation of the effects in our observations.

Furthermore, the inability to detect an association between TTNtv and DCM in African American individu-

als may be the result of a relatively modest sample size. Although we cannot conclude that there is no association between TTNtv and DCM in African Americans, we can safely exclude an association on the order of magnitude we report in individuals of European ancestry. Based on our case and control numbers in African Americans, we have a >99% power to detect an OR of 18, that which we report individuals of European ancestry, and >90% power to detect an OR of at least 7, with a 2-sided α of 0.05.

In conclusion, our genomics-first evaluation of TTNtv in 2 disparate clinical populations demonstrates that hiPSI TTNtvs in individuals of European ancestry are associated with worse cardiac function in the presence of DCM and reduced (low-normal) cardiac function in the absence of a DCM diagnosis. Identification of TTNtv carriers of European ancestry may thus alter clinical management strategies, because individuals with hiPSI TTNtvs, but without clinical evidence of DCM, may harbor unrecognized cardiac dysfunction and dysrhythmia, whereas those patients with hiPSI TTNtv-mediated DCM may be at risk for more severe disease. Conversely, we did not detect any significant association between TTNtvs and DCM among individuals of African ancestry from 2 independent cohorts, suggesting the OR for DCM in those individuals is much less. Further ancestry-stratified analyses are therefore warranted to confirm this finding and evaluate these trends among other ethnic populations.

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Authors

Christopher M. Haggerty, PhD*; Scott M. Damrauer, MD*; Michael G. Levin, MD; David Birtwell, MSE; David J. Carey, PhD; Alicia M. Golden, BS; Dustin N. Hartzel, BS; Yirui Hu, PhD; Renae Judy, MS; Melissa A. Kelly, MS; Rachel L. Kember, PhD; H. Lester Kirchner, PhD; Joseph B. Leader, BA; Lusha Liang, MD; Chris McDermott-Roe, PhD; Apoorva Babu, MS; Michael Morley, PhD; Zachariah Nealy, MD; Thomas N. Person, MS; Arichanah Pulenthiran, BS; Aeron Small, MD; Diane T. Smelser, PhD; Richard C. Stahl, BS; Amy C. Sturm, MS; Heather Williams, MSE; Aris Baras, MD; Kenneth B. Margulies, MD; Thomas P. Cappola, MD; Frederick E. Dewey, MD; Anurag Verma, PhD; Xinyuang Zhang, BS; Adolfo Correa, MD, PhD; Michael E. Hall, MD; James G. Wilson, MD; Marylyn D. Ritchie, PhD; Daniel J. Rader, MD; Michael F. Murray, MD; Brandon K. Fornwalt, MD, PhD†; Zoltan Arany, MD, PhD†; On behalf of the DiscovEHR and Penn Medicine Biobank Studies

Correspondence

Zoltan Arany, MD PhD, Director, Cardiovascular Metabolism Program, Perelman School of Medicine, University of Pennsylvania, Smilow Center for Translational Research, 3400 Civic Blvd, Philadelphia, PA 19104. Email zarany@pennmedicine.upenn.edu

Affiliations

Geisinger, Danville, PA (C.M.H., D.J.C., A.M.G., D.N.H., Y.H., M.A.K., H.L.K., J.B.L., Z.N., T.N.P., A.P., D.T.S., R.C.S., A.C.S., M.F.M., B.K.F.). Perelman School of Medicine, University of Pennsylvania, Philadelphia (S.M.D., M.G.L., D.B., R.J., R.L.K., L.L., C.M.-R., A. Babu, M.M., A.S., H.W., K.B.M., T.P.C., A.V., X.Z.,

M.D.R., D.J.R., Z.A.). Corporal Michael Crescenz VA Medical Center, Philadelphia, PA (S.M.D.). Regeneron Genetics Center, Tarrytown, NY (A. Baras, F.E.D.). Department of Medicine (A.C., M.E.H.) and Department of Physiology and Biophysics (M.E.H., J.G.W.), University of Mississippi Medical Center, Jackson.

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