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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. This work was published in *Circulation* in May of 2021. 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 2019.

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. Data with cardiotoxic stressors such as chemotoxic agents has revealed surprisingly little effect, compared to control animals. On the other hand, data indicated that repeated pregnancies (n=five) do worsen function in these animals, compared to controls. We have now generated reagents to test the impact of various pregnancy hormones on cardiac function in these *ttn* knock-in mice and are actively pursuing these studies.

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. We have also evaluated in detail tissue from these human hearts with TTN truncating variants (n=22), and have demonstrated conclusively that mutant protein can be found in these hearts, which has long been a controversial issue. This work is in press at *Science Translational Medicine*.

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 submitted and revised our work on the genetic findings from our multi-center PPCM Collaborative Network. This work is now published in *Circulation* (Goli et al. 2021). In brief, Women with PPCM were retrospectively identified from several US and international academic centers, and clinical information and DNA samples were acquired. Next-generation sequencing was performed on 67 genes, including *TTN*, and evaluated for burden of truncating and missense variants. The impact of *TTN*ts on severity of clinical presentation, and on clinical outcomes, was evaluated. 469 women met inclusion criteria. 10.4% of women with PPCM bore *TTN*ts (Odds ratio [OR]=9.4 compared with 1.2% in reference population; Bonferroni-corrected P [P^*] = 1.2×10^{-46}). We additionally identified overrepresentation of truncating variants in *FLNC* (OR=24.8, $P^*=7.0 \times 10^{-8}$), *DSP* (OR=14.9, $P^*=1.0 \times 10^{-8}$), and *BAG3* (OR=53.1, $P^*=0.02$), genes not previously associated with PPCM. This profile is highly similar to that found in non-ischemic dilated cardiomyopathy (DCM). Women with *TTN*ts had lower left ventricular ejection fraction (LVEF) on presentation than did women without *TTN*ts (23.5% vs 29%, $P=2.5 \times 10^{-4}$), but did not differ significantly in timing of presentation after delivery, in prevalence of preeclampsia, or in rates of clinical recovery. This study provides the first extensive genetic and phenotypic landscape of PPCM, and demonstrates that predisposition to heart failure is an important risk factor for PPCM. The work reveals a degree of genetic similarity between PPCM and DCM, suggesting that gene-specific therapeutic approaches being

developed for DCM may also apply to PPCM, and that approaches to genetic testing in PPCM should mirror those taken in DCM. Finally, the clarification of genotype/phenotype associations has important implications for genetic counseling.

In parallel work, we continued to leverage our large cohort of women with PPCM at the University of Pennsylvania to better understand the strikingly higher incidence of PPCM and worse outcomes in Black women (a group that is more highly represented in the US Armed Forces). We showed in 2017 that Black women with PPCM have persistent dysfunction at twice the rate of White women, and that even when recovery does occur, it takes twice as long. We then used geocoding and street-level US Census data to demonstrate that these racial inequities are in fact largely explained by socioeconomic inequities, most specifically education status. These observations are actionable and have high implications for clinical care, as they show that racial inequities in PPCM are not inherently genetic and thus can be addressed. This work was recently published in the American Heart Journal 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 2019 (see Appendix). In addition, 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. These data suggested that the repeated exposure to pregnancy hormones can trigger PPCM. We therefore generated multiple reagents to over-express pregnancy hormones in mice, via viral delivery to liver, and we are currently actively characterizing their impact on cardiac function in our *TTN* knock-in mice.

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. In parallel, we further leveraged our collaborator's large collection of post-transplant human hearts to identify 19 hearts with TTNtvs, and we developed mass spectrometry and electrophoretic methodologies to detect truncated TTN proteins (biochemical work with TTN protein is very challenging, because the protein is >4megadaltons) to demonstrate consistent presence of truncated TTN proteins in these hearts. This observation is of great importance, because truncated TTN protein has never been successfully detected before, and it was assumed not to exist. The work thus changes pathophysiological paradigms for both PPCM and DCM; explains why the mouse models insufficiently mirror the human disease; and expose new hypotheses about how TTNtvs cause cardiomyopathy (which we are addressing). This work is *in press* at Science Translational Medicine.

REPORTABLE OUTCOMES

Truncated titin proteins in dilated cardiomyopathy.

Quentin McAfee, Christina Yingxian Chen, Yifan Yang, Matthew Caporizzo, Michael Morley, Apoorva Babu, Sunhye Jeong, Jeffrey Brandimart, Kenneth C. Bedi Jr¹, Emily Flam, Joseph Cesare, Thomas P. Cappola, Kenneth Margulies, Benjamin Prosser, **Zolt Arany**.

Science Translational Medicine. 2021 *In press*

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Goli R, Li J, Brandimarto J, Levine LD, Riis V, McAfee Q, DePalma S, Haghighi A, Seidman JG, Seidman CE, Jacoby D, Macones G, Judge DP, Rana S, Margulies KB, Cappola TP, Alharethi R, Damp J, Hsich E, Elkayam U, Sheppard R, Alexis JD, Boehmer J, Kamiya C, Gustafsson F, Damm P, Ersbøll AS, Goland S, Hilfiker-Kleiner D, McNamara DM; IMAC-2 and IPAC Investigators, **Arany Z**.

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Getz KD, Lewey J, Tam V, Irizarry OC, Levine LD, Aplenc R, **Arany Z**

Am Heart J. 2021 Aug;238:27-32. PMID: 33857409

Women with peripartum cardiomyopathy have normal ejection fraction, but abnormal systolic strain, during pregnancy.

Tamrat R, Kang Y, Scherrer-Crosbie M, Levine LD, **Arany Z**, Lewey J.

ESC Heart Fail. 2021 May 4. PMID: 33943010

Peripartum Cardiomyopathy: JACC State-of-the-Art Review.

Davis MB, **Arany Z**, McNamara DM, Goland S, Elkayam U.

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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**.

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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 numerous major publications in Circulation, Hypertension, Science Translational Medicine, etc. 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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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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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 (intraquartile 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.

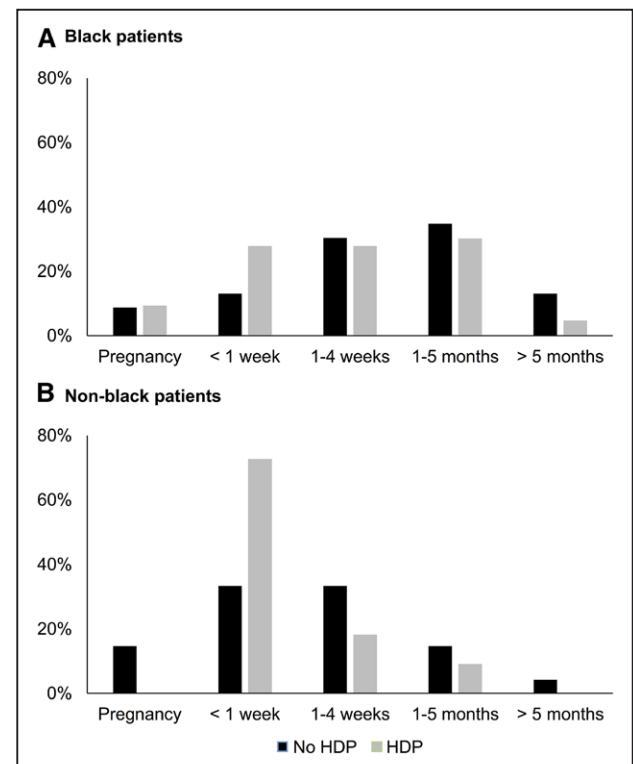


Figure. Timing of diagnosis by hypertensive disorder of pregnancy (HDP) and race. Timing refers to the postpartum period.

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.

THE PRESENT AND FUTURE

JACC STATE-OF-THE-ART REVIEW

Peripartum Cardiomyopathy

JACC State-of-the-Art Review



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ABSTRACT

Peripartum cardiomyopathy is a form of systolic heart failure affecting young women toward the end of pregnancy or in the months following delivery. Incidence is higher in African-American women and in women with older maternal age, hypertensive disorders of pregnancy, and multiple gestation pregnancies. Symptoms of heart failure mimic those of normal pregnancy, often resulting in a delay in diagnosis and preventable complications. Echocardiography showing decreased myocardial function is essential for the diagnosis. Medical management is similar to heart failure with reduced ejection fraction of other etiologies, but adjustments during pregnancy are necessary to ensure fetal safety. Variable outcomes include complete recovery, persistent heart failure, arrhythmias, thromboembolic events, and death. Subsequent pregnancy confers substantial risk of relapse and even death if there is incomplete myocardial recovery. Additional research about the etiology, optimal therapy including the use of bromocriptine, long-term outcomes, and duration of treatment after recovery are needed. (J Am Coll Cardiol 2020;75:207-21) © 2020 by the American College of Cardiology Foundation.

Peripartum cardiomyopathy (PPCM) is a form of systolic heart failure (HF) with reduced left ventricular ejection fraction (LVEF) affecting childbearing women during pregnancy or in the early postpartum period. Delays in diagnosis may occur because the symptoms and signs of PPCM can mimic the normal findings of late pregnancy and the peripartum period. Although some women have relatively mild disease and complete recovery, others experience significant morbidity and mortality. This clinical review will describe the definition, risk factors, etiology, pathophysiology, and prognostic factors and will provide recommendations for diagnosis, acute and chronic management, and important counseling considerations, including breastfeeding and subsequent pregnancies.

DEFINITION

PPCM is a diagnosis of exclusion in women presenting with HF due to left ventricular (LV) systolic dysfunction and should be considered when no other cause is evident. PPCM was previously defined as symptomatic HF presenting in the last month of pregnancy and up to 5 months postpartum (1,2); however, the definition has been broadened because women who presented prior to the last month of gestation were found to be clinically indistinguishable from patients with classically-defined PPCM (3). The 2010 Heart Failure Association of the European Society of Cardiology Working Group therefore revised the definition of PPCM to “an idiopathic cardiomyopathy presenting with HF secondary to LV systolic dysfunction towards the end of pregnancy or



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ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

BNP = brain natriuretic peptide

DCM = dilated cardiomyopathy

HF = heart failure

ICD = implantable cardioverter-defibrillator

LV = left ventricular

LVAD = left ventricular assist device

LVEF = left ventricular ejection fraction

PPCM = peripartum cardiomyopathy

in the months following delivery, where no other cause of heart failure is found” (4). The diagnostic criteria indicate that LVEF is <45% and there may or may not be ventricular dilatation (4,5). Outcomes are variable; women may have complete recovery, persistent myocardial dysfunction and HF, or rapid deterioration, leading to urgent need for temporary or durable mechanical circulatory support and cardiac transplantation (Central Illustration).

EPIDEMIOLOGY

Global estimates of the incidence of PPCM vary by regions, with reports as high as 1 in 100 deliveries in Nigeria (6) and 1 in 300 de-

liveries in Haiti (7), to as low as 1 in 20,000 deliveries in Japan (8). In the United States, reported incidence ranges from 1 in 1,000 to 1 in 4,000 (9–11) and may be increasing, due to the rise in maternal age, increased rates of multifetal pregnancies due to contemporary fertility techniques, and possibly to increased recognition of the disease. Many cases may moreover be unrecognized; thus, the true incidence is unknown.

RISK FACTORS

Several risk factors have been associated with PPCM. The incidence of PPCM is higher in women of African ancestry (10,12–15). In nationwide studies from the United States, >40% of the cases occurred in African-American women (9,10,16). Similarly, statewide studies report PPCM occurring 3 to 16 times as often in African-American women compared with white women (12–14). Pre-eclampsia and hypertension are strongly associated with PPCM (3,10,14,17–19). A meta-analysis of 22 studies including 979 cases of PPCM reported that pre-eclampsia was present in 22% of women with PPCM, compared with an average worldwide background rate of 5%, and other hypertensive disorders were present in 37% (18). Hypertension and pre-eclampsia can lead to HF and pulmonary edema due to predominant LV diastolic dysfunction. Although subclinical LV systolic dysfunction can be detected by speckle tracking strain imaging, LVEF is preserved (20–24). The majority of women with pre-eclampsia do not develop PPCM, which is only diagnosed when the systolic function is decreased. Multigestational pregnancies are reported in 7% to 14.5% of women with PPCM in the United States (17,18,25,26). A meta-analysis of 16 studies of PPCM showed an overall incidence of twin pregnancies in 9% of women, compared with the national average of 3% (18). Older maternal age also

HIGHLIGHTS

- Medications used to treat HF during pregnancy and lactation require special considerations.
- Severe HF may require advanced therapies and mechanical circulatory support.
- Subsequent pregnancies carry risk of relapse, and dedicated counseling and monitoring are essential.
- Future research about long-term outcomes, continued drug therapy, use of bromocriptine, device therapy, and genetics are needed.

appears to be associated with PPCM (3,12,14): one-half of cases of PPCM occur in women age >30 years, and 1 study reported that age >40 years had an odds ratio of 10 of developing the disease compared with women age <20 years (9). Although a minority of women with PPCM have a family history of cardiomyopathy (see the Genetics section), awareness of HF symptoms during and after pregnancy may improve detection of PPCM in these patients. Closer surveillance of women who are at highest risk during pregnancy could lead to earlier diagnosis and treatment.

PATHOPHYSIOLOGY

The etiology of PPCM is not fully understood and is likely multifactorial. Suggested but not proven mechanisms for the development of PPCM have included nutritional deficiencies (27,28), viral myocarditis (29,30), and autoimmune processes (31,32). Hemodynamic stress of pregnancy has been postulated as a potential etiology. However, the maximal cardiovascular changes occur in the second trimester (33), when most women with pre-existing cardiac disease develop symptomatic HF (34). In contrast, the majority of women with PPCM develop symptoms during late pregnancy or after the delivery (35).

The development of 2 vascular-hormonal animal models of pregnancy-associated cardiomyopathy suggested novel mechanisms for PPCM in humans. The first model was a STAT3 knockout mouse in which oxidative stress led to cleavage of the nursing hormone, prolactin. The 16-kDa prolactin fragment had vasculotoxic and pro-apoptotic properties and vascular and myocardial dysfunction (36). The mice treated with bromocriptine, a suppressor of prolactin secretion, had complete reversal of the

CENTRAL ILLUSTRATION Diagnosis, Management, and Outcomes for Peripartum Cardiomyopathy

Peripartum Cardiomyopathy (PPCM)

Definition:

- Non-ischemic cardiomyopathy with reduced LVEF (<45%)
- Commonly presents in the first months postpartum or towards the end of pregnancy

Risk Factors:

- African-American race, preeclampsia, hypertension, multigestational pregnancies, age >30 years

Symptoms:

- Heart failure symptoms can be confused with common symptoms of normal pregnancy

Management Options for PPCM



During Pregnancy:

- Beta-blockers, loop diuretics, hydralazine/isosorbide dinitrate, digoxin, low-molecular-weight heparin
- (No ACE/ARB/aldosterone receptor antagonists)
- MCS for severe heart failure/cardiogenic shock
- Consider early delivery if unstable



Delivery:

- Plan ahead with a Cardio-Obstetrics Team
- If unstable, consider hemodynamic monitoring and optimization
- Caution for fluid overload, especially after delivery



After Pregnancy:

- Heart failure management. Beta-blockers, enalapril, and spironolactone are compatible with breastfeeding.
- Anticoagulation for LV thrombus; consider if severe LV dysfunction (LVEF <35%)
- Consider a wearable cardioverter/defibrillator if severe LV dysfunction
- Discuss Contraception

Outcomes

Worse prognosis with lower LVEF, dilated LV, African-American race, and delayed diagnosis.

Long-term Outcomes

- After recovery, optimal duration of medication treatment is unknown
- In the case of stopping medications, wean gradually and observe closely
- Continue surveillance after recovery

Davis, M.B. et al. J Am Coll Cardiol. 2020;75(2):207-21.

Diagnostic criteria, symptoms, and risk factors can aid in the diagnosis. Medications need to be tailored to pregnancy and breastfeeding status. Short- and long-term outcomes are variable and serial follow-up is important. ACE = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; LV = left ventricle; LVEF = left ventricular ejection fraction; MCS = mechanical circulatory support; PPCM = peripartum cardiomyopathy.

cardiomyopathy. Of note, human prolactin has been shown to be more resistant to cleavage than prolactin in rats (37), and extrapolations from rodent models may be limited. A second vascular-hormonal mouse model of PPCM was developed by cardiac-specific genetic deletion of proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), leading to vasculotoxicity by activation of the 16-kDa prolactin fragment and decreased expression of proangiogenic vascular endothelial growth factor (VEGF). In this model, the cardiomyopathy could be reversed only partly with bromocriptine and required the addition of VEGF for complete recovery (38). VEGF is also strongly antagonized in late gestation by the placental secretion of soluble Fms-like tyrosine kinase 1 (sFlt1), an antagonist of VEGF and placental growth factor. Exogenous sFlt1 was sufficient to cause profound systolic dysfunction in mice lacking cardiac PGC-1 α , even in the absence of pregnancy. Moreover, sFlt1 levels are markedly elevated in women with preeclampsia, likely in part explaining why preeclampsia is such a strong risk factor for PPCM. Elevated sFlt1 levels have also been found in a subset of women with PPCM and predict worse outcomes (39). In summary, 2 mouse models and epidemiological data support the notion that PPCM is in large part a vascular disease, triggered by the hormonal milieu of the peripartum (40,41).

GENETICS

Genetic differences may explain why only relatively few women develop HF in response to the vascular insults of late pregnancy. Observations of familial clustering of PPCM, as well as ~6% co-occurrence with idiopathic dilated cardiomyopathy (DCM), suggested early on the possibility of a genetic contribution to the disease (42-48). In a German registry, 15% of women with PPCM had a family history of cardiac disease in a first-degree relative (defined as PPCM, DCM, sudden death, or arrhythmias) (49). Genetic studies have subsequently supported the notion that genetics contribute to PPCM. A genome-wide association study of 79 patients with PPCM identified a single-nucleotide polymorphism near the *PTH1H* gene (50), and this gene may regulate vascular homeostasis (51). Evaluation of rare pedigrees that include both PPCM and DCM identified likely pathogenic variants in genes known to contribute to DCM such as *TTN* and *BAG3*. In a definitive genetic study of 172 patients with PPCM not pre-selected for family history, targeted sequencing of 43 genes known to associate with DCM revealed a 15% prevalence of truncating variants (i.e., nonsense, missense, or splicing variants) in patients

with PPCM (52). Two-thirds of the truncating variants were in the *TTN* gene, with the majority of these in the A band of *TTN*. The *TTN* gene encodes the largest human protein, titin, a critical structural component of sarcomeres in cardiac and skeletal muscle. The prevalence and location of these mutations in *TTN* overlap with other forms of DCM, again suggesting a genetic overlap between these 2 diseases. In post hoc analyses, *TTN* variants identified in the well-characterized subset of patients derived from the IPAC (Investigations of Pregnancy Associated Cardiomyopathy) cohort correlated with lower LVEF at 1 year, suggesting that the presence of *TTN* truncating variant may presage worse outcome (52). Overrepresentation of *TTN* truncating variants have likewise been identified in cohorts of cardiotoxicity associated with chemotherapy (53) and alcohol use (54), suggesting that pregnancy may similarly represent a “second hit” for patients predisposed by genetic variance. Importantly, however, >90% of individuals with *TTN* truncating variants do not develop DCM or PPCM (55), indicating that additional environmental, genetic, or epigenetic factors are at play. The fact that most women with PPCM do not have a family history of cardiomyopathy and that PPCM does not always recur with a subsequent pregnancy indicates that any genetic origin is incompletely penetrant and that additional factors contribute to disease incidence. Further research in diverse populations is needed to study the interaction of genetic variants with comorbidities as well as vascular, hormonal, and hemodynamic insults.

DIAGNOSIS

TIMING AND CLINICAL PRESENTATION. The majority of women with PPCM are diagnosed after delivery, typically in the first month postpartum (3). Frequent delays in diagnosis occur due to under-recognition of this disease and the overlap in signs and symptoms of normal pregnancy with those of HF. Unfortunately, delays in diagnosis are associated with increased incidence of preventable complications and worse outcomes (3,56,57). Most women present with signs and symptoms of HF including shortness of breath on exertion, fatigue, orthopnea, paroxysmal nocturnal dyspnea, edema, and chest tightness. Physical examination often reveals tachypnea, tachycardia, elevated jugular venous pressure, pulmonary rales, and peripheral edema. A minority of patients will present with cardiogenic shock, severe arrhythmias, and thromboembolic complications.

DIAGNOSTIC TESTING. Echocardiography should be performed in any suspected case of PPCM as the

TABLE 1 Differential Diagnosis for Heart Failure During Pregnancy

Differential Diagnosis	Considerations
Takotsubo cardiomyopathy	Echocardiogram may show classic apical ballooning
Familial cardiomyopathy	Family history, genetic testing
Pre-existing cardiomyopathy	History of HF prior to pregnancy; prior echo studies with low LVEF before pregnancy
Recurrent peripartum cardiomyopathy	Ask about symptoms of HF that occurred after a prior pregnancy
Pre-eclampsia	Preserved systolic function on echocardiogram
Hypertrophic cardiomyopathy	Left ventricular hypertrophy, LVOT obstruction, preserved systolic function, genetic testing
Myocarditis	Consider if viral prodrome, histological diagnosis, fulminant presentation
Arrhythmogenic right ventricular cardiomyopathy	Consider with family history, genetic testing, echocardiographic findings
Left ventricular noncompaction	Echocardiographic and CMR findings
Chemotherapy-related cardiomyopathy	History of chemotherapy, particularly doxorubicin
Valvular heart disease	Echocardiographic findings; congenital aortic stenosis; mitral stenosis from rheumatic heart disease in endemic country. Patients with PPCM may also have valve disease, i.e., mitral regurgitation
Congenital heart disease	May be diagnosed for the first time during pregnancy by echocardiography
Tachycardia-arrhythmia mediated cardiomyopathy	Consider if specific underlying rhythm abnormality. Note that sinus tachycardia may be secondary to heart failure during pregnancy
Hypertensive heart disease	Left ventricular hypertrophy; less common in young people unless very longstanding history of hypertension
Ischemic heart disease	Cardiovascular risk factors; angina; prior CAD; consider SCAD and MINOCA diagnoses
Cardiomyopathy related to other systemic medical diseases	Consider in the appropriate context, i.e., systemic lupus erythematosus, antiphospholipid syndrome, hemochromatosis
Cardiomyopathy related to other acute conditions	May consider if patient has other conditions such as sepsis, treatment in intensive care unit, post-respiratory arrest
Pulmonary embolism	Dyspnea, tachycardia with preserved LVEF

CAD = coronary artery disease; CMR = cardiac magnetic resonance imaging; HF = heart failure; LVEF = left ventricular ejection fraction; LVOT = left ventricular outflow tract; MINOCA = myocardial infarction in non-obstructive coronary arteries; PPCM = peripartum cardiomyopathy; SCAD = spontaneous coronary artery dissection.

LVEF is typically <45% (4). In addition to systolic dysfunction, the echocardiogram may demonstrate LV and right ventricular dilatation and/or dysfunction, functional mitral and/or tricuspid regurgitation, pulmonary hypertension, and left atrial or biatrial enlargement. Intracardiac thrombus may occur (17,58), and the LV apex should be clearly visualized particularly when the LVEF is severely reduced. Levels of brain natriuretic peptide (BNP) and N-terminal pro-BNP, which do not change significantly during normal pregnancy and may be mildly elevated in the setting of pre-eclampsia, are usually markedly elevated in PPCM (59–62). The electrocardiogram may show nonspecific abnormalities, but a normal electrocardiogram does not rule out PPCM (63). Chest x-rays show pulmonary venous congestion. Cardiac magnetic resonance imaging provides accurate ejection fraction and chamber measurements when the echocardiogram is inadequate, but gadolinium is avoided during pregnancy. Endomyocardial biopsy is only indicated if there is suspicion for an alternative diagnosis, such as giant cell myocarditis, that would necessitate a different management plan.

DIFFERENTIAL DIAGNOSIS. PPCM is a diagnosis of exclusion. To avoid overdiagnosis, careful attention

to possible pre-existing heart disease including cardiomyopathies and valvular disease is important. Severe pre-eclampsia can cause HF related to diastolic dysfunction, but PPCM is only diagnosed in the presence of systolic dysfunction. Potential causes of pregnancy-related HF are listed in [Table 1](#).

PROGNOSIS AND OUTCOMES

ADVERSE OUTCOMES. PPCM is associated with adverse outcomes, including brain injury, cardiopulmonary arrest, pulmonary edema, thromboembolic complications, mechanical circulatory support, cardiac transplantation, and death. In a study of 182 women with PPCM, the major adverse event preceded the diagnosis of PPCM in one-half of the patients, and cerebrovascular events and cardiopulmonary arrest were associated with residual brain damage (57). LV thrombus has been identified in as much as 10% to 17% of initial echocardiograms (17,64), and thromboembolic complications have been reported in 5% to 9% of women (65,66). The increased incidence of thromboembolic events in PPCM is likely related to the hypercoagulable state of pregnancy, cardiac dilatation and dysfunction, venous stasis, bed rest, and the post-operative status after cesarean section.

PROGNOSTIC FACTORS. Of various prognostic factors that have been studied, LVEF at the time of diagnosis is the most reliable predictor of adverse events or long-term recovery (3,25,57,67-69). In the IPAC cohort, LVEF <30% was associated with lower rates of recovery and increased risk of adverse events (70). Despite severe LV dysfunction at the time of diagnosis, some women will recover; thus, initial LVEF is not sufficient for determining an early and possible premature need for advanced therapies such as durable left ventricular assist device (LVAD), implantable cardioverter-defibrillator (ICD), cardiac resynchronization therapy, or transplant (25). Additional predictors of worse outcome include LV dilatation (17,25,67,70-72), LV thrombus (17), right ventricular systolic dysfunction (73,74), and obesity (75). African-American ethnicity is strongly associated with lower rates of recovery, longer recovery time, more adverse outcomes, and higher mortality (14,15,70,76). Concomitant pre-eclampsia has been associated with lower 1-year survival, but higher rates of LV recovery in survivors (77). Biomarkers associated with adverse outcomes include troponin (78), NT-proBNP (79), and sFlt1 (39). The presence of late gadolinium enhancement by cardiac magnetic resonance imaging may indicate fibrosis associated with less myocardial recovery; however, most patients with PPCM do not demonstrate late gadolinium enhancement (80,81).

MORTALITY. Mortality estimates differ significantly based on racial groups, geographical region, and duration of follow-up. At 1-year follow-up, mortality rates in the United States ranged from 4% in the IPAC study (70) to 11% in a population of 96% African-American women (82). Two-year mortality rates have been reported as 0% to 16% in different studies in the United States (3,64,68), 15% in Haiti (7), and 28% in Africa (83). Less data are available about long-term outcomes (>5 years), but U.S. mortality estimates have ranged from 7% to 20% (mean follow-up 6.3 to 8.6 years) (14,84-86).

RECOVERY. PPCM has been associated with a higher rate of recovery than other forms of HF with reduced LVEF (64), and recovery frequently occurs within the first 3 to 6 months (3,17,70). Delayed recovery can also occur, even up to 2 years following diagnosis (68,85-88). In the United States, recovery rates have varied depending on the patient population and the definition of recovery. Of the 100 prospectively enrolled women in the IPAC study, 72% recovered to LVEF >50% at 12 months (70). A lower recovery rate (45%) was reported in a study of 55 patients (51%

African-American) at follow-up time of 29 months (17), and among 40 indigent patients (87.5% African-American) where only 35% recovered with an average time of 54 months (68). A recent retrospective analysis of 59 women reported 43% recovered to LVEF of $\geq 50\%$ by 12 months with a median time to recovery of 8 months (86).

MANAGEMENT

MEDICAL MANAGEMENT. Treatment of HF during pregnancy requires special modifications to ensure fetal safety. Following delivery, most HF medications are compatible with breastfeeding (Figure 1) (89-95).

Anticoagulation. Due to reports of increased incidence of LV thrombi and systemic thromboembolism in women with PPCM (9,40,57,96) and the hypercoagulable state of pregnancy and the early postpartum period (97), anticoagulation should be considered in the setting of severely decreased LVEF during late pregnancy and 6 to 8 weeks postpartum (40). Anticoagulation is suggested by the American Heart Association when the LVEF is <30% (98), whereas the European Society of Cardiology suggests using LVEF $\leq 35\%$ as the threshold (99). No published data are available to guide the decision of therapeutic versus prophylactic anticoagulation. Warfarin crosses the placenta and is avoided during pregnancy for indications other than anticoagulation of mechanical heart valves. Low-molecular-weight heparin does not cross the placenta and can be used during pregnancy (92). Both warfarin and low-molecular-weight heparin are considered safe with lactation. The novel anticoagulants have not been studied during pregnancy or lactation and are generally avoided.

EXPERIMENTAL TREATMENT. Bromocriptine is a dopamine agonist and inhibits the release of prolactin. It was originally marketed for lactation suppression, but due to the association with myocardial infarction (100-103), stroke (104,105), and seizures (105), it is no longer approved for this indication. The concept that PPCM is driven by the antiangiogenic and proapoptotic 16-kDa form of prolactin led to experimentation using bromocriptine to inhibit prolactin secretion to prevent the development of PPCM in a mouse model (36). A pilot study compared the outcome of 10 South African patients with PPCM receiving bromocriptine in addition to standard HF therapy to that of 10 patients receiving standard therapy alone (106). Patients treated with bromocriptine (2.5 mg twice daily for 2 weeks, followed by 2.5 mg daily for 6 weeks) had greater improvement in LVEF at 6 months (27% to 58%; $p = 0.012$) than the

FIGURE 1 Heart Failure and Anticoagulant Medications: Indications and Safety in Pregnancy and During Lactation

MEDICATION	DURING PREGNANCY	POTENTIAL ADVERSE EFFECTS	INDICATIONS	DURING LACTATION
HEART FAILURE MEDICATIONS				
Loop diuretics	Yes	Caution for hypovolemia or hypotension that may lead to decreased placental perfusion	For signs and symptoms of congestion and fluid overload.	Yes, but over-diuresis can lead to decreased milk production.
Beta blockers (metoprolol tartrate used most commonly)	Yes	IUGR; fetal bradycardia and hypoglycemia	For standard treatment of HF; consider treatment of women with subsequent pregnancy.	Yes
Hydralazine/nitrates	Yes	Caution with hypotension	Use for afterload reduction during pregnancy (instead of ACE-I/ARB) when needed.	Yes, but ACE-I/ARB typically chosen post-partum
Digoxin	Yes	No associated congenital defects	Can be used with symptomatic heart failure and/or systolic dysfunction during pregnancy, or afterwards per guidelines.	Yes
ACE-I/ARB	No	Anuria, oligohydramnios, fetal limb contractures, craniofacial deformation, pulmonary atresia, fetal hypocalvaria, intra uterine growth restriction, prematurity, patent ductus arteriosus, stillbirth, neonatal hypotension and death	Cannot use during pregnancy. After delivery, should be used as part of guideline-directed medical therapy for afterload reduction and LV remodeling.	Enalapril and captopril can be used
Aldosterone receptor antagonists	No	Spironolactone has been associated with antiadrenergic activity, feminization of male rat fetuses and permanent changes in reproductive tract in both sexes	As per guideline-directed medical therapy for heart failure.	Spironolactone can be used
Sacubitril-valsartan	No	Same as ACE-I/ARB	As per guideline-directed medical therapy for heart failure.	No information in human, present in rat milk
Ivabradine	Scant data in humans; would avoid due to concerns in animal studies	Scant data in humans, animal data suggest risk	As per guideline-directed medical therapy for heart failure.	No information in human, present in rat milk
ANTICOAGULANTS				
Low molecular weight heparin	Yes	Caution at time of delivery and with neuraxial anesthesia; does not cross placenta; consider the need for monitoring anti-Xa levels	For prevention and treatment of thromboembolic complications during pregnancy and as bridge to warfarin postpartum.	Yes
Warfarin	Avoid	Warfarin embryopathy and fetopathy	For prevention and treatment of thromboembolic complications postpartum.	Yes

Legend:

Green box	Data or experience to support use
Red box	Caution with using this medication
Blue box	Data is limited or inconclusive

Safety of medications need to be considered during pregnancy and lactation. ACE-I = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blocker; HF = heart failure; IUGR = intra-uterine growth restriction; LV = left ventricular; SSP = subsequent pregnancy.

control group (27% to 36%), and fewer experienced the composite endpoint ($n = 1$, 10%) (defined as death, New York Heart Association functional class III/IV, or LVEF $<35\%$ at 6 months) compared with the control group ($n = 8$, 80%; $p = 0.006$). A second African study compared the outcome of 48 patients with PPCM receiving HF therapy of furosemide and angiotensin-converting enzyme (ACE) inhibitors to an equal number of patients additionally receiving bromocriptine 2.5 mg twice daily for 4 weeks (107). LVEF was similar at entry but higher in the bromocriptine group at 2 weeks ($p = 0.01$) and at 3, 6, and 12 months ($p = 0.001$). Mortality at 6 months was high in both groups but lower in the bromocriptine-treated women (17% vs. 29%; $p = 0.0001$). The results of these 2 African studies and their general applicability were limited by the uncharacteristically high mortality rate in the control group compared with other studies (108). An observational registry of 115 German patients with PPCM reported that bromocriptine in addition to standard therapy was associated with a higher rate of improvement in LVEF, but there was no significant difference in overall rates of recovery (49). Recently, a randomized trial of 2 different regimens of bromocriptine (1 week in 27 patients vs. 8 weeks in 31 patients) in Germany found similar outcomes in both groups (109). Treatment was started on the average of 1.6 ± 1.6 months after the delivery, in addition to standard HF therapy including ACE inhibitors/angiotensin receptor blockers, beta-blockers, mineralocorticoid antagonists, and diuretic agents. The investigators postulated an association between bromocriptine and the favorable outcomes, but the absence of a control group not receiving bromocriptine makes the results inconclusive. No information is available for the use of this therapy in women with PPCM in the United States. Until more definitive information becomes available, bromocriptine should be considered experimental. The implications of not breastfeeding due to bromocriptine should be discussed with the patient. A randomized double-blind, placebo control study (REBIRTH [Randomized Evaluation of Bromocriptine In Myocardial Recovery Therapy]) to investigate the effect of bromocriptine on myocardial recovery and clinical outcome of 200 women with PPCM in the United States and Canada has been proposed by the IPAC group and is under evaluation. The 2018 European Society of Cardiology guidelines include a weak recommendation (Class IIb, Level of Evidence: B) for the use of bromocriptine (110). Due to the association with thrombotic complications, therapeutic anticoagulation is recommended in conjunction with bromocriptine.

TREATMENT OF SEVERE PPCM. Intravenous vasodilators, such as nitroglycerin, may be needed in the setting of acute decompensated HF during pregnancy. Nitroprusside is less desirable due to the theoretical risk of cyanide toxicity (92). Possible adverse effects of dobutamine were described in an observational study of 27 nonrandomized patients with PPCM and LVEF $\leq 25\%$ (111). The 7 women treated with dobutamine had worse outcomes, but there may have been selection bias in this small study. Levosimendan is an alternative inotropic medication (99), but was not shown to improve outcomes in a randomized study of 24 patients with PPCM (112), and is not available in the United States or Canada. A recent comparison of milrinone and levosimendan in 15 women with PPCM showed comparable hemodynamic improvement with both drugs (113).

Advanced therapies. A total of 60% of cases of cardiogenic shock during or shortly after pregnancy are caused by PPCM (114). Temporary mechanical circulatory support with intra-aortic balloon pump, percutaneous ventricular assist device therapy, and extracorporeal membrane oxygenation have been used successfully in PPCM and should be considered early in patients with hemodynamic instability despite inotropic support (115–119). Temporary or durable LVADs may also be needed. Of 99 women with PPCM who received LVAD, 6% recovered and 48% went on to cardiac transplantation (118). A study of 485 women with PPCM who received cardiac transplant between 1987 and 2010 reported higher rates of graft failure and lower age-adjusted survival, which may be explained by increased rejection, higher allo-sensitization, and higher pre-transplant acuity (120).

LABOR AND DELIVERY. Timing and mode of delivery in patients presenting with PPCM during pregnancy should be discussed with the patient and coordinated by a cardio-obstetrics team of experts from obstetrics, cardiology, maternal fetal medicine, anesthesiology, nursing, pharmacy, and social work (121). An attempt to stabilize the mother to avoid potential fetal complications of prematurity is reasonable. Hemodynamic instability despite medical therapy should prompt early delivery (or termination if prior to fetal viability). Stable patients are delivered vaginally unless there are obstetric reasons for cesarean section. Cesarean delivery is associated with a higher incidence of hemorrhage, infection, and thromboembolic complications (122). Detailed pre-delivery plans should involve the patient and an experienced multidisciplinary team. Unstable patients may benefit from invasive hemodynamic optimization prior to

FIGURE 2 Counseling and Management of Subsequent Pregnancies in PPCM

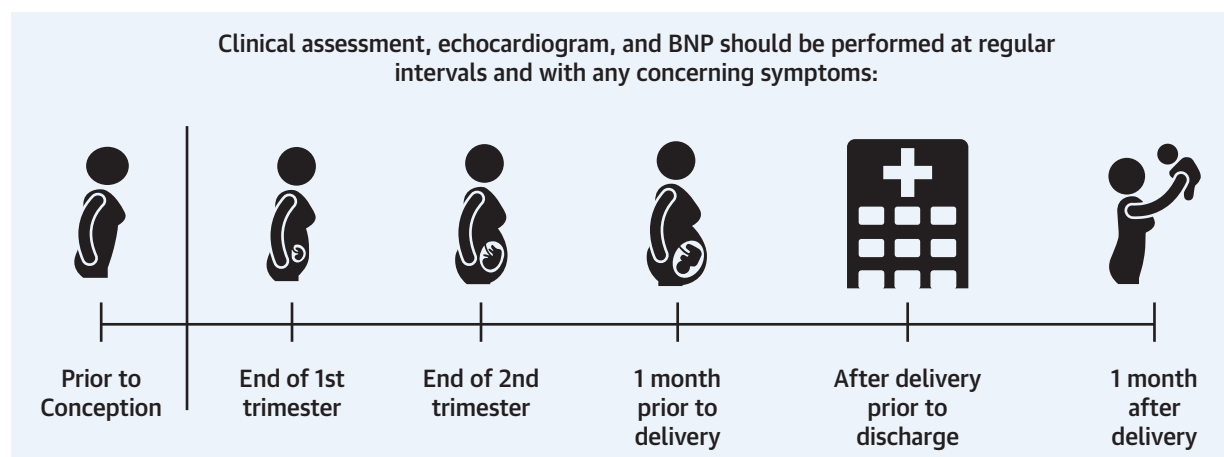
 Subsequent Pregnancy	Recovered (LVEF $\geq$ 50%)	Nonrecovered (LVEF <50%)
Preconception or First Visit	Preconception risk counseling and follow-up planning. Clinical and LVEF reassessment off renin-angiotensin blocking agents for 3 months. Baseline echocardiogram and BNP/NT-proBNP level.	Preconception risk counseling including discussion of alternative ways to build a family. If pregnant and not considering termination: Close follow-up planning, stop renin-angiotensin blocking agents and switch to hydralazine/isosorbide dinitrate. Baseline echocardiogram and BNP/NT-proBNP level.
Maternal Risks	~20% have a relapse Severe deterioration is rare Mortality unlikely Rate of subsequent recovery is high	Higher risk of relapse ~50% show further deterioration in LV dysfunction Increased morbidity and mortality Premature delivery and abortion more common
Medications	Continue beta blocker therapy (metoprolol tartrate preferred). Yield of starting prophylactic beta blocker therapy unclear. Diuretics and hydralazine/isosorbide dinitrate in case of clinical or LV functional deterioration.	Continue beta blocker therapy (metoprolol tartrate preferred). Hydralazine/Isosorbide dinitrate for hemodynamic and symptomatic improvement. Consider digoxin. Consider anticoagulation if severe LV dysfunction (LVEF <35%).
Follow-up	Close monitoring of symptoms during pregnancy and the postpartum period with repeat echocardiographic assessment of LV function and BNP/NT-proBNP level at the end of the 1st and 2nd trimesters, 1 month prior to delivery, after delivery prior to hospital discharge, 1 month postpartum, and at any time if symptoms develop.	
Labor and Delivery	Multidisciplinary team for planning; patient involved. Spontaneous vaginal delivery preferred unless fetal or maternal instability. Monitor for volume overload in the first 48 hours after delivery in cases of recurrent LV dysfunction.	Multidisciplinary team for planning; patient involved. Spontaneous vaginal delivery preferred unless fetal or maternal instability. Early delivery if further decrease in LV function and hemodynamic deterioration. Consider hemodynamic monitoring for optimization prior to delivery and monitoring during and after delivery. Monitor for volume overload in the first 48 hours after delivery.

Risks of a subsequent pregnancy differ based on the pre-conception recovery status. There is higher risk with nonrecovered myocardial function and pregnancy should be discouraged. Peripartum management options depend on the clinical status and myocardial function. ACE = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; LV = left ventricular; LVEF = left ventricular ejection fraction; PPCM = peripartum cardiomyopathy.

delivery and monitoring during delivery and the early postpartum period. Following delivery, removal of caval compression by the fetus, autotransfusion due to uterine contractions, and fluid mobilization and resorption contribute to an increase in venous return. The post-partum risk of fluid overload and pulmonary edema must be anticipated.

PRIOR TO HOSPITAL DISCHARGE

LACTATION. Breastfeeding confers multiple benefits for infants and mothers (123,124) and is recommended by the World Health Organization and the American Academy of Pediatrics (125). In developing countries where formula may be expensive and clean

FIGURE 3 Serial Monitoring During a Subsequent Pregnancy

Proposed outline for serial testing during a subsequent pregnancy. BNP = brain natriuretic peptide.

water is not readily available, not breastfeeding can pose a significant risk to the infant (126). Based on the prolactin hypothesis, the 2010 European statement on PPCM advised against breastfeeding (4). However, a small study of patients enrolled online in the United States indicated that women who breastfed actually had higher rates of recovery (26). Recent data from IPAC demonstrated that breastfeeding was not associated with adverse outcomes, inflammatory markers, or persistent myocardial dysfunction (127). The observation that breastfeeding seems to be safe in PPCM suggests that continued stimulation of prolactin secretion may not be harmful (128). Most HF medications can be given safely with breastfeeding (Figure 1) and should not be a reason to advise women against lactation.

SUDDEN DEATH PREVENTION. Only limited published data is available to guide the timing of ICD placement in women with PPCM. Premature placement should be avoided because a large proportion of women will recover to LVEF >35% within the first 6 months postpartum and will not meet criteria for ICD placement (129). Unfortunately, women with PPCM may experience cardiac arrest in the early months following diagnosis (130) and need to be protected. A recent analysis of administrative data of 9,841 hospitalizations with a primary diagnosis of PPCM revealed that 18.7% had an arrhythmia; of these, 4.2% had ventricular tachycardia, 1% had ventricular fibrillation, and 2.2% had cardiac arrest (131). In a small prospective study of women with

newly diagnosed PPCM, 3 of 7 patients with severely reduced LVEF who were compliant with a wearable cardioverter/defibrillator were found to have ventricular fibrillation that was appropriately shocked (130). A subsequent retrospective multicenter study by the same group reported that of 49 women with newly diagnosed PPCM and LVEF <35%, 12% (n = 6) had ventricular tachyarrhythmias (5 episodes of ventricular fibrillation, 2 with sustained ventricular tachycardia, and 1 with nonsustained ventricular tachycardia), and there were no inappropriate shocks (132). In contrast, a retrospective analysis of wearable cardioverter/defibrillators in 107 patients with PPCM found no shocks were delivered (either appropriate or inappropriate) during an average of 4 months of follow-up (133). Thus, despite conflicting data in small studies, and until more information becomes available, it may be reasonable to consider wearable cardioverter/defibrillators for women with new onset PPCM and severe LV dysfunction as a bridge to recovery or until an implantable ICD is indicated. Certain patients may benefit from cardiac resynchronization therapy. An anecdotal report demonstrated positive remodeling and improvement in LVEF in 2 women with PPCM treated with CRT after failing to improve on medical therapy (134).

CONTRACEPTION. Contraception should be discussed at the time of diagnosis or prior to hospital discharge. In the early postpartum setting with severe LV dysfunction, the increased risk of thromboembolism should dissuade the use of estrogen-containing

contraceptives. Progesterone-releasing subcutaneous implants or the Mirena intrauterine device are safe and effective choices. Injectable depot medroxyprogesterone acetate is less effective and is considered a second-line option. Nonhormonal barrier methods are less effective. Tubal ligation and vasectomy are other options. In a woman with persistent LV dysfunction, the risk of a subsequent pregnancy likely outweighs any risk associated with contraception. Therefore, women should be encouraged to select the method they will use most consistently. The importance of contraception should be emphasized by the cardiologist, as well as the obstetrician/gynecologist (135).

DURATION OF TREATMENT. In the presence of persistent cardiac dysfunction, cardiac medications should be continued indefinitely. After LV recovery, optimal duration of treatment is unknown. A rationale for continuation of medical therapy is supported by evidence of subclinical LV systolic dysfunction and anecdotal reports of late deterioration of LV function. Impaired LV global longitudinal and apical circumferential 2-dimensional strain were reported in 29 women with LV recovery (defined as LVEF $\geq 50\%$) at least 12 months after acute PPCM (136), supporting prior reports of decreased contractile reserve on dobutamine stress echocardiogram in women with PPCM and recovered LVEF (137). In addition, anecdotal reports have described deterioration of LV function after partial or complete recovery, even in the absence of a subsequent pregnancy (57,86,138). A recent study of patients with recovered DCM (LVEF $>50\%$) not related to pregnancy reported that 44% needed to restart cardiac medications within 6 months after discontinuation (139). It is not clear whether these findings can be extrapolated to patients with PPCM. If the patient is free from congestive symptoms, diuretic medications can be stopped. Additional HF medications, if stopped, should be weaned in a stepwise fashion with frequent clinical assessment and echocardiographic monitoring of LVEF (i.e., every 3 to 6 months). Reassessment of LV function is advised after drug discontinuation followed by annual clinical and echocardiographic assessment.

SUBSEQUENT PREGNANCY

The safety of a subsequent pregnancy is a frequent concern for patients and their families. Appropriate and accurate counseling is essential. The risks associated with a subsequent pregnancy depend primarily upon whether the myocardial function has fully recovered, and the pre-pregnancy LVEF is the

strongest predictor of outcomes (Figure 2). Detection of subclinical LV dysfunction by stress testing and strain imaging has been proposed (136,140), but further research about the predictive utility of these tests is needed.

RESIDUAL MYOCARDIAL DYSFUNCTION. If there is evidence of persistent myocardial dysfunction (i.e., LVEF $<50\%$), women should be advised on the reported high risk of recurrent HF, durable deterioration of cardiac function, and mortality. In a review of 93 women with persistent LV dysfunction (published between 2001 and 2010), nearly one-half had deterioration of LV function, which persisted in 39%, and 16% died (141). A more recent study of 16 European and African women with LV dysfunction prior to a subsequent pregnancy reported death in 25%, and only 31% recovered LV function $>50\%$ (142). Additionally, fetal outcomes tend to be worse among women with persistent LV dysfunction, with higher rates of stillbirth, abortion, and pre-term delivery (141). Based on these data, the 2018 European Society of Cardiology guidelines for the management of cardiovascular diseases during pregnancy discourage subsequent pregnancy if the LVEF is not $>50\%$ to 55% (110). After counseling, some women choose to proceed and should be followed closely during pregnancy.

RECOVERED MYOCARDIAL FUNCTION. Women who recover LVEF $>50\%$ have lower risk of complications during a subsequent pregnancy, but there is still increased risk of recurrent HF (141). An early study of 23 subsequent pregnancies in women with recovered LVEF reported no deaths, but 21% had a substantial ($>20\%$) decrease in LVEF and symptoms of HF, with 14% having persistent LV dysfunction (143). A prospective study of 18 women with subsequent pregnancies in Europe and Africa reported no mortality but an average of 15% reduction in LVEF after the delivery, which was persistent in almost one-half of them (142). More recently, a single-center study in the United States of 39 pregnancies in 24 women with LVEF of $\geq 50\%$ prior to subsequent pregnancy reported no maternal deaths and a relapse rate of 21% (LVEF nadir range of 35% to 45%) (144). All patients were reported to recover, although 3 women required admission to the intensive care unit. None of these patients were treated with bromocriptine, 79% breastfed their infants, and 44% were treated with beta-blockers (144). Pre-conception counseling should include discussion of the potential risk of recurrent myocardial dysfunction, which may persist after the pregnancy.

MONITORING AND TREATMENT DURING SUBSEQUENT PREGNANCY.

Our recommended protocol for monitoring women with a history of PPCM during a subsequent pregnancy is outlined in [Figures 2 and 3](#). In women with recovered LV function who are taking HF medications, ACE inhibitors/angiotensin receptor blockers, and aldosterone receptor antagonists should be discontinued prior to conception, and it may be prudent to ensure stability of LV function after at least 3 months off of these medications prior to considering the LV recovered. The prophylactic use of beta-blockers during subsequent pregnancies in women with recovered LVEF may be considered but there is a paucity of data to support its role. In the study by Codsì et al. (144), 19 of 43 women with subsequent pregnancies were treated with beta-blockers, 6 had a decrease in LVEF, and there were no instances of intrauterine growth restriction. In a recent series of patients with subsequent pregnancies, it was postulated that addition of bromocriptine to standard therapy led to higher rates of recovery and no deaths (142); however, this was a small, non-randomized study, and there was a significant difference in the baseline LVEF in the 2 arms of the study.

CONCLUSIONS

The diagnosis of PPCM should be considered in any pregnant or postpartum woman with symptoms concerning for HF. An elevated BNP level should always be followed by an echocardiogram to assess for systolic dysfunction. Prompt treatment with medications tailored for pregnancy and lactation may

prevent adverse outcomes. Limited studies suggest breastfeeding is safe. Acutely ill women should be managed by specialized multidisciplinary teams, and may require advanced HF therapies. Women considering a subsequent pregnancy should be counseled and monitored by physicians familiar with PPCM. Long-term follow-up is important, but the optimal duration of medications following recovery is unknown.

Despite many advances in our understanding of PPCM, questions remain about the pathogenesis and complex interaction of genetics with the vascular and hormonal milieu of late pregnancy. The role of bromocriptine remains unclear, and randomized clinical trials are needed for determining the risks and potential benefits. Important gaps in knowledge remain, such as the optimal anticoagulation strategy, timing of ICD implantation, risk prediction and management during a subsequent pregnancy, and the long-term duration of medications after myocardial recovery. Given the overall rarity of PPCM, collaboration among multiple centers will be needed to answer these questions.

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KEY WORDS dilated cardiomyopathy, peripartum cardiomyopathy, postpartum cardiomyopathy, pregnancy-associated cardiomyopathy



Neighborhood education status drives racial disparities in clinical outcomes in PPCM

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Background Peripartum cardiomyopathy (PPCM) disproportionately affects women of African ancestry. Additionally, clinical outcomes are worse in this subpopulation compared to White women with PPCM. The extent to which socioeconomic parameters contribute to these racial disparities is not known.

Methods We aimed to quantify the association between area-based proxies of socioeconomic status (SES) and clinical outcomes in PPCM, and to determine the potential contribution of these factors to racial disparities in outcomes. A retrospective cohort study was performed at the University of Pennsylvania Health System, a tertiary referral center serving a population with a high proportion of Black individuals. The cohort included 220 women with PPCM, 55% of whom were Black or African American. Available data included clinical and demographic characteristics as well as residential address georeferenced to US Census-derived block group measures of SES. Rates of sustained cardiac dysfunction (defined as persistent LVEF <50%, LVAD placement, transplant, or death) were compared by race and block group-level measures of SES, and a composite neighborhood concentrated disadvantage index (NDI). The contributions of area-based socioeconomic parameters to the association between race and sustained cardiac dysfunction were quantified.

Results Black race and higher NDI were both independently associated with sustained cardiac dysfunction (relative risk [RR] 1.63, confidence interval [CI] 1.13-2.36; and RR 1.29, CI 1.08-1.53, respectively). Following multivariable adjustment, effect size for NDI remained statistically significant, but effect size for Black race did not. The impact of low neighborhood education on racial disparities in outcomes was stronger than that of low neighborhood income (explaining 45% and 0% of the association with black race, respectively). After multivariate adjustment, only low area-based education persisted as significantly correlating with sustained cardiac dysfunction (RR 1.49; CI 1.02-2.17).

Conclusions Both Black race and NDI independently associate with adverse outcomes in women with PPCM in a single center study. Of the specific components of NDI, neighborhood low education was most strongly associated with clinical outcome and partially explained differences in race. These results suggest interventions targeting social determinants of health in disadvantaged communities may help to mitigate outcome disparities. (Am Heart J 2021;238:27–32.)

Peripartum cardiomyopathy (PPCM) is a sometimes severe form of cardiomyopathy occurring toward the end of pregnancy or months postpartum in women with previously normal hearts.^{1,2} The estimated incidence of PPCM is 1 in 2,000 pregnant women world-

wide, with hotspots such as Haiti and Nigeria.^{1,3} In the U.S., the prevalence of PPCM is higher in Black women than White women.^{4,5} Clinical outcomes in PPCM vary widely, with most women ultimately recovering cardiac function, but a significant minority of women developing worsening function, need for LVAD or cardiac transplant, or death.^{1,3} Again, Black women fare worse than White women: they are generally diagnosed later in the postpartum period and, when diagnosed, are less likely to have recovery of left ventricular (LV) function and more likely to experience adverse events compared to White women.^{6,7} We previously described the outcomes of 220 women with PPCM diagnosed and treated within the University of Pennsylvania Health System (Penn) and demonstrated that Black women were half as likely to recover, and took twice as long to do so when they did recover.⁶

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The reasons for differences in clinical outcomes between Black and White women with PPCM remain unclear, and may be related to underlying biology, genetics, or a complex interplay of individual and social factors. Social determinants of health impact the development of cardiovascular (CV) risk factors and disease, health behaviors, treatment patterns, and clinical outcomes in complex ways. Neighborhood disadvantage, for example, is associated with incident heart failure and risk of readmission, in addition to individual race/ethnicity and socioeconomic status.⁸⁻¹⁰ Neighborhood characteristics and associations with outcomes in PPCM have not previously been examined.

We therefore sought to determine: (1) if neighborhood level social determinants impact clinical outcomes in PPCM; and (2) to what extent the poor outcomes observed in Black women with PPCM may be attributed to neighborhood disadvantage. To do so, we leveraged our large, racially diverse cohort of PPCM patients, the majority of whom live in Philadelphia, a large city with noted differences in access to care and clinical outcomes based on race.¹¹ We further integrated our cohort with a spatially enabled data set to understand how neighborhood disadvantage impacts clinical outcomes in PPCM, and interacts with racial background.

Methods

The University of Pennsylvania Institutional Review Board approved the study and determined that informed consent was not required. We assembled a retrospective cohort of patients at Penn with a diagnosis of PPCM from January 1, 1986, through December 31, 2016 based on diagnostic codes (International Classification of Diseases, Ninth Revision codes 674.50-54) or echocardiographic evaluations obtained within 6 months of delivery.⁶ Patients with a history of congenital heart disease, valvular disease predating their PPCM diagnosis, history of radiation or cardiotoxic chemotherapy, or another explanation for their heart failure were excluded.

We abstracted demographic details, residential addresses, diagnosis, and clinical outcome information from the patient electronic medical records. We used ArcGIS Pro (Esri, Redlands, CA) to geocode addresses and identify corresponding Census block groups. A Census block group is the smallest geographic unit (average 1,500 people) in which sample data from decennial census and the American Community Survey are available. Using block groups as a proxy for neighborhoods, we spatiotemporally integrated a select set of variables from the 1990, 2000, and 2010 Census as well as the 2009 through the 2016 American Community Surveys.¹² A composite neighborhood concentrated disadvantage index (NDI) was computed for each patient as the sum of the proportions of unemployment, households receiving public assistance, persons below the federal poverty line,

adults without a high school diploma, female-headed households, renter-occupied residences, and residential tenure shorter than 1 year.¹³ Each of the components of the NDI were dichotomized at the threshold of their highest quartile for statistical analyses. Sustained cardiac dysfunction was defined as persistent LVEF <50%, cardiac transplant, LVAD placement, or death. Myocardial recovery was defined as at least one occasion of LVEF >50% on echocardiography.

Distributions of patient characteristics were compared by quartiles of block group level factors using χ^2 or Fisher exact tests. We examined the potential for a non-linear association between NDI and persistent cardiomyopathy with restricted cubic splines.¹⁴ We examined the cardiac outcomes (sustained cardiac dysfunction, and myocardial recovery) relative to NDI, and then separately to each component informing the NDI. Crude and multivariate log binomial regression models were used to estimate risk ratios (RR) and corresponding 95% confidence intervals (95% CI). Multivariable models were adjusted first for patient characteristics that were associated with the area-based exposures, or race. The proportional contribution of each area-based socioeconomic parameters to the effect of race on cardiac outcomes was quantified.¹⁵

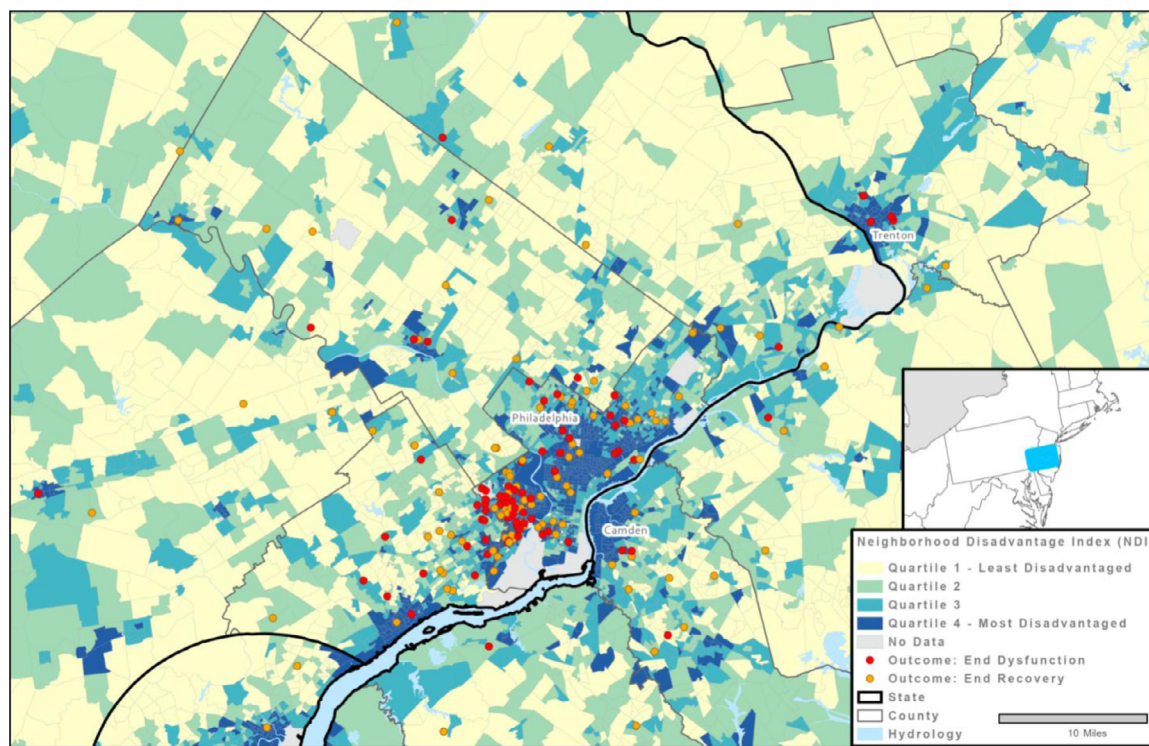
The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents. Sources of funding: ZA was supported by the Department of Defense (DOD W81XWH18-1-0503) and NIH (HL126797). KG was supported by the NIH (HL143153). JL was supported by the NIH (HL153667).

Results

The cohort of 220 women diagnosed with PPCM consisted of 121 Black women and 99 non-Black women. Demographic and clinical characteristics at presentation and clinical outcomes of this cohort have been described previously.⁶ Figures 1 and S1 present the relational geographic distribution of the patients to the greater Philadelphia Metropolitan Area. Neighborhoods with greater disadvantage are concentrated in Philadelphia, Camden, and Trenton.

Table S1 provides the demographics and clinical characteristics for the study population overall, and subdivided by quartiles of NDI, median household income, and proportion of residents with less than high school education, according to block group. Patients in neighborhoods with greater disadvantage were younger (28 years in quartile 4 (Q4) vs. 32 years in quartile 1 (Q1), $P = .002$), and more likely to be Black (89% in Q4 vs 16% in Q1, $P < .001$). Patients from more disadvantaged neighborhoods tended to have a lower nadir LVEF compared to those from less disadvantaged neighborhoods.

Figure 1



Relational geographic distribution of PPCM patients to the greater Philadelphia Metropolitan Area, distinguishing between patients who recovered (in yellow) versus those who did not (in red), and stratified by neighborhood disadvantage index (NDI). To protect the privacy of the patients, their locations were spatially anonymized using areal filters and random perturbation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

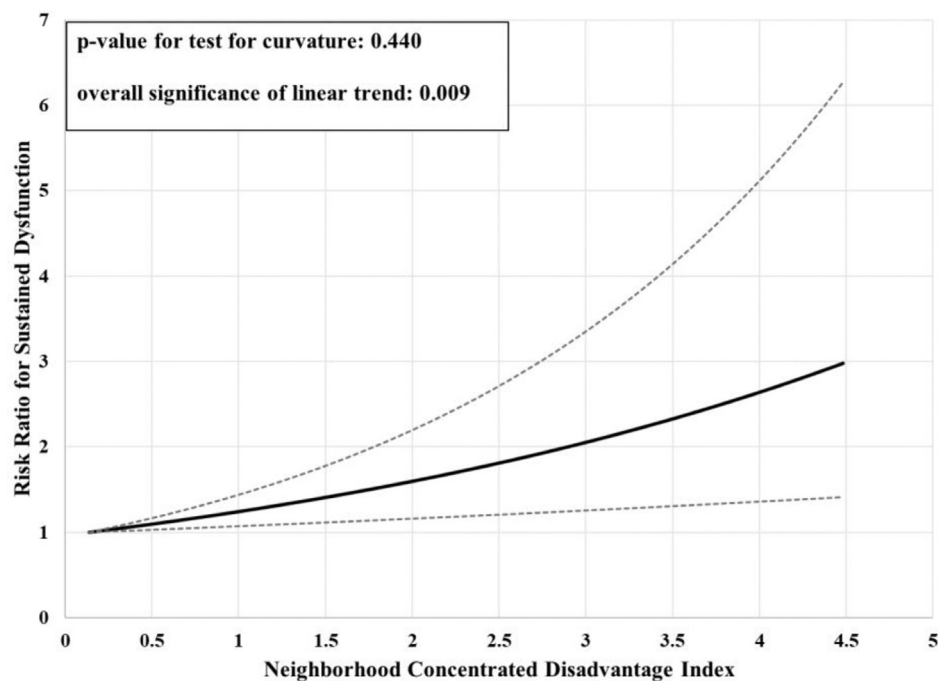
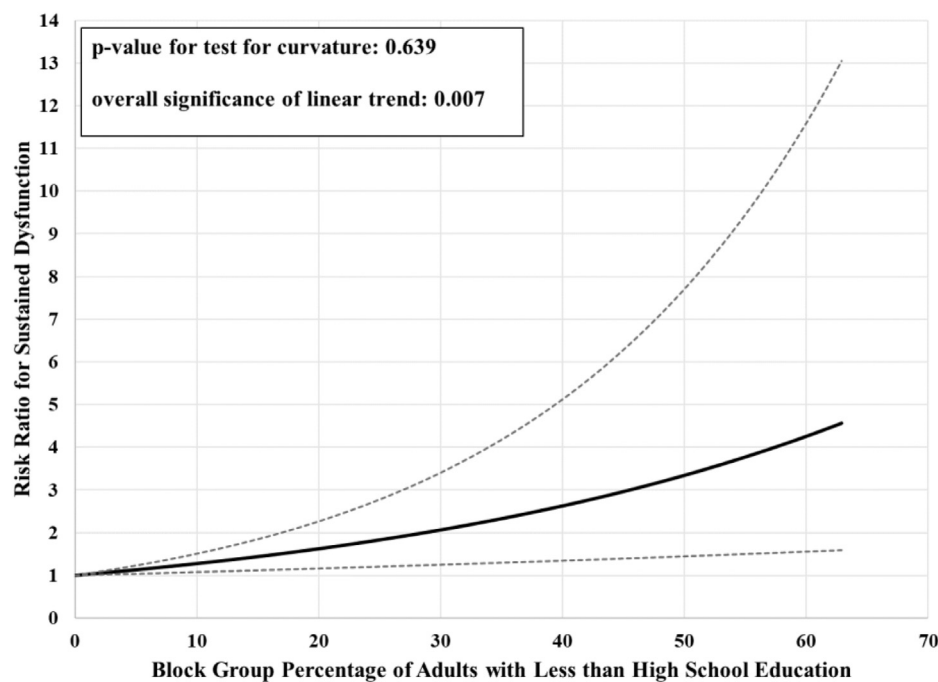
Overall, 36.3% ($n = 81$) of patients experienced sustained dysfunction. Both Black race (44% vs 28%; RR 1.63, 95% CI 1.13-2.36) and higher NDI (RR 1.29, 95% CI 1.08-2.53) were independently associated with sustained cardiac dysfunction (Table I). Restricted cubic spline regression revealed that the association between NDI and sustained dysfunction was linear (P value for test for curvature: 0.440; overall significance of linear trend 0.004; Figure 2). Every unit increase in NDI was associated with a 29% higher likelihood of sustained cardiac dysfunction. Effect estimates for both exposures were attenuated following adjustment for age, twin pregnancy, number of children, and timing of diagnosis, but only NDI remained statistically significant (Table I, M1).

Of the individual components of NDI, only area-based low education and high rental occupied housing were significantly associated with sustained cardiac dysfunction (Table I, first column), which persisted after adjusting for race (Table I, M2). Other components of NDI had adjusted risk ratios near null (Table I, M2). The effect estimate for race on sustained dysfunction was largely attenuated upon control for area-based low education: in fact,

low education explained approximately 45% of the effect of race on sustained dysfunction (Table I, M3 vs M2). In contrast, control for area-based low income had no impact on the effect estimate for race on sustained dysfunction (Table I, M5 vs M1). Overall comparable results were observed for myocardial recovery (Supplemental Table S2).

Discussion

We find here, in a large racially diverse cohort of women with PPCM, that the markedly worse average clinical outcome of Black women may be explained in large part by greater neighborhood disadvantage, specifically lower educational status at the block group level. The findings underscore the importance of social determinants of health as potential drivers of racial disparities in PPCM. Specifically, lower education status appears to impact PPCM outcomes independent of race, and may do so by creating barriers to timely access to high-quality medical care, communication with medical providers, understanding of symptoms, medication adherence, and

Figure 2**A.****B.**

Linear relationships between (A) neighborhood-concentrated disadvantage index and occurrence of sustained cardiac dysfunction, and (B) proportion of adults in block group with less than a high school education and occurrence of sustained cardiac dysfunction.

Table I.

	Crude RR (95% CI)	p-value	Adjusted RR (M1)	p-value	Adjusted RR (M2)	p-value	Adjusted RR (M3)	p-value	Adjusted RR (M4)	p-value	Adjusted RR (M5)	p-value
Black race	1.63 (1.13, 2.36)	0.009	1.34 (0.88, 2.03)	0.171	1.19 (0.74, 1.92)	0.474	1.17 (0.76, 1.81)	0.419	1.18 (0.74, 1.78)	0.527	1.34 (0.87, 2.06)	0.185
Neighborhood concentrated disadvantage index	1.29 (1.08, 1.53)	0.004	1.23 (1.00, 1.51)	0.021	1.17 (0.91, 1.49)	0.217						
Assessments for the Individual Components of the Neighborhood Concentrated Disadvantage Index ^a												
>22.3% adults with less than HS education	1.73 (1.25, 2.39)	<0.001	1.57 (1.10, 2.24)	0.014	1.49 (1.02, 2.17)	0.040						
>31.8% Renter Occupied Housing	1.70 (1.20, 2.42)	0.003	1.57 (1.07, 2.30)	0.020	1.50 (1.00, 2.25)	0.049						
>28.5% HH w. annual income below poverty line	1.23 (0.86, 1.77)	0.265	1.09 (0.75, 1.60)	0.647	1.00 (0.68, 1.46)	0.999						
>31.5% Female Headed Households	1.23 (0.86, 1.77)	0.264	1.00 (0.68, 1.47)	0.991	0.95 (0.65, 1.39)	0.946						
>16.3% adults unemployed	1.14 (0.78, 1.67)	0.482	0.98 (0.66, 1.45)	0.915	0.90 (0.60, 1.33)	0.585						
>10.2% adults on public assistance	1.10 (0.75, 1.62)	0.621	0.96 (0.65, 1.42)	0.827	0.90 (0.60, 1.34)	0.900						
>28.4% with residential tenure less than 1 year	1.11 (0.76, 1.63)	0.576	1.08 (0.74, 1.59)	0.682	1.10 (0.74, 1.64)	0.616						

^a Each of the block group-level components of the neighborhood disadvantage index were dichotomized at highest quartile for statistical analyses (i.e. Q4 vs Q1-Q3). The numeric thresholds presented for each of the NDI components represent the cutoff value defining the highest quartile.

M1 = adjusted for age, twin pregnancy, number of children, and timing of diagnosis

M2 = race model adjusted for age, twin pregnancy, number of children, timing of diagnosis, and neighborhood concentrated disadvantage index (NDI); Models for individual NDI component area-based exposures adjusted for age, twin pregnancy, number of children, timing of diagnosis, and race

M3 = race model adjusted for age, twin pregnancy, number of children, timing of diagnosis, and neighborhood-based low education

M4 = race model adjusted for age, twin pregnancy, number of children, timing of diagnosis, and neighborhood-based high renter occupied housing

M5 = race model adjusted for age, twin pregnancy, number of children, timing of diagnosis, and neighborhood-based high poverty

self-advocacy, especially in the context of structural racism. Race and socioeconomic class are highly correlated in the U.S. Racial disparities in maternal health have been documented independent of education of the individual.¹⁶ The experiences of racism impact the quality of care delivered, patient care engagement, and communication between health care providers and patients. Women from lower SES groups report higher rates of perceived discrimination based on race and insurance status, which may further adversely impact maternal health outcomes.^{17, 18}

Despite being the largest mixed-race PPCM cohort reported to date, limitations of our study include being single-center with variable duration of follow-up, which reduces generalizability. Due to relatively small numbers, the analyses do not include Latino or Asian women. Additionally, data were assessed only at the block group level, rather than individual level. Although area-based measures of socioeconomic status may serve as crude proxies to individual SES, they may also have distinct independent and synergistic impacts on PPCM outcomes. Nevertheless, the NDI and component area-based exposures examined do not account for psychological distress, food insecurity, or delays in medical care, all of which may directly impact outcomes for women with PPCM. Data on the use of in vitro fertilization, or on medical treatments for PPCM including type, intensity, or duration were not captured in the manual chart abstractions completed for the cohort and thus are not accounted for in the presented analyses.

In summary, our finding that low area-based education was associated with worse outcomes in women with PPCM suggests a potential role for strategies aimed at improving access to care, patient-provider communication, and in particular health education in mitigating such disparities.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL RESEARCH ARTICLE

Genetic and Phenotypic Landscape of Peripartum Cardiomyopathy

BACKGROUND: Peripartum cardiomyopathy (PPCM) occurs in $\approx 1:2000$ deliveries in the United States and worldwide. The genetic underpinnings of PPCM remain poorly defined. Approximately 10% of women with PPCM harbor truncating variants in *TTN* (TTNtvs). Whether mutations in other genes can predispose to PPCM is not known. It is also not known if the presence of TTNtvs predicts clinical presentation or outcomes. Nor is it known if the prevalence of TTNtvs differs in women with PPCM and preeclampsia, the strongest risk factor for PPCM.

METHODS: Women with PPCM were retrospectively identified from several US and international academic centers, and clinical information and DNA samples were acquired. Next-generation sequencing was performed on 67 genes, including *TTN*, and evaluated for burden of truncating and missense variants. The impact of TTNtvs on the severity of clinical presentation, and on clinical outcomes, was evaluated.

RESULTS: Four hundred sixty-nine women met inclusion criteria. Of the women with PPCM, 10.4% bore TTNtvs (odds ratio=9.4 compared with 1.2% in the reference population; Bonferroni-corrected P [P^*]= 1.2×10^{-46}). We additionally identified overrepresentation of truncating variants in *FLNC* (odds ratio=24.8, $P^*=7.0 \times 10^{-8}$), *DSP* (odds ratio=14.9, $P^*=1.0 \times 10^{-8}$), and *BAG3* (odds ratio=53.1, $P^*=0.02$), genes not previously associated with PPCM. This profile is highly similar to that found in nonischemic dilated cardiomyopathy. Women with TTNtvs had lower left ventricular ejection fraction on presentation than did women without TTNtvs (23.5% versus 29%, $P=2.5 \times 10^{-4}$), but did not differ significantly in timing of presentation after delivery, in prevalence of preeclampsia, or in rates of clinical recovery.

CONCLUSIONS: This study provides the first extensive genetic and phenotypic landscape of PPCM and demonstrates that predisposition to heart failure is an important risk factor for PPCM. The work reveals a degree of genetic similarity between PPCM and dilated cardiomyopathy, suggesting that gene-specific therapeutic approaches being developed for dilated cardiomyopathy may also apply to PPCM, and that approaches to genetic testing in PPCM should mirror those taken in dilated cardiomyopathy. Last, the clarification of genotype/phenotype associations has important implications for genetic counseling.

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The full author list is available on page 1861.

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■ peripartum period

Sources of Funding, see page 1861

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Clinical Perspective

What Is New?

- Women with peripartum cardiomyopathy bear a significantly high burden of loss-of-function variants in several genes, including *TTN*, *FLNC*, *DSP*, and *BAG3*.
- The identity and relative abundance of these variants is remarkably similar to that seen in idiopathic dilated cardiomyopathy, indicating that the genetic predisposition to peripartum cardiomyopathy and dilated cardiomyopathy may be the same.
- Although patients who have peripartum cardiomyopathy with *TTN* truncating variants present with a lower ejection fraction, no significant differences in rates of recovery were seen.

What Are the Clinical Implications?

- Genetic counseling and testing should be considered for women with peripartum cardiomyopathy, following guidelines developed for dilated cardiomyopathy.
- Gene-specific therapies established for dilated cardiomyopathy, such as low threshold for defibrillator devices in patients with *DSP* or *FLNC* variants, should be considered.

Peripartum cardiomyopathy (PPCM) is a rare disease manifesting as heart failure with reduced left ventricular ejection fraction (LVEF) that develops during the late peripartum or postpartum period.^{1,2} Women exhibit a range of presentations, including cardiogenic shock, and a range of outcomes, including the need for mechanical circulatory support or heart transplantation. The pathobiology of PPCM remains unclear, although there is a strong epidemiological relationship with preeclampsia,³ and evidence in rodents and clinical studies has implicated vasculohormonal pathways of late gestation, including prolactin, vascular endothelial growth factor, and soluble Fms-like tyrosine kinase 1, in the pathogenesis of PPCM.^{4,5} Genetic variation has been linked to the development of PPCM. PPCM incidence is higher in women of African American descent, and strikingly higher in populations from Haiti and Nigeria, suggesting a possible genetic contribution.^{2,6} A number of studies have reported familial clustering of PPCM, and co-occurrence, as well, with nonischemic dilated cardiomyopathy (DCM), a phenotypically similar disease, and which itself is linked to genetic variation.² Last, we showed previously that, among 172 women with PPCM, 15% bore truncating loss-of-function (LOF) variants (ie, frameshift, nonsense, and splice-altering) in the gene *TTN*, a prevalence significantly higher than in a reference population ($P=1.3\times 10^{-7}$), and similar to

a cohort of patients with DCM.⁷ At least a subset of PPCM thus has a genetic pathogenesis.

At least 2 critical questions were highlighted by previous studies. First, do LOF variants in genes other than *TTN* also predispose to PPCM? To date, PPCM cohorts have not been of sufficient size to allow assessment of the contribution of other DCM genes. And second, are there genotype/phenotype correlations between women with and without *TTN* truncating variants (TTNts)? To date, the largest PPCM group with both genotype and phenotype information was our post hoc analysis of 85 women from the IPAC cohort (Investigations in Pregnancy Associated Cardiomyopathy).⁷ In this small group, cardiac function at presentation was similar between women with and without TTNts, but at a 1-year follow-up, women carrying TTNts had worse cardiac function. By contrast, however, in the IMAC-2 group (Intervention in Myocarditis and Acute Cardiomyopathy 2), no differences were observed in ejection fraction at enrollment or follow-up ejection fraction.⁷ Thus, it remains unclear if TTNts in women with PPCM are associated with any aspects of clinical presentation or predict any clinical outcomes.

We therefore enrolled and examined a large, international cohort of 654 women with PPCM, who provided clinical information and DNA. Of these, 469 satisfied clinical and sequencing data quality metrics. We report here the identification of novel genetic determinants of PPCM and genotypic/phenotypic correlations.

METHODS

The authors declare that all supporting data are available within this article and its [Data Supplement](#).

Patient Recruitment and Exclusion

Eligible women with a diagnosis of peripartum cardiomyopathy were retrospectively recruited from international institutions, using the internationally accepted definition of PPCM, most recently updated by the Heart Failure Association of the European Society of Cardiology Working Group, as the development of heart failure and $\text{LVEF}\leq 45\%$ toward the end of pregnancy or in the months after delivery, where no other cause is identified.⁸ In the United States, patients carrying an *International Classification of Diseases, 9th Revision* code of 674.54 or *International Classification of Diseases, 10th Revision* code of O90.3 were recruited from the identified academic centers. Patients were excluded for lack of baseline (ie, at time of diagnosis) LVEF data or a baseline $\text{LVEF}>45\%$, and those with previous evidence of congenital heart disease or preexisting valvular heart disease were excluded, as well. Clinical and genetic data from these recruits were combined with data from the previously described cohort of 172 patients with PPCM.⁷ Chart reviews were performed for pertinent medical history including race and ethnicity, age at diagnosis, delivery date, mode of delivery, time from delivery to PPCM diagnosis, and history of gestational hypertension/preeclampsia/superimposed preeclampsia. Ancestry was

Table 1. Demographic and Clinical Characteristics, and Burden of Rare Protein-Truncating Variants, in Each Cohort of the Current Study

Characteristics	University of Pennsylvania	Japan	IPAC	IMAC-2	Germany	Chicago	Pitt
No. of patients	106	8	82	31	108	4	25
Age, y	30.49±6.7	30.63±3.5	29.89±6.3	31.29±6.8	33.85±5.2	23.50±8.6	29.67±5.6
European descent, n (%)	48 (46.1)	0	52 (63.4)	20 (63.5)	47 (90.4)	0	16 (64)
African descent, n (%)	53 (51.0)	0	26 (31.7)	11 (35.5)	3 (5.8)	3 (75)	9 (36)
Other race, n (%)	3 (2.9)	8	4 (4.9)	0	2 (3.8)	1 (25)	0
Left ventricular ejection fraction	27.2±12.4	29.1±10.1	29.5±9.2	27.2±7.4	26.7±9.0	32.9±6.0	27.8±10.2
Gestational hypertension or pre-eclampsia, n (%)	42/104 (40.4)†	6/8 (75)	32/82 (39)	NA	24/64 (37.5)†	1/3 (33.3)	8/25 (32)
All truncating variants	15	1	12	4	18	0	3
TTN truncating variants	9	1	10	4	13	0	0

(Continued)

established by patient self-report or by chart review. Cardiac data were reviewed for mode of PPCM diagnosis, ejection fraction, left ventricle end-systolic dimension, and left ventricle end-diastolic dimension at the time of diagnosis, presence of arrhythmias, use of cardiac medications, follow-up cardiac testing, and end point of PPCM diagnosis including but not limited to recovery, persistent dysfunction, left ventricular assist device, transplant, and death. The study was approved by the institutional review board at each study center. All patients provided written informed consent.

DNA Capture, Sequencing, and Analysis

DNA was extracted using Qiagen's Gentra Puregene kit; 1 µg of DNA was sheared with a Diagenode BioRuptor for 30 minutes set on high with 30 seconds on/off. Sheared DNA underwent library preparation using the Agilent SureSelect XT kit. Barcoded libraries were enriched for DCM-associated genes by using a custom Agilent SureSelect bait panel (Seidman DCMv5),⁷ which includes all 364 exons of *TTN*. Libraries were run on an Illumina NextSeq and variants were called using the Agilent SureCall.

Reference Population and Rare Variant Definition

The MyCode Community Health Initiative at Geisinger in Pennsylvania and New Jersey is a precision medicine initiative

that enrolls patient participants in a systemwide biobank designed to store blood and other samples for research use. We previously described TTN genotyping on the first 92 455 participants who underwent exomic sequencing,⁹ and use this population here as a control group of patients with TTNts but without a documented diagnosis of cardiomyopathy. The Genome Aggregation Database, GnomAD v2.1.1, was used as a reference population for pooled genetic analysis of the non-TTN genes. The data set spans 125 748 exome sequences and 15 708 whole-genome sequences from unrelated individuals. For all comparative analyses, we compared our sequencing data against the GnomAD v2 exomes for homogeneity of read depth and coverage, and used a minor allele frequency cutoff at $<1 \times 10^{-4}$ for rare variants, as used in previous studies in DCM and hypertrophic cardiomyopathy.¹⁰

Variant Analysis and Burden Testing

Protein-altering variants were defined as truncating (nonsense, frameshift, and splice site altering) or nontruncating (missense). We only included variants that mapped to regions in the GnomAD data frame with adequate coverage (defined as read depth ≥ 10 in at least 90% of individuals sequenced). All identified variants were passed through a filter for minor allele frequency $<1 \times 10^{-4}$ within the GnomAD database, and GnomAD flags including multinucleotide variant, low-complexity region, low-confidence predicted loss of

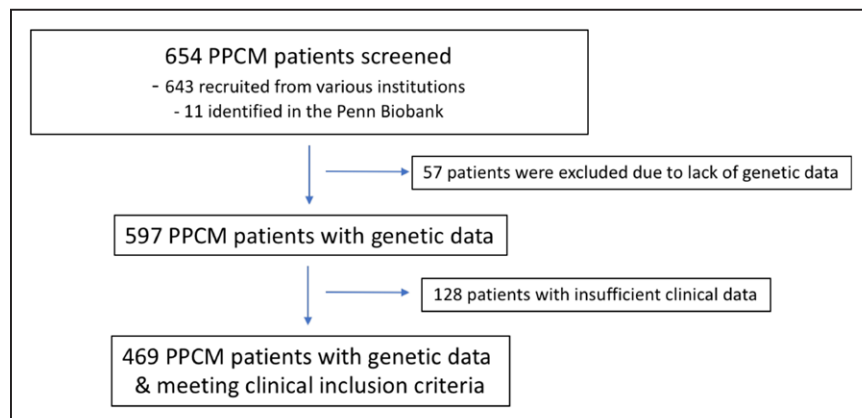


Figure 1. Inclusion criteria.
PPCM indicates peripartum cardiomyopathy.

Table 1. Continued

Hopkins	Washington University	Yale	Israel	Denmark	Total	USA	Non-USA	P value*
19	19	4	41	22	469	290	179	
32.47±4.8	27.67±6.9	27.75±5.9	32.15±6.3	30.59±6.7	30.94±6.4	30.13	32.64	1.3×10 ⁻⁵
12 (63.2)	10 (55.6)	1 (25)	35 (87.5)	22 (100)	263 (64.1)	159 (55.21)	62 (82.67)	2.62×10 ⁻⁵
5 (26.3)	8 (44.4)	2 (50)	5 (12.5)	0	126 (30.7)	118 (40.97)	5 (6.67)	1.87×10 ⁻¹⁹
2 (10.5)	0	1 (25)	0	0	21 (5.1)	11 (3.82)	8 (10.67)	
27.8±10.6	31.3±8.3	27.0±12.4	35±8.1	25.9±9.5	28.5	28.43	28.6	0.85
3/18 (16.7)†	9/19 (47.4)	1/3 (33.3)	6/41 (14.6)	10/22 (45.5)	142/389 (36.5)	96/254 (37.8)	46/135 (34.1)	0.51
4	2	0	7	4	70	40 (13.8)	30 (17.1)	0.46
2	0	0	6	4	49	25 (8.6)	24 (13.4)	0.14

Other race indicates non-White and non-Black.

IMAC-2 indicates Myocarditis and Acute Cardiomyopathy 2; and IPAC, Investigations in Pregnancy Associated Cardiomyopathy.

*P value United States vs non-United States by Student *t* test for continuous variables and by χ^2 test for discrete variables.

†Some patients missing data on hypertensive disorder.

function, and LOFTEE warning, as well. The National Center for Biotechnology Information ClinVar database was referenced for known pathogenic, unknown, or benign associations with cardiomyopathy listed in [Tables II and III in the Data Supplement](#).

Statistical Analysis

Comparisons of clinical and genetic characteristics were performed by using χ^2 and 2-sided Student *t* tests. Ejection fraction compared against time to diagnosis was evaluated using ANOVA and the Tukey method for multiple comparisons. Variant burden testing was performed for each gene, and case excess was defined by the difference in frequency between the PPCM cohort and GnomAD database. An odds ratio (OR) with 95% confidence interval was calculated using a presumed case and control ratio inferred by variant frequency of each gene in GnomAD. *P* values were adjusted by Bonferroni correction. All analyses were performed using Microsoft excel and R studio software.

RESULTS

Study Patients and DNA Sequencing

Six hundred fifty-four women with PPCM were identified and recruited from several US and international academic centers (Figure 1). Next-generation sequencing was performed on 67 genes, including *TTN* ([Table I in the Data Supplement](#)). Fifty-seven patients were excluded because of insufficient genetic data. Of the remaining 597 women, complete baseline cardiac function information was unavailable in 128, leaving 469 women with adequate genetic and clinical data. Demographic data ([Table 1](#)) indicated that 41% of US subjects were of African descent (compared with 13.4% of the US population,¹¹ OR, 4.43 [3.51–5.60], $P \leq 0.00001$), consistent with previous reports of elevated prevalence of PPCM in subjects of African descent. Of the total cases, 36.5%

were accompanied by gestational hypertension or pre-eclampsia (compared with estimated 5%–8% worldwide prevalence,¹² OR, 6.6–10.9 [5.4–13.4], $P \leq 0.00001$), again consistent with previous reports.³ No significant differences were noted in LVEF at presentation or presence of hypertensive disorder between US and non-US centers ([Table 1](#)). There was a higher prevalence of patients of African descent in US centers.

Genetic Variant Burden in PPCM

Variants were identified by next-generation sequencing of 67 genes previously associated with DCM using target capture methodology (see Methods). These sequence variants were filtered to include those with high sequence quality (read depth of ≥ 10 in 90% of sequenced individuals) and were rare in the reference GnomAD data set (mean allele frequency $< 1 \times 10^{-4}$). We focused on truncating variants, including nonsense, frameshift, and canonical splicing variants, because these variants are predicted to have a strong impact on protein function. Among the cohort of 469 women with PPCM, we identified 69 women who bore 70 different rare heterozygous truncating variants in 12 different genes ([Table 2](#) and [Table II in the Data Supplement](#)). There were no women bearing homozygous or compound heterozygous truncating variants. One woman bore a truncating variant in both MYH7 and FLCN ($P=0.81$ versus GnomAD, assuming a random distribution of truncating variants). The prevalence of 70 truncating variants in 469 women with PPCM (14.9%) demonstrates a 4.1-fold increased risk of disease compared with the GnomAD reference population (3.6%, Bonferroni-corrected $P [P^*]=3.5 \times 10^{-8}$).

Of the 70 truncating variants, 49 (70%) were in *TTN*, and are discussed further in the next section. Six truncating variants were in the desmoplakin (*DSP*) gene (OR, 14.9, compared with GnomAD; $P^*=1.0 \times 10^{-8}$), 4 were in

filamin C (*FLNC*, OR, 24.8; $P^*=7.0\times10^{-8}$), 1 was in *BAG3* (OR, 53.1; $P^*=0.02$), 2 were in each Myosin Heavy Chain 6 and 7 (*MYH6* and *MYH7*, $P^*>0.05$), and 1 was in *VCL* ($P^*>0.05$). Fifty-three of these variants were absent from the >125 000 exomes in GnomAD v2.1.1. This profile of genes with significant rare truncating variant enrichment is highly similar to that reported in a large cohort of patients with DCM,¹⁰ which identified significant variant enrichment in *BAG3*, *DSP*, *LMNA*, *TTN*, and *VCL* (Figure 2). Excess variant burden in *FLNC* was not reported in DCM, because *FLNC* is not included in most panels, including in that report. Of the 70 truncating variants identified in women with PPCM, 17 were identical to variants annotated as pathogenic or likely pathogenic for DCM in the ClinVar database (www.ncbi.nlm.nih.gov/clinvar).

The prevalence of missense variants in each of the 67 genes among the 469 women with PPCM was not significantly different from the burden found in GnomAD, after Bonferroni correction (Tables III and IV in the Data Supplement). *ANKRD1*, *DSP*, *LAMA2*, and *MYH7* reached nominal statistical significance. Excess burden of missense variants in *MYH7* has been reported in DCM.¹⁰ Two of the identified missense variants in *MYH7* were associated with dilated and hypertrophic cardiomyopathy in the ClinVar database.

There have been recent improvements in the prediction of noncanonical splicing variants that cause cryptic splicing, including the machine learning algorithm SpliceAI¹³ with a positive predictive value of $\approx 85\%$.¹⁴ Application of SpliceAI to our cohort revealed no splicing acceptor loss or donor loss variants in *TTN*, and 1 donor loss variant in *FLNC* ($P=0.022$ compared with prevalence in GnomAD). SpliceAI also identified 2 variants, 1 acceptor loss and 1 donor loss, in *DMD* ($P=0.008$ compared with GnomAD), in which we previously also noted a truncating variant in a woman with PPCM.⁷

Increased Risk of PPCM in Subjects With TTNtv

Forty-nine truncating variants were identified in *TTN* (10.45% of the cohort), OR=9.4 compared with 1.2% in GnomAD ($P^*=1.2\times10^{-46}$), and OR=14.5 compared with 0.8% in a collection of >60 000 exomes from volunteer participants in an outpatient clinical setting (Geisinger MyCode Community Health, ref 9, $P^*=1.1\times10^{-42}$). Some 19, 21, and 9 truncating variants were frameshift, nonsense, and canonical splice site variants, respectively, a distribution similar to that found in GnomAD, the Geisinger cohort, and various DCM cohorts (Table V in the Data Supplement and refs 9,10,15). Eighteen variants have previously been described, whereas 31 (63%) are new variants. Fifteen of the variants are identical to variants annotated as pathogenic or likely pathogenic for DCM in the ClinVar database.

TTN encodes 364 exons, only a subset of which are constitutively expressed in adult hearts.^{9,16} Exons expressed in a high percentage (>90%) of *TTN* transcripts are termed high Percent Spliced In (hiPSI); mutations in these hiPSI exons are likely to cause DCM.¹⁶ Of the 49 TTNtvs identified in women with PPCM, 46 (94%) were in hiPSI exons, a fraction similar to that found in the University of Pennsylvania Medical Biobank, representing a tertiary care center referral population enriched for disease prevalence (85%), but strongly enriched compared with the outpatient community Geisinger cohort⁹ and GnomAD reference cohorts (58%, $P=5.8\times10^{-6}$, and 51.2%, $P=5.1\times10^{-8}$, respectively). The burden of hiPSI TTNtvs was similar between geographic regions (Table 1), and between racial backgrounds (Table 3).

The location along the *TTN* gene of the 49 TTNtvs identified in women with PPCM is shown in Figure 3, and compared with the locations of TTNtvs identified in a primary outpatient cohort of 1040 patients with DCM from London and Singapore,¹⁰ and 2 reference cohorts: the University of Pennsylvania Medical Biobank, and the Geisinger cohort (the London/Singapore group only reported hiPSI TTNtvs). The majority of TTNtvs in women with PPCM mapped to the A band region of *TTN*, a distribution akin to that observed in the London/Singapore DCM cohort, and among patients diagnosed with DCM within the disease-enriched University of Pennsylvania Medical Biobank cohort, as well (dark red bars).

Clinical Characteristics of Patients Who Have PPCM With TTNtv

Patients who have PPCM with hiPSI (ie, likely pathogenic) TTNtvs presented at a similar age as did patients who have PPCM without TTNtvs (Table 3). The prevalence of women of African descent also did not differ between the 2 groups (24% and 31%, $P=0.5$), nor did the prevalence of TTNtvs in patients of African descent versus other descent (8.8% and 11%; $P=0.58$). In the reference GnomAD population, TTNtvs were found in 2.1% of persons of African descent ($P=1.3\times10^{-3}$) and in 1.1% of those of European descent ($P=5.4\times10^{-16}$). Patients with PPCM with hiPSI TTNtv, however, did have significantly lower LVEF at presentation than patients with PPCM without TTNtv (23.5% versus 29%, $P=2.5\times10^{-4}$; Table 3 and Figure 4A). We reported previously that LVEF tends to be lower with increasing time between delivery and diagnosis,¹⁷ and observed the same trend among the current cohort (Figure 4B; correlation coefficient -0.21 , $R^2=0.04$, $P=4.1\times10^{-5}$). The difference in presenting LVEF between patients who have PPCM with and without likely pathogenic TTNtvs was most pronounced among patients presenting in the first week after delivery (20.9% versus 31.3%, $P=2.3\times10^{-5}$; Figure 4B), compared with the subsequent 3 weeks (20.2% versus 31.3%; $P=0.035$), or thereafter (23.8% versus 25.2%;

Table 2. Prevalence of Rare Protein Truncating Variants in Peripartum Cardiomyopathy

Genes	Peripartum cardiomyopathy		Controls		Variant excess, %	Odds ratio (95% CI)	Nominal <i>P</i> value	Corrected <i>P</i> value*
	Cohort variants	Cohort frequency, %	Genome Aggregation Database variants	Genome Aggregation Database frequency, %				
<i>BAG3</i>	1	0.21	5	0.004	0.209	53.05 (6.19–454.96)	2.92×10 ⁻⁴	1.96×10 ⁻²
<i>DES</i>	1	0.21	28	0.025	0.189	8.72 (1.18–64.2)	3.36×10 ⁻²	1
<i>DSP</i>	6	1.28	108	0.087	1.192	14.9 (6.51–34.06)	1.55×10 ⁻¹⁰	1.04×10 ⁻⁸
<i>FKTN</i>	1	0.21	57	0.048	0.165	4.46 (0.62–32.28)	0.14	1
<i>FLNC</i>	4	0.85	41	0.035	0.818	24.75 (8.83–69.38)	1.05×10 ⁻⁹	7.02×10 ⁻⁸
<i>ILK</i>	1	0.21	59	0.048	0.166	4.48 (0.62–32.4)	0.14	1
<i>MYH6</i>	2	0.43	153	0.123	0.303	3.47 (0.86–14.05)	0.08	1
<i>MYH7</i>	2	0.43	94	0.075	0.351	5.7 (1.4–23.21)	1.50×10 ⁻²	1
<i>PLEC</i>	1	0.21	169	0.157	0.056	1.36 (0.19–9.73)	0.76	1
<i>TMPO</i>	1	0.21	87	0.069	0.144	3.08 (0.43–22.13)	0.26	1
<i>VCL</i>	1	0.21	59	0.047	0.166	4.54 (0.63–32.84)	0.13	1
<i>TTN</i>	49	10.45	1453	1.224	9.224	9.42 (6.97–12.71)	1.81×10 ⁻⁴⁸	1.21×10 ⁻⁴⁶
Summary	70	14.93	5103	3.619	11.306	2.25 (1.74–2.9)	5.18×10 ⁻¹⁰	3.47×10 ⁻⁸

The prevalence of truncating variants in patients with peripartum cardiomyopathy is compared with controls from the Genome Aggregation Database. Nominal *P* values are from odds ratios, and *corrected *P* values are adjusted for testing 67 genes with the Bonferroni method.

$P=0.58$). The prevalence of *TTN*ts did not correlate with time to diagnosis, despite the lower average LVEF in women presenting late after pregnancy.

We previously reported preliminary findings, in a limited post hoc analysis of a subset of 85 women with PPCM, that the presence of hiPSI, likely pathogenic *TTN*ts was associated with a lower prevalence of gestational hypertension and preeclampsia, and with worse clinical outcomes.⁷ In the current much larger cohort, these associations were not observed. Gestational hypertension and preeclampsia showed only a mild trend for lower prevalence among women with and without *TTN*tv (27% versus 38%; $P=0.21$). Recovery of LVEF (defined as >50%) was achieved in 68% and 60% of women with and without *TTN*tv, respectively ($P=0.28$), and adverse outcomes (death, advanced therapies including left ventricular assist device placement or heart transplantation, or persistent dysfunction with an LVEF≤35) occurred in 21% and 17%, respectively ($P=0.68$). Recovery of LVEF was overall less frequent in women of African versus European descent (53% versus 64%, $P=0.041$), but in neither group did *TTN*tv status affect rates of recovery ($P=0.73$ and 0.17, respectively). There was no significant difference in prevalence of hiPSI *TTN*tv between patients with follow-up and those lost to follow-up (Table VI in the Data Supplement).

DISCUSSION

We present here genotypic and clinical features in 469 patients with PPCM, the largest such cohort, and use this information to extend our understanding of

genotype-phenotype relationships in this disease. We confirm our previous observation that ≈10% of patients with PPCM carry a *TTN*tv and further identify a statistically significant excess burden of LOF variants in 3 genes previously associated with DCM, but not PPCM: *DSP*, *FLNC*, and *BAG3*.

The proteins encoded by these 4 genes have significantly different role in the cardiomyocyte. *TTN* is a major component of the sarcomere. *DSP* encodes desmoplakin, the primary force transducer between cardiac desmosomes and intermediate filaments. Truncating variants in *DSP* are associated with arrhythmogenic left ventricular cardiomyopathy.¹⁸ *FLNC* encodes for filamin C, a cytoskeletal protein expressed in the intercalated discs of cardiac cells. Truncating variants in *FLNC* have only recently been reported in DCM,^{19–22} and thus *FLNC* is still absent from most clinical genetic test panels. *BAG3* encodes a chaperone protein critical for chaperone-assisted selective autophagy.²³ Despite these differences, the profile of identified LOF variants in PPCM is remarkably similar to that seen in cohorts with DCM. The recent extensive analysis of a primary outpatient cohort of 1040 patients with DCM from London and Singapore revealed significant LOF burdens in *DSP*, *BAG3*, *VCL*, *LMNA*, and *MYH7*, in addition to the well-established prevalence of *TTN*ts.¹⁰ Excess LOF burdens in *VCL* and *MYH7* were also seen in our PPCM cohort but did not reach statistical significance after correction for multiple hypothesis testing, which may simply reflect insufficient statistical power. Moreover, the frequencies of LOF variants in each of these genes range widely (0.2%–10%) and

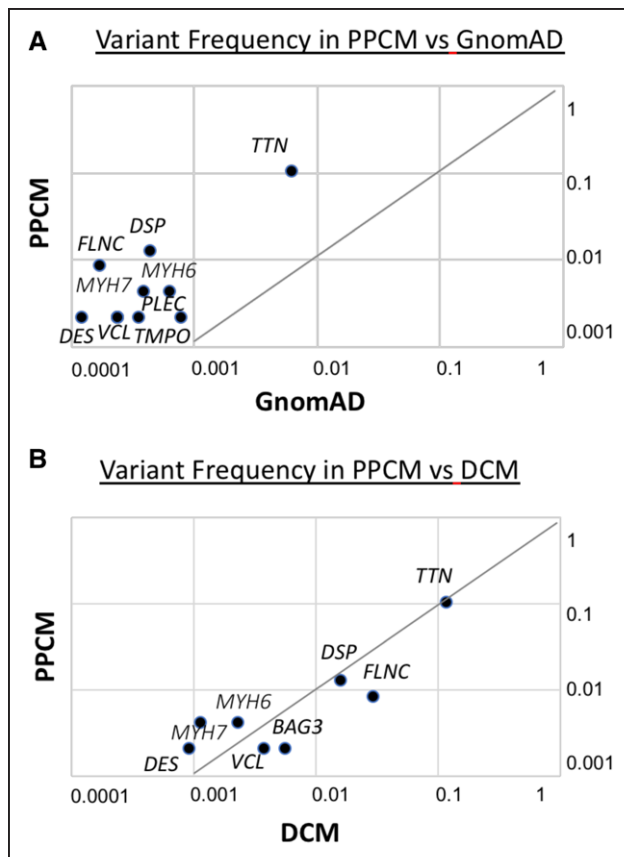


Figure 2. Comparison of the prevalence of rare truncating variants between peripartum cardiomyopathy (PPCM) and the Genome Aggregation Database (GnomAD, A), and between PPCM and nonischemic dilated cardiomyopathy (DCM, B).

Variant frequencies for DCM are from Mazzarotto et al¹⁰ except for *FLNC*, which is from Ader et al.²² Diagonal line represents the equivalence line.

are remarkably similar between PPCM and DCM (Figure 2). There is thus a high degree of genetic similarity between PPCM and DCM, both in which genes bear LOFs and in the frequency with which LOFs are found in each gene.

A simple model to explain these observations invokes a 2-hit mechanism, whereby a genetic background, for example, a TTNtv, predisposes to DCM in response to an additional cardiac stressor, for example, pregnancy in the case of PPCM. In keeping with this notion, a high burden of TTNtvs has also been noted in cardiomyopathy induced by alcohol and cancer therapy, 2 other prominent cardiac stressors.^{24,25} Also in keeping with this notion, the penetrance of most LOF variants is low, suggesting that in the absence of additional cardiac stressors, cardiac function is preserved even in the presence of a genetic predisposition. For example, 95% of subjects in the outpatient Geisinger cohort who were identified to carry hiPSI TTNtvs did not have DCM, or significant cardiac dysfunction on echocardiography.⁹ In the context of PPCM, pregnancy is clearly the predominant stressor, often accentuated by preeclampsia, which is associated with 30% to 50%

Table 3. Comparison of Clinical Characteristics Between Patients With Peripartum Cardiomyopathy With High-Percentage Spliced-in TTN Truncating Variants (TTNtvs) and Those Without

Characteristic	TTN truncating variant present (n=45)	TTN truncating variant absent (n=424)	P value
Age at diagnosis, y	30.40±7.04	31.03±6.28	0.41
Ejection fraction, %	23.51±8.99	29.08±10.02	2.52×10 ⁻⁴
Race/ethnicity, % (No./total No.)			0.53
Black	24.32 (9/37)	31.38 (116/372)	
White	67.57 (25/37)	63.98 (238/372)	
Other	8.11 (3/37)	4.84 (18/372)	
Gestational hypertension,* % (No./total No.)	25 (9/36)	37.5 (132/352)	0.14
Left ventricular end-diastolic diameter,† cm	5.71±1.33	5.68±0.77	0.93
Left ventricular end-systolic diameter,† cm	4.97±1.34	4.70±0.90	0.34
Recovery, % (No./total No.)	67.5 (27/40)	59.6 (211/354)	0.33
Negative outcome,‡ % (No./total No.)	21.74 (5/23)	17.42 (27/155)	0.59

Other indicates non-White and non-Black.

*Includes patients with gestational hypertension, mild and severe preeclampsia, and superimposed preeclampsia.

†Measured on transthoracic echocardiography from initial presentation.

‡Negative outcome counted as ejection fraction≤35 at 12-month follow-up, left ventricular assist device, heart transplantation, or death. P values are by the Student t test for continuous variables and by the χ^2 test for discrete variables.

of cases of PPCM. We have shown previously that pregnancy triggers a vascular stress on the heart, which is accentuated in preeclampsia, and likely contributes to the pathogenesis of PPCM.^{5,26}

The genetic similarities between PPCM and DCM have implications for the care of women with PPCM. There are currently no recommendations for genetic testing in women with PPCM, and such testing is not reimbursed. In contrast, genetic testing for DCM is widely performed and usually reimbursed. Current recommendations for genetic testing in DCM vary, but, in general, it is recommended that probands and affected family members undergo genetic counseling and genetic testing, especially if there is evidence of conduction disease or family history of sudden death.^{27–29} The presence of truncating variants in *FLNC*, for example, has been associated with malignant arrhythmias, and may warrant more aggressive antiarrhythmic management (eg, automatic implantable cardioverter defibrillator).^{19,30} The American College of Medical Genetics also recommends cascade testing of at-risk family members for pathogenic and likely pathogenic variants.²⁸ Our study, which indicates that PPCM and DCM are often manifestations of similar underlying genetic predispositions, therefore suggests that approaches taken to genetic testing in PPCM should mirror those taken in DCM.

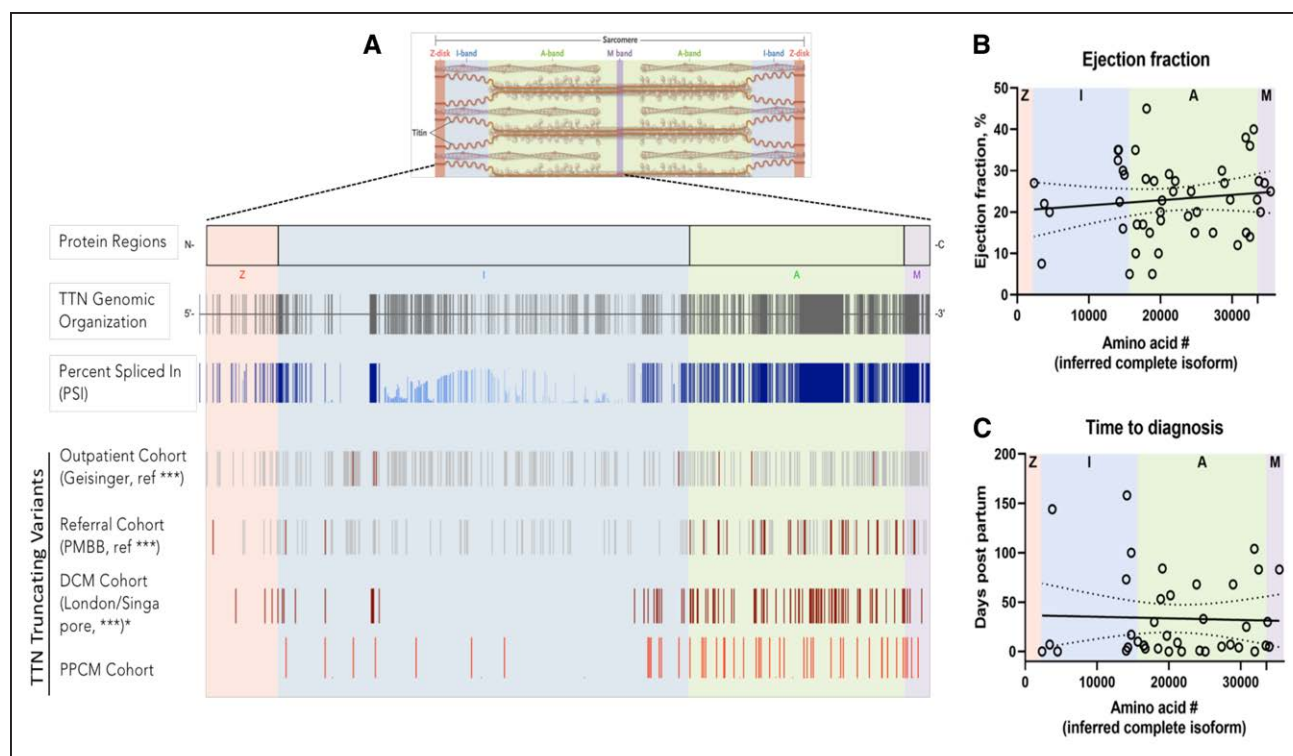


Figure 3. Genomic characteristics of truncating variants in *TTN*, and associated clinical phenotypes, in patients with peripartum cardiomyopathy.

A, Top. Titin is an integral part of the cardiac sarcomere, spanning from Z-disk to M-line. **Bottom.** Regions of the *TTN* gene and protein are designated according to their spatial distribution in the sarcomere: Z-disk, I-band, A-band, and M-band. The 364 exons of the *TTN* gene and their associated proportion spliced in (PSI) are shown, followed by the distribution of truncating variants in those exons identified in the Geisinger cohort (a general outpatient population), in the Penn Medicine Biobank (PMBB) cohort (a tertiary care referral population), the London/Singapore cohort of patients with idiopathic dilated cardiomyopathy (DCM; *only hiPSI TTNts are reported in this cohort), and the current peripartum cardiomyopathy (PPCM) cohort. Variants found in patients with DCM in the reference cohorts are designated in dark red. **B and C.** Lack of association between location of TTNtv and either left ventricle ejection fraction (**B**) or left time to diagnosis (**C**). Dark and dotted lines indicate linear regression and 95% confidence intervals, respectively. $P=0.41$ and 0.84 for difference from slope of zero in **A** and **B**, respectively. From the *New England Journal of Medicine*, Ware JS, Li J, Mazaika E, et al. "Shared genetic predisposition in peripartum and dilated cardiomyopathies," Volume 374. Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

Because TTNts were found with high frequency in PPCM subjects ($\approx 10\%$), we were able to assess if clinical features of PPCM differ between patients with and without TTNts. LVEF at presentation was significantly lower in patients with TTNts, suggesting that TTNts may confer an initially more aggressive disease than other causes of PPCM. This observation is similar to that seen in DCM. For example, among patients with DCM in the University of Pennsylvania Biobank, we found that LVEF was significantly lower in patients with TTNts than in those without TTNts.⁹ LVEF is the best known predictor of clinical outcomes in PPCM,² but, surprisingly, despite the lower ventricular function on presentation, the presence of TTNts in women with PPCM did not correlate with worse rates of recovery, with lower ejection fraction at 1 year, or with adverse outcomes. The apparent equivalent final recovery rates in the context of lower LVEF at presentation could reflect a higher rate of recovery in TTNtv carriers, as has been suggested in DCM.^{31–33} These observations differ from those we made, post hoc, in the much smaller group of 85 women from the IPAC study alone,⁷ underscoring the importance

of recruiting large cohorts such as the current one to avoid spurious conclusions.

The prevalence of PPCM is at least 4-fold higher in women of African descent than European descent.^{2,34,35} However, the prevalence of TTNts among PPCM subjects was similar in these 2 groups, and the prevalence of TTNts in the GnomAD reference population is also similar in these 2 groups, indicating that TTNts cannot explain the higher predisposition to PPCM in women of African descent. This difference could reflect biological differences inherent to pregnancy as a cardiac stressor, or environmental differences, including access to health care and social determinants of health during pregnancy.

Preeclampsia and hypertension strongly predispose to PPCM,³ but the prevalence of TTNts also was equivalent in PPCM cases with or without a hypertensive disorder. In the context of the 2-hit hypothesis described above, these observations suggest that pregnancy as a cardiac stressor affects TTNtv carriers and noncarriers equivalently, regardless of race or the presence of hypertension. In addition, the findings indicate that the mechanisms by

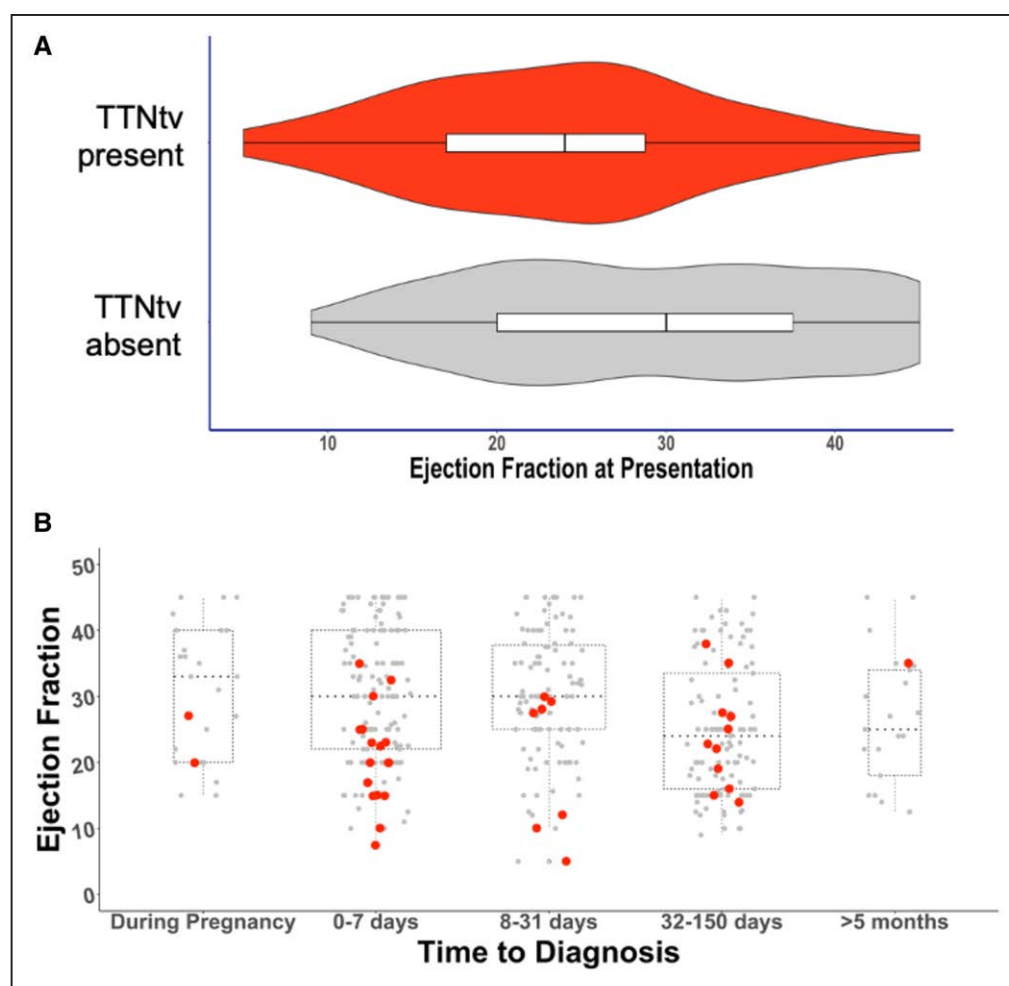


Figure 4. Impact of TTN truncating variants (TTNtv) on left ventricle ejection fraction at presentation, and time to diagnosis after delivery, in patients with peripartum cardiomyopathy (PPCM).

A, Comparison of left ventricular ejection fraction at the time of presentation between TTNtv-positive and -negative patients. $P=2.5 \times 10^{-4}$ by Student *t* test. **B**, Comparison of left ventricular ejection fraction with time to diagnosis after delivery. TTNtv-positive cases are indicated in red.

which gene mutations and hypertensive disorders stress the heart are distinct.

The mechanism by which TTNtv confer risk for PPCM or DCM remains unclear. We find that the location of truncation within the gene, and thus the predicted protein size, does not correlate with either LVEF at presentation, or time from delivery to diagnosis, indicating that the location of the TTNtv does not influence severity of disease. This observation suggests a pathogenic mechanism that is independent of truncated titin protein expression (eg, haploinsufficiency)³⁶ or, if integration of the truncated protein into the sarcomere contributes to disease, that integration of the N terminus is important, and that the loss of structures associated with the M-line is sufficient to cause disease.

Our study does have limitations. Because PPCM is a rare disease, our cohort size is necessarily limited, and lacks power to detect more subtle genetic predispositions. For example, we detected no significant increase in burden of missense variants in any gene tested, in contrast to much larger studies in DCM.¹⁰ Increasing

the number of PPCM cases studies will require a large international consortium. Our analyses are also limited to the panel of genes in our DNA capture platform. Whole exome sequencing or whole genome sequencing may elicit further genetic predispositions to PPCM, but such analyses will invariably suffer even further from power limitations. In addition, it is important to note that our conclusion of genetic similarity between PPCM and DCM is necessarily based on this limited panel of genes, previously associated with DCM, and thus genetic differences between DCM and PPCM may be uncovered with more comprehensive whole exome sequencing or whole genome sequencing. Our study is retrospective, and sampling bias may have occurred, although, considering the absence of dramatic phenotypic differences between TTNtv carriers and noncarriers, for example, it is unlikely that referral would have led to a bias with respect to genetic profile. Last, it is also worth noting that PPCM is a heterogeneous disease, and some women may have had some level of cardiac dysfunction before pregnancy.

Conclusions

In summary, we provide the first extensive genetic and phenotypic landscape of PPCM. We identify new truncating variants in *FLNC*, *DSP*, and *BAG3* with robust evidence of disease association. We report detailed associations between the highly prevalent truncating variants in *TTN* and clinical presentation and outcomes of PPCM. And we show that PPCM and nonischemic DCM closely share profiles of genetic predispositions, suggesting that approaches to genetic testing in PPCM should mirror those taken with DCM, and that pharmacological agents that are being developed for the treatment of DCM associated with mutations in these genes will also protect women with genetic PPCM.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Materials

Expanded Methods

Data Supplement Tables I–VI

Appendices I and II

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Women with peripartum cardiomyopathy have normal ejection fraction, but abnormal systolic strain, during pregnancy

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Abstract

We report a case series of six women with peripartum cardiomyopathy (PPCM) who incidentally underwent echocardiography prior to the clinical presentation of PPCM. For comparison, we identified controls, matched 2:1 on age, race, body mass index, gestational age, and hypertensive disorder. Among the six cases, all were diagnosed with PPCM during the post-partum period. Pre-PPCM echocardiograms were performed between 17.7 weeks of gestation and 13 days post-partum. Baseline left ventricular ejection fraction and size were normal and similar to the 12 matched controls ($60\% \pm 6.6\%$ vs. $61.4\% \pm 6.3\%$, $P = 0.63$) or left ventricular end-diastolic dimension ($4.6 \text{ cm} \pm 0.2 \text{ cm}$ vs. $4.5 \text{ cm} \pm 0.4 \text{ cm}$, $P = 0.689$). There was a trend towards a less negative (more abnormal) mean global longitudinal strain in cases compared with controls ($-14\% \pm 4\%$ vs. $-18.3\% \pm 4.5\%$, $P = 0.0658$). Mean global circumferential strain was significantly less negative (more abnormal) in cases compared with controls ($-21.5\% \pm 5\%$ vs. $-29.3\% \pm 7.6\%$, $P = 0.0329$). We conclude that women who develop PPCM have normal left ventricular ejection fraction during gestation preceding PPCM, indicating that the disease develops acutely in the peripartum period. Abnormal strain can be detected, however, suggesting that strain imaging could represent a screening method in populations at high risk for PPCM if confirmed in future studies.

Keywords Peripartum cardiomyopathy; Strain echocardiography; Heart failure; Maternal morbidity

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Introduction

Peripartum cardiomyopathy (PPCM) is a form of systolic heart failure diagnosed towards the end of pregnancy or in the months following delivery in women without pre-existing cardiac disease.¹ Contemporary cohorts in the USA demonstrate that 65% to 72% have recovery of left ventricular (LV) function; however, recovery rates vary by population, and many women experience adverse outcomes, including persistent heart failure, arrhythmias, thromboembolic complications, or death.^{1–5} Whether women have normal LV ejection fraction (LVEF) and LV size during pregnancy and prior to development of PPCM has been disputed and has been difficult to study given the low incidence of PPCM and the infrequent use of echocardiograms during pregnancy. In addition, LV

strain has emerged as a powerful tool to detect subclinical LV dysfunction in the setting of cardiotoxic chemotherapy, prior to a subsequent decrease in LVEF.⁶ Whether changes in LV strain indices precede the drop in LVEF in PPCM has not been studied and may hold predictive ability to identify women at risk. Our aims are to describe baseline LV function, including LVEF and strain indices, of peripartum women prior to their development of PPCM and to compare these measures to peripartum women without PPCM.

Case report

We identified six cases of pregnant women with echocardiograms performed during pregnancy or post-partum period

and prior to the development of PPCM. We identified cases from a previously established and updated retrospective cohort of women with PPCM treated within the University of Pennsylvania Health System, as previously described.³ We included women who had also had an echocardiogram performed during pregnancy/post-partum period prior to the diagnosis of PPCM (pre-PPCM echocardiogram). We also compared baseline echocardiographic parameters of peripartum women who subsequently developed PPCM (cases) to peripartum women without PPCM (controls). Controls without known cardiac disease were matched 2:1 on maternal age (± 5 years), race, body mass index, gestational age (± 4 weeks), and hypertensive disorder. Control echocardiograms were selected from women who had enrolled in prior clinical research studies at our health system evaluating echocardiographic differences between women with hypertensive disorders of pregnancy and normotensive pregnancies. The primary outcomes were LVEF, LV end-diastolic dimension, global longitudinal strain (GLS), and global circumferential strain (GCS). LVEF was measured using modified Simpson bi-plane method. Strain was measured using speckle tracking (TomTec Imaging Systems, Unterschleissheim, Germany), as described previously.^{7,8} All echocardiograms were analysed by study staff (R.T. and Y.K.). Continuous variables were presented as means and standard deviations and assessed using the Student's *t*-test given normality of the data. Categorical variables were assessed using Fisher's exact test. We used univariate exact conditional regression to analyse outcomes to account for matched data.

The timing of pre-PPCM echocardiograms ranged from 17.7 weeks of gestation to 13 days post-partum. Indications for the pre-PPCM echocardiograms are presented in *Table 1*. For Cases 1, 2, and 3, indications for echocardiogram were first degree atrioventricular block, palpitations, and chronic hypertension, respectively. Case 4 had a known small, restrictive ventricular septal defect (VSD), and an echocardiogram was performed during pregnancy for routine screening. Of note, at the time of her subsequent PPCM diagnosis, it was thought that the drop in LVEF was not related to her known prior VSD. Case 5 had an echocardiogram performed as part of a workup for hypoxia and tachycardia, and she was ultimately diagnosed with a pulmonary embolism. Case 6 had an echocardiogram performed due to chest pain, and she was ultimately diagnosed with and treated for pericarditis.

Peripartum cardiomyopathy was diagnosed post-partum in all cases, ranging from 6 to 100 days after delivery. The timing between the pre-PPCM echocardiograms and PPCM diagnosis echocardiograms ranged from 19 to 222 days. At the time of PPCM diagnosis, LVEF ranged from 24% to 45%. The majority of the cases (5/6) ultimately had LVEF recovery (LVEF $\geq 50\%$). Genetic results were available for 4/6 cases, and none had a titin-truncating mutation.

The mean age was 32.8 years, and the majority (4/6) were black. There were no significant differences in baseline

Table 1 Cases of peripartum cardiomyopathy with echocardiograms performed prior to diagnosis

Case	Medical history	Pre-PPCM echocardiogram ^a			At time of PPCM diagnosis			Recovery	
		GA or days post-partum	LVEF	Clinical indication	Days post-partum	LVEF	Days between echocardiograms	Recovery to LVEF ≥ 50%	TTN mutation
1	T2 IDDM	17.7 weeks	52%	First degree AVB on ECG	73 days	33%	222	Yes	No
2	Crohn's disease	19.7 weeks	68%	Palpitations	9 days	37%	121	Yes	N/A
3	T1DM, chronic HTN	20.1 weeks	54%	Screening given chronic HTN	93 days	36%	153	No	No
4	Small restrictive VSD	32.9 weeks	67%	Screening given known VSD	6 days	45%	42	Yes	No
5	Asthma, rheumatoid arthritis	Post-partum 1 day	61%	Shortness of breath due to PE	20 days	29%	19	Yes	N/A
6	Asthma, hyperlipidaemia	Post-partum 13 days	58%	Chest pain, diagnosed with pericarditis	100 days	24%	87	Yes	No

AVB, atrioventricular block; ECG, electrocardiogram; GA, gestational age; HTN, hypertension; LVEF, left ventricular ejection fraction; N/A, not available; PE, pulmonary embolism; PPCM, peripartum cardiomyopathy; T1DM, type 1 diabetes mellitus; T2 IDDM, type 2 insulin-dependent diabetes mellitus; TTN, titin; VSD, ventricular septal defect.

characteristics between the matched cases and controls (Table 2). There was no significant difference in pre-PPCM LVEF ($60\% \pm 6.6\%$ vs. $61.4\% \pm 6.3\%$, $P = 0.63$) or LV end-diastolic dimension ($4.6 \text{ cm} \pm 0.2 \text{ cm}$ vs. $4.5 \text{ cm} \pm 0.4 \text{ cm}$, $P = 0.689$) between cases and controls. Strain analysis was technically able to be performed in 5/6 cases and all controls. There was a trend towards a less negative (more abnormal) mean GLS in cases compared with controls ($-14\% \pm 4\%$ vs. $-18.3\% \pm 4.5\%$, $P = 0.0658$). Mean GCS was significantly less negative (more abnormal) in cases compared with controls ($-21.5\% \pm 5\%$ vs. $-29.3\% \pm 7.6\%$, $P = 0.0329$) (Table 2).

Discussion

While it has been often assumed that women have normal LVEF and LV dimensions during pregnancy and prior to development of PPCM, this has not been previously confirmed with serial echocardiograms. Our findings support the conclusion that LVEF and LV dimensions are largely normal during pregnancy and early post-partum period in women who go on to develop PPCM and that changes in LVEF and LV dimensions occur acutely at the time of or shortly before the diagnosis of PPCM. Our study suggests that subclinical dysfunction, as measured by GLS and GCS, may be present prior to the development of symptomatic heart failure and LV dysfunction in women with PPCM. Therefore, subclinical strain abnormalities identified during pregnancy may help to identify women at increased risk of developing PPCM. Current thinking on the pathophysiology of PPCM suggests that hormones of late gestation, including those increased by pre-eclampsia, cause microvascular damage to the heart, leading to

PPCM.^{1,9} This abnormal hormonal milieu may manifest its cardiotoxic effect as subclinical dysfunction prior to causing an overt drop in LVEF, analogous to the cardiotoxic effect of chemotherapy.⁶ If our results are confirmed in larger cohorts, strain imaging could serve as a screening target in populations at increased risk for developing PPCM.

Cardiovascular disease, including PPCM, is a leading cause of maternal morbidity and mortality, disproportionately impacts women of African ancestry, and contributes to significant racial disparities in maternal outcomes observed in the USA.¹⁰ A limited echocardiogram during pregnancy to identify clinical or subclinical abnormalities in women with risk factors for PPCM, such as African ancestry, advanced maternal age, multi-gestational pregnancies, or hypertensive disorders, may identify women who need closer follow-up during pregnancy and the post-partum period to monitor for early signs or symptoms of heart disease. This is especially important for women who experience barriers to accessing care and may mistake cardiac symptoms for normal pregnancy symptoms.¹¹ Further research should examine the feasibility of obtaining limited maternal cardiac views during routine pregnancy care, such as the second trimester foetal ultrasound scan.

Our study has limitations to consider. First, normative values for strain during pregnancy have not been established, although some studies suggest that strain worsens during the third trimester.^{12,13} Average strain rates in our control cohort are similar to those reported in the literature. We aimed to account for pregnancy-related changes in strain by matching on gestational age and other clinical factors, although it was not possible to match on all relevant factors. Second, our sample size is small, and therefore, our results need to be confirmed in a larger

Table 2 Clinical and echocardiographic characteristics of peripartum women who subsequently develop peripartum cardiomyopathy and matched controls

	Cases (n = 6)	Controls (n = 12)	P-value
Baseline characteristics^a			
Age at delivery, mean (SD)	32.8 (8.3)	30.0 (8.3)	0.505
Race, n (%)			1.000
White	2 (33.3)	4 (33.3)	
Black	4 (66.7)	8 (66.7)	
BMI, mean (SD)	31.1 (4.9)	30.0 (4.7)	0.645
GA or days PP at time of echocardiogram	17.7 weeks to 13 days PP	19.1 weeks to 2 days PP	
Hypertensive disorder at time of echocardiogram, n (%)	2 (33.3)	3 (25.0)	1.000
GA at delivery, mean (SD)	35.5 (4.5)	36.6 (4.2)	0.619
Echocardiographic parameters, mean (SD)^b			
LV ejection fraction (%)	60.0 (6.6)	61.4 (6.3)	0.630
LV end-diastolic dimension (cm)	4.6 (0.2)	4.5 (0.4)	0.689
Global longitudinal strain (%)	-14.0 (4.0)	-18.3 (4.5)	0.066
Global circumferential strain (%)	-21.5 (5.0)	-29.3 (7.6)	0.033

BMI, body mass index; GA, gestational age; LV, left ventricular; PP, post-partum.

^aAnalysed using the Student's *t*-test for continuous variables and Fisher's exact test for binary variables.

^bAnalysed using univariate conditional exact regression.

study. Third, we do not have longitudinal strain data to determine whether these changes in strain persist or resolve over time in women with subsequent LV recovery. Finally, echocardiograms are not standard of care during pregnancy, and our cases and controls may represent selection bias. Pre-eclampsia and hypertension can impact strain values and are associated with PPCM, for which we aimed to control through matching. To our knowledge, the other indications for pre-PPCM echocardiograms (e.g. restrictive VSD and pulmonary embolism) do not significantly affect LV strain values.^{14–16}

Peripartum cardiomyopathy is a leading cause of maternal mortality in the post-partum period, especially among black women, and delayed diagnosis leads to worse clinical outcomes. Therefore, efforts focused on closer monitoring and earlier treatment of those at risk have the potential to improve maternal cardiovascular health and lessen racial disparities.

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Conflict of interest

None declared.

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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.

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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%, $P=7.36e-08$) or healthy volunteers (0.7%, $P=3.42e-06$) and enriched in comparison with ancestry-matched reference populations ($P=5.87e-14$). Because cardiac status is not recorded for TCGA participants, these data provide conservative estimates of the burden of TTNtv in CCM. Further support that TTNtv contribute to CCM is derived from a mouse model of CCM: anthracycline-treated $Ttn^{tv/+}$ 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

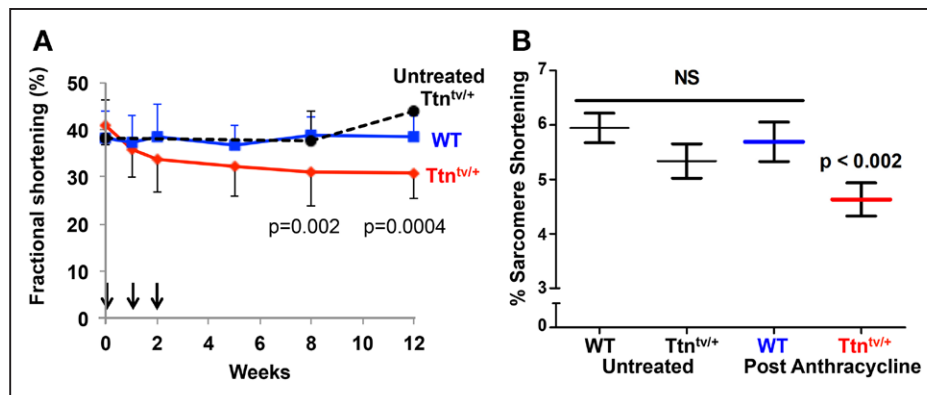


Figure. Persistent cardiac dysfunction in *Ttn^{tv/+}* mice after anthracycline treatment.

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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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.

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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

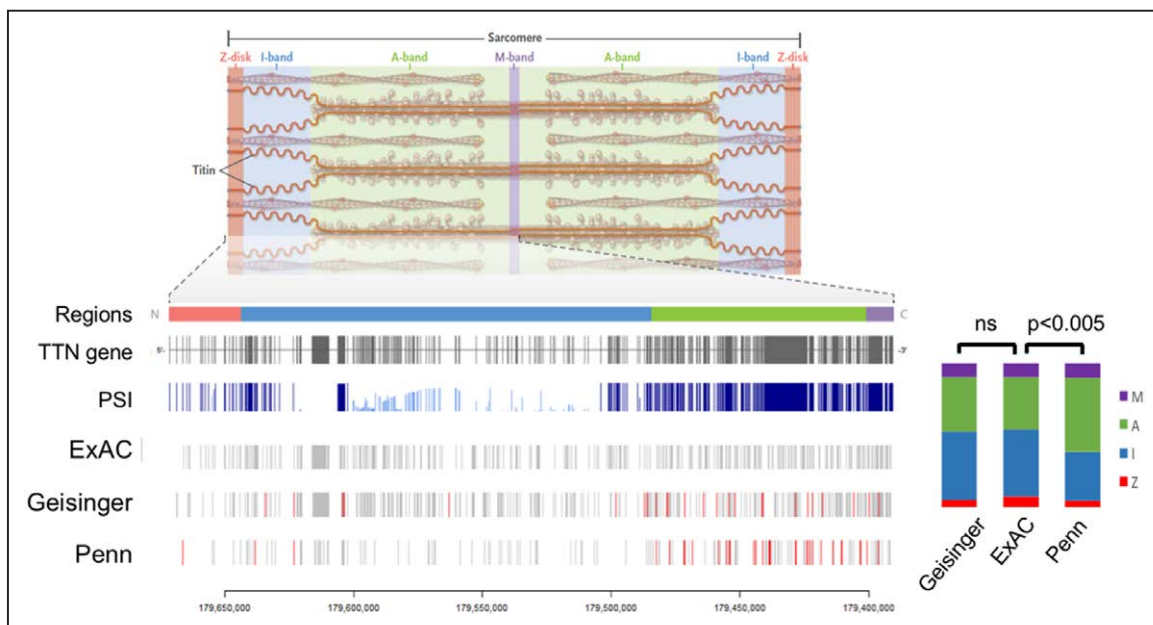


Figure 1. Location within the *TTN* gene of identified variants in the Geisinger and Penn cohorts.

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 I 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 II 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

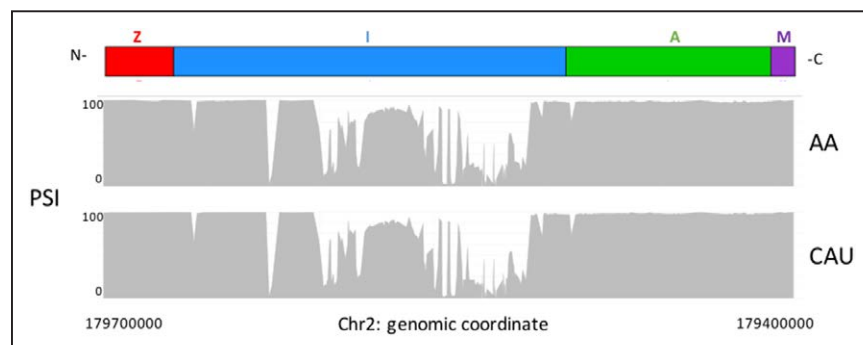


Figure 2. Proportion spliced in (PSI) exons in adult hearts from Caucasian (CAU) and African Americans (AA), explanted after cardiac transplantation for dilated cardiomyopathy.

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

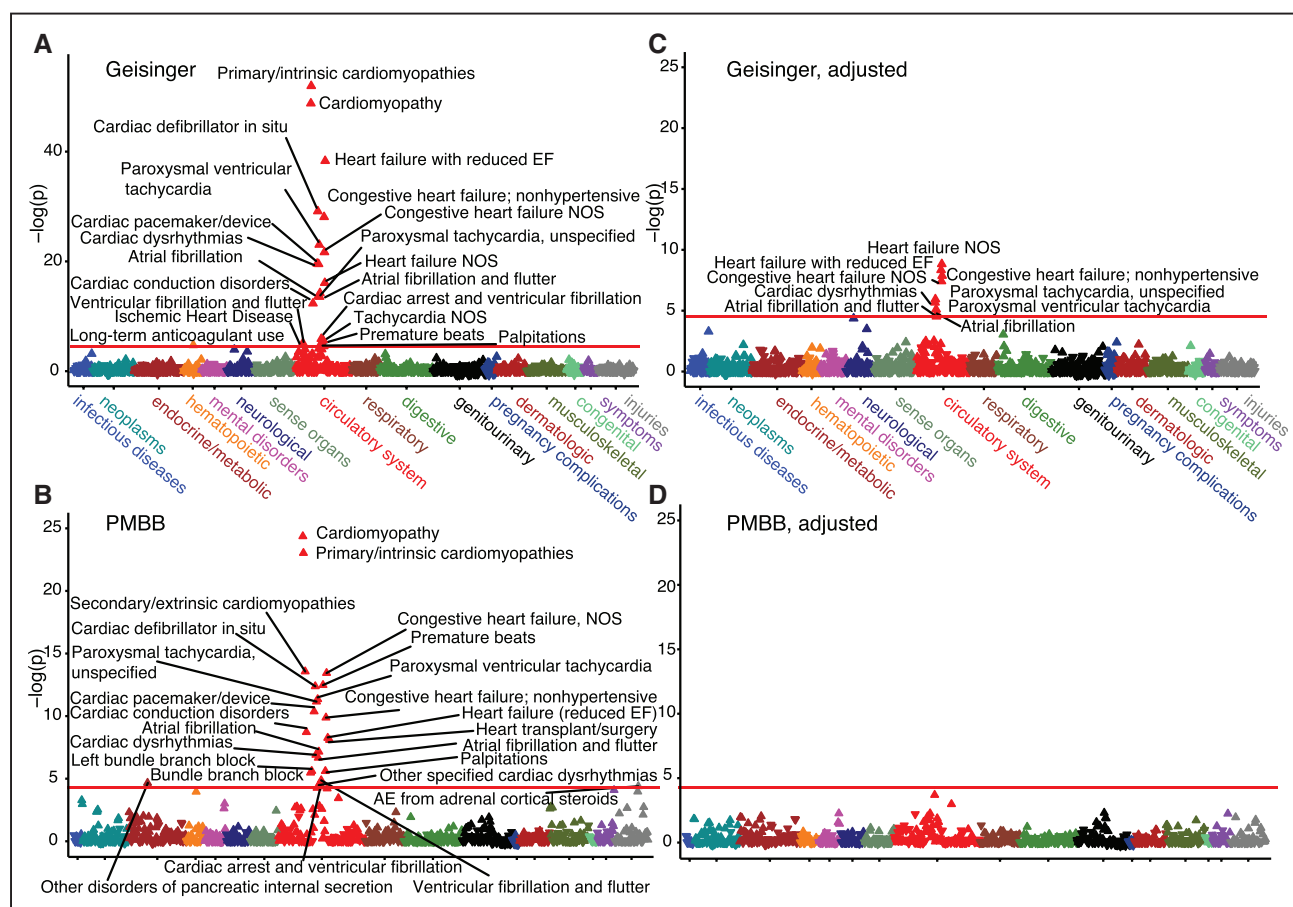


Figure 3. Manhattan plots representing the Phenome-wide Association findings.

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

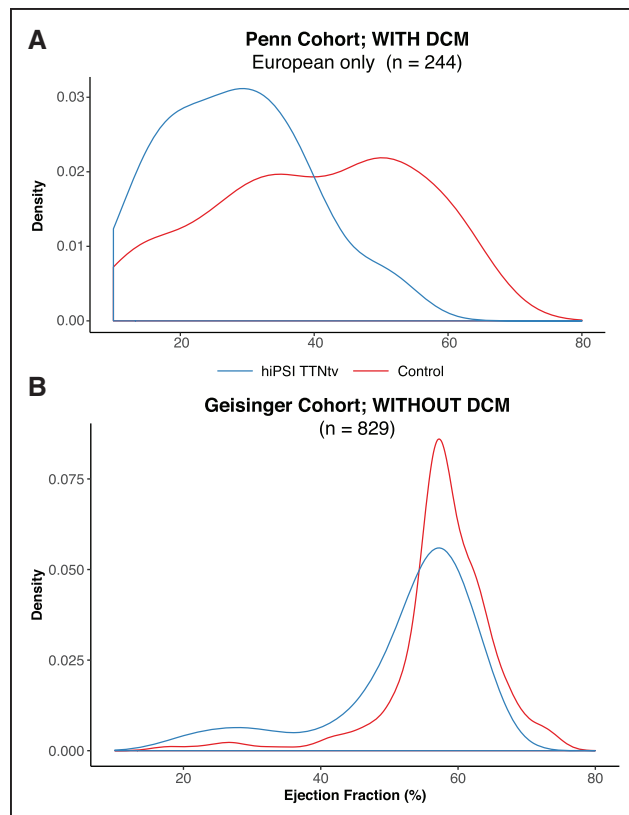


Figure 4. Density plots comparing left ventricular ejection fraction (LVEF) between hiPSI TTNtv (blue) and controls (red) among patients with (Penn, A) and without (Geisinger, B) dilated cardiomyopathy (DCM) diagnoses.

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.

ARTICLE INFORMATION

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Truncated titin proteins in dilated cardiomyopathy

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One Sentence Summary: We provide the first evidence that truncating mutations in *TTN* – the leading cause of inherited dilated cardiomyopathy – result in both appreciable quantities of

truncated titin proteins and reduced full length titin in patient hearts, supporting combined dominant-negative and haploinsufficiency contributions to disease.

Abstract: Truncating variants in *TTN* (TTNtv), which encodes the sarcomeric protein titin, are the most common known cause of non-ischemic dilated cardiomyopathy (DCM), but how TTNtv cause disease has remained controversial. Efforts to detect truncated titin proteins in affected human DCM hearts have failed, suggesting the currently favored paradigm that disease is caused by haploinsufficiency, but reduced amounts of titin protein have not been demonstrated. We leverage here a collection of 184 explanted post-transplant DCM hearts to show, using specialized electrophoretic gels, western blotting, allelic phasing, and unbiased proteomics, that truncated titin proteins can quantitatively be detected in human DCM hearts. The sizes of truncated proteins correspond to that predicted by their respective TTNtv; the truncated proteins are encoded by the TTNtv-bearing allele; and no degradation fragments from protein encoded by either allele are detectable. In parallel, full length titin is less abundant in TTNtv+ than in TTNtv- DCM hearts. Disease severity or need for transplantation did not correlate with TTNtv location. Transcriptomic profiling revealed few differences in splicing or allelic imbalance of the *TTN* transcript between TTNtv+ and TTNtv- DCM hearts. Studies with isolated human adult cardiomyocytes revealed no defects in contractility in cells from TTNtv+ compared to TTNtv- DCM hearts. Together, these data unequivocally demonstrate the presence of truncated titin protein in human DCM, demonstrate for the first time reduced amounts of full-length titin protein in TTNtv+ DCM hearts, and support combined dominant-negative and haploinsufficiency contributions to disease.

Introduction

Titin, encoded by the gene *TTN*, is a large protein that spans the entire sarcomere, from the Z-disk to the M-band, and is essential for sarcomere integrity and function (1). Heterozygous variants in *TTN* that are predicted to lead to truncated titin proteins (*TTN*-truncating variants, or TTNtvs) can be found in 10-20% of patients with idiopathic or familial dilated cardiomyopathy (DCM), compared to a population prevalence of ~1% (2-6), making TTNtvs the most common known cause of DCM. More recent studies have also shown a high prevalence of TTNtvs in cardiomyopathy induced by alcohol (7), pregnancy (8), and cancer therapies (9), suggesting that TTNtv-bearing individuals are susceptible to cardiomyopathy in response to numerous insults.

How TTNtvs mechanistically give rise to disease has remained controversial. Various hypotheses have been proposed, ranging from haploinsufficiency, whereby insufficient titin protein is generated from the intact allele to compensate for the TTNtv allele (a process that might be aggravated by cardiac insults requiring accelerated titin production, e.g. alcohol toxicity, cardiotoxic drugs, or pregnancy), to “poison peptide”, whereby TTNtv protein, i.e. the protein encoded by the TTNtv-bearing allele, incorporates into the sarcomere causing dysfunction (2, 10). Determining whether TTNtv proteins are present in adult human DCM hearts is paramount to distinguishing between these mechanisms, and to understanding how TTNtvs cause heart disease. TTNtv proteins can be detected in immature induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) (11), as well as in skeletal muscle of some patients bearing TTNtvs (12), and work with polysomes has demonstrated that mRNA originating from the TTNtv allele can be found associated with ribosomes, suggesting that the allele can be translated (13). However, to date, repeated efforts to detect TTNtv proteins in affected human DCM hearts have failed (2, 4, 14). Moreover, proteomic studies in a rat model bearing an A-band TTNtv demonstrated complete

absence of protein encoded by the TTNtv-bearing allele (13). The current leading theory is thus that haploinsufficiency of titin, rather than dominant-negative actions of the truncated peptide, leads to heart disease (2). However, reduced amounts of titin mRNA or protein expression in TTNtv human hearts have not been observed to date (2, 4, 14), challenging this theory. In sum, strong evidence in adult human hearts for any mechanism is lacking.

In addition, the functional impact of TTNtv on cardiomyocyte function also remain undefined. Work with iPSC-CMs has demonstrated that mutant titin protein results in sarcomere insufficiency and impaired responses to stress (11), yet the extent to which these findings in immature iPS cells reflect the impact of TTNtv on adult cardiomyocytes remains unclear. To better understand the impact of TTNtv on human hearts, we therefore leveraged unique access to a large collection, accumulated over the past 20 years, of hearts collected post-transplantation, as well as an ongoing procurement of such hearts, to evaluate comprehensively the impact of TTNtv on cardiac TTN mRNA and protein, and on intact adult cardiomyocyte contractility.

Results

Identification and characterization of TTNtv in post-transplant DCM hearts

To evaluate the impact of TTNtv on human hearts, we analyzed 184 hearts explanted from patients with non-ischemic dilated cardiomyopathy (DCM), and 14 non-failing discarded donor hearts. Capture-based exome sequencing of *TTN* revealed 25 truncating variants in the DCM hearts (11 nonsense, 9 frameshift, 5 splice site; table S1). Previous work demonstrated that, of the 364

exons encoded by *TTN*, only TTNtvs located in exons predicted to be spliced into the adult TTN transcripts (percent spliced in [PSI]>0.9, or hiPSI) are likely pathogenic (4, 5). Of the 25 TTNtvs identified above, 22 were located in hiPSI exons (12% prevalence, $P < 2 \times 10^{-23}$ compared with 0.5% prevalence of hiPSI TTNtvs in reference gnomAD population). This prevalence is comparable to that reported in other DCM cohorts not requiring cardiac transplantation (4, 6). 7 of the TTNtvs (all hiPSI) are identical to variants annotated as pathogenic, or probably pathogenic, for DCM in the ClinVar database (www.ncbi.nlm.nih.gov/clinvar). DNA from the 14 non-failing hearts encoded no TTNtvs. No demographic differences or differences in cardiac morphology or hemodynamics were apparent between TTNtv- and TTNtv+ patients. All 22 hiPSI TTNtvs were located in the C-terminal region of the I band, A band, or M band region (fig. 1A), akin to the distribution seen in DCM not requiring cardiac transplantation (fig. 1A) (5, 6). Moreover, neither the age of patient at time of transplant (fig. 1B) nor pre-transplant left ventricular ejection fraction (LVEF, fig. 1C) correlated with the location of the TTNtv in the protein, suggesting that the location of the TTNtv does not influence severity of disease.

Table 1. Patient statistics

	TTNtv-DCM (n=158)	TTNtv+DCM (n=22)	p-value
Age (yrs)	51.77 ± 11.06	54.59 ± 6.62	0.25
Male (%)	93(59)	16(73)	0.21
African American (%)	71(45)	6(27)	0.12
Weight (kg)	78.52 ± 17.28	84.56 ± 20.43	0.14
Height(cm)	171.46 ± 10.53	174.43 ± 10.62	0.22
BMI (kg/m ²)	26.56 ± 4.67	27.67 ± 5.38	0.31
Heart Weight (grams)	504.56 ± 148.24	475.73 ± 96.00	0.38

History of comorbidity			
Atrial fibrillation (%)	69(44)	13(59)	0.18
VT/VF (%)	77(49)	10(45)	0.75
Diabetes mellitus (%)	41(26)	5(23)	0.77
Hypertension (%)	89(56)	10(45)	0.34
Echocardiography	(n=108)	(n=18)	
LVEF (%)	16.26 ± 7.40	15.38 ± 6.25	0.60
LVEDD (cm)	6.68 ± 1.35	6.77 ± 1.06	0.80
LVESD (cm)	6.10 ± 1.22	5.99 ± 1.03	0.73

Table 1: Clinical and echocardiographic characteristics of 22 hiPSI TTNtv positive (TTNtv+) DCM patients, versus 158 TTNtv negative (TTNtv-) DCM patients, BMI: body mass index. LVEF: left ventricle ejection fraction. LVEDD: left ventricle end-diastolic dimension. LVESD: left ventricle end-systolic dimension. For echocardiographic data, n represents the number of patients with complete echocardiography records.

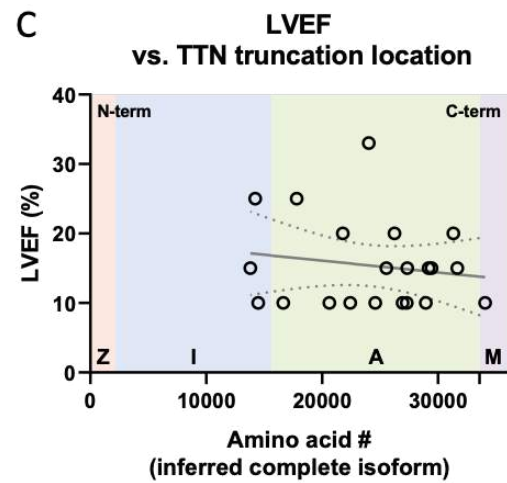
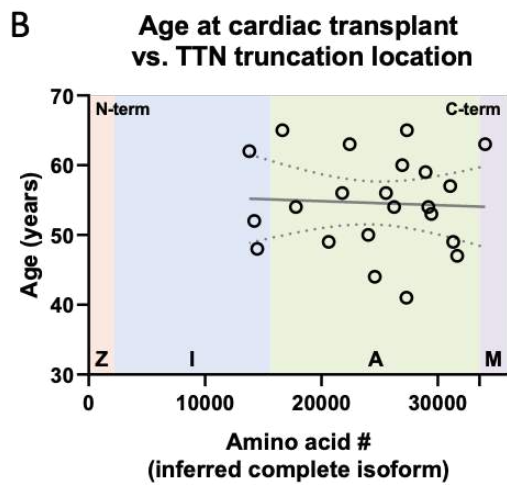
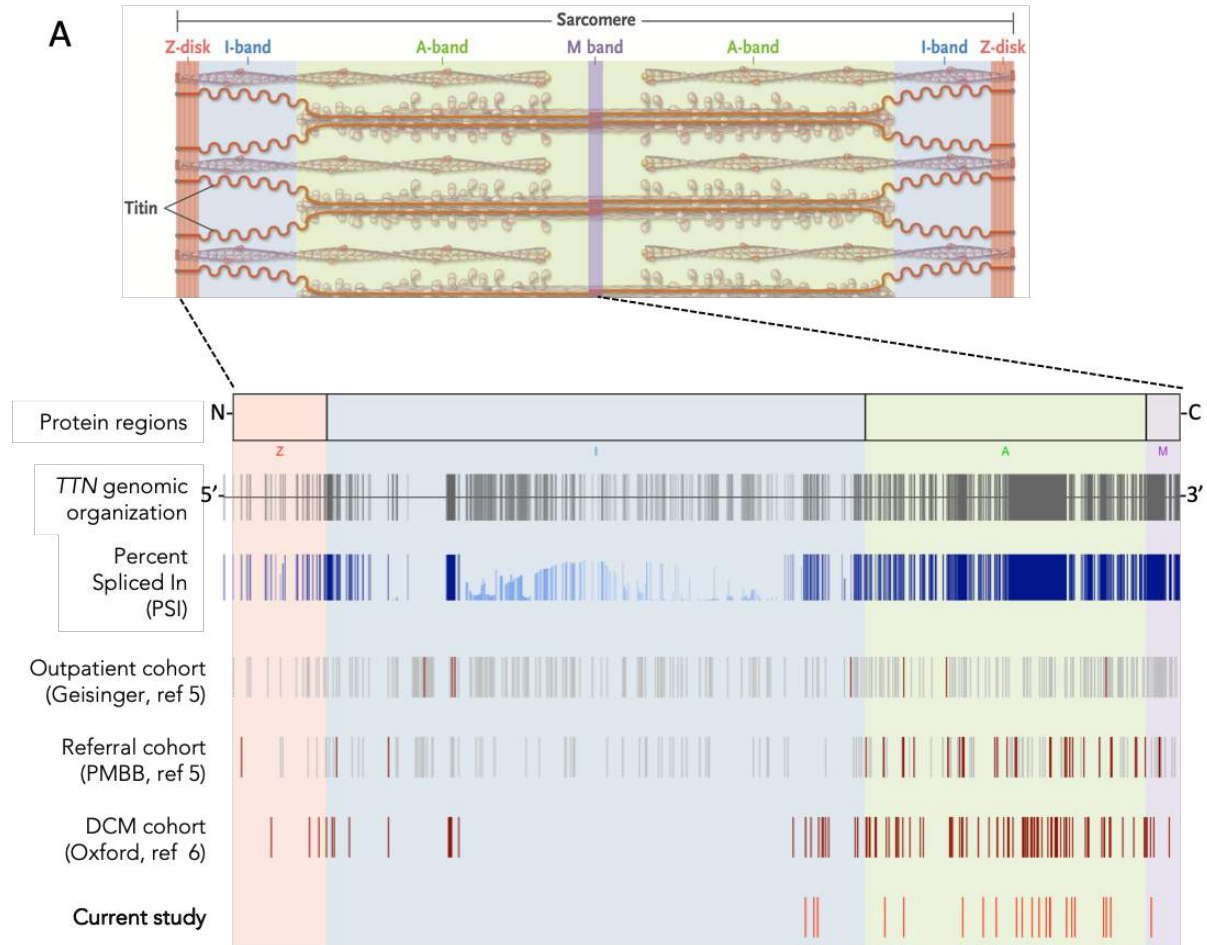


Fig. 1. Genomic characteristics of 22 hiPSI TTNtv, and associated clinical phenotypes, in DCM patients who underwent cardiac transplantation. (A): Top: Titin is an integral part of the cardiac sarcomere, spanning from Z-disk to M-line. Bottom: Regions of the *TTN* gene and protein are designated according to their spatial distribution in the sarcomere: Z-disk, I-band, A-band, M-band. The 364 exons of the *TTN* gene and their associated percent spliced in (PSI) are shown, followed by the distribution of truncating variants in those exons identified in the Geisinger cohort (a general outpatient population) (5), in the Penn Medicine Biobank (PMBB) cohort (a tertiary care referral population) (5), a cohort of 1040 patients with idiopathic dilated cardiomyopathy (DCM) from London and Singapore (6), and the variants identified in the current study. For the latter two, only hiPSI variants are noted. Variants found in patients with DCM are designated in red. (B-C): Lack of association between location of TTNtv and either age (B) or left ventricular ejection fraction (LVEF; C) at the time of cardiac transplantation. Dotted lines indicate a 95% confidence interval about a linear regression, indicated by a solid line.

Impact of TTNtv on TTN mRNA allelic balance and splicing, and genomic mRNA expression

To investigate the impact of TTNtv on mRNA expression, we examined genome-wide RNA sequencing of myocardium from 138 of the 184 DCM hearts, of which 19 had hiPSI TTNtv, and 10 non-failing hearts. As noted above, the 364 exons of *TTN* are variably spliced, and alterations in *TTN* splicing caused by the presence of TTNtv could in principle cause disease. However, we identified no differences in *TTN* exon usage between TTNtv+ and TTNtv- DCM hearts (fig. 2A), suggesting that *TTN* alternative splicing is not an important driver of TTNtv-induced pathology. We next tested if the presence of a TTNtv affects allelic balance, for example by leading to nonsense-mediated decay of the truncated transcript. To do this, we reconstructed each of the two

individual alleles for each patient, based on the results of exomic sequencing and by phasing known haplotypes (see methods section). With this approach, we detected no evidence of significant allelic imbalance in DCM, compared with non-failing hearts, and only marginal allelic imbalance in TTNtv+ DCM hearts compared with TTNtv- DCM hearts (fig. 2B-C). What little allelic imbalance was seen in TTNtv+ DCM hearts did not correlate significantly with age at transplant, ejection fraction, or location of truncation in *TTN* (Fig. S1A). Thus, the presence of TTNtvs appears to have little impact on the level of expression or splicing of *TTN*.

We next investigated the impact of TTNtvs on global mRNA expression. Principal component analysis (PCA) of the RNAseq datasets differentiated both TTNtv+ and TTNtv- DCM hearts from non-failing hearts, but did not differentiate between the two forms of DCM (fig. 2D). After Bonferroni correction, only one gene (TPM3; tropomyosin α -3 chain) (fig. S1B) was significantly altered in TTNtv+ compared to TTNtv- DCM hearts. Of the top 20 most highly expressed genes, which mainly encode sarcomeric proteins, only *TTN* expression was nominally significantly reduced in TTNtv+ compared to TTNtv- DCM hearts (fig. S1C). This mild reduction in *TTN* transcript may reflect the impact of mild allelic imbalance. Thus, no gross transcriptional differences are apparent between TTNtv+ and TTNtv- DCM hearts. A limitation of these studies is that mRNAs were acquired from terminally failing hearts, affected by a range of medications and devices, which may lead to the null effects by introducing large variability.

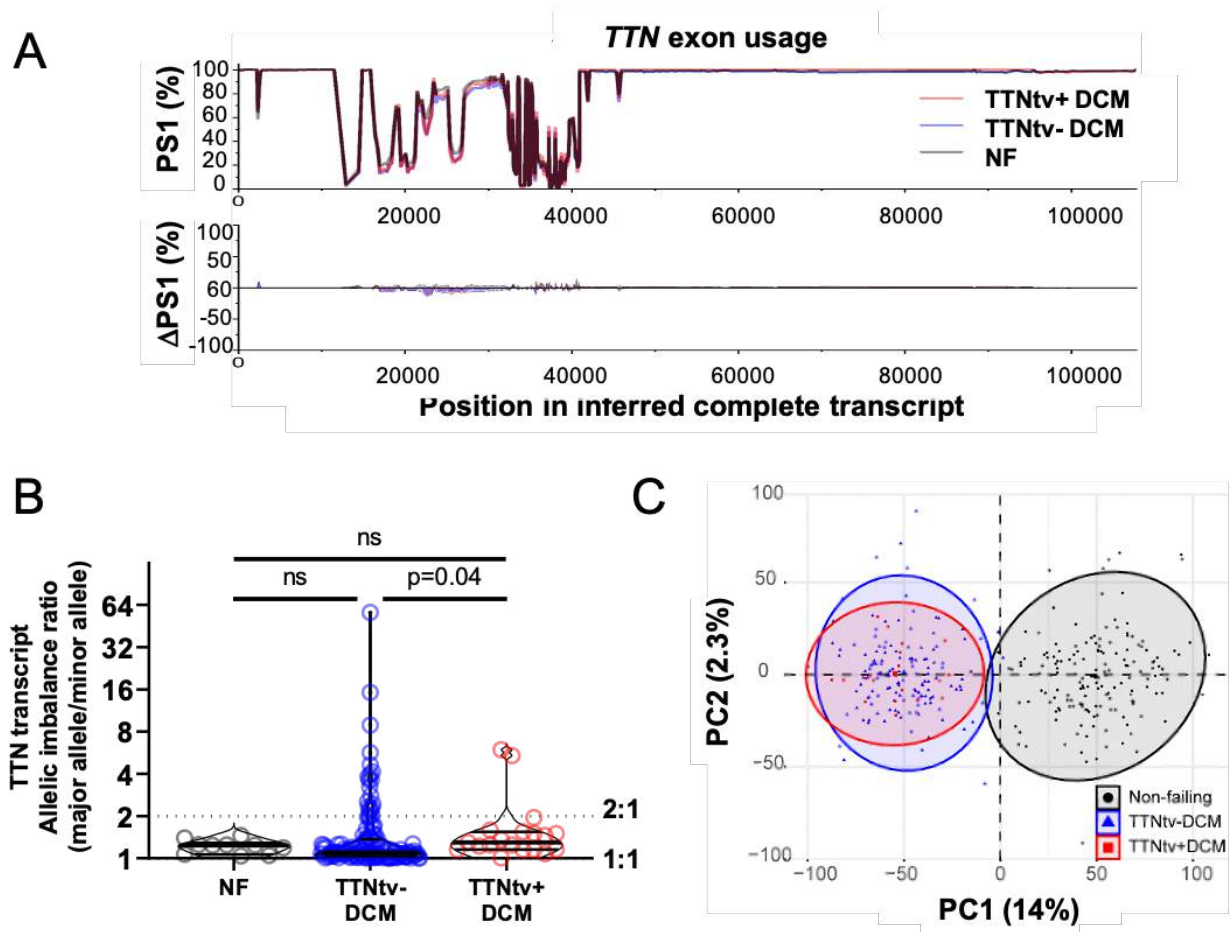


Fig. 2. Transcriptomic evaluation of hiPSI TTNtv+ versus TTNtv- post-transplant DCM hearts. (A): Top panel: minimal changes in *TTN* percent spliced in (PSI) exons between TTNtv+ DCM (n=19), TTNtv- DCM (n=119), and nonfailing (NF) hearts (n=10). Bottom panel: change in PSI (Δ PSI) compared between types. Red shading: TTNtv+ DCM minus NF. Blue shading: TTNtv- DCM minus NF. Grey trace: TTNtv+ DCM minus TTNtv- DCM. Of note: splicing patterns of *TTN* do differ in Black vs White populations (5). **(B):** mildly increased allelic imbalance in TTNtv+ versus TTNtv- DCM heart ($P = 0.04$, n=10, 129, and 19 in NF, TTNtv- DCM, and TTNtv+ DCM respectively), shown as allelic imbalance expressed as the ratio of the more common allele over the less common allele. P value determined by Kruskal-Wallis ANOVA. Error bars report median and interquartile ranges: NF: 1.24 (1.06-1.29), TTNtv- DCM (1.03-1.37), TTNtv+

DCM (1.15-1.54) (C): global transcriptomic profiling principal component analysis separated non-failing (n=166) from DCM hearts, but not TTNtv+ (n=143) from TTNtv- DCM (n=19) hearts.

Impact of TTNtv on human adult cardiomyocyte contractile function

Experimental data with iPSCs have indicated that TTNtv cause impairment of sarcomerogenesis, (11) leading to the model that TTNtv may impair the ability to maintain or restore sarcomeres damaged by stresses such as pregnancy, alcohol, or chemotherapy (8, 9, 15). To identify putative defects of sarcomere function in adult DCM hearts with TTNtv, we isolated adult left ventricular cardiomyocytes (ACMs) from three DCM patients with hiPSI TTNtv via collagenase perfusion at the time of transplant, as described previously (16), and compared to a group of similarly treated TTNtv- DCM and non-failing (NF) controls (fig. 3). Consistent with previous results (16), TTNtv- DCM ACMs showed impaired contractile function upon electrical stimulation when compared to NF controls. Surprisingly, ACMs from TTNtv+ DCM hearts had at least equal or better fractional shortening, contraction and relaxation velocities, and contraction and relaxation times compared with TTNtv- DCM hearts, in fact more closely approximating the values of NF ACMs (fig. 3). Interestingly, TTNtv+ ACMs had shorter resting sarcomere lengths than either TTNtv- or NF ACMs. These data thus suggest that TTNtv do not impair sarcomerogenesis in adult human hearts, in contrast to the case in iPSCs (11).

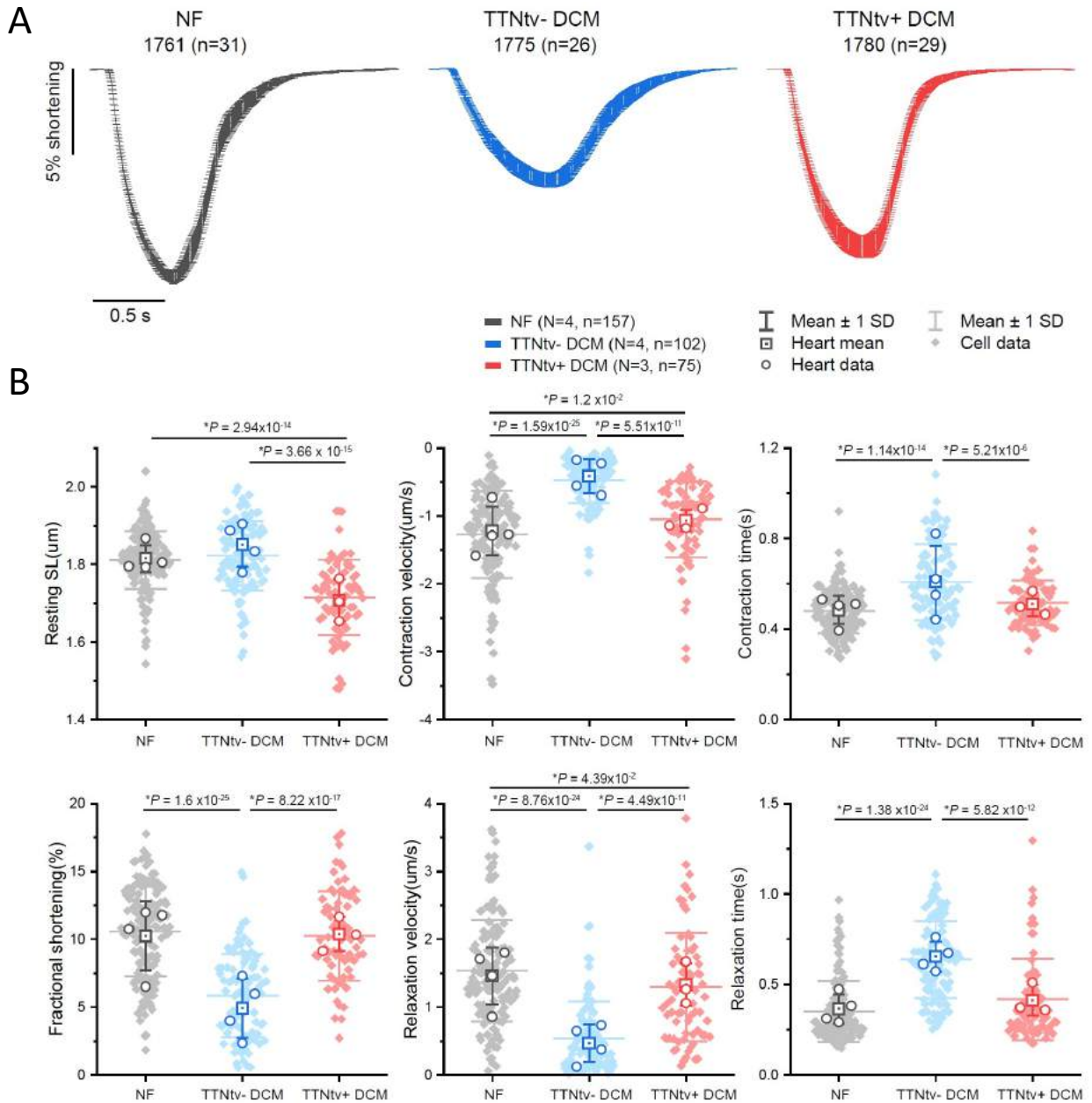


Fig. 3. Contractility of intact adult cardiomyocytes isolated from TTNtv+, TTNtv-, and non-failing (NF) human hearts. ACM unloaded shortening was assessed from 4 NF donor hearts, 4 TTNtv- post-transplant hearts, and 3 hiPSI TTNtv+ post-transplant hearts. **A:** Representative average ACM fractional shortening upon 0.5 Hz electrical stimulation from a NF, TTNtv-, and TTNtv+ heart. Patient ID is followed (in parenthesis) by n = number of myocytes assessed per heart, data presented as mean shortening $\pm$ SD. **B:** Quantification of the indicated contractile parameters of pooled data from all assessed hearts. N = number

of hearts per group, n = number of cells evaluated per group. Statistical significance was determined by One-Way ANOVA with post-hoc Bonferroni comparisons between myocytes (closed diamonds). Between heart comparisons (open circles) were also performed, yielding similar results, and are reported in Data File S2, along with full contractility parameters for each individual heart.

In light of this absence of overt worsening of contractility in TTNtv+ ACMs, we also sought to investigate if other pathways were differentially affected between TTNtv+ and TTNtv- DCM hearts. Alterations in autophagy have been suggested to contribute to disease progression in TTNtv+ DCM, including the observations of increased LC3B-II and p62 proteins in rat models of TTNtv+ DCM (17, 18). However, western blotting studies of proteins and post-translational modifications involved in autophagy demonstrated no convincing differences in LC3, p62, or other proteins in TTNtv+ DCM compared to TTNtv- DCM hearts (fig. S2). Thus, alterations in autophagy that occur in DCM appear not to be specific to TTNtv+ DCM.

Detection of abundant peptides encoded by the TTNtv-bearing allele in TTNtv hearts

The observed alterations in resting sarcomere length in TTNtv+ vs. TTNtv- hearts suggested that truncated titin proteins may be present and affecting cardiomyocyte function. To date, however, efforts at detecting truncated titin protein in human TTNtv+ DCM hearts have failed (4, 5, 14). Thus, evidence existing up to this point has been taken to support the paradigm that DCM induced by TTNtvs is caused by haploinsufficiency, and not dominant-negative actions of a truncated peptide (2). To probe more deeply into this question, and to test which, if not both, of these mechanisms are potentially at play, we sought to use proteomics to detect titin protein in TTNtv+ human DCM hearts. Proteins were prepared from 10 hiPSI and 2 loPSI TTNtv+ DCM hearts,

separated by SDS-agarose gel electrophoresis, extracted from the gel (excluding the highly abundant myosin heavy chain band), subjected to trypsin or chymotrypsin fragmentation, and analyzed by unbiased proteomics (fig. 4A).

We first tested the hypothesis that truncated titin may be actively cleaved or degraded into fragments that cause cellular proteotoxicity. However, we detected no titin peptides among proteins smaller than 200kD (i.e. myosin size) in 6 of the samples, and less than 1 in 100,000 peptides mapped to titin in the remaining samples (fig. 4B). No difference in abundance of such peptides was seen in pathogenic hiPSI vs non-pathogenic loPSI TTNtv+ DCM hearts.

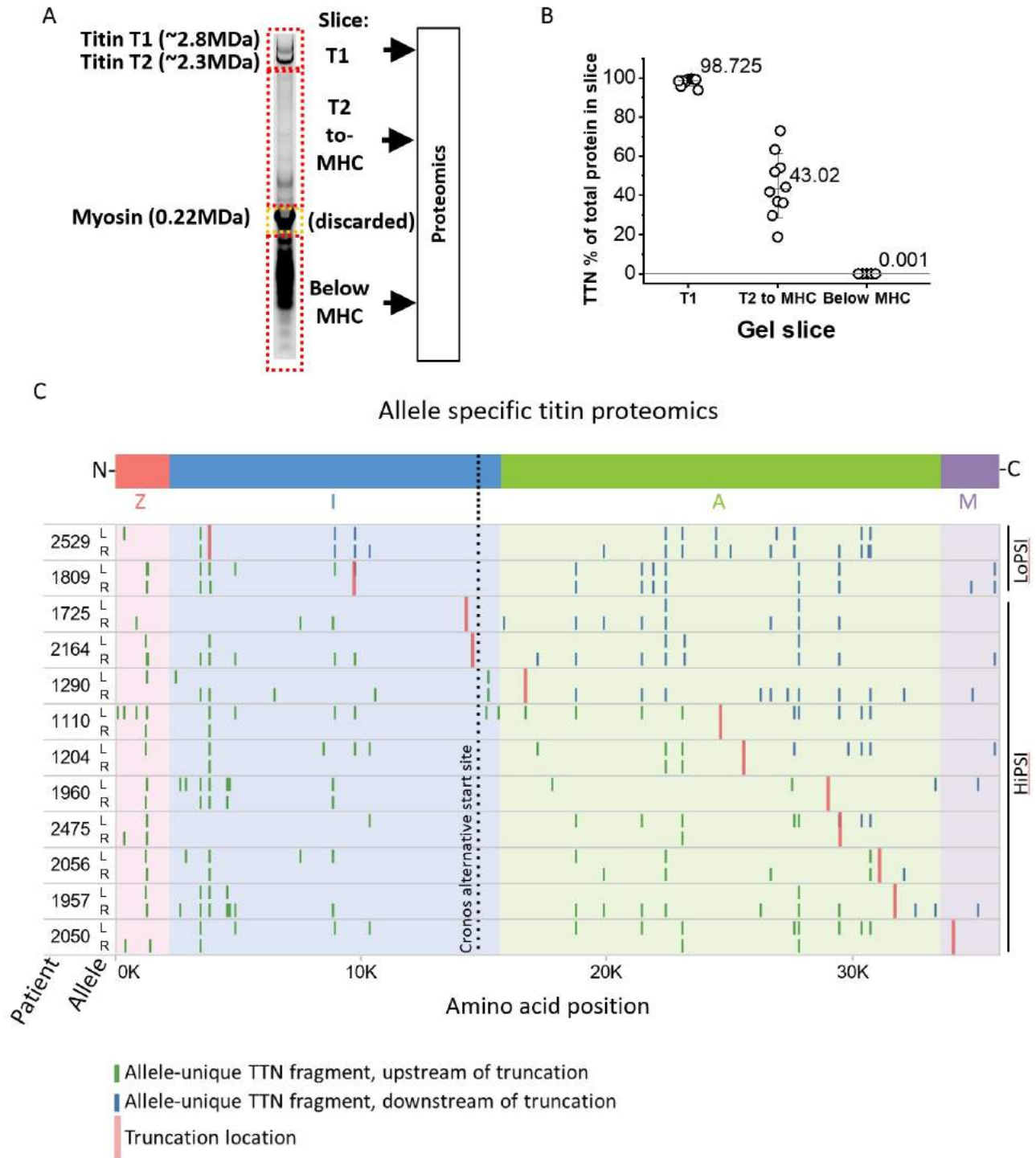


Fig. 4. Proteomic detection of truncated titin proteins encoded by the allele bearing TTNtv in TTNtv heretozygous DCM hearts. (A): experimental scheme. Modified electrophoresis was used to excise three slices (labeled T1, T2-to-MHC, and below MHC), discarding the abundant myosin heavy chain (MHC).

Slices were then subjected to proteomic evaluation. **(B)**: relative abundance of peptides mapped to the *TTN* inferred complete isoform in each slice. **(C)**: Mapping of identified *TTN* peptides to the titin protein predicted to be encoded by either allele (arbitrarily labeled L and R), using haplotype phasing and captured genomics sequences (one allele bears the *TTN*tv, but cannot be ascribed to L or R based on phasing). Patients 2529 and 1809 bear non-pathogenic loPSI *TTN*tv; the remainder are hiPSI *TTN*tv. The Cronos alternative transcriptional start site is indicated.

We next tested if truncated titin could be detected among the larger proteins. The presence of heterozygous missense single nucleotide variants (SNVs) allowed assignment of peptide fragments to one allele or the other of each patient (arbitrarily labeled L and R allele, fig. 4C). Of note, there was a non-significant trend for higher total number of *TTN* missense variants per individual, and number of rare (<1%) missense variants, in *TTN*tv vs non-*TTN*tv individuals (13.5 vs 11.4, $P=0.07$; and 5.6 vs 4.4, $P=0.07$; respectively). The role of *TTN* missense variants in DCM remains controversial (19, 20). Here, we used the missense variants as markers to identify the allelic source of detected peptides. Reconstruction of each complete *TTN* allele in each patient was achieved by phasing of the missense SNVs (see methods). We identified 10 hiPSI *TTN*tv samples with sufficient number of missense variants to allow separate detection of proteins encoded by the two parental alleles. We also included 2 loPSI *TTN*tv samples to test the widely accepted notion that the reason loPSI variants are not pathogenic is because full length protein is readily made by skipping the affected exon. Strikingly, in all 12 hearts, including all 10 samples with hiPSI *TTN*tv, peptide fragments could be assigned to both alleles, unambiguously demonstrating that the *TTN*tv-bearing alleles are translated into protein (fig. 4C). In 7 of the 10 hiPSI hearts, fragments downstream of the truncations could only be assigned to one allele, consistent with translation

proceeding up to and stopping at the truncation site. The frequency of detection of peptides assigned to the allele bearing the TTNtv in these 7 hearts was roughly half that assigned to the non-TTNtv allele upstream of the truncating site (4.1 vs 9.4 per sample, $P = 0.007$), indicating that the truncated protein is less abundant than full length titin (note that the TTNtv could not be mapped to the L or R allele, and that assignment of the allele that bears the TTNtv is thus inferred). In 1 of the 10 hiPSI hearts (#2050), with the most C-terminal TTNtv, no peptides were detected downstream of the truncation in either allele, although in this case as well, one allele mapped 42% as many peptides upstream of the truncation site as the other allele, again suggesting that the truncated protein is less abundant than full length titin. Interestingly, in the remaining 2 of the 10 hiPSI cases (#1725 and #2164), peptides could be assigned to both alleles even downstream of the predicted truncation, and in both these cases the TTNtv occurs 5' to Cronos, a known alternative transcriptional start site for *TTN* (fig. 4C), suggesting that these peptide fragments may be generated from the Cronos transcript. Finally, in the 2 cases with a loPSI TTNtv (#2529 and #1809), fragments were detected from both alleles, both upstream and downstream of the truncation site, and at equivalent frequency, consistent with exclusion of the truncation-encoding exon. Together, these proteomic data indicate that fragments of truncated titin, encoded from the allele bearing a TTNtv, are present and abundant in TTNtv+ DCM hearts.

Detection of intact TTNtv protein in TTNtv+ hearts

The proteomic data above demonstrate the presence of titin at a molecular weight >200kD (i.e. in the gel slices containing proteins larger than myosin) but cannot be used to infer the presence of intact titin. Therefore, to determine if intact TTNtv protein is present in TTNtv+ DCM hearts, as

well as to confirm the presence of TTNtv protein by methods orthogonal to proteomics, we size-separated protein from TTNtv⁺ and TTNtv⁻ DCM hearts, using modified SDS-agarose gel electrophoresis, followed by protein detection using either Coomassie blue staining or two-color fluorescent immunoblotting. Cardiac titin is typically detected in Coomassie stains as three clear bands, N2BA (2.9MDa), N2B (2.7MDa), and a Cronos-derived protein (2.3MDa)(21). Strikingly, in most samples from TTNtv⁺ hearts, additional bands were clearly visible (fig. 5A, top panel). Immunoblotting with antibodies targeting the N- and C-terminal regions of titin demonstrated that all of these shorter titin proteins contained the N-terminus (fig. 5A, middle panel), while none of them contained the C-terminus (fig. 5A, lower panel). Moreover, the observed bands migrated with a predicted size closely corresponding to the predicted molecular weight of the N2B isoform bearing the respective TTNtv (fig. 5A, middle panel, red arrowheads). The more slowly migrating bands did not as reliably correspond to the predicted N2BA molecular weights, suggesting different processing of these isoforms. No distinct N-terminus-containing proteins were detected in any sample below the predicted N2B TTNtv molecular weight, with the exception of Novex-3, a known 0.6MDa cardiac titin isoform present in all samples. The intensity of the readily quantifiable lower N2B-derived TTNtv bands was considerably lower than that of full length TTN proteins, ranging from 35% of the sum of total of N2B+N2BA to nearly undetectable (average 4%), indicating the abundance of TTNtv proteins is less than that of intact titin (fig. S3). Upper N2BA-derived TTNtv bands, if present, were not quantified, due to overlap with full length isoforms, and therefore some TTNtv band intensity is unaccounted for in this analysis. These data demonstrate conclusively the presence of TTNtv proteins, at the predicted size, in TTNtv⁺ DCM hearts.

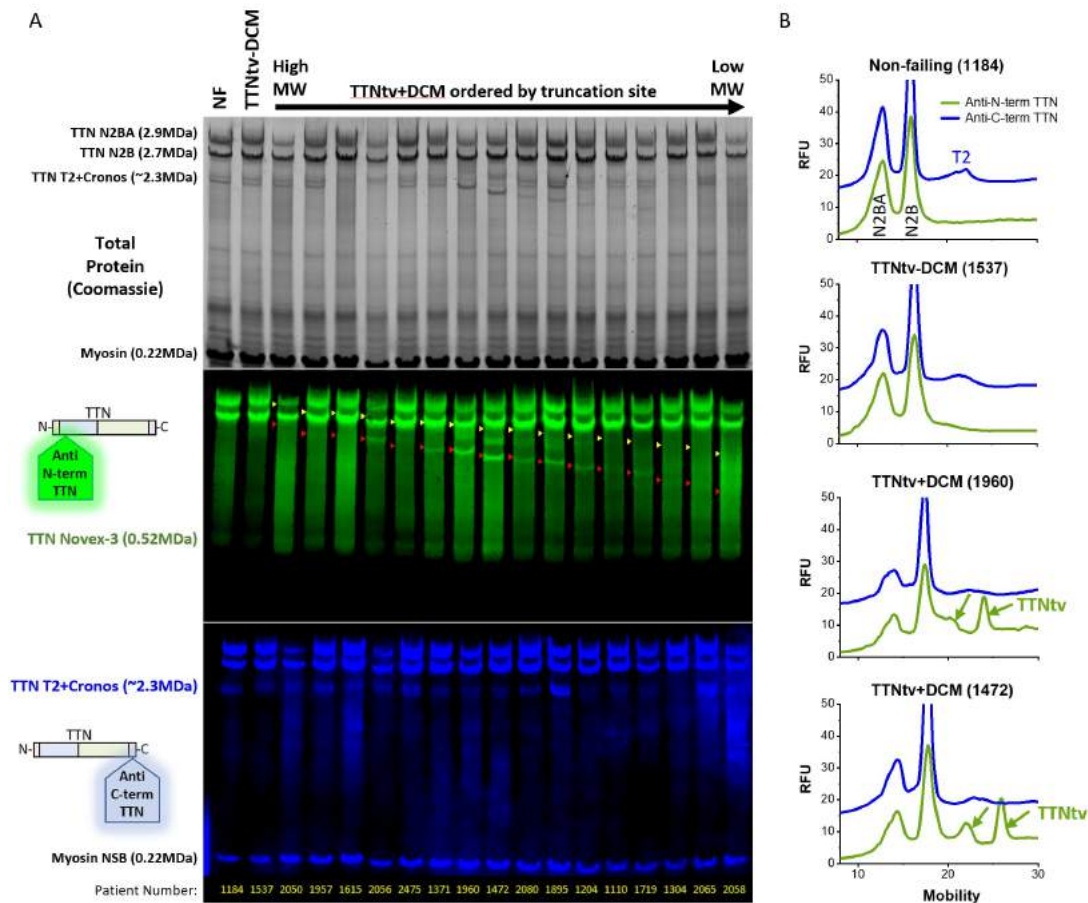


Fig. 5: Detection of truncated titin proteins at predicted locations in TTNtv+ DCM hearts by gel electrophoresis and simultaneous two-color Western blotting. (A): Total protein Coomassie staining (top), and Western blotting with antibodies directed against the N-terminus (middle, green) and C-terminus (bottom, blue) of titin protein, of 16 TTNtv+ DCM hearts, ordered by size of predicted truncated titin protein. A nonfailing (NF) and a TTNtv- DCM heart are shown for comparison. TTN Novex: protein encoded by an alternatively spliced isoform that does not contain the epitope recognized by the C-terminus antibody. TTN Cronos: a TTN isoform encoded by the transcript originating at the alternative start site Cronos, and which does not contain the epitope recognized by the N-terminus antibody. Threshold and gamma are adjusted per channel for clarity of display. Yellow and red arrowheads represent the predicted mobilities for truncated N2B and N2BA, respectively, based on the truncation site location within the

genome. The lowermost band in the blue TTN C-terminus channel is nonspecific binding (NSB) to myosin heavy chain. **(B)**: Traces of Western blot lane fluorescent intensities with N- (green) and C- (blue) terminus antibodies with control NF and TTNtv- DCM hearts (top two) and two sample TTNtv+ DCM hearts (bottom two).

TTNtv proteins loosely associate with sarcomere-containing cellular fractions

To begin to address where in the cell TTNtv proteins can be found, we devised a novel method (fig S4A) whereby cryo-preserved cardiac tissue was first physically crushed on liquid nitrogen into a grossly cell-scale (~20-100µm) powder (fig. S4B). The frozen powder was then added to relaxing buffer containing 0.5% Triton X-100 thus lysing cardiomyocytes and liberating soluble and membrane bound proteins, but leaving large sarcomeric complexes intact and sedimentable by gentle centrifugation. Non-sarcomeric proteins subjected to this protocol were found in the supernatant (e.g. GAPDH), while sarcomeric or sarcomere-associated proteins were found in the pellet (e.g. myosin heavy chain, desmin, and full length titin, as well as Cronos titin) (fig S4C). 99.7% of full-length titin partitioned to the sarcomere-associated fraction. In three evaluated TTNtv+ DCM hearts, TTNtv protein was largely (~80%) found in the pellet (fig. S4C-D), suggesting active incorporation of truncated titin into the sarcomere, although it cannot be ruled out that truncated titin is instead in non-sarcomeric aggregates that are also found in the pellet. Interestingly, despite >80% of truncated titin protein being retained in the sarcomere-containing fraction, TTNtv protein were 49-fold more likely than full-length titin to dissociate from the sarcomere-containing fraction and partition to the soluble fraction. Thus, if truncated titin proteins do incorporate into the sarcomere, they are more loosely bound there than either full-length titin or Cronos titin. These data also demonstrate for the first time the consistent presence of appreciable

quantities of Cronos titin protein in adult myocardium, and that Cronos titin is tightly associated with sarcomere-containing fractions, consistent with its incorporation into the sarcomere.

Evidence of reduced full length TTN protein expression in TTNtv+ hearts

Finally, we sought to address the question of whether TTNtv+ patients demonstrate evidence of reduced total TTN protein expression, i.e. to what extent does full length titin, encoded by the non-truncating allele, compensate quantitatively for the absence of full length titin translated from the TTNtv allele, despite observed equivalent transcription of both alleles (i.e. allelic balance, shown above), and active translation from the transcript that encodes the TTNtv. We therefore quantified full length titin proteins in TTNtv+ hearts, measured from Coomassie stained gels or western blots (fig. 6 and S5). The sum of full length N2A and N2B isoforms was ~30% lower in TTNtv+ hearts, compared to TTNtv- hearts (fig. 6 and S5). This reduction was apparent whether detected TTN protein amounts were normalized to total tissue protein or to individual intracellular structural proteins (MHC, dystrophin or desmin). Thus, full length titin protein, expressed from the non-TTNtv-bearing allele, does not fully compensate for truncated titin in TTNtv+ DCM hearts.

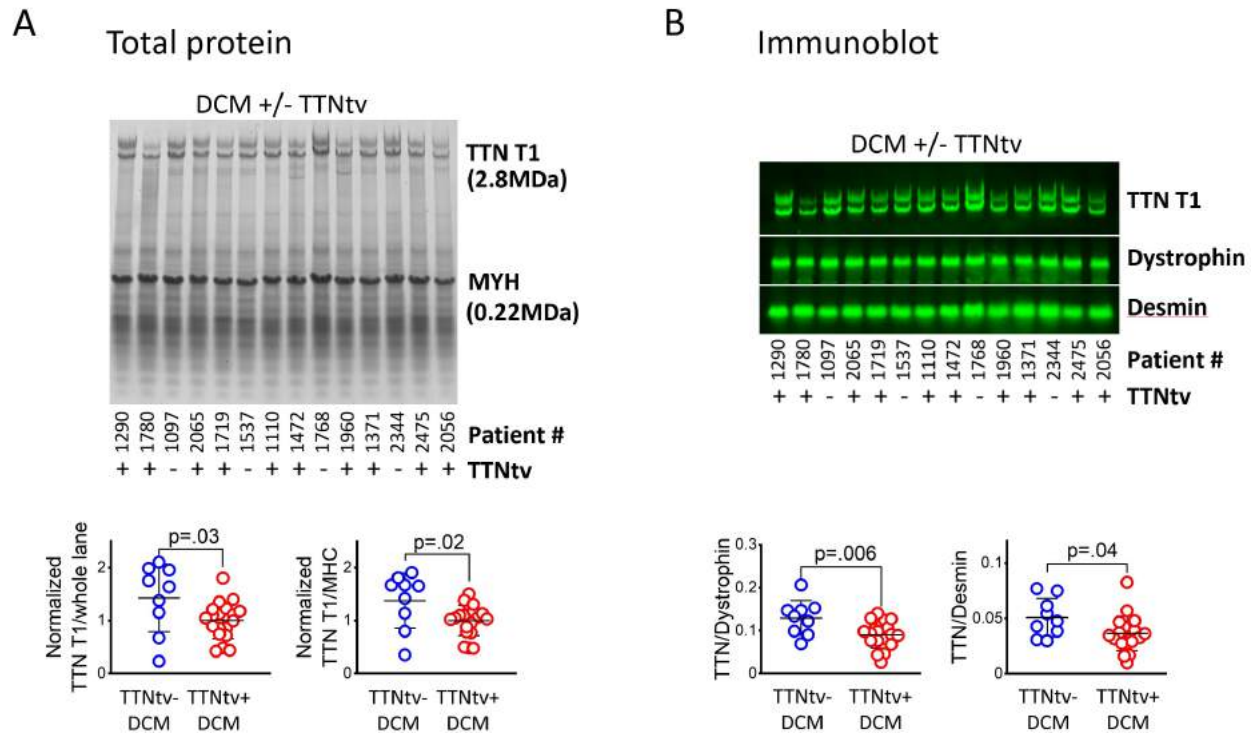


Fig. 6: Evidence of reduced titin protein in TTNtv+ DCM myocardium. Sample SDS-agarose gel and immunoblot of LV myocardium total protein samples from DCM hearts with and without TTNtv (**A**): SDS-agarose gel stained with Coomassie blue, with total protein fluorescence detected at 700nm. TTN relative abundance is expressed as normalized TTN total protein (T1, i.e., N2BA+N2B) band intensity divided by either whole lane or myosin heavy chain (MYH) band intensity. (**B**): Immunoblot of replica gel of samples presented in A, probed for titin, dystrophin, and desmin. TTN relative abundance is expressed as TTN total protein (T1, i.e. N2BA+N2B) band intensity divided by dystrophin or desmin band intensity. Quantifications of A and B are from gels displayed in this figure as well as additional gels present in supplemental figure S5. For both gels and blots there are a total of 8 TTNtv- and 20 TTNtv+ hearts. Image gamma was adjusted for clarity of display. Statistical significance was determined using a One-Way ANOVA followed by a post-hoc Tukey correction.

Discussion

We show here unequivocally, using independent orthogonal methods, i.e., unbiased proteomics, modified gel electrophoresis, and immunological detection, that truncated titin proteins, encoded by the allele containing a TTNtv, are present and relatively abundant in hearts from TTNtv+ DCM patients. This conclusion reverses the current paradigm that these polypeptides are absent in TTNtv+ hearts. The presence of truncated titin protein might suggest that a dominant negative or “poison peptide” mechanism contributes to DCM in these patients. Importantly, we also provide here for the first-time quantitative evidence of reduced amounts of titin protein in TTNtv+ hearts. We thus propose that disease initiation may be multifactorial, involving both haploinsufficiency, as a consequence of reduced amounts of titin protein, and poison peptide mechanisms.

At least two possible poison peptide mechanisms can be envisioned: first, incorporation of the truncated proteins into the sarcomere, as suggested by our fractionation studies (fig. S4), may directly lead to sarcomeric dysfunction. Importantly, we show here that the size of the truncated protein does not appear to impact disease severity, as reflected in the need for transplantation (typically reflecting more severe disease), or LVEF or age at time of transplantation (fig. 1). Thus, if integration of the truncated protein into the sarcomere contributes to disease, it can be inferred that integration of the N-terminus is important, and that the loss of structures associated with the M-line is sufficient to cause disease. The C-terminus of titin encodes a putative kinase domain, mutations in which are thought to cause skeletal muscle disease (12), and the absence of which in TTNtv protein could thus also be contributing to disease (2). On the other hand, integration of TTNtv protein into the sarcomere may not occur, or may not be required for disease, as suggested

by the intact contractility of TTNtv+ ACMs (fig. 3). For example, the TTNtv protein may be actively targeted for cleavage by the many proteases known to target titin, leading to disrupted protein homeostasis. Arguing against this model, however, is the absence of detectable small titin fragments in TTNtv+ hearts (fig. 4A), or of significantly altered signatures of protein clearance such as macroautophagy in TTNtv+ hearts compared with TTNtv- hearts (fig S2). Importantly, the disease penetrance of TTNtvs is low, indicating that pathogenicity almost certainly requires additional “hits” in addition to a TTNtv. For example, we have previously shown, in a large outpatient population, that 95% of subjects with TTNtvs, including some annotated as pathogenic in ClinVar, showed no signs of cardiomyopathy (5). This concept is consistent with observations by us and others that TTNtvs are also prominently found in cohorts of pregnancy-induced, alcohol-induced, and oncotherapy-induced cardiomyopathies (9, 22, 23), all representing potential “second hits”. How these various cardiac stressors mechanistically interact with TTNtvs to cause disease remains unclear, but it is possible that they may synergize with a poison peptide mechanism, for example by altering proteostasis.

We also show here that the presence of TTNtvs appears to have little to no effect on mRNA processing. We used allelic phasing to show that there is little to no *TTN* allelic imbalance in excess to that seen in TTNtv- hearts, nor any evidence for significant alternative splicing of *TTN* in TTNtv+ hearts. In light of the significantly lower detection of TTNtv protein compared to full length titin, these observations imply that either mRNA transcripts that contain TTNtvs are translated less efficiently, or there is increased degradation of TTNtv protein, as suggested above. Ribosome profiling studies have suggested that translation of the TTNtv-bearing mRNA is intact (13), supporting the notion that TTNtv proteins are actively degraded.

Our contractility data with isolated ACMs are surprising. TTNtv+ ACMs appear to more closely preserve a non-failing contractility profile when compared to TTNtv- ACMs. These data differ from data acquired in TTNtv+ iPSC-derived CMs (11), in which contractility was significantly affected, and more closely approximate data seen with isolated myofilaments from human TTNtv+ hearts (14), in which myofilament kinetics were more similar to NF than TTNtv- samples. We did note a uniquely decreased resting sarcomere length in TTNtv+ ACMs. This could be due to structural changes to the sarcomere, increased diastolic calcium concentrations, or elevated myofilament calcium sensitivity, the latter of which would be consistent with the increased fractional shortening observed in these cells relative to TTNtv- ACMs. Loss of ejection fraction in the context of seemingly preserved contractility could reflect other contextual factors, such as afterload, preload, and extracellular milieu. We also note that contractility was assessed in unloaded myocytes, and thus may be insensitive to potential effects of TTNtvs on load-dependent regulation of myofilament function. The data also underscore the imperative to study adult cardiomyocytes. Two important limitations to these ACM studies are the possibility of cell ascertainment bias, if for example the presence of TTNtvs accelerates the demise of poorly functioning cells during the cellular isolation procedure, and the fact that we did not perform cellular challenges, e.g. with imposed mechanical load or adrenergic stimulation. Further studies in loaded, intact myocardial tissue preparations from TTNtv+ hearts will complement these single cell and myofilament level examinations. In addition, it is also important to note that all of these cells and mRNAs were acquired from terminally failing hearts, and the pathophysiology of advanced heart failure may have masked earlier differences in the pathogenicity of TTNtv- vs TTNtv+ cardiomyopathy.

Our data also support the conclusion that the presence of TTNtv does not increase the likelihood of more severe disease, compared to TTNtv- DCM patients. No differences in demographic or echocardiographic characteristics were apparent in TTNtv+ versus TTNtv- patients undergoing cardiac transplant. The 12% prevalence of hiPSI TTNtvs in our transplant cohort was no higher than that seen in cohorts of predominantly non-transplanted patients (e.g., 11% in both a primary outpatient clinic DCM cohort, and a secondary diagnostic referral DCM cohort (6)), indicating that TTNtvs do not increase the likelihood of undergoing transplantation, an indicator of disease severity. Finally, global transcriptomic profiling revealed almost no significant differences between TTNtv+ and TTNtv- DCM hearts. Together, these data demonstrate that TTNtvs contribute to disease progression, but suggest that a common pathogenic pathway is shared between most forms of DCM, regardless of their TTNtv genetic status.

Limitations of our study include the limited number of hearts available. For example, we did not have tissue from hearts bearing TTNtvs in the N-terminal hiPSI exons, which are also likely pathogenic. The high variability, for example in the observed amount of truncated titin protein, also renders some analyses under-powered. And, as noted above, the fact that all hearts are in end-stage heart failure precludes conclusions about the early stages of pathogenesis, and may introduce ascertainment bias.

DCM is a multifactorial disease, for which to date no disease-specific therapies exist. Neurohormonal blockade and volume control remain the mainstay of therapy, but the prognosis of

patients with DCM remains poor, with 5-year mortality approaching 50%. Our data, unambiguously demonstrating the presence of truncated titin proteins in TTNtv+ DCM hearts, suggest that, in the large subset of DCM patients with TTNtvs, targeting the truncated titin protein may provide a therapeutic opportunity.

Materials and Methods

Procurement and Characterization of Human Hearts

Procurement of human myocardial tissue was performed under protocols and ethical regulations approved by Institutional Review Boards at the University of Pennsylvania and the Gift-of-Life Donor Program (Pennsylvania, USA) and as described(16). Briefly, failing human hearts of non-ischemic origin were procured at the time of orthotopic heart transplantation at the Hospital of the University of Pennsylvania following informed consent from all participants. Only hearts from patients with non-ischemic dilated cardiomyopathy were included; exclusion criteria included coronary artery disease, valvular disease, toxic agents, and anatomical abnormalities. Non-failing (NF) hearts were obtained at the time of organ donation from cadaveric donors. In all cases, hearts were arrested in situ using ice-cold cardioplegia solution and transported on wet ice. TTNtv hearts were identified by genomic sequencing and dilated cardiomyopathy were classified by clinical diagnosis. The values reported for LVEF for DCM patients in the table are the last recorded LVEF measurement before heart transplant. These are uniformly derived via echocardiography. The most frequently employed measurement approach is the modified Simpson's method (biplane method of discs).

Human Left Ventricular Myocyte Isolation and Contractility Measurements

Human left ventricular myocytes were isolated as described previously (16). For this study myocytes were isolated from 11 hearts (4 nonfailing, 4 TTNtv- DCM, and 3 hiPSI TTNtv+; patients 1725, 1780 and 1719) for functional studies. Isolated myocytes were also used as part of separate, unrelated experiments that required short term (48hr) culture and viral transduction with a control or experimental adenovirus; in the current manuscript, only cells transduced with control virus were used for analysis. Culture medium consisted of F-10 (1X) Nutrient Mixture (Ham) [+] L-Glutamine (Life Technologies, 11550-043) supplemented with insulin-transferrin selenium-X (Gibco, 51500-056), 20 mmol/L HEPES, 1 μ g μ L⁻¹ Primocin (Invivogen, ant-pm-1), 0.4 mmol/L extra CaCl₂, 5% FBS, and 25 μ mol/L cytochalasin D (Cayman, 11330). Viable myocytes were concentrated by gravity and the proper amount of medium was added in culture so that neighboring cells were not in direct contact. Control viral constructs were permitted to express for 48 hours with a multiplicity of infection = 100-200.

Assessment of contractility by unloaded shortening was performed as in (16). Briefly, prior to contractility measurement, cultured human myocytes were transferred to fresh warm medium without cytochalasin D. Contractility was measured in a custom-fabricated cell chamber (IonOptix) mounted on an LSM Zeiss 880 inverted confocal microscope using either a 40x or 63x oil 1.4 numerical aperture objective and transmitted light camera (IonOptix MyoCam-S). Experiments were conducted at room temperature and field stimulation was provided at 0.5 Hz with a cell stimulator (MyoPacer, IonOptix). After 10-30 seconds of pacing to achieve steady state, five traces were recorded and analyzed. Sarcomere length was measured optically by Fourier transform analysis (IonWizard, IonOptix). All experiments were performed and analyzed blind to genotype.

Tissue DNA and RNA sequencing

DNA was extracted using Qiagen's Gentra Puregene kit. 1µg of DNA was sheared with a Diagenode BioRuptor for thirty minutes set on high with thirty seconds on/off. Sheared DNA underwent library prep using Agilent's SureSelect XT2 kit. Barcoded exomes were enriched for DCM associated genes using the Seidman DCM version 5.2 bait panel, which includes all 364 exons of *TTN*. Libraries were run on an Illumina NextSeq and variants were called using Agilent's SureCall. Total RNA was extracted using the Qiagen MiRNeasy kit with DNase on-column treatment; quality of RNA was verified by Agilent Bioanalyzer. 1µg of RNA underwent library prep using Illumina's TruSeq Stranded mRNA kit. RNAseq libraries were run on an Illumina HiSeq2000. Reads were aligned using STAR and normalized with SVA. Variants were identified with the use of the Genome Analysis Tool Kit (GATK) HaplotypeCaller tool on the basis of GATK Best Practices. All but one identified hiPSI truncating variant, a 5bp deletion in patient 2065, were confirmed by means of traditional Sanger sequencing. A close review of NextGen sequencing results of patient 2065 showed extensive coverage (>100) at that site, with >40% prevalence of the 5bp deletion, strongly suggesting that the failure to confirm by Sanger sequencing results from amplification artifact. This case was therefore included in subsequent analyses.

DNA exome sequencing phasing and TTN allelic imbalance analysis

To discriminate *TTN* alleles on maternal and paternal chromosomes, we phased the genomes of 210 subjects using EAGLE2 (version 2.4.1) provided by the Sanger Institute (<https://www.sanger.ac.uk/science/tools/sanger-imputation-service>)(24). Due to the lack of intron information, we used a reference-based phasing method, using the haplotype reference consortium,

which consists of 64,976 haplotypes at 39,235,157 SNPs (<http://www.haplotype-reference-consortium.org/>). Only phased heterozygous SNPs were used for the subsequent analyses. In the allelic counting step, RNA-seq reads were aligned to the human reference genome hg19 and Samtools (version 1.10) mpileup (<http://www.htslib.org/doc/samtools-mpileup.html>) was used to generate a “pileup” of RNA-seq bases (or indels) aligned on each given heterozygous SNP on *TTN*. A customized script was then used to parse the heterozygous variations and compute how many reads were aligned to each haplotype (i.e., allele_0 and allele_1) on *TTN*. For each heterozygous SNP, the allelic imbalance was computed as $\text{number_of_bases_matched_on_allele0}/(\text{number_of_bases_matched_on_allele0} + \text{number_of_bases_matched_on_allele1})$. An allelic imbalance equal to 0.5 indicates exact 1:1 allelic ratio.

Myocardial protein isolation and gel electrophoresis

Previously frozen tissue samples stored at -80°C were handled on dry ice or liquid nitrogen. Roughly crushed fragments of myocardium free of epicardial fat of approximately 10-15mg in mass were selected and further crushed on liquid nitrogen into a fine powder using a stainless steel rod as a pestle and a 2ml stainless steel microfuge tube as a mortar. Cryo-powdered heart tissue was resuspended in 40 volumes of titin homogenization buffer prepared according to (25, 26) and containing 8mol/L urea, 2 mol/L thiourea, 3% SDS, 50m mol/L tris pH 6.8, a small amount of phenol red, and 75mM freshly added dithiothreitol. Tissue was then homogenized with a Potter-Elvehjem homogenizer until no fragments were visible, diluted 1:1 with 50:50 glycerol with 2x Roche Complete Mini protease inhibitor tablets, and briefly homogenized again. Samples were incubated for 30 minutes at 37°C, and then spun at ~20Kxg for 5 minutes at 10°C, and the pellet

discarded. Supernatants were aliquoted and stored at -80°C. For use, samples were thawed, briefly vortexed, and electrophoresed.

For SDS-Agarose electrophoresis, total myocardial protein prepared as above was separated according to (26) with modifications according to a protocol kindly provided by the Granzier laboratory. The running buffer was 50mmol/L tris, 0.384 mol/L glycine, (pH 8.3). Gels were cast from running buffer containing 1% Sea-Kem Gold agarose (Lonza) plus 30% glycerol. The gel was retained within the plates by a folded segment of nylon mesh placed between the plates. Fresh 2-mercaptoethanol was added to the upper buffer chamber at a dilution of 1:1000 just before sample loading. The gel apparatus was nearly completely filled with pre-chilled running buffer and placed in a slurry of ice during the run, which was conducted at a constant current of ~16mA per gel.

For total protein staining of SDS-agarose electrophoresis gels, gels were stained with GelCode blue (Thermo) according to manufacturer's instructions, and imaged on an Odyssey CLX (LiCor) infrared imager at 700nm.

For immunoblotting of titin from SDS-agarose gels, protein was transferred to a nitrocellulose membrane using a Bio-Rad semi-dry transfer apparatus with a discontinuous buffer system, according to a protocol kindly provided by the Granzier laboratory. The anode I solution contained 20mM tris, 0.05% SDS, 10% methanol, pH 10.4. Anode II solution contained 25m mol/L tris, 0.05% SDS, 10% methanol, pH 10.4, and the cathode buffer solution contained 25m mol/L tris, 0.05% SDS, 10% methanol, 40m mol/L CAPS, pH 10.4, each with 10m mol/L 2-mercaptoethanol

added just prior to assembly. The gel was soaked for 20 minutes prior to transfer in cathode buffer plus 10mM 2-mercaptoethanol. Proteins were transferred at 1.3mA per square centimeter of gel for one hour. Gels were stained post-transfer with GelCode blue to verify absence of detectable untransferred protein, and membranes were stained with Ponceau-S to verify even transfer. Membranes for fluorescent imaging were blocked and probed using Intercept (Li-Cor) blocking buffer and standard blotting methods. Fluorescent blots were imaged using a Li-Cor Odyssey FC infrared imager. Fluorescent gels and blots were quantified using ImageStudio (LiCor).

For routine SDS-PAGE analysis and western blotting of non-titin proteins, proteins prepared as for titin gels were run on standard 4-20% SDS-PAGE gels, transferred to a nitrocellulose membrane, stained for total protein using Ponceau S for transfer verification, probed according to standard immunoblotting methods, treated with chemiluminescent substrate, and exposed to film. Band quantifications were made using Image Studio or Empiria (Li-Cor) and normalized to indicated internal controls.

Myofibrillar physical isolation and immunohistochemistry:

Cryo-crushed human myocardial tissue powder produced as described above was weighed in frozen form then directly combined with 20 volumes (w/v) of modified skinning/relaxing buffer (27) composed of 20mM imidazole, pH 7.0, 7.8mM Na⁺·ATP, 12mM magnesium propionate, 97.6 mM potassium propionate, and 0.5% Triton x-100, as well as Roche Complete Mini EDTA free protease inhibitor tablets used at 2 fold indicated concentration. After brief agitation the mixture was incubated for approximately 15 minutes on ice, and then spun at 5000 x g for 5

minutes to partition soluble components to the supernatant and insoluble myofibrillar and other components to the pellet. A ~300µm pore size nylon mesh was used to exclude oversize fragments. The pellet was washed in the same buffer, and both supernatant and pellet were solubilized in titin solubilizing buffer, bringing the final volume to 80-fold the original tissue input mass in titin solubilization buffer and 50:50 (v:v) glycerol, as described above. Complete partitioning of soluble (GAPDH) and insoluble markers (TTN T1, desmin, and myosin) were used to evaluate separation of soluble from insoluble components.

For cryo-crush immunohistochemistry, cryo-crushed myocardium was added directly to neat methanol at -20°C and incubated for 8 minutes, quick spun to pellet myocardial fragments, and resuspended in Seablock (ThermoFisher) for continued processing for immunofluorescent staining, mounting, and imaging using standard methods.

Protein Sequence Analysis by LC-MS/MS

Hearts included in proteomic analysis were chosen by sample availability and preference for the largest number missense mutations to facilitate sensitive detection by proteomics and separation of proteins encoded by the two parental alleles. Excised gel bands were cut into approximately 1 mm³ pieces. Gel pieces were then subjected to a modified in-gel trypsin and chymotrypsin digestion procedure. (28) Gel pieces were washed and dehydrated with acetonitrile for 10 min. followed by removal of acetonitrile. Pieces were then completely dried in a speed-vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate solution containing 12.5 ng/µl modified sequencing-grade trypsin (Promega, Madison, WI) at 4°C. After 45 min., the excess trypsin solution was removed and replaced with 50m mol/L ammonium bicarbonate solution to just cover

the gel pieces. Samples were then placed in a 37°C room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed-vac (~1 hr). The samples were then stored at 4°C until analysis.

Proteomics was performed by the Taplin Biological Mass Spectrometry Facility. On the day of analysis the samples were reconstituted in 5 - 10 µl of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-phase HPLC capillary column was created by packing 2.6 µm C18 spherical silica beads into a fused silica capillary (100 µm inner diameter x ~30 cm length) with a flame-drawn tip. (29) After equilibrating the column each sample was loaded via a Famos auto sampler (LC Packings, San Francisco CA) onto the column. A gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid).

As peptides eluted they were subjected to electrospray ionization and then entered into an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein databases with the acquired fragmentation pattern by the software program, Sequest (Thermo Fisher Scientific, Waltham, MA)(30). All databases include a reversed version of all the sequences and the data was filtered to between a one and two percent peptide false discovery rate. Files containing raw proteomic have been uploaded to the PRIDE Database.

Statistics

Statistics were performed using OriginPro, Graphpad Prism, or Excel, except for bioinformatics, as noted. Multiple comparisons were made using a One-way ANOVA followed by a Tukey or Bonferroni multiple comparison correction. In the case of fig 2, multiple comparisons were made using a Kruskal-Wallis test followed by a Dunn's test.

Supplementary Materials

Fig. S1. Connected to fig. 2. Transcriptomic evaluation of hiPSI TTNtv-positive versus TTNtv-negative post-transplant DCM hearts.

Fig. S2. Connected to fig. 4. Evidence of altered autophagy activation in TTNtv+ DCM hearts.

Fig. S3. Connected to fig. 5. Detection of truncated titin proteins in TTNtv+ DCM hearts by gel electrophoresis and Western blotting.

Fig. S4. Evidence that TTNtv proteins loosely associate with sarcomere-containing cellular fractions

Fig. S5. Connected to fig. 6. Evidence of titin protein haploinsufficiency in TTNtv+ DCM myocardium.

Table S1. Identified TTNtv's in post-transplant DCM cohort.

Data file S1. Individual level data for figures where $n < 20$

Data file S2. Contractility parameters and statistical analysis segregated by heart.

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Truncated titin proteins in dilated cardiomyopathy

Supplementary Material

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List of Supplementary Materials

- Fig. S1. Connected to fig. 2. Transcriptomic evaluation of hiPSI TTNtv-positive versus TTNtv-negative post-transplant DCM hearts.
- Fig. S2. Profiling of autophagy proteins in hiPSI TTNtv+ and TTNtv- DCM hearts.
- Fig. S3. Connected to fig. 5. Quantification of TTNtv as a fraction of full length (T1) titin by Western blotting.
- Fig. S4. TTNtv proteins loosely associate with sarcomere-containing cellular fractions.
- Fig. S5. Connected to fig. 6. Evidence of titin protein haploinsufficiency in TTNtv+ DCM myocardium, additional gels supporting quantifications found in figure 6A.
- Table S1. Identified TTNtvs in post-transplant DCM cohort.
- Data file S1. Individual level data for figures where $n < 20$
- Data file S2. Contractility parameters and statistical analysis segregated by heart.

Supplementary Materials:

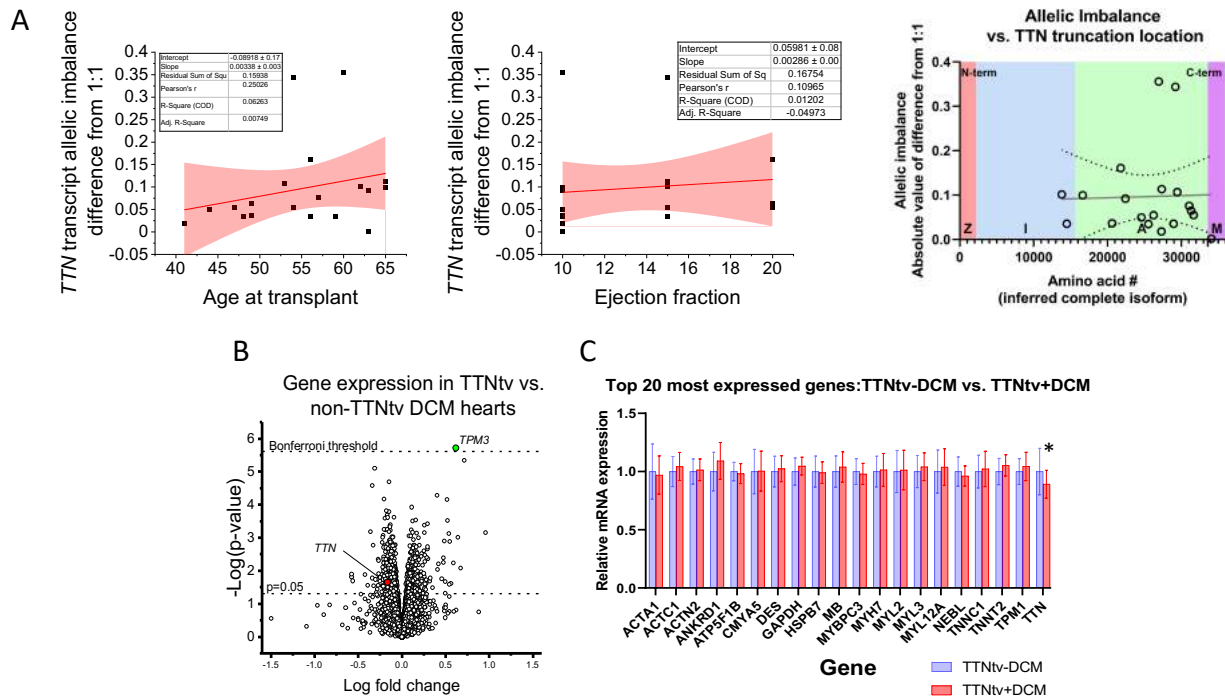


Fig. S1. Connected to fig. 2. Transcriptomic evaluation of hiPSI TTNtv+ versus TTNtv- post-transplant DCM hearts. (A): Lack of association between allelic imbalance in TTNtv+ DCM hearts and location of TTNtv and either age or left ventricular ejection fraction (LVEF) at the time of cardiac transplantation, or location of the truncation within the *TTN* gene. Dotted lines indicate a 95% confidence interval about a linear regression, indicated by a solid line. **(B):** Volcano plot of individual genes from global transcriptomic profiling depicted in fig. 2D, with TTNtv+ DCM normalized to TTNtv- DCM hearts. **(C)** Top 20 most highly expressed genes from TTNtv+ DCM and TTNtv- DCM hearts. Error bars indicate +/- 1SD. *nominal $P < 0.05$.

B

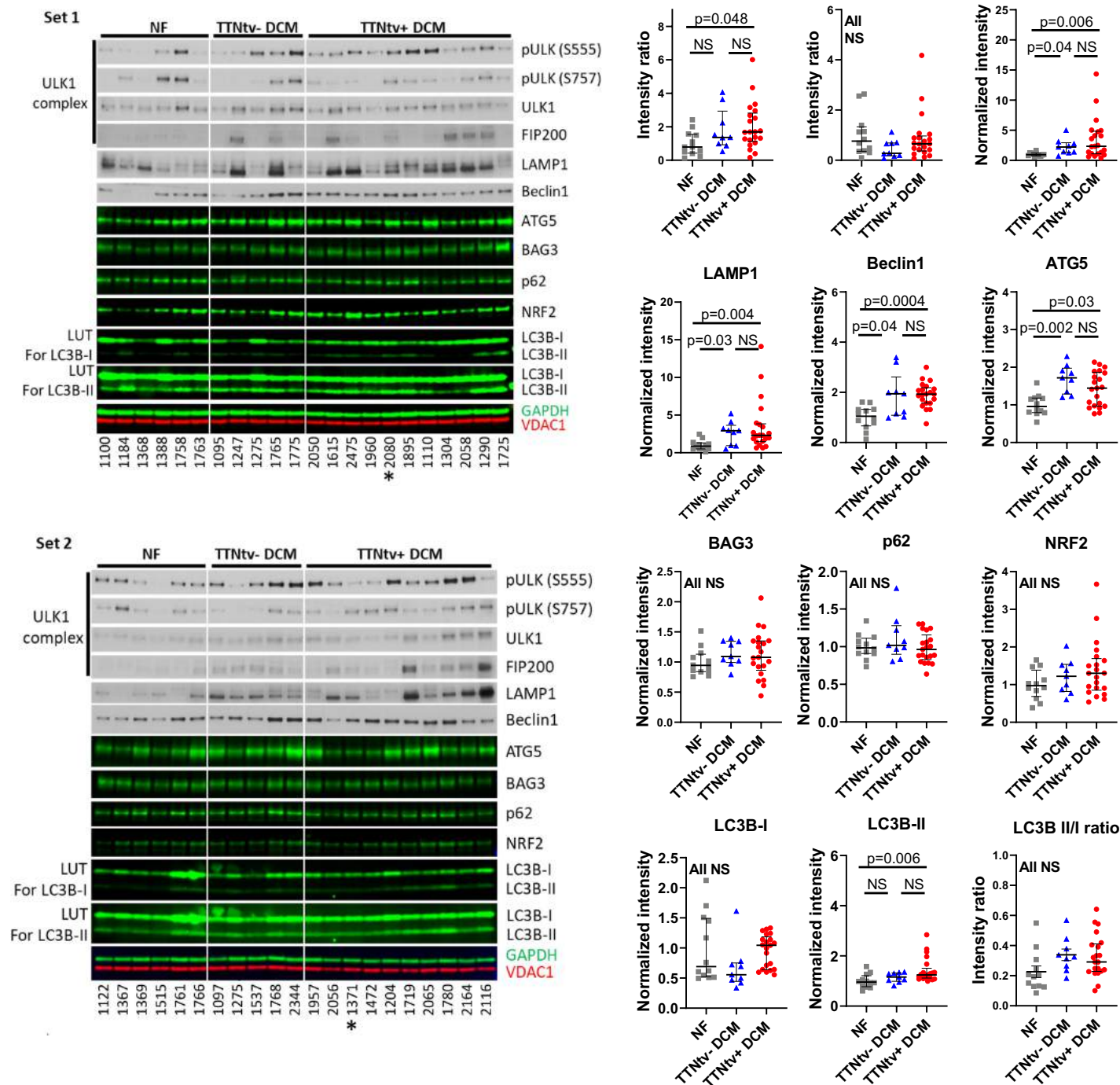


Fig. S2. Profiling of autophagy proteins in hiPSI TTNtv+ and TTNtv- DCM hearts. (A): Immunoblots of replica gels of the indicated proteins and post-translational modifications, in non-failing (NF), TTNtv+, and TTNtv- DCM hearts, detected by either fluorescent or film-based methods. ULK1: Unc-51 like autophagy activating kinase. FIP200: FAK family kinase-interacting protein of 200 kDa. ATG5: Autophagy

related 5. BAG3: BCL2 associated athanogene 3. LC3: Microtubule-associated protein 1A/1B-light chain 3. NRF2: nuclear factor erythroid 2-related factor 2. LAMP1: Lysosomal Associated Membrane Protein 1. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase. VDAC: Voltage dependent anion channel 1. **(B):** Quantification of immunoblots from A. Quantifications are either normalized per sample to the average intensity intracellular (fibrosis-insensitive) markers GAPDH and VDAC1, or are ratios of bands within the same lane. The total number of unique patients represented is 12 NF, 9 TTNtv- DCM, and 21 TTNtv+ DCM. Patient 1275 is duplicated between the two sets, and omitted from quantifications. Patients 1371 and 2080, indicated with an asterisk (*) are patients with relatively higher transcript allelic imbalance, shown in fig. 2b. Error bars indicate median $\pm$ 1SD.

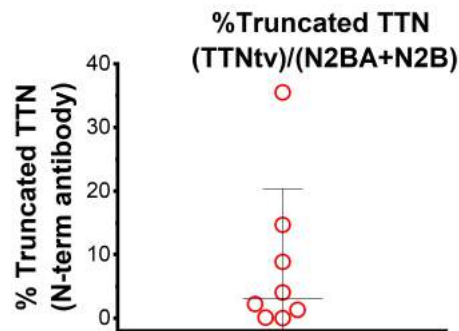
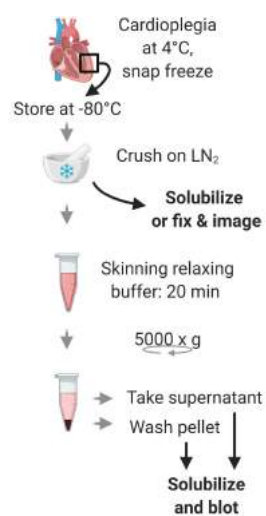
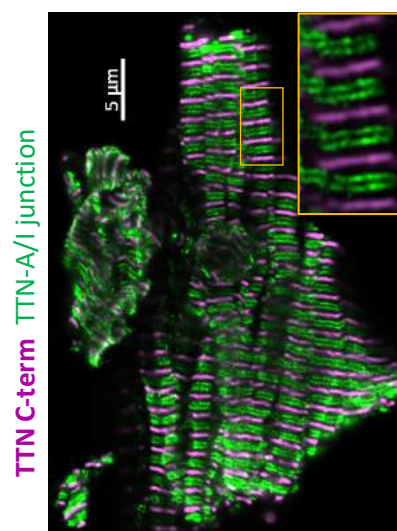


Fig. S3. Connected to fig. 5. Quantification of TTNtv as a fraction of full length (T1) titin by Western blotting. TTNtv bands appearing in the N-terminal TTN channel of the middle panel of fig. 6A, middle panel, expressed as % truncated TTNtv/(N2BA + N2B). Error bars indicate median $\pm$ 1SD.

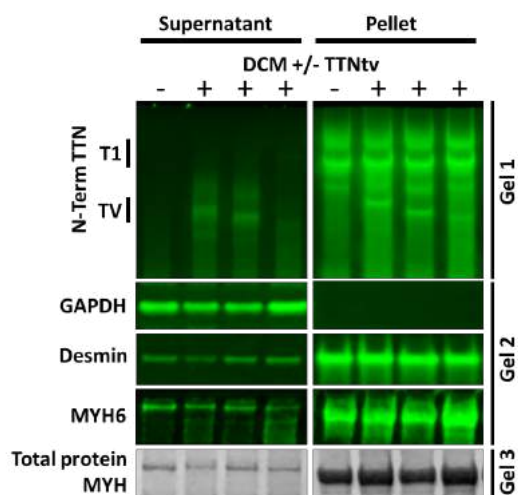
A



B



C



D

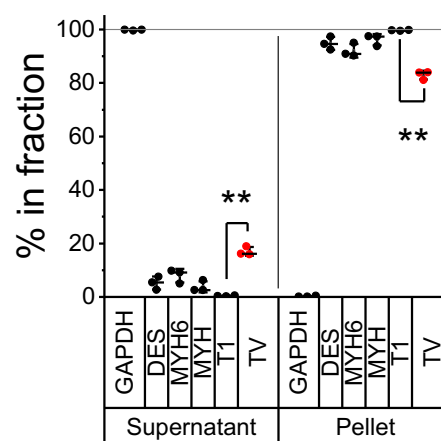


Fig. S4. TTNtv proteins loosely associate with sarcomere-containing cellular fractions. (A) Schematic of simplified cryo-crush method for preparation of cardiac tissue for separation of soluble and sarcomere containing fractions. (B) Airy-scan image and inset of fragment distribution showing structural organization of sarcomere in alcohol fixed cryo-crush prepared myocardium. Green: TTN C-terminus; Magenta: TTN A/I junction. (C) Immunoblot and coomassie stained SDS-agarose replica gels of cryo-crush prepared myocardium treated with relaxing/skinning buffer and separated by centrifugation. Samples from left to right are from patients 1725, 1960, 1472, and 1895. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) is used as a soluble fraction marker. Desmin, MYH6 (myosin heavy chain 6) and MYH (total protein) are used as a sarcomeric and insoluble fraction marker. N-terminal TTN immunostaining marks both full length and truncated titin. (D) Quantification of band intensities from TTNtv+ patients in C. For panels C and D, “T1” band intensity is the total intensity of TTN N2BA and N2B. “TV” indicates intensity of the major TTNtv band present at the expected molecular weight. Error bars indicate median $\pm$ 1SD.

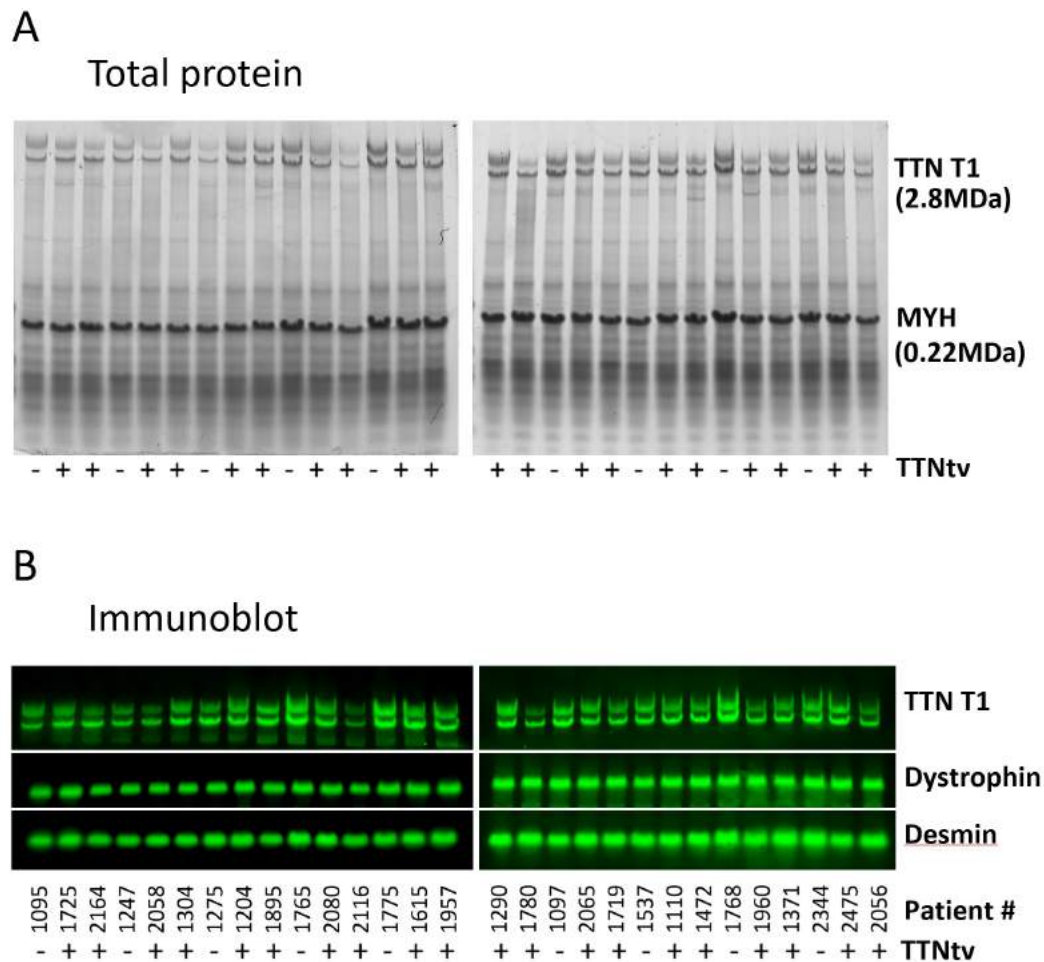


Fig. S5 Connected to fig. 6. Evidence of titin protein haploinsufficiency in TTNtv+ DCM myocardium, additional gels supporting quantifications found in figure 6A. TTNtv- DCM and TTNtv+ DCM samples run in an alternating pattern across replica gels and immunoblots. (A): Sample

SDS-Agarose gel of LV myocardium total protein samples from DCM hearts with and without TTNtv, stained with Coomassie blue, with total protein fluorescence detected at 700nm. **(B)** Replica immunoblots of samples presented in A. Figure S5A and S5B right panels are from fig. 6 and are reproduced here for completeness. Threshold and gamma are adjusted for clarity of display.

Table S1. Identified TTNtv's in post-transplant DCM cohort.

See attached file

Data file S1. Individual level data for figures where n<20

See attached file

Data file S2. Contractility parameters and statistical analysis segregated by heart

See attached file