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Supplementary Materials for

Disruption of cardiac thin filament assembly arising from a mutation in *LMOD2*: A novel mechanism of neonatal dilated cardiomyopathy

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Supplementary Materials

Additional Clinical Details

The proband is a girl born at 39 weeks and 5 days gestation after a pregnancy complicated only by maternal vaginitis. Prenatal ultrasounds were unremarkable. The patient's mother tested positive for group B streptococcus during pregnancy and was appropriately treated. The infant was born via cesarean section due to fetal distress with late decelerations and tachycardia on fetal monitoring. The delivery was complicated by the presence of thick meconium. APGARS were 6 and 7 at 1 and 5 minutes of life, respectively. Birth growth parameters were appropriate with weight at 21st, height at 40th, and head circumference at 23rd percentiles. She was sent to the neonatal intensive care unit for evaluation and was found to have severe biventricular dysfunction on echocardiogram, and she also had an open atrial septal defect.

Her diagnostic work-up included metabolic testing was essentially unremarkable (Supplemental Table 1). Urine organic acids analysis initially showed lactate which subsequently normalized when her perfusion improved. Total serum carnitine was slightly low. This was thought to be secondary to early dietary insufficiency and the level improved on low-dose levocarnitine therapy. Ultimately, levocarnitine was stopped and serum levels have remained normal. Serum acylcarnitine profile, plasma amino acids and urine amino acids were not concerning.

Initial diagnostic work-up to evaluate for non-cardiac features of disease included: an abdominal ultrasound demonstrating normal abdominal organs, ascites and left pleural effusion, and a brain ultrasound with bilateral grade 1 hemorrhages.

On genetic testing, in addition to the *LMOD2* mutation, a second variant of uncertain significance was identified in the proband through exome sequencing, *OBSCN* (c.12860G>T, p.Gly4287Val); haploinsufficiency of *OBSCN* has recently been reported to be possibly associated with dilated cardiomyopathy (*35*). This change was thought to be benign as it is inherited from the patient's asymptomatic father and is carried by her asymptomatic sister. A nonsense variant at this residue is present in control populations, and the variant results in a conservative amino acid change that is not likely to impact secondary protein structure.

Brief post-transplant hospital complications

The patient's post-transplant course was complicated by an episode of possible acute rejection which was managed with steroids. She developed multifactorial respiratory failure due to infection, post-surgical diaphragm paresis, and tracheomalacia. She had episodes of acute chronic respiratory failure in the setting of *Klebsiella* sepsis and parainfluenza infections. She ultimately required tracheostomy placement at 1 year of age for failure to wean from ventilatory support. She also required placement of a gastrostomy tube and ultimately a jejunostomy tube due to persistent gagging and emesis with feeds. She was discharged at 16 months and remains home in her family's care at 30 months of age. She has motor and cognitive delays thought secondary to her previous LVAD-related stroke and extensive post-operative complications. In both motor and cognitive domains, she functions at ~12-15 month level at 30 months of age.

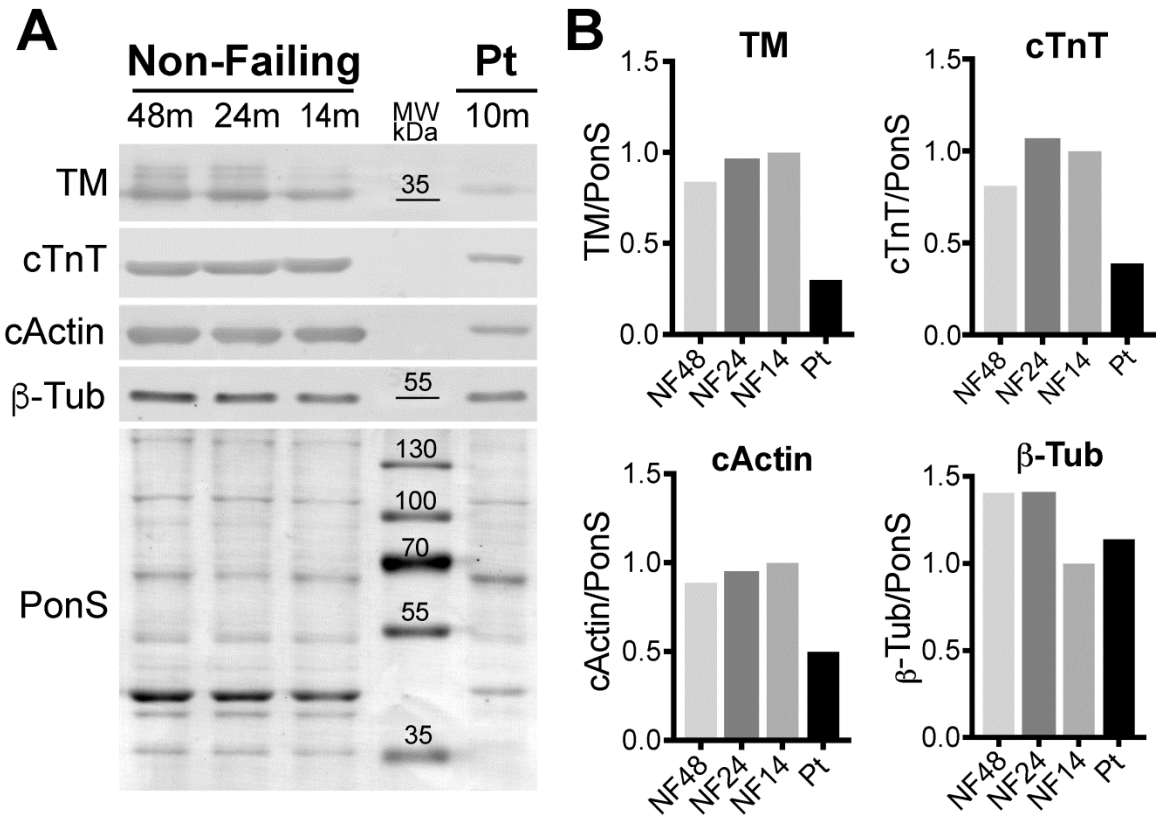

Fig. S1. The levels of thin filament proteins are reduced in the patient's LV. A, Western blots of tropomyosin (TM), cardiac troponin T (cTnT), cardiac actin and β-tubulin (β-tub) in LV of non-failing donor hearts at 48, 24 and 14 months of age and the patient's explanted heart (10 months). Total protein was stained with Ponceau S. **B**, Relative protein levels following normalization to total protein. Note, the 14-month non-failing donor heart (closest in age to the patient) was set to 1.

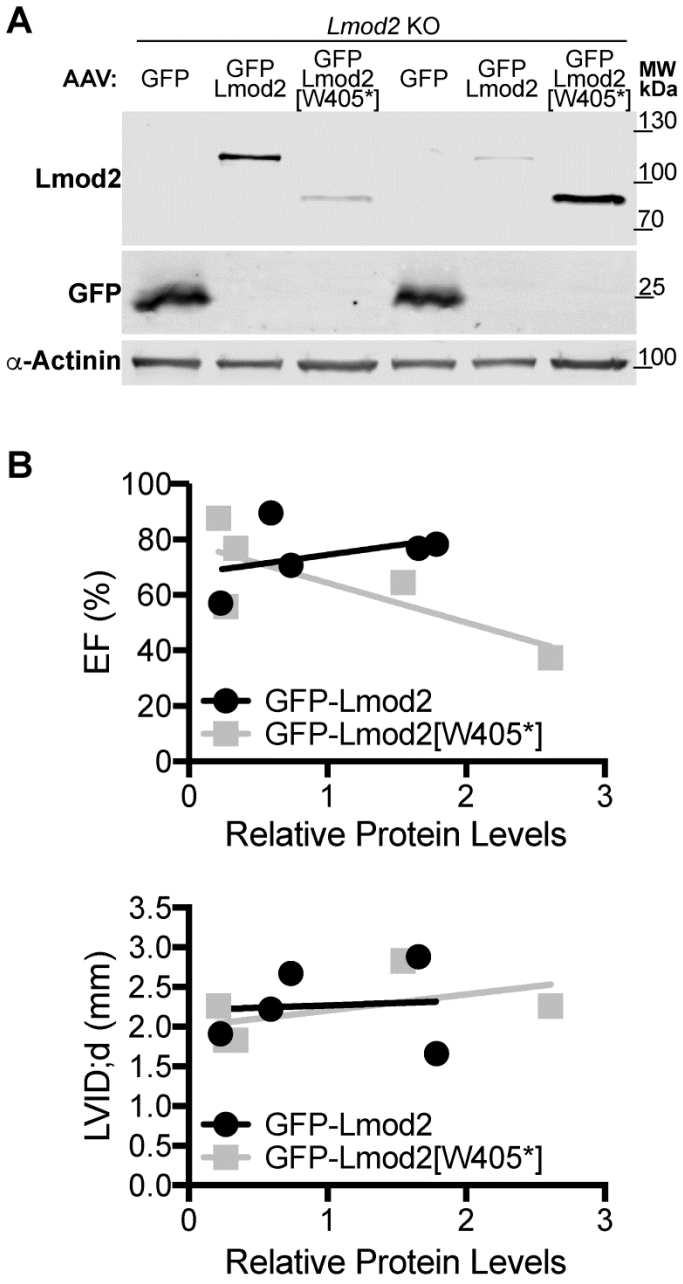

Fig. S2. Even low levels of GFP-Lmod2 or GFP-Lmod2[W405*] expression prevents onset of DCM in *Lmod2* knockout mice. A, Representative western blots of Lmod2, GFP, and α-actinin in LV of mice injected with adeno-associated virus expressing GFP, GFP-Lmod2 and GFP-Lmod2[W405*]. **B**, Cardiac systolic performance (top, ejection fraction-EF) and LV internal diameter in diastole (bottom), determined by echocardiography, is plotted against the relative protein expression of GFP-Lmod2 or GFP-Lmod2[W405*]. The slopes of all linear regression lines are not significantly different from zero.

Table S1. Initial diagnostic evaluations.

INFECTIOUS TESTING (all negative)	METABOLIC TESTING (unremarkable except where indicated)
EBV	Serum acylcarnitine profile
CMV	Urine organic acids: Elevated lactate, collected while patient poorly perfused
HHV6	Plasma amino acids
Adenovirus	Total serum carnitine: 12.7 nmol/mL (normal >25) as neonate with nutritional deficiency 57.1 nmol/mL at 16 months of age while off of supplementation
HSV	Creatine kinase
Toxoplasmosis	Serum lactate / pyruvate
Rubella	
Parainfluenza	
Influenza A/B	

Table S2. Summary of mechanics data for cells isolated from nonfailing donor hearts at 48, 24, and 14 months of age and the patient's explanted heart (10 months). SL, sarcomere length; Max Force, mean force at maximum calcium activation; EC_{50}, concentration of calcium at half-maximal force; Hill Coefficient, measure of cooperativity. All values are means ± SEM; n = 7, 8, 8, 4 cells/cell clusters (NF48, NF24, NF14, Pt, respectively). Values with different letters are significantly different from each other ($P<0.05$), determined by one-way ANOVA with Tukey's post hoc test.

	SL (μm)	**Max Force (mN/mm^2)**	**EC_{50}**	**Hill Coefficient**
NF48	1.85	21.321 ± 2.907^{A}	1.815 ± 0.082^{A}	6.905 ± 0.576^{A}
NF24	1.82	17.686 ± 1.572^{A}	1.687 ± 0.046^{A}	6.365 ± 0.438^{A}
NF14	1.86	18.640 ± 2.210^{A}	1.731 ± 0.088^{A}	5.072 ± 0.514^{A}
Pt	1.78	2.303 ± 0.523^{B}	4.372 ± 1.037^{B}	1.722 ± 0.296^{B}

Table S3. Echocardiography data of *Lmod2* knockout mice injected with adeno-associated virus. *Lmod2* knockout mice were injected with adeno-associated virus expressing GFP, GFP-Lmod2, or GFP-Lmod2[W405*]. Echocardiography measurements include: LV internal diameter (LVID), LV anterior wall thickness (LVAW), LV posterior wall thickness (LVPW), eccentricity (LV end diastolic diameter/combined wall thickness), ejection fraction (EF) and heart rate (HR). d, diastole; s, systole; mm, millimeter; bpm, beats per minute. Measurements are means ± SD. Values with different letters are significantly different from each other ($P<0.05$), determined by one-way ANOVA with Tukey's post hoc test. n = 5-6.

AAV:	**GFP**	**GFP-Lmod2**	**GFP-Lmod2[W405*]**
n	6	5	5
LVID;d (mm)	3.29 ± 0.59^{A}	2.27 ± 0.51^{B}	2.20 ± 0.41^{B}
LVID;s (mm)	2.97 ± 0.76^{A}	1.37 ± 0.35^{B}	1.36 ± 0.46^{B}
LVAW;d (mm)	0.69 ± 0.05^{B}	0.88 ± 0.10^{A}	$0.71 \pm 0.05^{C,B}$
LVAW;s (mm)	0.79 ± 0.10^{A}	1.20 ± 0.20^{B}	1.06 ± 0.03^{B}
LVPW;d (mm)	0.64 ± 0.14^{A}	0.92 ± 0.12^{B}	$0.82 \pm 0.07^{A,B}$
LVPW;s (mm)	0.69 ± 0.22^{A}	1.21 ± 0.13^{B}	1.18 ± 0.10^{B}
Eccentricity (LVID;d/WT)	2.55 ± 0.59^{A}	1.35 ± 0.32^{B}	1.43 ± 0.19^{B}
EF (%)	26.48 ± 17.76^{A}	74.48 ± 11.91^{B}	64.40 ± 19.39^{B}
HR (bpm)	564 ± 53	577 ± 69	563 ± 40