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Attenuated hypertrophic response to pressure overload in a lamin A/C haploinsufficiency mouse

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Abstract

Inherited mutations cause approximately 30% of all dilated cardiomyopathy cases, with autosomal dominant mutations in the *LMNA* gene accounting for more than one third of these. The *LMNA* gene encodes the nuclear envelope proteins lamins A and C, which provide structural support to the nucleus and also play critical roles in transcriptional regulation. Functional deletion of a single allele is sufficient to trigger dilated cardiomyopathy in humans and mice. However, whereas *Lmna*^{-/-} mice develop severe muscular dystrophy and dilated cardiomyopathy and die by 8 weeks of age, heterozygous *Lmna*^{+/-} mice have a much milder phenotype, with changes in ventricular function and morphology only becoming apparent at one year of age. Here, we studied 8 to 20 week old *Lmna*^{+/-} mice and wild-type littermates in a pressure overload model to examine whether increased mechanical load can accelerate or exacerbate myocardial dysfunction in the heterozygotes. While overall survival was similar between genotypes, *Lmna*^{+/-} animals had a significantly attenuated hypertrophic response to pressure overload as evidenced by reduced ventricular mass and myocyte size. Analysis of pressure-overload induced transcriptional changes suggested that the reduced hypertrophy in the *Lmna*^{+/-} mice was accompanied by impaired activation of the mechanosensitive gene *Egr-1*. In conclusion, our findings provide further support for a critical role of lamins A and C in regulating the cellular response to mechanical stress in cardiomyocytes and demonstrate that haploinsufficiency of lamins A and C alone is sufficient to alter hypertrophic responses and cardiac function in the face of pressure overload in the heart.

Keywords

lamin; hypertrophy; mechanical stress; pressure overload; mechanotransduction; transverse aortic constriction

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Introduction

Approximately 30% of dilated cardiomyopathy cases are caused by inherited mutations [1]. Autosomal dominant mutations in the *LMNA* gene that encodes the nuclear envelope proteins lamins A and C account for more than one third of these cases [2,3], and the *LMNA* mutation carriers have a significantly poorer cumulative survival compared with other dilated cardiomyopathy patients [4]. Mutations in the *LMNA* gene are also linked to the autosomal dominant form of Emery-Dreifuss muscular dystrophy, while the X-linked form of the disease is caused by mutations in the integral membrane protein emerin that directly binds to lamin [5–7]. Both Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy are associated with severe cardiac symptoms that include conduction-system defects, arrhythmias, left ventricular dilation, and a high incidence (> 40%) of sudden cardiac death [4,8–10]. Interestingly, mutations in the *LMNA* gene are also responsible for a variety of other human diseases referred to as laminopathies. These diseases, which include limb-girdle muscular dystrophy, Charcot-Marie-Tooth disease, Dunnigan-type familial partial lipodystrophy, and Hutchinson-Gilford progeria syndrome, can also affect cardiac and skeletal muscle, although generally to a lesser extent [11].

Mammalian cells express two types of lamins, A-type lamins and B-type lamins. Lamins A and C, the major A-type lamins, are alternatively spliced isoforms of the *LMNA* gene and are expressed in most but not all differentiated cells [12]. Lamins A and C are type V intermediate filaments that provide the main structural support for the nucleus [13–15], are involved in coupling the nuclear envelope to the cytoskeleton [16–20], and also interact with several transcriptional regulators such as c-Fos, MOK2, retinoblastoma protein (pRb), and sterol response element binding protein 1 (SREBP1) [21–24].

Lamin A/C-null (*Lmna*^{−/−}) mice are indistinguishable from their littermates at birth but, by 3–4 weeks of age, develop muscular dystrophy and rapidly progressing dilated cardiomyopathy and die by 8 weeks [25,26]. The dilated cardiomyopathy in *Lmna*^{−/−} mice is characterized by left ventricular dilation and reduced systolic contraction, but—interestingly—a lack of hypertrophic gene activation [26,27]. Cells derived from *Lmna*^{−/−} mice often have misshaped nuclei and obvious ultrastructural damage [25,28], similar to fibroblasts from laminopathy patients [29–32]. We previously reported that *Lmna*^{−/−} mouse embryo fibroblasts have increased nuclear fragility and impaired activation of the mechanosensitive genes *Egr-1* and *Irf-1*, making them more susceptible to strain-induced necrosis and apoptosis [33]. Heterozygous *Lmna*^{+/-} cardiac myocytes display an intermediate phenotype in terms of nuclear abnormalities and have mildly impaired contractility compared to wild-type cells, but these phenotypes are less severe than in *Lmna*^{−/−} cells [26]. Recent longitudinal studies on *Lmna*^{+/-} mice found atrioventricular (AV) conduction defects in 10-week old *Lmna*^{+/-} animals and atrial and ventricular arrhythmias caused by early onset apoptosis in AV nodal cardiomyocytes [34]. Conduction system defects persisted but did not progress in aged *Lmna*^{+/-} mice. In 10-week old *Lmna*^{+/-} mice, cardiac morphology and contractility were completely normal [34]. In contrast, 50-week old *Lmna*^{+/-} mice developed dilated cardiomyopathy, even in animals without conduction system defects [34].

The milder cardiac phenotype in the heterozygous mice compared to *Lmna*^{−/−} animals is consistent with results from other mouse models of laminopathies, in which homozygous expression of *Lmna* N195K [35] or *Lmna* H222P [8] mutations is required to cause a muscular phenotype. Interestingly, in humans, Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy are primarily caused by heterozygous *LMNA* mutations predicted to result in dominant negative missense proteins [36], while milder, late-onset forms primarily affecting the heart and muscles in the limb girdle arise from lamin A/C haploinsufficiency caused by mutations predicted to result in truncated proteins [37,38].

The severity of the cardiac symptoms in Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy suggests that the myocardium is especially sensitive to functional defects in nuclear envelope proteins and particularly to lamin A/C haploinsufficiency [3,4,8–10,26]. This increased susceptibility of the heart could at least in part arise from an increased mechanical sensitivity of myocytes due to lamin A/C haploinsufficiency. Here, we applied a pressure overload model in heterozygous *Lmna*^{+/-} mice that express reduced levels of lamin A/C to examine whether increased mechanical load is sufficient to accelerate or exacerbate myocardial dysfunction caused by abnormal nuclear structure and function in *Lmna*^{+/-} mice. We found that *Lmna*^{+/-} animals have a significantly attenuated hypertrophic response to pressure overload compared to wild-type littermates as evidenced by reduced ventricular mass and myocyte size, and that the reduced hypertrophy is accompanied by impaired activation of mechanosensitive genes.

Methods

Mice

Lamin A/C deficient mice (*Lmna*^{-/-}) were generated by targeted deletion of the *Lmna* gene, as described previously [25]. Heterozygous (*Lmna*^{+/-}) and wild-type (*Lmna*^{+/+}) mice were crossed to produce litters consisting of *Lmna*^{+/-} and *Lmna*^{+/+} animals that were used for the pressure overload studies. Genotype was determined by polymerase chain reaction of genomic tail DNA, as described previously [25].

Transverse aortic constriction

Pressure overload was induced by transverse aortic constriction (TAC), as described previously [39]. In brief, a 6-0 silk suture was placed around the transverse aorta between the left common carotid artery and the brachiocephalic trunk and tied tightly around both the aorta and a 27-gauge needle. The needle was then carefully removed, yielding a reproducible degree of constriction. Sham-operated controls consisted of age-matched littermates that underwent an identical surgical procedure but without placement of the suture. *Lmna*^{+/-} animals and wild-type littermates were randomly assigned to treatment groups (non-operated, sham, or TAC). TAC or sham-surgery was performed at 8–10 weeks of age by an operator blinded to genotype. Mice were anesthetized with pentobarbital (30–70 mg/kg, i.p.). The study protocol was approved by the Harvard Medical School Standing Committee on Animal Research.

Echocardiographic and hemodynamic analyses

Cardiac morphology and function were evaluated by echocardiography 1–2 days prior to TAC or sham-surgery and at 4, 8, and 12 weeks post surgery by an observer blinded to genotype. Light anesthesia was induced by intraperitoneal injection of pentobarbital. Ultrasound image sequences were acquired using a Sonos 4500 (Philips) and a 15-MHz transducer. Left ventricular (LV) mass was calculated as described [38]. Hemodynamic parameters were acquired in animals at 12 weeks post TAC or sham surgery using a close-chest invasive method through the right carotid artery with a Millar pressure catheter, as described previously [39]. Data were analyzed by PowerLab Chart5 (ADInstruments, Inc., Colorado Springs, CO) by an observer blinded to genotype and treatment.

Histological analysis

Paraffin-mounted histologic sections were stained with Picro-sirius red or Periodic acid-Schiff (PAS). Picro-sirius red-stained sections were quantified for percent area stained positive for collagen. Staining, scanning, and quantification were carried out in a blinded manner using 5 randomly-selected sections from the heart, and the amount of fibrosis was expressed as an average for each animal. Myocyte cross-sectional area was determined based on PAS staining

and analyzed with ImagePro (Media Cybernetics, Inc., Silver Spring, MD) software. Apoptosis of cardiac myocytes was detected by triple staining with TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling), anti- α -sarcomeric actin antibody (Sigma), and DAPI using the ApopTag Plus Fluorescein In Situ Apoptosis Detection Kit (Chemicon, Temecula, CA).

Gene expression analysis

To quantify expression of pressure overload induced genes, hearts from TAC-operated animals and sham-operated controls were excised and snap frozen in liquid nitrogen at 1 week or 3 weeks after surgery. RNA was extracted with Trizol reagent (Sigma), transcribed into cDNA with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) using random primers, and subsequently analyzed by RealTime PCR on an Applied Biosystems 7300 Real Time PCR System using SYBR Green (Applied Biosystems) and primers for *Egr-1* (left: 5'-cctatgagcacctgaccaca-3'; right: 5'-tcgtttggctgggataactc-3') and *GAPDH* (left: 5'-gtgcagtgccagcctcgctc-3'; right: 5'-ggcagcaccagtgatgcag-3'). Results were normalized to expression of *GAPDH* and presented as fold-increase relative to sham-operated animals based on the $\Delta\Delta C_t$ method.

Western blot analysis

Protein lysates were extracted from snap frozen left ventricles as described previously [33], with the following modifications. RIPA buffer was supplemented with fresh phenylmethylsulphonyl fluoride (PMSF), protein inhibitor (both Sigma), and NaCl (final concentration: 490mM) and sonicated for 15 seconds to extract lamins. Samples were probed with primary antibodies against lamin A/C (Santa Cruz Biotechnology), Phospho-p44/42 MAP Kinase (Erk1/2) and total p44/42 MAP Kinase (both Cell Signaling), and β -actin (Sigma). Horseradish-conjugated secondary antibody (BioRad) was used for detection by chemiluminescence.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (s.e.m.). Statistical analysis was carried out with GraphPad Prism software. Comparisons between two groups were done by unpaired t-test with Welch's correction to allow for unequal variances; comparisons between more than two groups were performed by ANOVA. One-way ANCOVA analysis was performed using a web-based calculator provided by Vassar College (<http://faculty.vassar.edu/lowry/VassarStats.htm>). For all experiments, a *P*-value of less than 0.05 was considered significant. All experiments were performed in a double-blinded manner, *i.e.*, neither the person performing the experiments nor the person analyzing the data were aware of genotype or treatment.

Results

Despite the presence of one functional allele, mouse embryo fibroblasts derived from animals heterozygous for lamin A/C (*Lmna*^{+/-}) have reduced levels of lamins A and C [14,25]. To examine if a similar haploinsufficiency is also found *in vivo*, we assessed protein levels in lysates derived from the left ventricles of 9-week old *Lmna*^{+/-} mice and wild-type littermates. Consistent with the findings in mouse embryo fibroblasts and recent observations [34], we found that protein expression of lamins A and C in left ventricles of *Lmna*^{+/-} mice was 2 to 3-fold lower compared to tissues from matching wild-type littermates (Fig. 1).

***Lmna*^{+/-} mice have reduced hypertrophy in response to pressure overload**

Lmna^{-/-} and—to a lesser degree—*Lmna*^{+/-} mice have left ventricular myocytes with elongated and lobulated nuclei and central displacement and fragmentation of heterochromatin [26]. These abnormalities are less severe in myocyte nuclei of the left atrium and even less pronounced in hepatocyte nuclei [25], indicating that mechanical load could directly contribute to nuclear defects. Left ventricular pressure overload is an established animal model to study mechanically induced signaling pathways and hypertrophy in the heart [40–42]. Therefore, to study the *in vivo* effects of reduced lamin level (*i.e.*, haploinsufficiency) on mechanotransduction in the heart, we induced left ventricular pressure overload model by transverse aortic constriction (TAC) in ~9-week in *Lmna*^{+/-} mice and wild-type littermates (*Lmna*^{+/+}). To confirm the success of TAC surgery, we measured the pressure gradient between left and right carotids immediately after TAC surgery in a subset of animals. The average pressure gradient was 50.0 ± 4.1 mmHg ($N=6$), with no significant difference between *Lmna*^{+/-} animals and wild-type littermates. Cardiac function and cardiac dimensions in banded and in non-operated controls were monitored by echocardiographic analysis prior to surgery and at 4, 8, and 12 weeks following TAC surgery (Table 1).

As expected, hearts excised from wild-type animals at 12 weeks after TAC surgery showed a $81.3 \pm 15.2\%$ increase in heart weight normalized by body weight compared to sham- and non-operated controls. Interestingly, *Lmna*^{+/-} mice had a significantly smaller increase ($39.7 \pm 9.5\%$) in normalized weight (Fig. 2A), indicating that the *Lmna*^{+/-} animals developed milder hypertrophy in response to left ventricular pressure overload. To address whether the observed differences could be caused by variations in body weight, we compared the body weight of *Lmna*^{+/-} and wild-type littermates before and after TAC surgery. Importantly, there was no statistically significant difference in body weight between genotypes at any time point (Table 1), demonstrating that observed differences in normalized heart weight were due to differences in cardiac remodeling but not due to changes in body weight. We further confirmed this by employing an analysis of covariance (ANCOVA) as described by Packard and Boardman [43], with the heart weight as the dependent variable and body mass as the covariate to adjust for variation in body weight between individual animals. The adjusted means revealed an even more distinct difference between the increase in heart weight of *Lmna*^{+/-} (28.6% increase) and wild-type controls (64.3% increase) in response to pressure overload (adjusted mean heart weights after TAC were 0.23 vs. 0.18 g for wild-type and *Lmna*^{+/-}, respectively; adjusted mean heart weights in sham-operated animals were 0.14 vs. 0.14 g).

Similarly, echocardiographic analysis of left ventricular (LV) mass demonstrated that while *Lmna*^{+/-} and wild-type littermates had increased LV mass in response to pressure overload at 4, 8, and 12 weeks after surgery compared to sham- and non-operated animals, the hypertrophic response in *Lmna*^{+/-} mice was consistently lower than in wild-type littermates (Fig. 2B). Additional echocardiographic parameters such as anterior and posterior wall thickness showed similar trends, although they were not always statistically significant (Table 1).

To confirm these observations on the cellular level, we evaluated cardiac myocyte cross-sectional area based on periodic-acid Schiff (PAS) stained histological preparations of excised heart sections (Fig. 3A). We found that even though LV cardiac myocytes in *Lmna*^{+/-} and wild-type littermates were significantly larger after 12 weeks of pressure overload than cells from sham- and non-operated controls, the average cardiac myocyte cross-sectional area was significantly larger in the samples from the pressure overload wild-type animals than from *Lmna*^{+/-} mice (Fig. 3B). A similar trend, albeit not quite statistically significant ($P = 0.08$), was observed at the 8-week time point. Importantly, there was no difference in the cardiac myocyte cross-section area in sham- and non-operated animals between genotypes at any of the time points (Fig. 3B).

Since the reduced hypertrophic response to pressure overload in the *Lmna*^{+/-} mice could alter cardiac function, we evaluated hemodynamic parameters in TAC- and sham-operated animals at 12 weeks after surgery. As expected, peak LV pressure was significantly increased in wild-type mice subjected to pressure overload compared to sham controls (Fig. 4A). In contrast, *Lmna*^{+/-} animals showed an attenuated increase in LV pressure, suggesting that the reduced myocyte hypertrophy was associated with an inadequate adaptive response in the pressure generated by the LV. Interestingly, the *Lmna*^{+/-} mice also showed an abnormal response in the diastolic relaxation parameter (minimum *dP/dt*): *Lmna*^{+/-} animals subjected to 12 weeks of pressure overload had a significantly slower LV relaxation rate than wild-type littermates (Fig. 4B). No significant differences in maximum *dP/dt* and cardiac output were found between genotypes. A full overview of hemodynamic parameters is given in Table 2.

In wild-type animals, pressure overload results in increased myocardial and perivascular fibrosis [39]. To examine whether the attenuated increase in left ventricular pressure after TAC surgery could affect pressure overload induced fibrosis in *Lmna*^{+/-} animals, we quantified collagen deposition by Sirius-Red stain of histological sections from hearts excised at 8 and 12 weeks after surgery. *Lmna*^{+/-} mice and wild-type littermates had significantly increased myocardial (Fig. 5A) and perivascular (Fig. 5B) fibrosis compared to sham- and non-operated animals after 12 weeks of pressure overload, but perivascular fibrosis was significantly more extensive in wild-type animals than in *Lmna*^{+/-} animals following TAC surgery (Fig. 5B, C). A similar trend was observed after 8 weeks of pressure overload, although the difference was not quite significant (data not shown). Overall, these data are consistent with the idea that damage caused by increased ventricular pressure is responsible for TAC-induced perivascular fibrosis, and that the lower LV pressure in *Lmna*^{+/-} animals, accompanied by attenuated myocytes hypertrophy, results in reduced levels of perivascular fibrosis in these animals.

***Lmna*^{+/-} mice lack pressure overload induced activation of mechanosensitive genes**

We had previously reported that mouse embryo fibroblasts derived from two different animal models of Emery-Dreifuss muscular dystrophy, *i.e.*, *Lmna*^{-/-} mice and Emerin-null mice (*Emd*^{-Y}), had reduced expression of the mechanosensitive genes *Egr-1* and *Iex-1* in response to mechanical strain [33,44]. To examine if impaired activation of mechanosensitive genes could also be responsible for the attenuated hypertrophy in the *Lmna*^{+/-} mice in response to pressure overload, we examined expression of the mechanosensitive immediate-early genes *Egr-1* and *Iex-1* in the myocardium at 1 and 3 weeks post TAC surgery. Consistent with previous reports [45], we found that in wild-type hearts, *Egr-1* expression was increased at 1 week of pressure overload (Fig. 6), but returned to normal at 3 weeks after TAC (data not shown). Importantly, this induction of *Egr-1* at 1 week of pressure load was absent in *Lmna*^{+/-} hearts (Fig. 6). A similar, although not statistically significant trend, was found for *Iex-1* expression. *Egr-1* has been suggested as a key master regulator in multiple pathological cardiovascular processes and *Egr-1* null mice have reduced ventricular and myocyte hypertrophy in response to left ventricular pressure overload [46]. Thus, the lack of mechanosensitive activation of *Egr-1* might be attributed to the attenuated ventricular and cardiomyocyte hypertrophy in the *Lmna*^{+/-} pressure overload model.

Recent studies suggested that increased activation of the mitogen activated protein kinase (MAPK) signaling cascade is responsible for the cardiac phenotype observed in mice homozygous for the *Lmna* H222P mutation and in mice lacking emerin [47–50]. However, consistent with previous findings in *Lmna*^{+/-} mice [34], we found that levels of phosphorylated Erk44/42 MAPK were normal in sham and non-operated *Lmna*^{+/-} mice, and pressure overload induced activation of MAPK was similar in *Lmna*^{+/-} animals and wild-type controls (data not shown).

Lmna^{+/-} myocytes are not more prone to apoptosis in response to pressure overload

Ruptured nuclei have been detected in skeletal muscle biopsies from Emery-Dreifuss muscular dystrophy patients [32]. Similarly, loss of lamins A and C causes more deformable and fragile nuclei in *Lmna*^{-/-} mouse embryo fibroblasts, resulting in increased fractions of apoptotic and necrotic cells after mechanical stimulation [33], and *Lmna*^{-/-} mice have a significantly higher apoptotic index in left ventricular tissue at 4–6 weeks of age than wild-type littermates [26]. To evaluate the effect of pressure overload on cell viability in our lamin A/C haploinsufficiency model, we quantified the fraction of apoptotic cells in the left ventricle of TAC or sham operated animals by deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL). Pressure overload resulted in increased fractions of apoptotic cells compared to sham-operated animals, with no statistically significant difference between genotypes (1.56 ± 0.26 vs. 0.05 ± 0.02 and 1.12 ± 0.35 vs. 0.43 ± 0.15 TUNEL positive myocytes per 1,000 cells for wild-type and *Lmna*^{+/-} mice respectively, N = 8 for each group). These data are consistent with our earlier findings that *Lmna*^{+/-} mouse embryo fibroblasts have, compared to cells from *Lmna*^{-/-} mice, only moderately impaired nuclear mechanics and that their sensitivity to mechanical stress is indistinguishable from wild-type cells. These data suggest that reduced levels of lamin A/C may be sufficient to provide at least mechanical stability to the nucleus [14], and *Lmna*^{+/-} myocytes are not more prone to apoptosis in response to pressure overload.

Discussion

One of the perplexing questions in the nuclear envelope field has been why mouse models for muscular laminopathies require homozygous expression of the mutant protein [8,25,26,35], even though the majority of *LMNA* mutations in humans are dominant negative. The reason for this discrepancy remains unclear, but a plausible theory is that the short life-span of the mouse does not allow the phenotype to penetrate; this theory is consistent with the late onset and slow progression for nuclear envelope cardiomyopathies and muscular dystrophies. The recent findings by Wolf and colleagues [34] that *Lmna*^{+/-} mice develop late onset dilated cardiomyopathy further support this notion and provide new insights into the possible role of haploinsufficiency in the development of cardiac defects in laminopathies. To examine if increased mechanical stress could accelerate or exacerbate the cardiac phenotype in younger *Lmna*^{+/-} mice, we studied cardiac function and morphology in a pressure overload model. We found that *Lmna*^{+/-} mice have an attenuated hypertrophic response to pressure overload, associated with impaired activation of a mechanosensitive immediate-early gene, *Egr-1*.

At this point, we cannot conclusively pinpoint the molecular mechanism responsible for the impaired activation of mechanosensitive genes. However, one intriguing explanation could be provided by a recent report that lamins A and C directly interact with the transcription factor c-Fos and can suppress Activating Protein 1 (AP-1) activity by sequestering c-Fos at the nuclear envelope [24]. Since c-Fos can down-regulate *Egr-1* and other immediate-early genes [51], reduced levels of lamin A/C in *Lmna*^{+/-} mice may be sufficient to allow more (now non-sequestered) c-Fos to enter the nuclear interior and result in the attenuated gene expression of *Egr-1* in response to pressure overload compared to wild-type animals.

Another possibility is that differences in sub-cellular mechanics further contribute to the deficient mechanotransduction signaling in *Lmna*^{+/-} (and even more so in *Lmna*^{-/-}) cells. Lamins A and C are the main contributors to nuclear stiffness [14], and impaired nuclear mechanics could contribute to defects in cellular mechanosensing [52]. Furthermore, lamins directly interact with nesprins, SUN1/2, and emerin, nuclear envelope proteins that are directly involved in physically connecting the nuclear envelope to the cytoskeleton [16–20]. Complete loss of lamin A/C and mutations associated with the premature aging disease Hutchinson-Gilford progeria syndrome have been previously shown to result in abnormal nuclear cytoskeletal coupling and reduced cytoskeletal stiffness [20,33], and cardiac myocytes from

Lmna^{-/-} mice show disrupted desmin filaments around the nucleus [26]. Understanding the possible relationship between nuclear structure and how physical deformations of this central organelle may directly transduce mechanical stimuli into biochemical signals is an exciting area of current research, and more work is needed to elucidate the proposed role of the nucleus as a cellular mechanosensors [53].

Interestingly, our results suggest that while transcriptional regulation—particularly of mechanosensitive genes—is sensitive to haploinsufficiency of lamins A and C, it appears that lower levels of lamin A/C are sufficient to protect cardiac myocytes from direct mechanical induced damage, as pressure overload induced apoptosis in cardiac myocytes was indistinguishable from that in wild-type littermates, and *Lmna*^{+/-} mice showed comparable levels of myocardial fibrosis. A previous study also found no evidence for increased apoptosis in cardiomyocytes in the ventricular free wall in non-operated animals [34]. The concept that reduced levels of lamin A/C are sufficient to provide structural stability to the nucleus is supported by our previous findings that *Lmna*^{+/-} mouse embryo fibroblasts have, in contrast to *Lmna*^{-/-} cells, no increased rates of apoptosis or necrosis in response to repetitive cyclic strain application [14,33]. Nonetheless, the trend towards lower LV pressure in the *Lmna*^{+/-} animals might have precluded small differences in mechanical sensitivity. In fact, despite the overall failure of *Lmna*^{+/-} myocytes to adequately respond to pressure overload, the attenuated increase in LV pressure, combined with the reduced cardiac hypertrophy, could provide an explanation for the surprising finding that post-surgery survival was comparable between *Lmna*^{+/-} mice and wild-type litter mates (data not shown).

Thus, we have to account for the possibility that differences in nuclear stability may contribute to the late onset of dilated cardiomyopathy in the *Lmna*^{+/-} animals. A longitudinal study on *Lmna*^{+/-} mice found that in 1-year old animals, ventricular function was significantly worse than in wild-type littermates, even in animals without any defects in the cardiac conduction system [34]. Another group, studying homozygous *Lmna*^{-/-} mice, suggested that the primary pathophysiological mechanisms for the dilated cardiomyopathy in these animals was disrupted force transmission between the myocyte desmin network and the nucleus, and that defects in nuclear function prevented these mice from developing compensatory hypertrophy, thereby accelerating disease progression [26]. Unfortunately, we could not study the effect of pressure overload on cardiac function in *Lmna*^{-/-} mice, as these animals die between 4 and 8 weeks of age and are too small and too young for the TAC procedure.

Even though the fact that different heterozygous mutations in the *LMNA* gene cause distinct human diseases demonstrates that haploinsufficiency alone cannot account for the disease pathobiology, the effect of decreased levels of lamins A and C on cardiac function still remains an important research topic, especially since functional deletion of a single *LMNA* allele is sufficient to trigger dilated cardiomyopathy in mice and humans [34,37,38]. The relevance of lamin levels on cardiac function is further supported by the finding that expression of lamins A and C, but not B1, is reduced in cardiac myocytes from normally aged mice compared to younger animals [54]. Interestingly, levels of lamin C are significantly decreased in explanted hearts from patients with ischemic cardiomyopathy [55], further illustrating the need to better understand the effect of reduced lamin levels on cardiac function.

In conclusion, while much work remains to explore the multiple functions of lamins in nuclear structure and transcriptional regulation, our findings provide further support for a critical role of lamins A and C in regulating the cellular response to mechanical stress and demonstrate that haploinsufficiency of lamins A and C alone is sufficient to alter hypertrophic responses and cardiac function in the face of pressure overload in the heart.

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Figure 1.

Lmna^{+/-} mice have reduced levels of lamin A and C in the left ventricle. Western blot analysis of protein lysates from hearts excised from 9-week old *Lmna*^{+/-} mice and wild-type littermates (*Lmna*^{+/+}) showed markedly reduced expression of lamins A and C in *Lmna*^{+/-} animals. β -actin was used as loading control.

Figure 2.

Attenuated cardiac hypertrophy in response to pressure overload in *Lmna*^{+/-} mice. **(A)** Weight of heart excised from TAC-operated, sham-operated, or unoperated *Lmna*^{+/-} and wild-type (*Lmna*^{+/+}) mice after 12 weeks of pressure overload normalized to body weight for each animal. **(B)** Increase in left ventricular (LV) mass compared to pre-surgery values during pressure overload (TAC) and in sham- and unoperated controls based on echocardiographic measurements. For *Lmna*^{+/-} and wild-type animals, LV mass increased significantly compared to sham-operated controls at 4, 8, and 12 weeks post surgery ($P < 0.0001$ for wild-type; $P < 0.01$ for *Lmna*^{+/-} mice). Differences in LV mass increase between TAC-operated *Lmna*^{+/-} and wild-type mice were not quite significant at 4 and 8 weeks ($P = 0.076$ and $P = 0.105$,

respectively) but reached significance at 12 weeks post surgery ($P = 0.018$). *, denotes $P < 0.05$ at 12 weeks post TAC; **, denotes $P < 0.01$ at 12 weeks post TAC; ***, denotes $P < 0.001$ at 12 weeks post TAC.

Figure 3. *Lmna*^{+/-} myocytes have an attenuated hypertrophic response to pressure overload. **(A)** Representative histological preparations of left ventricles from *Lmna*^{+/-} and wild-type littermates (*Lmna*^{+/+}) after 12 weeks of transverse aortic constriction (TAC) or sham-surgery with Periodic acid Schiff (PAS) staining. Scale bar 20μm. **(B)** Myocyte cross-sectional area in left ventricle from TAC or sham-operated animals based on PAS staining. For each animal, myocyte size was determined from cross-sectional area measurements of ~50 myocytes in 5 independent myocardial sections. Results are based on the average from 6–12 animals per group. *, denotes $P < 0.05$; **, denotes $P < 0.01$.

Figure 4.

Hemodynamic analysis of left ventricular pressure and relaxation. **(A)** Pressure in the left ventricle (LV) after 12 weeks of pressure overload determined by a close-chest invasive method through the right carotid artery with a Millar pressure catheter. Wild-type mice showed a significant (*, $P < 0.05$) increase in LV peak pressure at 12 weeks after transverse aortic constriction (TAC). In *Lmna*^{+/-} animals, the increase in LV pressure was not significant compared to sham operated animals (n.s., $P = 0.20$). LV pressure in sham-operated animals was indistinguishable ($P = 0.94$) between genotypes. **(B)** Minimum dP/dt in *Lmna*^{+/-} and wild-type littermates after 12 weeks of pressure overload (TAC) or sham surgery. Differences in minimum dP/dt in sham operated animals were not statistically significant between genotypes.

($P = 0.18$). *, denotes $P < 0.05$ vs. *Lmna*^{+/-} sham and wild-type TAC, respectively. All results are based on 10–13 animals per group.

Figure 5.

Lmna^{+/-} myocytes have attenuated perivascular fibrosis in response to pressure overload. Left ventricular myocardial interstitial fibrosis (A) and perivascular (B) fibrosis in TAC or sham-operated animals based on histological assessment after 12 weeks of transverse aortic constriction (TAC) or sham surgery. For each animal, fibrosis was quantified as Sirius Red-positive area relative to myocardial area in 5 different cross-sections, assessing 5 areas in each section. Results are based on the average of 6–13 animals per group. Differences between sham- or unoperated animals between genotypes were not statistically significant (*n.s.*). *, denotes $P < 0.05$; **, denotes $P < 0.01$. (C) Representative histological preparations of left ventricles from *Lmna*^{+/-} and wild-type littermates (*Lmna*^{+/+}) after 12 weeks of TAC or sham-surgery stained with Sirius Red. Scale bar 20μm.

Figure 6.

Impaired activation of the mechanosensitive gene *Egr-1* in response to pressure overload in *Lmna*^{+/-} mice. Myocardial gene expression of *Egr-1* in *Lmna*^{+/-} mice and wild-type littermates (*Lmna*^{+/+}) after 1 week of pressure overload was quantified by Real Time PCR, normalized to expression of *GAPDH*, and presented as fold-increase compared to sham-operated controls. Results are based on 3–6 animals per group.

Table 1

Echocardiographic parameters for TAC mice

		Before		4 weeks		8 weeks		12 weeks	
		Lmna+/+	Lmna+/-	Lmna+/+	Lmna+/-	Lmna+/+	Lmna+/-	Lmna+/+	Lmna+/-
# of animals	TAC	44	44	20	17	20	17	20	17
	SHAM			16	21	16	20	16	21
Heart rate, bpm	TAC	606 ± 10	555 ± 10	596±19	600±16	593±19	600±17	612±18	628±12
	SHAM			628±19	614±10	634±11	618±9	623±12	641±12
Body weight, g	TAC	24.1±0.6	24.8 ± 0.7	27.0±1.0	24.6±0.8	31.0±1.4	28.1±1.1	32.9±1.5	30.6±1.4
	SHAM			28.1±1.3	29.4±1.1	31.1±1.5	34.0±1.4	34.8±1.8	37.5±1.4
Anterior wall thickness, mm	TAC	1.14±0.01	1.14±0.01	1.44±0.03	1.34±0.03*	1.45±0.03	1.38±0.03	1.47±0.04	1.40±0.04
	SHAM			1.24±0.03	1.19±0.02	1.24±0.03	1.22±0.02	1.23±0.03	1.23±0.02
Posterior wall thickness, mm	TAC	0.99±0.01	1.01±0.02	1.23±0.04	1.21±0.04	1.25±0.04	1.18±0.04	1.31±0.04	1.21±0.05
	SHAM			1.03±0.03	1.03±0.02	0.99±0.02	1.07±0.03*	0.99±0.02	1.06±0.02
End-systolic dimension, mm	TAC	1.09±0.03	1.05±0.02	1.41±0.11	1.15±0.07	1.42±0.15	1.27±0.10	1.57±0.20	1.30±0.11
	SHAM			1.15±0.05	1.09±0.03	1.18±0.05	1.15±0.04	1.18±0.05	1.17±0.03
End-diastolic dimension, mm	TAC	2.78±0.04	2.79±0.04	2.95±0.10	2.80±0.07	3.01±0.13	2.87±0.11	3.11±0.17	2.89±0.09
	SHAM			2.84±0.06	2.95±0.04	2.93±0.07	2.78±0.07	2.96±0.05	3.01±0.06
LV mass, g	TAC	103.1±2.6	102.7±2.9	163.0±9.0	141.4±9.7	171.1±11.0	147.2±10.5	187.4±12.7	151.0±8.7*
	SHAM			117.7±6.8	119.6±3.6	118.3±4.6	115.2±4.8	120.7±5.6	129.4±5.1
LV mass/body weight	TAC	4.31±0.10	4.30±0.10	6.07±0.31	5.74±0.31	5.69±0.44	5.28±0.33	5.94±0.50	5.02±0.29
	SHAM			4.20±0.16	4.14±0.16	3.86±0.12	3.46±0.15	3.52±0.14	3.49±0.10
Fractional shortening, %	TAC	60.8±0.9	62.1±0.6	53.3±2.5	59.4±1.6*	54.3±2.7	56.2±1.9	52.3±3.3	55.7±2.4
	SHAM			59.9±1.2	62.9±0.9	59.5±1.4	58.4±1.0	60.2±1.35	60.9±0.8

*, denotes $P < 0.05$ vs. Lmna+/+ TAC

Table 2

Hemodynamic parameters for TAC mice, 12 weeks after surgery

		Lmna+/+	Lmna+/-
# of animals	<i>TAC</i>	10	13
	<i>SHAM</i>	13	11
Heart rate, bpm	<i>TAC</i>	536±22	480±21
	<i>SHAM</i>	551±12	558±21
LV Peak pressure, mm Hg	<i>TAC</i>	133.4±8.9	116.0±7.0
	<i>SHAM</i>	105.3±7.1	104.6±4.8
LV End-systolic pressure, mm Hg	<i>TAC</i>	117.8±9.2	102.5±6.2
	<i>SHAM</i>	95.7±5.6	91.0±5.9
LV End-diastolic pressure, mm Hg	<i>TAC</i>	3.5±0.7	4.5±0.9
	<i>SHAM</i>	6.0±1.3	5.1±0.9
dP/dt maximum, mm Hg/s	<i>TAC</i>	7372±503	6261±837
	<i>SHAM</i>	9332±1116	9549±807
dP/dt minimum, mm Hg/s	<i>TAC</i>	8230±526	6348±689*
	<i>SHAM</i>	8674±683	10447±1051
Cardiac output, µL/min	<i>TAC</i>	5270±591	4548±617
	<i>SHAM</i>	5522±504	6330±919

*, denotes $P < 0.05$ vs. Lmna+/+ TAC