Lamina-Associated Polypeptide 2α Loss Impairs Heart Function and Stress Response in Mice

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Rationale: Lamina-associated polypeptide (LAP)2α is a mammalian chromatin-binding protein that interacts with a fraction of A-type lamins in the nuclear interior. Because mutations in lamins and LAP2α lead to cardiac disorders in humans, we hypothesized that these factors may play important roles in heart development and adult tissue homeostasis.

Objective: We asked whether the presence of LAP2α was required for normal cardiac function.

Methods and Results: To study the molecular mechanisms of the disease, we analyzed heart structure and function in complete and conditional Lap2α−/− mice as well as Lap2α−/−/Mdx mutants. Unlike conditional deletion of LAP2α in late embryonic striated muscle, its complete knockout caused systolic dysfunction in young mice, accompanied by sporadic fibrosis in old animals, as well as deregulation of major cardiac transcription factors GATA4 and myocyte enhancer factor 2c. Activation of compensatory pathways, including downregulation of β-adrenergic receptor signaling, resulted in reduced responsiveness of the myocardium to chronic β-adrenergic stimulation and stalled the progression of LAP2α-deficient hearts from hypertrophy toward cardiac failure. Dystrophin deficiency in an Mdx background resulted in a transient rescue of the Lap2α−/− phenotype.

Conclusions: Our data suggest a novel role of LAP2α in the maintenance of cardiac function under normal and stress conditions. (Circ Res. 2010;106:346-353.)

Key Words: lamins ■ LAP2α ■ dilated cardiomyopathy ■ β-adrenergic receptors

Dilated cardiomyopathy (DCM) is a primary myocardial disease characterized by dilation and impaired contraction of one or both heart ventricles. One of the genes most frequently involved in the development of DCM is LMNA, which encodes the nuclear intermediate filament proteins lamin A and lamin C.1 Mutations in lamin A/C cause the most severe forms of DCM, posing a high risk of heart failure in symptomatic patients.1 Besides heart muscle disease, mutations in LMNA cause a variety of pathological conditions in skeletal muscle, skin, nerve, bone, and adipose tissue, known as laminopathies,2 emphasizing the importance of the search for molecular disease mechanisms.

Recently, research on laminopathies has focused on lamin A/C–interacting proteins, whose mutations have been linked to a similar spectrum of human disorders.3 One of the best studied lamin A/C–binding partners is lamina associated polypeptide (LAP)2α, an unusual splice variant of the mammalian LAP2 gene.4 All LAP2 proteins (α, β, γ, δ, ε, ξ) share a common chromatin-binding structural motif called the LEM (LAP2-emerin-MAN1) domain at their N terminus. The C terminus of most LAP2 variants comprises a transmembrane region, which targets them to the inner nuclear membrane, where they serve mainly structural roles.5 LAP2α lacks the common LAP2 transmembrane domain and possesses an additional chromatin-binding region at its C-terminal end, which mediates targeting to the nuclear interior.6 In the nucleoplasm LAP2α specifically interacts with a fraction of lamin A/C via its unique C-terminal tail.7 Together, LAP2α and lamin A/C influence various nuclear processes, such as epigenetic chromatin regulation, gene expression, and signal transduction.7 In particular, LAP2α–lamin A/C complexes have been found to control the balance between proliferation and differentiation of early progenitor cells in regenerative tissues by affecting the E2F/retinoblastoma pathway.8

Interestingly, a mutation in LAP2α (c. 2068C>T, p. R690C), which lowers its binding affinity for lamin A/C in vitro, has also been linked to DCM.9 Because changes in both LAP2α and lamin A/C cause pathological heart conditions, we hypothesized that an intact LAP2α–lamin A/C complex may be necessary for proper cardiac function and that...
mislocalization or absence of one of the components would lead to disease.

Most Lmna transgenic mice generated so far show complex phenotypes, including various stages of heart failure.10,11 To see whether the presence of LAP2α is also important for normal cardiac output, we analyzed heart structure and function in previously generated Lap2α−/− mice.6 Here, we describe a new mouse model of cardiomyopathy and provide novel insights into mechanisms governing heart development and tissue homeostasis.

Methods

Mice

Lap2α−/− mice were kept on a mixed Mus musculus C57BL/6×129 genetic background.4 Compound mutant Lap2α−/−/Mdx animals were obtained by crossing Lap2α−/− mice with the Mdx line.12 Generation of conditional LAP2α knockout (KO) mice is described in Online Figure I (available in the Online Data Supplement at http://circres.ahajournals.org). All histological and physiological analyses were done by observers blinded for the genotype, as well as localization, of lamin A/C and other LAP2 isoforms in absence of the gene product, we performed Western blot, semiquantitative PCR, and immunofluorescence analyses of heart muscle tissue. LAP2α is absent from cardiac tissue of Lap2α−/− mice. A, Immunofluorescence analysis of Lap2α−/− and Lap2α+/+ heart tissue. B, LAP2α mRNA and protein are absent from heart muscle tissue of Lap2α−/− mice, whereas the expression of lamin A/C and alternative LAP2 splice variants remains unaltered. Western blot and semiquantitative RT-PCR analyses; samples were normalized for endogenous γ-tubulin and GAPDH content, respectively (n=3 male and 3 female littermate pairs).

Results

LAP2α-deficient mice used in this study were generated by Cre recombinase–mediated excision of the Lap2α-specific exon 4 of the Lap2 gene in the germline.8 To confirm the absence of the gene product, we performed Western blot, semiquantitative PCR, and immunofluorescence analyses of Lap2α−/− heart muscle tissue. LAP2α was detectable neither at an mRNA nor a protein level, whereas the expression, as well as localization, of lamin A/C and other LAP2 isoforms were not significantly altered (Figure 1 and Online Figure II).

Absence of LAP2α Causes Ventricular Systolic Dysfunction in Mice

The symptoms in LAP2α− and most lamin A/C–linked human cardiomyopathies appear predominantly in adults.5,9 In mouse lamin A/C–linked laminopathy models, however, heart defects develop in young animals and cause early mortality.10,11 As Lap2α−/− mice are grossly indistinguishable from their WT littermates and have a normal life expectancy,8 we analyzed heart function in young, as well as old animals. Echocardiography in 10-week-old Lap2α−/− mice revealed ventricular systolic dysfunction, characterized
by significantly decreased left ventricular FS% and percentage ejection fraction values. Moreover, enlarged left atria in male Lap2α-deficient mice emphasized the defective cardiac phenotype, indicating a possible left ventricular and left atrial volume overload (Figure 2 and Online Table I). The FS% and percentage ejection fraction values remained similarly depressed in old Lap2α−/− male mice (aged 10 to 12 months), suggesting that the functional defect was not progressive. Interestingly, cardiac parameters in Lap2α−/− females appeared largely comparable to WT at both ages (Online Table I).

**Old Lap2α−/− Mice Present Only Sporadic Cases of Cardiac Fibrosis**

To see whether the functional defect in Lap2α−/− hearts was accompanied by structural changes, we performed gravimetric and histological analyses. Lap2α−/− hearts had normal morphology and similar heart weight/body weight indexes compared to WT littermates in newborn, young, and old animals (data not shown). Furthermore, no overt histological pathologies were detectable in young Lap2α−/− mice (Figure 3A). Old Lap2α−/− hearts, however, showed high phenotypic variability in the degree of fibrosis, as assessed by the extent of interstitial collagen deposition in the left ventricle. Although the overall difference in the extent of cardiac fibrosis between Lap2α−/− and WT mice was found to be statistically insignificant (Online Figure III), of 11 Lap2α−/− tested mice, 18% developed extensive subendocardial fibrosis of the left ventricle (Figure 3B). In addition to fibrosis, 1 mouse presented regions of extremely thin, transparent myocardium, which collapsed in the absence of internal blood pressure (Figure 3C). In contrast, WT animals did not exhibit signs of increased fibrosis at any age.

**Deregulated Expression of Major Cardiac Transcription Factors in Male Lap2α-Deficient Mice**

In an attempt to identify the molecular mechanisms leading to systolic dysfunction in Lap2α−/− mice, we analyzed the expression of several markers connected to myocardial remodeling. Two major cardiac transcription factors, GATA4 and MEF2c, as well as their downstream targets (brain natriuretic peptide [BNP] and proteins involved in serum response factor–mediated transcription of immediate early genes), and sarcomeric and muscle-specific genes, myocardin A and STARS [striated muscle activator of Rho signaling], showed deregulated expression patterns in male Lap2α−/− mice (Figure 4A and 4B). Whereas MEF2c expression was repressed in newborn Lap2α−/− hearts and reached WT levels by the age of 10 weeks, GATA4, myocardin A and STARS were downregulated only in old hearts. In addition, consistent with the reported cooperative regulation of BNP expression by both GATA4 and MEF2c, BNP mRNA levels were significantly lower in Lap2α-deficient hearts compared to WT at all ages (Figure 4A). The expression of other MEF2c and GATA4 targets, such as α- and β-myosin heavy chain and atrial natriuretic factor was not significantly changed in Lap2α−/− mice (data not shown). The expression of the aforementioned factors in female Lap2α−/− hearts was comparable to WT (data not shown). Our data show that loss of Lap2α affects the expression of GATA4 and MEF2c, as well as some of their downstream targets, which in turn may influence the expression of a plethora of genes involved in cardiovascular development and stress-induced hypertrophic growth.

Because old Lap2α−/− mice showed occasional fibrosis, we analyzed the expression of known fibrotic markers in WT and Lap2α-deficient hearts (Figure 4C). Interestingly, loss of...
LAP2α caused a downregulation of connective tissue growth factor, an inducer of fibroblast proliferation and extracellular matrix synthesis, but affected only mildly the expression of its activator, the transforming growth factor β2 (TGFβ2) mRNA content. Quantitative PCR and semiquantitative PCR analyses of heart tissue (n=4 to 5 male littermate pairs of each age). One outlier pair was excluded according to Grubb’s test. Samples were normalized for endogenous GAPDH levels in semiquantitative PCR and hypoxanthine-guanine phosphoribosyltransferase (HPRT) levels in quantitative PCR and analyzed according to Pfaffl method. Each KO sample was compared to its respective WT littermate sample. Values are mean KO/WT expression ratios±SE. *P<0.05 (ANOVA).

**Lap2α−/− Mice Show a Blunted Response to Chronic ISO Infusion**

GATA4 and MEF2c play important roles during cardiomyocyte hypertrophy. To see whether their deregulation in Lap2α−/− hearts affects their response to cardiac hypertrophic stimuli, we subjected Lap2α−/− mice to chronic infusion of the β-adrenergic agonist ISO for 7 days. To detect ISO-induced changes in heart function and structure, we performed echocardiography before and after the treatment, as well as gravimetric and histological analyses at the end of the experiment. As shown by the heart weight/body weight indexes, ISO administration caused a similar degree of cardiac hypertrophy in Lap2α+/+ and Lap2α−/− mice (Figure 5A), indicating that the hypertrophic growth response is not grossly affected by the loss of LAP2α. Similarly, the extent of subendocardial fibrosis caused by chronic ISO infusion was comparable in Lap2α+/+ and Lap2α−/− mice (Online Figure IV).

In addition to hypertrophy, ISO treatment caused dilation of left heart ventricles, as shown by echocardiography (Figure 5B and Online Table II). In WT animals, systolic and diastolic left ventricular diameters and the corresponding ventricular volumes exhibited a significant increase, indicating the progression from cardiac hypertrophy toward heart failure. In contrast, left ventricles of Lap2α−/− mice were already slightly enlarged at the baseline and showed only milder additional dilation on ISO treatment, reaching end point sizes similar to WT. Heart weight/body weight indexes and echocardiography parameters of sham (PBS)-treated animals were not altered by the procedure (Online Table II). These data point to a blunted cardiac stress response in Lap2α-deficient mice.

FS values in treated animals were only slightly affected, showing that chronic ISO infusion did not significantly impair left ventricular systolic function either in Lap2α+/+ or Lap2α−/− mice (Figure 5B and Online Table II). This is in agreement with previous studies.

The preserved basal systolic function in ISO-induced cardiac hypertrophy has been associated with downregu-
ulation of β-adrenergic receptor (β-AR)–mediated inotropic responses. An ISO-induced downregulation of β-AR has also been shown to cause a blunted reactivity to its subsequent readministration. Therefore, we hypothesized that the reduced responsiveness of Lap2α−/− myocardium to ISO might also be a consequence of similar desensitization of the β-AR signaling pathway. To test this, we analyzed the expression of β2-AR in Lap2α-deficient and WT mice at baseline, as well as after ISO treatment. As expected, Lap2α−/− hearts showed lower baseline expression levels of β2-AR mRNA and protein in young and old mice (Figure 5C and Online Figure V). Importantly, the difference in relative β2-AR mRNA levels in untreated Lap2α KO versus WT hearts increased with age (Figure 5C), suggesting that the downregulation of β-AR signaling in Lap2α−/− mice is an aging-dependent phenomenon and may be a consequence of heart function impairment. ISO treatment caused an additional downregulation of β2-AR mRNA in Lap2α−/− hearts, albeit not as extensive as in the WT situation (≈71% in WT versus ≈39% in Lap2α−/−), resulting in comparable endpoint expression levels in both genotypes (Figure 5C).

Development of β-AR–mediated hypertrophy is associated with the activation of the fetal cardiac transcriptional program, including the expression of embryonic transcription factors atrial natriuretic factor and GATA4. In accordance with this, atrial natriuretic factor and GATA4 mRNA levels were upregulated in ISO-treated hearts of both Lap2α−/− and Lap2α+/− mice (Figure 5D). However, consistent with the reduced β-AR levels in old Lap2α−/− mice, the increase in ISO-induced atrial natriuretic factor expression was significantly lower in Lap2α-deficient myocardium compared to WT. Relative GATA4 levels were also substantially lower in ISO-induced hypertrophy. To address this question, we generated conditional KO mice by crossing Lap2αflflMdx−/−Neo animals with mice expressing Cre recombinase under the control of striated muscle-specific muscle creatine kinase (Mck) promoter (Online Figure I).40 In this system, the expression of Cre recombinase and the consequent deletion of Lap2α are turned on in heart and skeletal muscle during later stages of mouse embryonic development and striated muscle differentiation. Lap2αflflMdx−/−Neo/Mck-Cre+ mice were born at Mendelian ratios and did not demonstrate any overt phenotype. Lap2α mRNA and protein levels were significantly reduced in Lap2αflflMdx−/−Neo/Mck-Cre+ hearts, indicating an efficient recombination in the myocardium (Figure 7A). In contrast, other tissues like spleen, which do not express MCK,31 had normal levels of Lap2α (our unpublished data). Echocardiography in Lap2αflflMdx−/−Neo/Mck-Cre− mice showed normal heart function (Figure 7B and Online Table IV) and histological, as well as morphometric, analyses did not reveal any pathological heart phenotype (Figure 7C and data not shown). In accordance, the expression levels of MEF2c and GATA4 mRNA were similar in Lap2αflflMdx−/−Neo/Mck-Cre+ and Lap2α−/− hearts (data not shown). Thus, Lap2α expression in late-embryonic and adult cardiomyocytes is dispensable for normal heart function, but may be important during earlier stages of heart development (before embryonic day 13.5 when the Mck promoter becomes active) or in nonstriated muscle cells of the heart.

**Discussion**

In this study, we describe a new mouse model of cardiomyopathy caused by the absence of LAP2α, a major binding
partner of lamin A/C in the nucleoplasm. Together with previous studies linking lamin A/C and lamin-binding inner nuclear membrane proteins, emerin and nesprin, to congenital heart disorders, our data suggest a major role of A-type lamina complexes in normal heart function.

A mutation in the α-specific exon 4 of the human LAP2 gene has previously been linked to familial DCM. Here, we show that the absence of LAP2α in mice also leads to a heart disease. Interestingly, only male LAP2α–/– mice showed a heart defect, whereas female animals exhibited normal cardiac function implicating gender-specific factors in the development of the disease. Similar gender-related phenotype variations were described in other cardiomyopathy mouse models, including mice carrying the H222P-Lmna mutation. Although the exact background of this phenomenon is still unknown, gender-based differences in cardiac dysfunction were linked to the activity of steroid hormones. At present, no data about the risk of heart failure in male versus female carriers of LAP2α mutations in humans are available.

In an attempt to disclose the molecular pathways of the disease, we analyzed the expression of factors involved in the development and remodeling of the myocardium. LAP2α–/– mice showed deregulated expression of major heart transcription factors, MEFC214 and GATA4,17 as well as some of their downstream targets, at different life stages. Both GATA4 and MEFC2 play essential roles in embryonic heart development and hypertrophic growth. Because MEFC2 is required for normal heart development (see review14), its downregulation in the absence of LAP2α might compromise the early stages of cardiac development in LAP2α–/– mice.

The appearance of systolic dysfunction in young LAP2α–/– mice and the delayed decrease in GATA4 levels only in old mice suggests that this deregulation may be a consequence rather than the cause of the disease. Despite lower GATA4 expression levels, LAP2α–/– hearts were able to undergo hypertrophic growth. In support of our data, mice with reduced GATA4 levels (4D mice) show a similar heart function defect at baseline, as well as the ability to grow under hypertrophic conditions. Accordingly, 4D mice develop fibrosis only after pressure overload as a consequence of increased stress-induced cardiomyocyte death.

GATA4 and MEFC2 synergistically activate the expression of BNP, a cardiac hormone involved in the regulation of blood pressure and fluid-electrolyte balance, which also plays a role in the inhibition of cardiac fibroblast proliferation and extracellular matrix production. Decreased levels of BNP in the absence of LAP2α could potentially explain the observed occurrence of cardiac fibrosis in LAP2α–/– mice. Interestingly, BNP-deficient mice (Nppb−/−), which exhibit normal heart morphology and hypertrophic growth after ventricular pressure overload, show a higher incidence of fibrosis (≈50%) in male versus female mice at the age of 15 weeks.37

The blunted response of LAP2α-deficient hearts to chronic ISO infusion points to the existence of compensatory pathways activated in response to changes in cardiac function in LAP2α–/– mice. The attenuated hypertrophic growth and lack of fibrosis observed in β-AR KO mice after pressure overload indicate that the downregulation of β-AR signaling found in LAP2α–/– hearts might be a part of this process.

The variability in the extent of fibrosis at baseline, as well as after chronic ISO infusion, might be explained by the observed deregulated expression of pro–connective tissue growth factor and antifibrotic (BNP) factors in LAP2α–/– mice. The observed downregulation of connective tissue growth factor in old LAP2α-deficient hearts might be a part of an extensive compensatory mechanism activated in response to loss of LAP2α to protect the myocardium from further tissue deterioration and loss of function.

Because LAP2α is highly expressed in proliferating tissues and only weakly in postmitotic tissues, such as heart muscle, we hypothesized that LAP2α might be required during cardiac development and/or postnatal myocardial remodeling, mediated by putative cardiac stem cells. Therefore, we generated conditional KO mice that lose LAP2α during later stages of embryonic development and adult striated muscle differentiation. LAP2α–/– mice showed normal heart function and normal levels of GATA4 and MEFC2, supporting the model according to which the defect in complete KO mice might arise during early embryonic development and at early stages of muscle differentiation, before the Mck promoter becomes active, and/or it might be a consequence of LAP2α loss from heart stem- and nonstriated muscle cells.

We have previously shown that LAP2α retains a subfraction of the nuclear lamin A/C pool inside the nucleoplasm in proliferating skin fibroblasts and intestinal cells. In contrast, nucleoplasmic lamin A/C is lost in nondividing differentiated stem cells. Moreover, the expression of LAP2α was significantly higher in nondividing tissues, whereas it was decreased in postmitotic tissues, such as heart muscle,29 we hypothesized that LAP2α might be required during cardiac development and/or postnatal myocardial remodeling. The observed deregulated expression of pro–connective tissue growth factor and antifibrotic (BNP) factors in LAP2α–/– mice after chronic ISO infusion points to the existence of compensatory pathways activated in response to changes in cardiac function in LAP2α–/– mice. The attenuated hypertrophic growth and lack of fibrosis observed in β-AR KO mice after pressure overload indicate that the downregulation of β-AR signaling found in LAP2α–/– hearts might be a part of this process. The variability in the extent of fibrosis at baseline, as well as after chronic ISO infusion, might be explained by the observed deregulated expression of pro–connective tissue growth factor and antifibrotic (BNP) factors in LAP2α–/– mice. The observed downregulation of connective tissue growth factor in old LAP2α-deficient hearts might be a part of an extensive compensatory mechanism activated in response to loss of LAP2α to protect the myocardium from further tissue deterioration and loss of function.
cells, implicating the nucleoplasmic pool of lamin A/C in regulation of the transition from proliferating to the differentiated state. The localization of lamin A/C has also been shown to change in cardiomyocytes during aging and development, going from being mainly nucleoplasmic to the nuclear periphery. The elongated shape and different orientation of cardiomyocyte nuclei within heart tissue, however, precluded the detection of potential changes in lamin A/C localization in LAP2a-deficient tissue. In view of the lack of heart defects in muscle-specific conditional LAP2a KO mice, there is a possibility that a potential mislocalization of lamin A/C in LAP2a-deficient cardiomyocyte precursor or nonstriated muscle cells may be the primary cause of the Lap2α−/− cardiac defect. Alternatively, lack of LAP2α may change the function, rather than the localization of lamin A/C, such as binding to epigenetic modifiers and components of different signaling cascades, which in turn may lead to cardiomyopathy. This model is consistent with previous reports that have linked lamin A/C–related cardiomyopathies to changes in signaling pathways, such as transforming growth factor β, phosphoinositide 3-kinase and mitogen-activated protein kinase, which influence MEF2c and GATA4 expression during development and hypertrophic growth and are also affected by β-AR signaling.

In summary, we show that the absence of LAP2α causes a baseline ventricular systolic dysfunction in male mice and activates compensatory pathways that prevent further decline of heart function under chronic stress conditions. The origins of these defects, which could lie in impaired proliferation and/or differentiation of early embryonic cardiomyocytes, resident cardiac stem cells, or nonmuscle cardiac tissue, remain to be discovered.

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Disclosures

None.

References


