Cardiomyopathy Associated with Microcirculation Dysfunction in Laminin α4 Chain-deficient Mice*§

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Laminin α4 chain is a component of extracellular matrix (ECM) laminin-8 and -9 and serves dual roles as a structure protein and as a signaling molecule. The abundance of laminin α4 chain transcripts in the heart suggests an important role of this protein in cardiovascular development and function. In this study, we demonstrate that laminin α4-deficient mice gradually develop cardiac hypertrophy with impaired function. We show that depletion of laminin α4 chain did not alter the levels of dystrophin-glycoprotein complex (DGC) components or affect cell membrane integrity. No alteration in integrin β1D protein was observed in terms of expression level or distribution pattern, indicating that the postnatal development of cardiac hypertrophy and cardiomyopathy in these mice is unlikely associated with the stability of sarcolemmal DGC and integrin complexes. Moreover, cardiomyocytes isolated from Lama4−/− mutant hearts maintained their contractility in vitro. In contrast, elevated levels of hypoxia-inducible factor 1α (Hif1α) and vascular endothelial growth factor A (Vegfa) transcripts, along with multiple foci of cardiomyocyte degeneration and fibrosis suggested sustained cardiac ischemia. Electron microscopy confirmed malformed blood vessels and wide pericapillary ECM spaces, suggesting the presence of microcirculation abnormalities in Lama4−/− mutant hearts. We thus conclude that mutation in the laminin α4 chain leads to abnormal cardiovascular ECM structure that cause insufficient oxygen supply to the heart and the subsequent ischemic cardiac phenotype observed. Our study links the genetic deficiency of an ECM protein to cardiomyopathy and implies a novel pathway of idiopathic cardiomyopathy in human.

1 The abbreviations used are: ECM, extracellular matrix; ANF, atrionatriuretic factor; BNP, brain natriuretic peptide; DGC, dystrophin glycoprotein complex; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hif, hypoxia-inducible factor; RT, reverse transcription; Vegf, vascular endothelial growth factor; %FS, percent fractional shortening; LVM, left ventricular mass; DG, dystroglycan; SG, sarcoglycan; Dys, dystrophin; Bis-Tris, 2-(bis(hydroxymethyl)amino)-2-(hydroxymethyl)propane-1,3-diol.

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ECM proteins, such as collagens, perlecans, nidogens, and agrin. Additionally, they impact cell differentiation and phenotype maintenance by participating in cell signaling via interactions with integrins and the DGC. Five laminin α chains, three β chains, and three γ chains recombine to form at least 14 distinct laminin isoforms. The five laminin α chains are expressed in a tissue-specific manner with some overlapping patterns (1, 2). Several laminin α chains have been proven to be functionally indispensable. Mutations in the laminin α2 chain cause congenital muscular dystrophy both in mice (3, 4) and humans (5), despite a compensation by α1 (4) and α4 chains (6). Mutations in the laminin α5 chain cause embryonic lethality in mice with developmental defects of several organs (7).

Lama4, the gene encoding the laminin α4 chain, is developmentally regulated during embryogenesis. In adult mice, Lama4 transcripts are most abundant in the heart. By immunostaining, the laminin α4 protein is localized mainly in basement membranes of blood vessel of an adult heart and in the peripheral sarcolemma of cardiomyocytes (8). To explore in vivo function of Lama4, the gene was disrupted in mice. Mice null for Lama4 survived embryonic development. About 20% of the homozygous mutants died at the perinatal stage (9), but those surviving the first week after birth were grossly indistinguishable from their littermates. However, mutant mice had an increased frequency of sudden death upon stress, such as exposure to anesthesia. In this study, we show that Lama4−/− mutant mice gradually develop cardiac hypertrophy and cardiac dysfunction. We further investigated the pathways by which laminin α4 protein deficiency leads to cardiomyopathy.

MATERIALS AND METHODS

Animals and Breeding—The generation of Lama4 null mice has been described elsewhere (9). Heterozygous mutant mice were backcrossed to C57BL/6 mice (Charles River, Uppsala, Sweden) for seven generations. Homozygous knock-out mice were obtained through breeding of two heterozygous mice.

Immunostaining—Immunofluorescence staining was performed on cryostat sections. To make results comparable, 10-μm-thick cryostat sections from paired mutant and control heart samples were placed on the same slide. Antibody against α-dystroglycan (α-DG) was a kind gift from Kevin Campbell, University of Iowa; antibodies against β-DG, β-sarcoglycan (β-SG), and dystrophin (Dys) were purchased from Novo, Castra, UK; laminin α1 was a kind gift from Lydia Sorokin, University of Erlangen-Nuremberg, Erlangen, Germany; laminin α2 polyclonal antibodies were kindly provided by Peter Yurchenko, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ; polyclonal antibody against the laminin α3 chain was a kind gift from Daniel Aberdam, INSERM U385, Nice,
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France; polyclonal laminin α5 chain antibody was kindly provided by Jeffrey Miner, Washington University, St. Louis, MO; polyclonal antibody against the LG1–3 modules of laminin α4 chain was a gift from Rupert Timpl, Max Planck Institute for Biochemistry, Martinsried, Germany; integrin β1D antibody was a kind gift from Robert Ross, University of California at San Diego, San Diego, CA. Hifα monoclonal antibody was from Novus Biologicals; fluorescein isothiocyanate secondary antibodies were from Dako. Signals were visualized under a fluorescence microscope (Leica) and recorded using Spot Advanced (Diagnosticsoft).

Western Blots Analysis—Total protein extract was prepared with methods described previously (10). Protein concentration was determined by DC protein assay (Bio-Rad) following the manufacturer’s instructions. A protein sample of 50 μg was separated on a 4–12% NuPAGE Bis-Tris polyacrylamide gel (Invitrogen), blotted to a PVDF membrane (Bio-Rad). After incubation with primary antibodies, and subsequently horseradish peroxidase-conjugated secondary antibodies (Zymed Laboratories Inc.), signals were developed with luminol reagent (Santa Cruz Biotechnology) and exposed to Kodak film.

Reverse Transcription (RT)-PCR and Northern Blot Analyses—Mouse heart tissue was homogenized in TRIzol solution (Invitrogen), and the RNA was isolated following the manufacturer’s instructions. Probes for Northern blot hybridization were purified PCR products amplified with the following primers: atrionatriuretic factor (ANF), 5′-cggtgctgacaagatgatc-3′ (sense) and 5′-gctcaacgtgcctgaatc-3′ (antisense); brain natriuretic peptide (BNP), 5′-caagacctgtataaggc-3′ (sense) and 5′-cttgacgctcaagctga-3′ (antisense); Hsp70, 5′-caagctggtgatccgca-3′ (sense) and 5′-gaagatgccgcttcgat-3′ (antisense). RT-PCR was performed using the one-step RT-PCR kit (Invitrogen) to detect mRNA expression as described previously (14, 15). Contractile properties were assessed using an IonOptix MyoCam® system with the Ion-Wizard SarcLen Data Acquisition software (IonOptix Inc., Milton, MA). Contraction amplitude was measured as the percentage of shortening of sarcomere length. Half-relaxation time was measured as the interval from the peak contraction to 50% relaxation.

RESULTS—All data were evaluated by the two-tailed Student’s t test.

Expression Profile of Other Laminin α Chains in Mouse Heart Deficient for the Laminin α4 Chain—We have demonstrated previously the abundance of laminin α4 chain transcripts in the heart. On immunostaining, the protein is localized to the basement membrane of blood vessels and in the peripheral sarcolemma of cardiomyocytes (8), indicating a critical role of this protein in cardiovascular development. Surprisingly, most Lama4 null mice survived embryonic and perinatal stages. To explore whether compensation of other laminin α chains occurs in Lama4−/− mice, we examined by immunohistochemistry the expression profile of all five laminin α chains in wild type and Lama4−/− mouse hearts. To ensure a reliable comparison, the thickness of the sections was tightly controlled. Sections from a mutant heart and its littermate control heart were placed on the same slide and stained together. A minimum of four pairs of samples were examined with each antibody. In wild type control hearts, laminin α4 chain was expressed in the basement membrane of blood vessels and in the peripheral sarcolemma of cardiomyocytes (Fig. 1A). The expression was abolished in Lama4−/− mutant hearts (Fig. 1B). Laminin α1 or α3 chains were not detectable in either wild type or mutant heart (data not shown). The laminin α2 chain was highly expressed along sarcolemma of cardiomyocytes (Fig. 1C). However, no significant difference was observed between wild type and Lama4−/− mutant mouse heart in terms of expression level or distribution pattern (Fig. 1, C and D). In wild type mouse heart, the laminin α5 chain was strongly expressed in large vessels and weakly expressed in capillaries and in the sarcolemma of cardiomyocytes (Fig. 1E and supplemental Fig. 1). In contrast, the expression of this chain in mutant hearts was highly elevated in the margin of cardiomyocytes and in basement membrane of capillaries (Fig. 1F). Thus, the laminin α5 chain has partially compensated for the loss of the laminin α4 chain in Lama4−/− hearts. Moreover, loss of the laminin α4 chain did not alter total laminin concentration in the heart as the staining intensities of laminin β1 and β2 chains were comparable between mutants and controls (data not shown).

Progressive Cardiac Hypertrophy—We have observed that adult Lama4−/− mice had increased frequency of sudden death under non-invasive procedures such as induction of anesthesia. Upon autopsy, enlarged hearts were observed in mice that had sudden death. To determine whether the enlarged heart was a common finding in these mice,
we measured body weight and heart weight of Lama4−/− mutant animals and their littermate controls from different age groups, seven to eight mice from each group were used in this study. At 16 weeks of age, both heart weight and body weight were comparable between Lama4−/− mutant and their littermate control mice. Heart weight/body weight ratio was 5.32 ± 0.43 mg/g (mean ± S.E.) in mutant mice, no significant difference compared with that in their littermate controls (4.96 ± 0.58 mg/g, mean ± S.E., p > 0.05). At the age of 40 weeks, there was no significant difference in body weight between mutants and controls (24.55 ± 2.03 g in mutants versus 25.28 ± 2.76 g in littermate controls, mean ± S.E., p > 0.05). In contrast, heart weight was significantly higher in mutant mice. Heart weight/body weight ratio was 6.25 ± 0.22 mg/g in mutant mice, significantly higher than 5.03 ± 0.40 mg/g in their littermate control mice (mean ± S.E., p < 0.001, Fig. 2a).

To quantify the size of cardiomyocytes, we measured the cross-section area of the cells. Cryostat sections were stained with antibodies against laminin α2, α4, and α5 chains. Cytochrome c oxidase staining was used to highlight the cell membrane. Sections were subsequently counterstained with Hoechst dye to mark the nuclei. Cross-sections of cardiomyocytes with visible nuclei were marked (Fig. 2b), and the area was measured using ImageJ (NIH software). Three mutant mice and three age- and sex-matched control mice were used in this study. One-hundred cells from three hearts were measured. There was a significant increase in cardiomyocyte size, as presented by average cross-section area, which was 131.43 ± 3.68 μm² in mutant heart and 106.95 ± 5.93 μm² in wild type control heart (mean ± S.E., p < 0.01, Fig. 2c).

Progressive Cardiac Dysfunction and Cardiac Arrhythmia—To evaluate cardiac function of mice deficient in the laminin α4 chain, we performed echocardiography on Lama4−/− mutant and control mice of different age groups. At 16–20 weeks of age, echocardiography on four mutant mice and four controls did not reveal any marked abnormalities (data not shown). However, at 36–40 weeks of age, Lama4−/− mutant mice showed significant abnormalities in cardiac function. Figure 3a is representative echocardiographic images of a Lama4−/− mutant mouse and its littermate control mouse, showing a larger left ventricular end diastolic and end systolic diameters in the mutant mouse compared with the control. The severity of the phenotype varied among individual animals. To gain a statistical evaluation of the phenotype, 12 mutant and 12 age-matched control mice were studied. Fig. 3, b–f, is a cumulative presentation of this study. Overall, heart rate in the Lama4−/− mutant showed no significant difference from control mice (482 ± 27 beats/min in mutant mice versus 564 ± 9 beats/min in control mice, mean ± S.E., p > 0.05, Fig. 3b). There was a significant increase in left ventricular end diastolic diameter (LVEDD) in mutants (4.21 ± 0.11 mm) compared with that in controls (3.95 ± 0.08 mm, mean ± S.E., p < 0.05, Fig. 3c) and an increase in left ventricular end systolic diameter (LVESD, 2.98 ± 0.16 mm in mutants versus 2.51 ± 0.10 mm in controls, mean ± S.E., p < 0.01, Fig. 3d). %FS was reduced in mutant mice, (29.58 ± 2.46) in comparison with 36.6 ± 1.55 in controls (mean ± S.E., p < 0.01, Fig. 3e). Echocardiography also revealed a significantly increase in LVM, which was 101 ± 4.50 mg in mutants compared with 88.3 ± 2.88 mg in controls, mean ± S.E., p < 0.05, Fig. 3f.

During echocardiography 6 out of 12 mutant mice had intermittent occurrence of bradycardia and sinus arrhythmia (data not shown), while none of the 12 controls had such abnormalities.

Retention of Integrin β1D and DGC Proteins and Intact Sarcolemma—The G domain of all laminin α chains physically interacts with the integrin receptors or with α-dystroglycan. To find out whether loss of the laminin α4 chain alters the expression of integrins or components of DGC, we examined the expression and distribution by Western blot analysis and immunofluorescence staining of β1D integrin and the DGC components α-DG, β-DG, β-SG, and Dys. Adult hearts from 24–40-week-old Lama4−/− mutant mice and their age- and sex-matched controls were analyzed. Western blot analysis revealed that the protein levels of β1D integrin, α-DG, β-DG, and β-SG were comparable between mutant and controls (Fig. 4a). On immunostaining, α-DG, β-DG, β-SG, and Dys (Fig. 4b) and β1D integrin (Data not shown) were observed along the sarcolemma in both wild type control hearts and laminin α4-deficient hearts. However, there was an uneven distribution of α-DG, β-DG, and β-SG in the Lama4−/− hearts (Fig. 4b, arrows) in comparison with that in control hearts. Staining patterns of Dys and β1D integrin were comparable in mutant and control hearts. Blood vessels were not stained with any of the above antibodies. To find out whether the altered distribution of DGC proteins affected sarcolemma integrity, we performed the Evans blue dye permeability assays on mice at 4, 16, and 40 weeks of age. Five mutant and five controls from each age group were used in the study. We did not observe any marked increase in cell permeability in Lama4−/− mutant mice of any age group (data not shown), indicating that the sarcolemma integrity of Lama4−/− mutant mice was not impaired.

Abnormal Extracellular Architecture and Multiple Foci Cardiomyocyte Degeneration—Hematoxylin and eosin staining of the heart did not reveal remarkable pathology in Lama4−/− hearts prior to 16 weeks of age (data not shown). Gradually, mutant hearts started to display widening of extravascular space, cardiomyocyte degeneration, and fibrosis as visualized in Fig. 5, a–d. HE staining of hearts from 40-week-old control mice (Fig. 5, a and c) and Lama4−/− mutant mice (Fig. 5, b and d). Wide intercellular space, interstitial and perivascular fibrosis, and cardiomyocyte degeneration were observed in mutant hearts. Electron microscopy, performed as described previously (16), revealed an early onset of ultra structural abnormalities in Lama4−/− heart. EM studies of 4–8-week-old mice showed normal sarcosome structure and Z disc organization. However, the intercellular space in the mutant heart was significantly enlarged (Fig. 5, f and h) compared with its littermate control hearts (Fig. 5, e and g). Moreover, mutant cardiomyocytes had an uneven cell border (Fig. 5, e and g), and the distance between myocyte
FIGURE 3. Impaired cardiac function in Lama4−/− mutant mice at the age of 36–40 weeks. a, M-mode echocardiography showing dilated left ventricle end diastole and systole in mutant heart in comparison with control heart. b–e, cumulative evaluation of cardiac function in 12 mutant and 12 age-matched control mice, showing heart rate (HR) in beats/min, LVIDd in mm, LVIDs in mm, %FS and LVM in mg. Filled bar, Lama4−/−; empty bar, wild type control, mean ± S.E.; *, p < 0.05; **, p < 0.01.
determine whether loss of the laminin Lα4 chain had led to intrinsic mechanical abnormalities in cardiomyocytes, we isolated cardiomyocytes from Lα4−/− mutant and control animals 16–20 weeks of age. A total of about 100 cells of each genotype group, isolated from three mutant and three control mice, were measured. Our results showed that single cell contractility was not impaired in mutant mice. On the contrary, there was an increase in percentage of sarcomere length shortening ($\Delta SL/SL_{0}$), 9.52 ± 0.37% in mutant versus 7.95 ± 0.25% in controls (mean ± S.D., $p < 0.001$), and the time of both peak contraction and relaxation was decreased; consequently, the maximum rate of contraction ($-dSL/dt$) was increased in mutant cells, $-3.54 \pm 0.16 \mu m/s$ in mutants versus $-2.77 \pm 0.10 \mu m/s$ in controls (mean ± S.D., $p < 0.001$), and the maximum rate of relaxation ($dSL/dt$) was also increased (3.09 ± 0.14 $\mu m/s$ in mutants versus 2.28 ± 0.10 $\mu m/s$ in controls, mean ± S.D., $p < 0.001$) indicating an improvement in contraction in the cardiomyocytes selected (Fig. 7a).

**Up-regulation of Hypoxia-induced Genes**—Blood vessel abnormality and interstitial fibrosis of the heart, as revealed by histology, pointed to the existence of long standing hypoxia in Lα4−/− mutant hearts. To verify this, we collected hearts from mutant and control animals at the age of 16–20 weeks when no marked hypertrophy was observed. Semi-quantitative RT-PCR analyses were performed. Hypoxia-inducible factor 1 (Hif1) is a transcriptional activator that is expressed in response to hypoxia. Of the two subunits, Hif1α is the hypoxia-responsive subunit, while Hif1β is a constitutively expressed nuclear translocator protein that is irresponsive to hypoxia (17). Vascular endothelial growth factor (Vegf) is a family of inducible factors that controls the growth of capillaries and angiogenesis (18). The expression of Vegfa has been shown to be activated by oxygen tension through Hif (17, 19). In Lα4−/− heart, there was a significant increase in transcript levels of Hif1α and Vegfa, while the levels of Hif1b remained unchanged (Fig. 7, b and c). An increase in Hif1α protein level in Lα4−/− mutant heart (supplemental Fig. 2) was also observed. We further performed Northern blot analyses and found in mutant hearts a significant increase in ANF transcripts, a representative marker of sustained cardiac hypoxia, cardiac hypertrophy, or heart failure. Expression BNP, an early responsive marker to hypoxia, was not significantly increased in Lα4−/− mutant hearts (Fig. 7, d and e). We did not observe any increase in transcript levels of Heat shock protein 70 (Hsp70) and Hsp27 (Fig. 7, a–c).
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DISCUSSION

We demonstrated in this study that the laminin α4 chain, a constituent of dominant laminin isoforms in blood vessel basement membranes and sarcolemma of the heart, is necessary in maintaining cardiac structure and function. Disruption of the gene resulted in cardiac hypertrophy, cardiomyocyte degeneration, and cardiac dysfunction. As the laminin α4 chain serves both as a structural and a signaling molecule, we investigated further the pathways of laminin α4 deficiency leading to cardiomyopathy.

Normally, DGC and integrins physically interact with the G domain of the α chain of laminin isoforms, thus serving as two families of receptors for all laminin α chains. It has been hypothesized that the DGC protects the sarcolemma from contraction-induced damage (21). We have reported previously (8), as well as observed in this study that by immunostaining, that the laminin α4 chain is present along sarcolemma from neonatal stage to early adulthood (Fig. 1a and supplemental Fig. 1). Depletion of this protein leads to the up-regulation of the laminin α5 chain. Talits and his colleagues (22) have reported that G domains in different laminin α chains have different affinity to dystroglycan. The laminin α4 chain is a poor ligand for DGC compared with G domains of the laminin α2 chain (22). This could be one explanation why laminin α4 chain depletion has not affected DGC contents or sarcolemma integrity, as the laminin α4 chain is not a critical laminin component that interacts with α-DG. Moreover, it has also been observed that laminin deficiency seems to affect myocyte survival through a mechanism independent of sarcolemma integrity, different from that in dystrophin-deficient myopathy animal models. In a comparative study of sarcolemma integrity, laminin α2-chain-deficient mice and dystrophin (mdx)-deficient mice both have developed severe muscular dystrophy with massive myocyte degeneration. However, the former have little Evans blue accumulation in their skeletal muscles (21, 23), while the later exhibit significant Evans blue accumulation in skeletal muscle fibers (23).

To explore whether laminin α4 chain deficiency affects cardiomyocyte contractile properties, we found that, on the contrary to impaired function, there was an improvement in the contractile property in Lama4−/− mutant cardiomyocytes. It is most likely that as a subpopulation of cardiomyocytes undergo degeneration, the remaining cells have grown in size and improved their contractile function as a compensatory response.

Integrin β1D is a striated muscle-specific integrin β subunit. Integrin β1D has been implicated in cardiac hypertrophy (24, 25) and cardiomyocyte survival (26). It has been shown that depletion of this subunit leads to cardiomyopathy and cardiomyocyte cell death (26). We demonstrated by Western blot and immunohistochemical analyses that the expression of integrin β1D remained unaltered in Lama4−/− mutant mice, suggesting that integrin signaling is unlikely responsible for the cardiomyocyte degeneration and cardiac phenotype observed in these mice. The fact that depletion of this laminin α chain did not affect integrin β1D content nor impair sarcolemma permeability could be due to the fact that the laminin α4 chain constitutes major laminin isoforms of blood vessel rather than that of myocytes in later adulthood.

Our data point to the fact that hypoxia/ischemia is responsible for the cardiomyopathy phenotype observed in Lama4−/− mice. First, morphologically as shown by electron microscopy, widening of extracellular matrix space and enlarged distance between myocyte and its adjacent capillary vessels may have caused insufficient oxygen and nutrient supply to cardiomyocytes. Second, in an environment independent of blood supply, cardiomyocytes retain their contractile function. Third, multiple foci of degenerative change and their generalized distribution pat-
tern in the heart are in agreement with a causative defect in the microcirculation. The hypertrophy of cardiomyocytes is likely to be a compensatory response to chronic cell loss resulted from hypoxia/ischemia. Fourth, overexpression of hypoxia-inducible markers, such as Hif1a, Vegfa, and ANF supports the presence of long standing hypoxia, as has been suggested previously (27).

The laminin α4 chain constitutes laminin-8 and -9, the ECM of blood vessels. As structural components, laminin-8 and -9 interact with other ECM components, and as signaling molecules, they interact with integrins α3β1 and α6β1, convey signals from outside to inside the cell, and regulate endothelial cell attachment, migration, and proliferation (28, 29), activities different from that in muscle cells.

The laminin α4 chain also contributes to blood vessels of other organs such as skeletal muscles. However, no marked structural or functional phenotype was observed in skeletal muscle (30) or other organs of these mice. The fact that the heart is the most oxygen-demanding organ, consuming twice as much oxygen as the brain, and 43 times as much as resting skeletal muscles (31), makes it the most vulnerable organ to hypoxia.

The involvement of blood vessel dysfunction in the development of cardiomyopathy has been studied in other mouse models (32, 33). Disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle perturbs vascular function, which initiates cardiomyopathy. Loss of δ-sarcoglycan (dSG) in these animals was mainly associated with irregularities of the coronary vasculature. In contrast to these models, the structure of coronary vessels is not impaired in Lama4−/− mutant mice, as demonstrated by microfil perfusion experiment (supplemental Fig. 3). This observation is in agreement with the expression pattern of the laminin α4 chain in hearts from wild type mice. As shown in supplemental Fig. 1, laminin α4 protein is readily detectable in capillaries; however, it is weakly stained or absent from larger vessels. Laminin α5 seems to be the dominant laminin α chain surrounding larger blood vessels. Remarkable pathology observed in Lama4−/− deficient mice was the irregularity of capillaries and enlargement of space between

![FIGURE 7](image_url)

**FIGURE 7.** a, single cell contractility measurement from cardiomyocytes isolated from Lama4−/− mutant heart and control hearts at the age of 16–20 weeks, showing an increase in percentage of sarcomere length (SL) shortening (ΔSL/SL0) and an increase in both dSL/dt shortening and dSL/dt relaxation in Lama4−/− mutant cardiomyocytes. ****, p < 0.01. b, semiquantitative RT-PCR analyses showing levels of Hif1a, Vegfa, and GAPDH transcripts in Lama4−/− mutant and control hearts at the age of 16–20 weeks. c, relative expression levels of Hif1a, Vegfa, and Hif1b; d, Northern blot analyses of ANF, BNP, Hsp70 transcripts in Lama4−/− mutant and control hearts at the age of 16–20 weeks; e, relative expression levels of ANF, BNP, and Hsp70. Empty bars, control hearts; filled bars, Lama4−/− mutant hearts; y axis in e and e, relative levels of expression presented by ratio of absolute expression levels of genes to that of GAPDH; ***, p < 0.05.
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myocyte and its adjacent capillary vessels, which might cause insufficient oxygen supply and subsequently cardiac phenotype. The cardiac dysfunction, myocyte degeneration, and subsequent compensatory hypertrophy observed in these mice could all be explained by microcirculation dysfunction.

We have demonstrated in this study that up-regulation of laminin α5 chain contributes to the survival of Lama4−/− mutant mice. However, this compensation is incomplete. Mice lacking the laminin α4 chain have developed cardiomyopathy, despite compensation of other α chains. So far, the laminin α4 chain has not been linked to any human disorder. Our study provides the first evidence in an animal model that a mutation in this gene might predispose to cardiomyopathy with defective microvascular circulation and chronic myocardial ischemia in certain subpopulations. Screening of this gene for mutation in patients with familial cardiomyopathy might link this gene to this human disorder.

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