Lmo7 is dispensable for skeletal muscle and cardiac function

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Submitted 22 June 2015; accepted in final form 6 July 2015

Lao DH, Esparza MC, Bremner SN, Banerjee I, Zhang J, Veevers J, Bradford WH, Gu Y, Dalton ND, Knowlton KU, Peterson RL, Liber RL, Chen J. Lmo7 is dispensable for skeletal muscle and cardiac function. Am J Physiol Cell Physiol 309: C470–C479, 2015. First published July 8, 2015; doi:10.1152/ajpcell.00177.2015.—Emery-Dreifuss muscular dystrophy (EDMD) is a degenerative disease primarily affecting skeletal muscles in early childhood as well as cardiac muscle at later stages. EDMD is caused by a number of mutations in genes encoding proteins associated with the nuclear envelope (e.g., Emerin, Lamin A/C, and Nesprin). Recently, a novel protein, Lim-domain only 7 (lmo7) has been reported to play a role in the molecular pathogenesis of EDMD. Prior in vitro and in vivo studies suggested the intriguing possibility that Lmo7 plays a role in skeletal or cardiac muscle pathophysiology. To further understand the in vivo role of Lmo7 in striated muscles, we generated a novel Lmo7-null (lmo7−/−) mouse line. Using this mouse line, we examined skeletal and cardiac muscle physiology, as well as the role of Lmo7 in a model of muscular dystrophy (EDMD). Using the dystrophin-deficient mdx mouse model. Our results demonstrated that lmo7−/− mice had no abnormalities in skeletal muscle morphology, physiological function, or regeneration. Cardiac function was also unaffected. Moreover, we found that ablation of lmo7 in dystrophin-deficient mdx mice had no effect on the observed myopathy and muscular regeneration exhibited by mdx mice. Molecular analyses also showed no changes in dystrophin complex factors, MAPK pathway components, and Emerin levels in lmo7 knockout mice. Taken together, we conclude that Lmo7 is dispensable for skeletal muscle and cardiac physiology and pathophysiology.

Lim-domain only 7; X-linked muscular dystrophy; skeletal muscle; cardiac muscle

MUSCULAR DYSTROPHIES COVER a genetically diverse group of inherited disorders. Afflicted patients may share a number of symptoms, such as loss of muscle function, defects in ambulation, posture, as well as cardiac and respiratory abnormalities (19, 20). A wide range of proteins are associated with muscular dystrophies, including plasma membrane, myofilamentous, and Z-line proteins. Recent studies have indicated that muscular dystrophies can be caused by a number of mutations in genes encoding proteins associated with the nuclear envelope (e.g., Lamin A/C, Emerin, and Nesprin encoded by lmaa, emd, and syne1, respectively) and their interacting protein partners (3, 6, 20, 30, 34, 35). Disease-causing mutations in the genes that encode these proteins alter the cellular functions of skeletal muscle (e.g., regeneration, force transmission) and cardiomyocytes, resulting in a variety of observed pathologies (3, 4, 11, 35). Indeed, understanding these proteins is key to unlocking their role(s) both in the physiology of skeletal and cardiac muscle, as well as in the pathology of muscular dystrophies.

Recently, a novel protein, Lim-domain only 7 (lmo7) has been reported to play a role in the molecular pathogenesis of muscular dystrophies (22). Lmo7 encodes three splice variants of calculated molecular weights of ~196 kDa (P200), of ~157 kDa (P150), and of ~100 kDa (P100) (23, 31). Lmo7 belongs to the single Calponin-homology (CH) domain-containing protein family, with a single actin-binding CH domain at the amino terminal end (13). In addition, Lmo7 also contains a PDZ domain and a carboxyl-terminal LIM domain, both of which have been described as protein-protein interacting domains (23).

Initial examination of the biological function of Lmo7 reported that this protein played a role in adhesion junction formation in epithelial cells (23). Holaska et al. (15) reported that Lmo7 directly interacts with the nuclear membrane protein Emerin and is required for emd expression. These data suggest that Lmo7 might be relevant in X-linked Emery-Dreifuss muscular dystrophy (EDMD).

Subsequent studies reported that Lmo7 is required for proper skeletal muscle differentiation (9). These studies suggested that Lmo7 functions by activating the expression of key myogenic differentiation genes (e.g., pax3, myoD) in C2C12 cells (9). Furthermore, these studies also suggested that Emerin binding to Lmo7 inhibits Lmo7 function as a transcription factor, important for skeletal muscle function (9). In vivo studies, using an engineered 800-kb deletion of ubiquitin COOH-terminal hydrolase L3 (uchl3) and lmo7 in mouse, resulted in defects including decreased viability, postnatal growth, and degeneration of muscle and retina (28).

Lmo7 has also been suggested to impact cardiac muscle function. Newborn et al. (21) reported that Lmo7 is misexpressed in the dilated cardiomyopathic heart of a patient with an lmaa-E161K laminopathy. In zebrafish, in vivo analyses of lmo7-morpholino knockdown manifest defects in heart development and functions including bradycardia, arrhythmia, and delocalization (24). These studies also raise the intriguing possibility that Lmo7 plays a role in skeletal and/or cardiac muscle pathophysiology.

Recently, Mull et al. (22) reported that lmo7 mutant mice exhibit an EDM-like phenotype. Despite the key findings of this study, the gene-trap system utilized was only shown to delete a single isoform of the gene. Therefore, we generated a novel lmo7-null (lmo7−/−) mouse with deletion of all three isoforms. Using these mice, we examined skeletal and cardiac muscle physiology, as well as the role of Lmo7 in a model of muscular dystrophy and regeneration using the dystrophin-deficient mdx mouse model (1, 6). Despite loss of all Lmo7 isoforms, our results demonstrated that lmo7−/− mice had no...
abnormalities in skeletal muscle morphology, physiological function, or regeneration. Cardiac function was also unaffected. Moreover, we found that dual lmo7−/−/mdx knockout (KO) had no effect on the observed myopathy and muscular regeneration exhibited by mdx mice. Molecular analyses also showed no changes in dystrophin glycoprotein complex (DGC) factors, MAPK pathways, and Emerin levels in our lmo7 KO model. Taken together, we conclude that Lmo7 is dispensable for skeletal muscle and cardiac physiology and pathophysiology.

MATERIALS AND METHODS

Gene targeting and generation of Lmo7 KO mice. lmo7 floxed mice were generated as described previously for other genes (25, 34). Briefly, genomic DNA was isolated from R1 embryonic stem (ES) cells and used to generate the lmo7-targeting construct containing loxP sites and a neomycin resistance gene (Fig. 1A). A pBluescript II KS+ vector and the 5' arm of homology consisting of a 3.5-kb NotI-NheI fragment were fused with the first loxP site upstream of Lmo7 exon 14, followed by neomycin flanked by FRT sites. The 3' arm of homology was a 4.1-kb SalI-Acc65I fragment located downstream of the second loxP site (Fig. 1A). Sequencing verification of the targeting construct was performed. The construct was then linearized with NotI and electroporated into R1 ES cells.

Southern blot confirmation of construct integration. Genomic DNA was extracted from G418-resistant ES cell clones, and Southern blot confirmation of target construct integration was performed as previously described (7). Briefly, ES cell DNA was digested with BamHI and analyzed by Southern blot analysis. A probe of 234-bp fragment.

Fig. 1. Generation of lmo7−/− mice. A: targeting strategy. A restriction map of the relevant genomic region of lmo7 (top), targeting construct (middle), and the mutated locus following recombination (bottom). DTA, diphtheria toxin A chain gene; Neo, neomycin resistance gene. B: detection of wild-type (WT) (+) and mutated (-) alleles by Southern blot analysis with the probe diagrammatically represented in A following digestion with BamHI. The 15-kb and 5-kb bands represent WT and mutated alleles, respectively. C: genotyping of lmo7 mutant mice by PCR analysis of DNA isolated from WT and knockout (KO) mouse tails. D: schematic diagram of Lmo7 protein domains depicting the locations of the epitopes of the NH2-terminal and COOH-terminal 863 antibodies used in E. E: Western blot analyses of lmo7 KO and WT adult mouse heart (10 µg protein loaded) using sera against the NH2-terminus of Lmo7 (left) or sera 863 against the COOH terminus of Lmo7 (right). Arrowheads indicate the different P100, P150, and P200 Lmo7 isoforms. IB, immunoblot. F: real-time PCR confirmation of global lmo7 deletion from gastrocnemius/soleus cDNA using bmo7 primer pair as detailed in Table 1. *P = 0.027 (n = 3).
was generated via PCR using mouse genomic DNA and specific lmo7 primers (forward: GCGAATCTGGAAGAGCTG; reverse: GTCGT; reverse: CCTGGGCAAAGAGAAATGTG) and KO allele-specific primers (forward: GCCAGAATCTAGTTGCTG; reverse: ACGAGTGCACACCCCTATTAA).

Animal procedures. All animal studies were performed using Laboratory Animal Care and Use Committee-approved protocols and conformed to the Guide for the Care and Use of Laboratory Animals, 8th Edition, published by the National Academies Press (US), 2011.

RT-PCR analyses. Total RNA was isolated from mouse skeletal muscle and heart tissue using Trizol reagent (Invitrogen) according to the manufacturer’s instructions. For RT-PCR analysis, first-strand cDNA synthesis was performed using random primer (Promega) and M-MLV Reverse Transcriptase (Promega). Primers for RT-PCR (Table 1) were designed and optimized using control murine cDNA as previously described (2). RT-PCR reactions were performed using Sso-Fast EvaGreen real-time PCR master mix in 96-well low-profile PCR plates in the CFX96 Bio-Rad thermocycler (Bio-Rad).

Protein isolation and Western blot analysis. Total protein extracts were prepared from whole heart and gastrocnemius/soleus skeletal muscle tissue with RIPA buffer (1.75 g NaCl, 2 ml NP-40, 1 g deoxycholic acid, 1 ml 20% SDS, and 6.7 ml 1.5 M Tris, pH 8, adjusted to 200 ml with H2O) containing protease inhibitor cocktail (Roche). Frozen tissue samples were weighed and suspended in 1:9 (wt:vol) of ice-cold RIPA buffer. Tissues were ground with a sterile plastic mini-pestle (Sigma) until the tissue was completely homogenized. Concentration of the protein samples was determined by Bradford dye method and facilitated fiber area quantification. Tile scan images were taken of the sections using a Leica DM6000 microscope (Leica Microsystems) equipped with a Leica DFC365 FX camera (Leica Microsystems) using a ×10 objective and DAPI and CX2 filter sets. Fiber cross-sectional areas (CSA) and number of fibers containing centralized nuclei were counted automatically using a custom-written macro in ImageJ (NIH) from 6–12 randomly selected fields per section. Filtering criteria were applied to ensure measurement of actual muscle fibers. Only regions of interest (ROIs) with fiber areas between 50 μm2 and 4,000 μm2 and circularity values >0.3 were measured to eliminate neurovascular structures and optically fused fibers. Once measured, the same ROIs were then assessed for the presence of centralized nuclei on the DAPI channel. A fiber was considered to contain a centralized nucleus if an area between 10 μm2 and 100 μm2 was contained within it and it did not make contact with the edge of the fiber.

Isometric stress. Isometric stress of the fifth toe muscle of the multibulled extensor digitorum longus (EDL) was measured as

<table>
<thead>
<tr>
<th>Table 1. Primer sequences used for PCR analysis</th>
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<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>lmo7</td>
</tr>
<tr>
<td>α-MHC</td>
</tr>
<tr>
<td>β-MHC</td>
</tr>
<tr>
<td>ANF</td>
</tr>
<tr>
<td>actα-1</td>
</tr>
<tr>
<td>proCol-1α1</td>
</tr>
<tr>
<td>proCol-3α1</td>
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<td>18S</td>
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ANF, atrial natriuretic factor; α-MHC, α-myosin heavy chain; β-MHC, β-myosin heavy chain; actα-1, α-skeletal actin 1; proCol-1α1, procollagen-α1 type I; proCol-3α1, procollagen-α1 type III.
described previously (33). Briefly, muscles were secured in a testing apparatus, and muscle length was adjusted by laser diffraction to a sarcomere length of ~3.0 μm. Maximum isometric tension was then measured by applying a 400-ms train of 0.3-ms pulses delivered at 100 Hz while muscle length was held constant. This measurement was repeated three times at 2-min intervals. This protocol was performed on both EDL per mouse, and the measurements were averaged. Muscle mass and slack fiber length were used to compute physiological cross-sectional area (PCSA) of each muscle to normalize the isometric tension values as previously described (27).

Echocardiography. Echocardiography was performed as previously described (37). Briefly, mice were anesthetized with 5% isoflurane for 30 s and then maintained on 0.5% isoflurane throughout analyses (Fig. 1C). Western blot analysis demonstrated that Lmo7 in vivo, we generated global lmo7-null mice by floxing exon 14, a shared exon containing 574 nucleotides within all isoforms, of the lmo7 gene (Fig. 1A). Deletion of exon 14 leads to a frame shift, resulting in a premature stop codon. Targeted ES cells were confirmed by Southern blot analyses (Fig. 1B). Heterozygous lmo7+/− mice were subsequently backcrossed 10 generations with C57BL/6J and then intercrossed to generate homozygous null mutant mice (lmo7−/−) (Fig. 1C). Western blot analysis demonstrated

**Table 2. Genotypes of offspring from lmo7+/− intercrosses show expected Mendelian ratios**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed (n)</th>
<th>Expected</th>
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<tr>
<td>lmo7+/+</td>
<td>20% (21/106)</td>
<td>25%</td>
</tr>
<tr>
<td>lmo7+/-</td>
<td>44% (47/106)</td>
<td>50%</td>
</tr>
<tr>
<td>lmo7−/−</td>
<td>36% (38/106)</td>
<td>25%</td>
</tr>
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</table>

Fig. 2. Loss of lmo7 does not alter growth, protein expression of dystrophin glycoprotein complex (DGC) components and Emerin, and MAPK signaling. A: growth curve. Body mass (g) of wild-type (WT) and lmo7 null (KO) mice was measured every week from the age of 4 wk to 1 yr. Data represent means ± SD (n ≥ 12). No significant change was observed for all time points. Western blot (B) and corresponding densitometric analysis (C) of DGC components in WT and KO gastrocnemius/soleus tissue lysates (n = 3 per genotype). GAPDH served as a loading control. D: Western blot analysis of Emerin and phospho-ERK1/2 (pERK1/2) in WT and KO gastrocnemius/soleus tissue lysates (n = 3). GAPDH served as a loading control. E and F: densitometric analysis of Emerin and pERK1/2, respectively.
that deletion of exon 14 successfully targeted the deletion of the three Lmo7 isoforms, ∼200 kDa (P200), ∼150 kDa (P150), and ∼100 kDa (P100), in lmo7 KO mice (Fig. 1E).

In WT adult heart, the NH2-terminal antibody detects all three Lmo7 isoforms, albeit the P100 isoform at a very low level, whereas the COOH-terminal antibody only detected isoforms P200 and P150 (Fig. 1, D and E). Unfortunately, Western blot analysis using gastrocnemius/soleus skeletal muscle tissue samples did not work with the Lmo7 antibodies. However, in consistence with our Western blot

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**Fig. 3.** Skeletal muscle morphology of WT, Lmo7 knockout (KO), mdx, and double KO (DKO) mice. Histological hematoxylin and eosin (H and E) staining of cross sections of tibialis anterior of 3.5-wk-old (A) and 6-wk-old (B) WT, Lmo7 knockout (KO), mdx, and DKO mice. Asterisks indicate areas of degeneration with inflammatory cell infiltration accompanied by regeneration of centrally nucleated fibers (arrowheads). Arrow indicates eosinophilic fiber. Scale bar = 100 μm. C: histological H and E staining of longitudinal sections of the tibialis anterior of 6-mo-old WT and lmo7 KO mice. Scale bar ×20 = 100 μm, ×40 = 50 μm.
data of whole heart, no Lmo7 mRNA was detected in lmo7 KO adult gastrocnemius/soleus skeletal muscle (Fig. 1F).

lmo7−/− mice have normal growth patterns and survival rates. lmo7−/− mice were born at expected Mendelian ratios, were fertile, and survived until adulthood (Table 2). A previous study reported that lmo7-null mice exhibit growth retardation with a reduced body mass evident at 24 wk of age (22). Thus we monitored the body mass of WT and lmo7−/− mice from the age of 4–52 wk. No significant difference in body mass was observed over the examined time course (Fig. 2A). Together, these data suggest that loss of lmo7 did not alter growth or survival in our murine model.

lmo7−/− mice display normal protein expression levels of DGC components and Emerin and unaltered MAPK signaling. Lmo7 has previously been shown to colocalize with dystrophin (9), suggesting a potential role for Lmo7 in the regulation of dystrophin and/or other DGC components. The expression level of dystrophin appeared normal in skeletal muscle of lmo7−/− mice compared with WT controls (Fig. 2, B and C). Furthermore, loss of Lmo7 did not affect the expression of the DGC components, β-dystroglycan and β-sarcoglycan (Fig. 2, B and C). These data suggest that Lmo7 is dispensable for DGC protein expression.

Lmo7 has been reported to directly interact with the nuclear membrane protein, Emerin, as well as regulate Emerin expression (15). However, we observed no significant difference in Emerin protein expression between lmo7−/− KO and WT skeletal muscle (Fig. 2, D and E).

It has also been recently reported that Lmo7 activates MAPK signaling in mouse skeletal muscle (22). Thus we
examined the phosphorylation of the classical MAP kinases, ERK1/2, in our lmo7-null model (Fig. 2, D and F). We found no significant change in the phosphorylation of ERK1/2 in lmo7 KO mouse skeletal muscle compared with WT controls. These data suggest that Lmo7 is dispensable for both Emerin protein expression and ERK1/2 activation.

Loss of lmo7 does not cause skeletal muscle morphological abnormalities or alter those observed in the dystrophin-deficient (mdx) model. It has been reported that Lmo7 is expressed in myogenic precursors and that Lmo7 is required for proper myoblast differentiation (9). Thus we next examined whether Lmo7 affects muscle regeneration, which is mediated by satellite cells (18). A model used to study muscle regeneration in vivo is the dystrophin-deficient mdx mouse, an X-chromosome-linked muscular dystrophy mouse model (1, 6, 26, 29). Briefly, the mdx mouse carries a spontaneous mutation in exon 23 of the dystrophin gene, introducing a premature stop codon (5, 29). The pathology of the mdx mouse is characterized by histologically well-defined stages. Necrotic or apoptotic processes in combination with inflammation emerge at ~3 wk of age (5). Regeneration of affected muscle tissue is most prominent from the age of 6 wk and continues while alternating with ongoing degeneration (1, 8).

To investigate the role of Lmo7 in muscle regeneration, we crossed lmo7<sup>−/−</sup> with the mdx mouse model to generate the lmo7<sup>−/−</sup>;mdx double KO (DKO) mouse. DKO mice were viable, and their appearance was indistinguishable from WT, lmo7<sup>−/−</sup>, and mdx mice (data not shown). To investigate the general morphology, cross sections of TA muscles were stained with hematoxylin and eosin (H and E). No morphological abnormalities were observed in skeletal muscle of lmo7<sup>−/−</sup> compared with WT (Fig. 3, A–C).

Prior observation (5) and our data demonstrate that, at 3.5 wk, mdx murine skeletal muscles exhibit focal areas of fiber degeneration (necrosis, apoptosis) with inflammatory cell infiltration (Fig. 3A). Moreover, these mice present with eosinophilic fibers accompanied by fiber regeneration, characterized by the presence of centrally nucleated fibers (Fig. 3A). Upon examination, DKO mice at 3.5 wk of age were observed to have skeletal muscle morphology (eosinophilic fibers, centralized nuclei) similar to that of mdx murine skeletal muscles (Fig. 3A). Furthermore, at 6 wk of age, mdx and DKO muscles also showed similar phenotypic morphology (Fig. 3B), as well as a marked increase in centralized nuclei fibers compared with those at 3.5 wk. Taken together, these data indicate that lmo7 loss does not cause muscle abnormalities or alter the mdx phenotype.

Loss of lmo7 does not affect skeletal muscle fiber CSA or fiber size distribution. Even if the regeneration process is not affected by Lmo7, it is possible that fiber growth and maturation, which is also dependent on satellite cells, could be affected. Thus we investigated the fiber size and fiber size distribution of TA and soleus muscles within all four experimental groups. No significant differences in average fiber CSA of TA (Fig. 4A) and soleus (Fig. 4B) muscle were observed among all four groups. Furthermore, analyses of lmo7<sup>−/−</sup> fiber size distribution in TA and soleus (Fig. 4E) yielded identical distributions to those of WT control (Fig. 4C). In both WT and lmo7<sup>−/−</sup>, the soleus showed a more restricted fiber size distribution compared with TA muscles. As expected, fiber size distribution was significantly altered in mdx and DKO mice compared with WT and lmo7<sup>−/−</sup> controls. Indeed, mdx and DKO have a wider range of fiber sizes and a drastic increase of small fibers in both TA and soleus muscles. Interestingly, fiber size distribution pattern was not changed between mdx and DKO mice (Fig. 4, D and F). Taken together, these data demonstrate that Lmo7 is dispensable for establishing both fiber size and fiber size distribution.

Loss of lmo7 does not alter the percentage of centralized nuclei. Prior clinical investigations demonstrated that the percentage of centralized nuclei is directly related to muscle fiber regeneration (16, 32). Analyses in murine and human models supports the notion that centralization of nuclei reveals that fiber regeneration has taken place (17, 36). Thus, to investigate the regeneration history in our experimental conditions, the percentage of centralized nuclei was determined (Fig. 5, A and B). In WT mice, ~3–4% of nuclei were centralized in both TA and soleus muscles. No significant difference was observed between WT and lmo7<sup>−/−</sup> mice. mdx mice have been shown to have increased centralized nucleated muscle fibers compared with WT controls (1, 6, 8). Examination of mdx and DKO mice found that lmo7 loss did not alter the observed percentages (~60% in TA and ~40% in soleus) between these two models. These data indicate that Lmo7 is dispensable for nuclei centralization and thus, presumably, fiber regeneration.

Loss of lmo7 does not impact isometric stress production within skeletal muscle. To gain insights into a potential physiological effect of Lmo7, we investigated the force-generating capacity of EDL muscles of experimental groups. Examination
of these data revealed no significant difference in isometric stress production between WT and lmo7−/− mice. mdx mice were observed in prior studies to have lower isometric stress compared with WT controls (12). Indeed, we observed lower isometric stress production between these models. The lower stresses are, in part, a byproduct of the fact that both mdx and DKO muscles have increased noncontractile tissue and thus weigh more, which drives up their PCSA value, leading to a decreased stress calculation. Examination of mdx and DKO mice, however, showed no significant differences in isometric stress production between these models. Together, these data find Lmo7 dispensable for isometric stress production within skeletal muscle.

lmo7−/− mice do not exhibit altered adult cardiac function. Prior studies found that loss of lmo7 leads to decreased cardiac function (22). Thus we sought to determine the effect of lmo7 loss on cardiac function in our KO model. Echocardiographic analyses of lmo7−/− mice at 6, 9, and 12 mo of age did not demonstrate any significant difference in left ventricular chamber dimensions and fractional shortening (Table 3). Furthermore, real-time PCR analyses did not show significant changes in cardiac fetal gene and fibrosis markers at 12 mo of age (Fig. 6, A–F). Moreover, we also performed surface electrocardiogram analysis on lmo7−/− and WT control mice and did not observe any electrophysiological abnormalities (data not shown). Together, these data indicate that Lmo7 is dispensable for basal cardiac function.

**DISCUSSION**

Characterization of factors that drive muscular dystrophies are critical for understanding both disease development and progression. A key group of proteins associated with muscular dystrophies are those localized to the nuclear membrane (e.g., Emerin, Lamin A/C, and Nesprin) and their interacting partners (3, 6, 20, 30, 34, 35). Recent studies have identified a novel protein, Lmo7, which is suggested to play a role in muscular dystrophies are critical for understanding both disease development and progression. A key group of proteins associated with muscular dystrophies are those localized to the nuclear membrane (e.g., Emerin, Lamin A/C, and Nesprin) and their interacting partners (3, 6, 20, 30, 34, 35). Recent studies have identified a novel protein, Lmo7, which is suggested to play a role in muscular dystrophy (15, 28). Mull et al. (22) reported that lmo7−/− mutant mice have an EDMD-like phenotype. Their lmo7−/− mutant mice presented with growth retardation, decreased fiber size, impaired neuromuscular functions, and cardiac impairment. At the molecular level, this model was shown to have lower levels of phospho-MAPK (22). Examination of these data, however, indicate the deletion of only one isoform of Lmo7 would be sufficient to cause these phenotypes.

### Table 3. Echocardiographic measurements

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<tr>
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<th>6 Months</th>
<th>9 Months</th>
<th>12 Months</th>
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<tr>
<td></td>
<td>WT</td>
<td>Lmo7 KO</td>
<td>WT</td>
</tr>
<tr>
<td>BW, g</td>
<td>26 ± 2</td>
<td>28 ± 3</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>565 ± 43</td>
<td>573 ± 50</td>
<td>557 ± 70</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.60 ± 0.32</td>
<td>3.59 ± 0.28</td>
<td>3.59 ± 0.18</td>
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<tr>
<td>LVIDs, mm</td>
<td>2.40 ± 0.38</td>
<td>2.39 ± 0.32</td>
<td>2.24 ± 0.16</td>
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<tr>
<td>IVSd, mm</td>
<td>0.75 ± 0.06</td>
<td>0.73 ± 0.08</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.69 ± 0.04</td>
<td>0.7 ± 0.09</td>
<td>0.7 ± 0.07</td>
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<tr>
<td>FS, %</td>
<td>33.5 ± 5.9</td>
<td>33.5 ± 5.7</td>
<td>37.5 ± 4.1</td>
</tr>
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</table>

Values are means ± SD in wild-type (WT) and lmo7−/− knockout (KO) mice; n = 9. BW, body weight; HR, heart rate; LVID, left ventricular (LV) inner diameter; IVS, interventricular septum; LVPW, LV posterior wall thickness; FS, fractional shortening; d, diastole; s, systole.
lmo7. It has been reported that there are multiple mouse Lmo7 isoforms encoded by the single lmo7 gene (23, 31). Therefore, to understand the role of this factor in physiology and pathophysiology, we generated our own lmo7\(^{-/-}\) mouse model, resulting in deletion of all three isoforms. In contrast to prior studies, our lmo7\(^{-/-}\) mouse model did not demonstrate any abnormalities in skeletal muscle, cardiac function, or molecular impairment of key pathways.

Previous studies of lmo7 loss suggested postnatal growth retardation, a common muscular dystrophic phenotype as a result of degenerating muscles (22, 28). Growth analyses of our lmo7-null mice observed no defects or growth retardation. Previous studies also suggested that loss of Lmo7 led to decreased fiber size and impaired neuromuscular functions (22). Histological examination of skeletal muscle in our lmo7\(^{-/-}\) mice showed that fiber size average and fiber size distribution were not changed, indicating that loss of Lmo7 allows normal muscle fiber size development. Furthermore, assessment of skeletal muscle physiological function, via isometric stress measurements, showed no significant change in response to loss of lmo7. In contrast to previous studies, our data indicate that Lmo7 is dispensable for skeletal muscle function and does not drive a skeletal myopathic phenotype.

In addition to skeletal defects, cardiac functional impairment is present within muscular dystrophies (20). Prior studies have shown that Lmo7 can impact cardiac function in both murine and zebrafish models (22, 24). Thus we examined the role of Lmo7 in cardiac function. Via echocardiography, we monitored cardiac function of our lmo7\(^{-/-}\) mice until 1 yr of age. Physiological analyses found no significant difference in cardiac function within our lmo7 loss model. Molecular analyses also found no significant changes in expression of cardiac fetal gene and fibrosis markers at 12 mo of age. Together, these data demonstrate that Lmo7 is dispensable for proper cardiac function.

An additional hallmark of skeletal myopathies is dysregulation of skeletal muscle regeneration (5, 26). Dedecic et al. (9) reported that Lmo7 was required for proper myoblast differentiation in vitro. Thus we investigated the role of Lmo7 in muscle regeneration. Examination of WT, lmo7-null, mdx, and DKO mice found no significant differences in fiber size distribution pattern or percentage of fibers with centralized nuclei between mdx and DKO mice. These data strongly suggest that Lmo7 does not play an important role in muscle regeneration.

Despite the lack of an in vivo or in vitro phenotype, we also sought to rule out a molecular phenotype, given that Lmo7 has functions in a number of key pathways. Indeed, Holaska et al. (15) reported that Lmo7 is required for Emerin expression. Thus we investigated Emerin expression level in our lmo7\(^{-/-}\) mice. Interestingly, we did not observe any significant difference in Emerin expression between lmo7\(^{-/-}\) and WT mice, suggesting that Lmo7 is not required for Emerin expression. Prior studies also observed changes in the phospho-MAPK levels. Unlike Mull et al. (22), we did not observe any significant difference in phospho-MAPK level. Prior studies also found that Lmo7 colocalized with dystrophin complex members, suggesting a role of Lmo7 in regulating this complex (9). Examination of this complex in our lmo7-deficient model found no change in expression of the members of this complex (dystrophin, β-dystroglycan, and β-sarcoglycan). Taken together, our data find that Lmo7 is not required for the regulation of these key pathways.

Recently, lmo7\(^{-/-}\) mutant mice were described to exhibit an EDMD-like phenotype (22). These findings are difficult to explain in light of our current investigation. However, some differences in the analyses are evident and should be pointed out. 1) Our mutant strain is on the C57BL/6J genetic background, and the mutant strain from Mull et al. (22) is on the C57BL/6N genetic background. 2) Our Lmo7 KO mice were generated by gene-targeting approach, whereas Mull and colleagues used the gene-trap methodology. 3) In the current study, between 12 and 24 mice were used to monitor growth, whereas significantly fewer mice were used by Mull et al. (22), potentially amplifying minor differences. 4) In addition, it is possible that differences in observed phenotypes between the two lmo7\(^{-/-}\) mutant mouse lines might be a result from variables such as environment, diet, and infectious state, which may differ between the two mouse-housing facilities. Taken together, these differences might culminate in substantial differences in the perception of Lmo7 phenotype and in a rather different interpretation. Despite these deviations, our current investigation found that lmo7\(^{-/-}\) mice exhibit no observable changes in skeletal muscle and no basal heart phenotype. Thus we conclude that Lmo7 is dispensable for skeletal muscle and basal cardiac function.

ACKNOWLEDGMENTS

We are grateful to Dr. Jun Miyoshi (Osaka, Japan) for the generous gift of the sera 863 against the Lmo7 COOH terminus.

GRANTS

J. Chen is funded by grants from the National Heart, Lung, and Blood Institute, and the National Institute of Arthritis and Musculoskeletal and Skin Diseases. J. Chen is an American Heart Association Endowed Chair. R. Lieber is funded by NIH grants AR40050, R24HD050837, and P30AR061303 and the Department of Veterans Affairs. The UCSD School of Medicine Microscopy Core is supported by grant no. NS047101.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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