Laminin α1 reduces muscular dystrophy in *dy*^{2J} mice

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Abstract

Muscular dystrophies, including laminin α2 chain-deficient muscular dystrophy (LAMA2-CMD), are associated with immense personal, social and economic burdens. Thus, effective treatments are urgently needed. LAMA2-CMD is either a severe, early-onset condition with complete laminin α2 chain-deficiency or a milder, late-onset form with partial laminin α2 chain-deficiency. Mouse models *dy*^{3K}/*dy*^{3K} and *dy*^{2J}/*dy*^{2J}, respectively, recapitulate these two forms of LAMA2-CMD very well. We have previously demonstrated that laminin α1 chain significantly reduces muscular dystrophy in laminin α2 chain-deficient *dy*^{3K}/*dy*^{3K} mice. Among all the different pre-clinical approaches that have been evaluated in mice, laminin α1 chain-mediated therapy has been shown to be one of the most effective lines of attack. However, it has remained unclear if laminin α1 chain-mediated treatment is also applicable for partial laminin α2 chain-deficiency. Hence, we have generated *dy*^{2J}/*dy*^{2J} mice (that express a substantial amount of an N-terminal truncated laminin α2 chain) overexpressing laminin α1 chain in the neuromuscular system. The laminin α1 chain transgene ameliorated the dystrophic phenotype, restored muscle strength and reduced peripheral neuropathy. Thus, these findings provide additional support for the development of laminin α1 chain-based therapy for LAMA2-CMD.

Introduction

Congenital muscular dystrophy with laminin α2 chain-deficiency (LAMA2-CMD) is a severe form of muscular dystrophy caused by mutations in the *LAMA2* gene encoding laminin α2 chain. Together with β1 and γ1 chains, laminin α2 forms the heterotrimeric protein laminin-211 that is highly expressed in skeletal muscle, heart and Schwann cell basement membranes. The biological functions of laminin α2 chain are to a large extent exerted by its binding to cell surface receptors on muscle and Schwann cells and major receptors are dystroglycan and integrin α7β1. By genotype-phenotype correlations it has been established that complete lack of laminin α2 chain leads to severe muscular dystrophy whereas partial absence leads to a milder disease course. The clinical manifestations of complete laminin α2 chain-deficiency include marked hypotonia at birth, widespread muscle weakness, proximal joint contractures, scoliosis, delayed motor milestones and respiratory failure. Individuals with partial laminin α2 chain-deficiency often have later onset of proximal muscle weakness and delayed motor milestones, but unlike patients with complete deficiency, they achieve independent ambulation [1–5].

There are mouse models for laminin α2 chain-deficiency that satisfactorily represent the clinical heterogeneity of LAMA2-CMD. The *dy*^{3K}/*dy*^{3K} mouse completely lacks laminin α2 chain and is the most severely affected LAMA2-CMD mouse model with a median survival of three weeks. In contrast, the *dy*^{2J}/*dy*^{2J} mouse model has slightly reduced expression of a truncated laminin α2 chain that lacks the N-terminal domain involved in laminin polymerization. Accordingly, *dy*^{2J}/*dy*^{2J} mice display relatively mild muscular dystrophy and survival rate of several months [4].

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Lama2 mutations also interrupt peripheral myelination as immature Schwann cells lacking laminin α2 chain fail to proliferate and differentiate correctly during axonal sorting. Therefore, nerves in dy^{3K}/dy^{3K} and dy^{2J}/dy^{2J} mice (as well as other LAMA2-CMD mouse models) contain large bundles of amyelinated axons and display peripheral neuropathy [6–8]. Hind leg lameness is particularly evident in the dy^{2J}/dy^{2J} mouse model as these mice have longer life span compared to other LAMA2-CMD mouse models.

Several different approaches to prevent muscular dystrophy have been tested in animal models for LAMA2-CMD [9–29]. Among these approaches, laminin α1 chain overexpression in dy^{3K}/dy^{3K} mice constitutes one of the most complete rescues of dystrophic and LAMA2-CMD-associated symptoms in multiple tissues (skeletal muscle, heart and peripheral nerve), with maintenance of good health of animals and a near normal life span (up to 2 years versus three weeks for dy^{3K}/dy^{3K} mice) [11–13,30–32]. However, whether transgenic laminin α1 chain also reduces the milder form of LAMA2-CMD in dy^{2J}/dy^{2J} mice, in which laminin α2 chain is still expressed, remains unclear. It might be that the transgenically expressed laminin α1 chain is not able to compete with the defective endogenous truncated form of laminin α2 chain for heterotrimeric chain assembly. Therefore, we have generated dy^{2J}/dy^{2J} mice overexpressing laminin α1 chain and demonstrate that laminin α1 chain significantly decreases disease severity.

Results

Localization of basement membrane components in dy^{2J}/LMα1 muscles

To generate dy^{2J}/dy^{2J} mice overexpressing laminin α1 chain we bred the previously described laminin α1 transgenic mice (line 12 with β-actin promoter) [11] with heterozygous $dy^{2J}/+$ mice. Dy^{2J}/dy^{2J} animals carrying the transgene (denoted dy^{2J}/LMα1) displayed robust laminin α1 chain expression in the skeletal muscle basement membrane, neuromuscular junction, myotendinous junction and in the cardiac basement membrane (Fig. 1 and data not shown). A more patchy expression of laminin α1 chain was noted in the sciatic nerve as earlier reported for transgenic line 12 [12] (Fig. 1). We have previously demonstrated that laminin α1 chain is robustly overexpressed and assembled into a laminin-111 heterotrimer in skeletal muscle of dystrophin-deficient *mdx* mice that express normal levels of laminin α2 chain [31]. Altogether, these data indicate that laminin α1 chain is able to compete with the defective endogenous truncated form of laminin α2 chain for heterotrimeric laminin-111 assembly.

Subsequently, we analyzed if overexpression of laminin α1 chain affected the expression of other laminin chains and integrin α7β1. Laminin α2 chain expression in dy^{2J}/dy^{2J} muscle was variable: stronger in dystrophic areas and somewhat weaker, yet not drastically decreased in healthier muscle areas (Fig. 2, a laminin α2 chain antibody against L4b domain (N-terminus) was used). However, we noted no obvious difference in laminin α2 chain expression between wild-type and dy^{2J}/LMα1 muscles (triceps is shown in Fig. 2; quadriceps, hamstring and diaphragm: data not shown). We observed a similar pattern using another laminin α2 chain antibody raised against globular domains 1–3 at the C-terminus region of the molecule (data not shown). Also, laminin γ1 chain was expressed in similar amounts (Fig. 2). It is well established that laminin α4 chain expression is enhanced at the sarcolemma in LAMA2-CMD [11,17,33] and we found a partially normalized expression in dy^{2J}/LMα1 muscle (Fig. 2). Similarly, laminin α5 chain is upregulated upon laminin α2 chain loss/reduction [10,11,33] and we also noted its decreased expression in dy^{2J}/LMα1 muscle at the sarcolemma (laminin α4 and α5 subunits are normally expressed in blood vessels within muscle tissue) (Fig. 2). Laminin β2 chain, on the other hand, has been shown to be vastly reduced in skeletal muscle with complete laminin α2 chain-deficiency and moderately reduced in patients with partial laminin α2 chain-deficiency [11,34]. In dy^{2J}/dy^{2J} and dy^{2J}/LMα1 muscle we found similar expression of laminin β2 chain (Fig. 2). Integrin α7B has been shown to be greatly reduced in LAMA2-CMD with complete laminin α2 chain-deficiency [35–37] but in dy^{2J}/dy^{2J} muscle, integrin α7B expression was reported to be highly irregular and variable [37]. Indeed, we noted uneven integrin α7B staining in dy^{2J}/dy^{2J} muscle fibers that was either negative, weakly positive or strongly positive and a similar expression pattern was found in dy^{2J}/LMα1 skeletal muscle (Fig. 2).

Since polymerizing forms of laminin are present in dy^{2J}/LMα1 muscle due to laminin α1 overexpression, we anticipated restoration of basement membranes. Muscle ultrastructure was examined by electron microscopy. In agreement with previous studies [16], basement membranes in 8-week-old dy^{2J}/dy^{2J} muscle were absent or had a patchy appearance. Notably, the laminin α1 transgene greatly restored the basement membrane (Fig. 3).

Laminin α1 chain improves function of the neuromuscular system in dy^{2J}/dy^{2J} mice

We next assessed the overall health status of dy^{2J}/LMα1 animals by investigating if laminin α1 chain contributed to enhanced body weight, improved locomotive behavior and increased muscle strength. As shown in Fig. 4A and Videos 1 and 2, 8-week-old dy^{2J}/LMα1 mice could not be outwardly distinguished from wild-type littermate controls, while dy^{2J}/dy^{2J} mice were easily distinguishable from control mice

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