

mouse with accompanying loss of all of the sarcoglycans as well as sarcospan, with at least minor preservation of merosin, the dystroglycans and dystrophin, reproducing the clinical picture seen in LGMD2E. The histological changes in this animal model were also a prototype for the clinical counterpart, including the prominence of skeletal muscle fibrosis.(14) Dressman et al. (25) injected the transverse abdominal muscle using rAAV2.CMV.SGCB. Expression persisted for 21 months and muscle fibers were protected from recurrent necrosis. The use of self-complementary AAV to enhance transgene expression,16 a muscle-specific promoter to better target skeletal muscle (20, 26) and the optimization of a human β -sarcoglycan gene (hSGCB) has also been described.

[0009] Functional improvement in patients suffering from LGMD and other muscular dystrophies require both gene restoration and reduction of fibrosis. There is a need for methods of reducing fibrosis that may be paired with gene restoration methods for more effective treatments of LGMD and other muscular dystrophies.

SUMMARY

[0010] Described herein are gene therapy vectors, e.g. AAV, expressing the β -sarcoglycan gene and methods of delivering β -sarcoglycan to the muscle to reduce and/or prevent fibrosis; and/or to increase muscular force, and/or to treat a β -sarcoglycanopathy in a mammalian subject suffering from muscular dystrophy.

[0011] In addition, the disclosure provides for combination therapies and approaches using gene therapy vectors to deliver β -sarcoglycan to address the gene defect observed in LGMD2E and gene therapy vectors delivering miR-29 to further suppress fibrosis.

[0012] In one aspect, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan. In some embodiments, the polynucleotide sequence encoding β -sarcoglycan comprises a sequence e.g. at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence set forth in SEQ ID NO: 1 and encodes protein that retains β -sarcoglycan activity. In some embodiments, the polynucleotide sequence encoding β -sarcoglycan comprises the nucleotide sequence set forth in SEQ ID NO: 1. In some embodiments, the polynucleotide sequence encoding β -sarcoglycan consists the nucleotide sequence set forth in SEQ ID NO: 1.

[0012a] In one aspect, the present disclosure refers to a composition comprising the recombinant AAV vector as described herein. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in the manufacture of a medicament for treating muscular dystrophy in a subject. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in the manufacture of a medicament for increasing muscular force and/or muscle mass in a mammalian subject suffering from muscular dystrophy. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in the manufacture of a medicament for reducing fibrosis in a subject suffering from muscular dystrophy. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in the manufacture of a medicament for reducing contraction-induced injury in a subject suffering from muscular dystrophy. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in the manufacture of a medicament for treating β -sarcoglycanopathy in a subject.

[0012b] In a further aspect, the present disclosure refers to a composition comprising the recombinant AAV vector as described herein for use in therapy for reducing fibrosis in a mammalian subject in need thereof. In a still further aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein or the composition as described herein in combination with a second recombinant AAV vector comprising a polynucleotide sequence comprising miR29C in the preparation of a combination therapy for reducing fibrosis in a mammalian subject in need thereof.

[0013] In another example, a recombinant AAV vector described herein comprises a polynucleotide sequence encoding β -sarcoglycan that is at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least 90%, 91%, 92%, 93%, or 94% and even more typically at least 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO: 2, and the protein retains β -sarcoglycan activity.

[0014] In another example, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding functional β -sarcoglycan that comprises a nucleotide

sequence that hybridizes under stringent conditions to the nucleic acid sequence of SEQ ID NO: 1, or a complement thereof.

[0015] The term “stringent” is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989). More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6x SSC 0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

[0016] Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO₄, (SDS), ficoll, Denhardt’s solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid Hybridisation: A Practical Approach, Ch. 4, IRL Press Limited (Oxford, England). Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids.

[0017] In another example, the recombinant AAV vectors described herein may be operably linked to a muscle-specific control element. For example the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, muscle creatine kinase (MCK), tMCK (truncated MCK), myosin heavy chain (MHC), MHCK7 (a hybrid version of MHC and MCK), C5-12

(synthetic promoter), murine creatine kinase enhancer element, skeletal fast-twitch troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypoxia-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

[0018] In some embodiments, the muscle-specific promoter is MHCK7 (SEQ ID NO: 4). An exemplary rAAV described herein is pAAV.MHCK7.hSCGB which comprises the nucleotide sequence of SEQ ID NO: 3; wherein the MCHK7 promoter spans nucleotides 130-921, a SV40 chimeric intron spans nucleotides 931-1078, the β -sarcoglycan sequence spans nucleotides 1091-2047 and the poly A spans nucleotides 2054-2106.

[0019] In some embodiments, the muscle-specific promoter is tMCK (SEQ ID NO: 6). An exemplary rAAV described herein is pAAV.tMCK.hSCGB which comprises the nucleotide sequence of SEQ ID NO: 5; wherein the tMCK promoter spans nucleotides 141-854, an SV40 chimeric intron spans nucleotides 886-1018, the β -sarcoglycan sequence spans nucleotides 1058-2014 and the poly A spans nucleotides 2021-2073.

[0020] The AAV can be any serotype, for example AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., Molecular Therapy, 22(11): 1900-1909 (2014).

[0021] Compositions comprising any of the rAAV vectors described herein are also contemplated.

[0022] Methods of producing a recombinant AAV vector particle comprising culturing a cell that has been transfected with any recombinant AAV vector described herein and recovering recombinant AAV particles from the supernatant of the transfected cells are also provided. Viral particles comprising any of the recombinant AAV vectors described herein are also contemplated

[0023] Methods of reducing fibrosis in a mammalian subject in need thereof is also provided. In this regard, the method comprises administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject. In some embodiments, the mammalian subject suffers from muscular dystrophy. In some embodiments, administration of an AAV vector described herein (or composition comprising an AAV vector described herein) reduces

fibrosis in skeletal muscle or in cardiac muscle of the subject. These methods may further comprise the step of administering a second recombinant AAV vector comprising a polynucleotide sequence comprising miR29C.

[0024] The term “muscular dystrophy” as used herein refers to a disorder in which strength and muscle bulk gradually decline. Non-limiting examples of muscular dystrophy diseases may include Becker muscular dystrophy, tibial muscular dystrophy, Duchenne muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, sarcoglycanopathies, congenital muscular dystrophy such as congenital muscular dystrophy due to partial LAMA2 deficiency, merosin-deficient congenital muscular dystrophy, type 1D congenital muscular dystrophy, Fukuyama congenital muscular dystrophy, limb-girdle type 1A muscular dystrophy, limb-girdle type 2A muscular dystrophy, limb-girdle type 2B muscular dystrophy, limb-girdle type 2C muscular dystrophy, limb-girdle type 2D muscular dystrophy, limb-girdle type 2E muscular dystrophy, limb-girdle type 2F muscular dystrophy, limb-girdle type 2G muscular dystrophy, limb-girdle type 2H muscular dystrophy, limb-girdle type 2I muscular dystrophy, limb-girdle type 2I muscular dystrophy, limb-girdle type 2J muscular dystrophy, limb-girdle type 2K muscular dystrophy, limb-girdle type IC muscular dystrophy, rigid spine muscular dystrophy with epidermolysis bullosa simplex, oculopharyngeal muscular dystrophy, Ullrich congenital muscular dystrophy, and Ullrich scleroatonic muscular dystrophy. In some embodiments, the subject is suffering from limb-girdle muscular dystrophy. In some embodiments, the subject is suffering from limb-girdle muscular dystrophy type 2E (LGMD2E).

[0025] The term “fibrosis” as used herein refers to the excessive or unregulated deposition of extracellular matrix (ECM) components and abnormal repair processes in tissues upon injury including skeletal muscle, cardiac muscle, liver, lung, kidney, and pancreas. The ECM components that are deposited include collagen, e.g. collagen 1, collagen 2 or collagen 3, and fibronectin.

[0026] In another example, described herein is a method of increasing muscular force and/or muscle mass in a mammalian subject comprising administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject.

[0027] In any of the methods of the disclosure, the subject may be suffering from muscular dystrophy such as limb-girdle muscular dystrophy or any other dystrophin-associated muscular dystrophy.

[0028] Also provided is a method of treating muscular dystrophy in a mammalian subject comprising administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject. In some embodiments, the muscular dystrophy is limb-girdle muscular dystrophy. Any of the methods described herein may further comprise the step of administering a second recombinant AAV vector comprising a polynucleotide sequence comprising miR29C.

[0029] Combination therapies are also contemplated. In this regard, any of the foregoing methods described here may further comprise administering a second recombinant AAV vector comprising a polynucleotide sequence comprising miR29C. In some embodiments, the polynucleotide comprising miR29C is operably linked operably linked to a muscle-specific control element. For example the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, muscle creatine kinase (MCK), tMCK (truncated MCK), myosin heavy chain (MHC), MHCK7 (a hybrid version of MHC and MCK), C5-12 (synthetic promoter), murine creatine kinase enhancer element, skeletal fast-twitch troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypoxia-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE). In some embodiments, the second recombinant vector comprises a polynucleotide sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 8, as described in U.S. Provisional Application No. 62/323,163 (the disclosure of which is incorporated herein by reference in its entirety).

[0030] In combination therapy methods described herein in which both an rAAV vector expressing β -sarcoglycan and an rAAV vector expressing miR29c are administered to the mammalian subject, the rAAV vectors may be administered concurrently, or administered consecutively with the rAAV vector expressing β -sarcoglycan being administered immediately before or after the rAAV expressing miR29c. Alternatively, the AAV vector expressing β -sarcoglycan is administered within about 1-24 hours (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours) after administering the rAAV expressing miR-29 or the methods of the disclosure are carried out wherein the AAV vector expressing the β -sarcoglycan is administered within about 1-24 hours (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours) before

administering the rAAV expressing miR-29. In some embodiments, the AAV vector expressing β -sarcoglycan is administered within about 1-5 hours (e.g., 1, 2, 3, 4 or 5 hours) after administering the rAAV expressing miR-29 or the methods of the disclosure are carried out wherein the AAV vector expressing the β -sarcoglycan is administered within about 1-5 hours (e.g., 1, 2, 3, 4 or 5 hours) before administering the rAAV expressing miR-29c.

[0031] In any of the methods of the disclosure, the rAAV is administered by intramuscular injection or intravenous injection. In addition, in any of the method of the disclosure, the rAAV is administered systemically, such as parental administration by injection, infusion or implantation.

[0032] The compositions of the disclosure are formulated for intramuscular injection or intravenous injection. In addition, the compositions of the disclosure are formulated for systemic administration, such as parental administration by injection, infusion or implantation.

[0033] In addition, any of the compositions formulated for administration to a subject suffering from muscular dystrophy (such as limb-girdle muscular dystrophy or any other dystrophin-associated muscular dystrophy). In some embodiments, the composition may further comprise a second recombinant AAV vector comprising a polynucleotide sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 8.

[0034] In any of the uses of the disclosure, the medicament is formulated for intramuscular injection or intravenous injection. In addition, in any of the uses of the disclosure, the medicament is formulated for systemic administration, such as parental administration by injection, infusion or implantation. In addition, any of the medicaments may be prepared for administration to a subject suffering from muscular dystrophy (such as limb-girdle muscular dystrophy or any other dystrophin associated muscular dystrophy). In some embodiments, the medicament may further comprise a second recombinant AAV vector comprising a polynucleotide sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 8.

[0035] The foregoing paragraphs are not intended to define every example of the disclosure, and additional examples are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph,

or section of this document. The disclosure includes, as an additional example, all embodiments of the disclosure narrower in scope in any way than the variations defined by specific paragraphs above. For example, where certain examples of the disclosure that are described as a genus, it should be understood that every member of a genus is, individually, an example of the disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0036] Figures 1A-1D demonstrate that AAV mediated β -sarcoglycan expression restores dystrophin-associated proteins and protects membrane integrity. (a) Self-complementary AAV vector containing the codon-optimized human β -sarcoglycan gene (hSGCB) driven by the muscle-specific tMCK promoter. The cassette also contains a chimeric intron to augment processing and polyadenylation signal for stability. (b) Immunofluorescence staining with anti- β -SG antibody shows high levels of sarcolemmal staining of the SGCB transgene in 5-week-old mice both 6 and 12 weeks post injection. $\times 20$ images shown. Percentage of fibers expressing beta-sarcoglycan per TA muscle averaged $88.4 \pm 4.2\%$ after 6 weeks ($n=9$, 4 male, 5 female) and $76.5 \pm 5.8\%$ after 12 weeks ($n=6$, 4 male, 2 female). Protein expression confirmed in the western blot with gamma-tubulin blot shown for a loading control. (c) AAV delivery of β -sarcoglycan leads to restoration of other members of the sarcoglycan complex; α -sarcoglycan, dystrophin. $\times 20$ images. (d) scAAVrh.74.hSGCB protects *sgcb*^{-/-} membranes from damage. Image showing a large area of Evans blue-positive fibers (red) juxtaposed to a cluster of β -sarcoglycan-positive fibers that have been protected from Evans blue dye incorporation. $\times 40$ image is shown.

[0037] Figures 2A-2D shows the histological analysis of β -SG-deficient treated skeletal muscle. scAAVrh.74.hSGCB treatment normalizes histological parameters of *sgcb*^{-/-} mice. Hematoxylin & Eosin staining and Picrosirius Red staining were performed on TA muscle from *sgcb*^{-/-} mice along with normal control C57/BL6 mice and scAAVrh.74.hSGCB-treated mice followed by quantification of histological parameters and % collagen staining. (a) H&E