

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, the present disclosure provides a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan, wherein the polynucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO: 1. In one example, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan. In one ~~aspect~~example, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan that comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 2. In one ~~aspect~~example, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan wherein the polynucleotide sequence comprises a nucleotide sequence at least 95% identical to SEQ ID NO: 1. In some ~~embodiments~~examples, 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~~examples, the polynucleotide sequence encoding β -sarcoglycan comprises the nucleotide sequence set forth in SEQ ID NO: 1. In some ~~embodiments~~examples, 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, wherein administration of the recombinant AAV vector or the composition results in an increase in muscular force and/or muscle mass in the subject. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein, wherein administration of the recombinant AAV vector or the composition results in a reduction in fibrosis in the subject suffering. In another aspect, the present disclosure refers to the use of the recombinant AAV vector as described herein, wherein administration of the recombinant AAV vector or the composition results in a reduction in contraction-induced injury in the 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 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 the muscle of a subject in need thereof. In a further aspect, the present disclosure refers to a composition comprising the recombinant AAV vector as described herein for use in treating muscular dystrophy in a 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 encoding miR29C in the preparation of a combination therapy for reducing fibrosis in the muscle of a subject in need thereof.

(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~~^{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

[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, ~~hypozia~~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

What is claimed is:

~~1. A recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan that comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 2.~~

~~2. A recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan wherein the polynucleotide sequence comprises a nucleotide sequence at least 95% identical to SEQ ID NO: 1.~~

~~31. The A recombinant AAV vector of claim 2, comprising a polynucleotide sequence encoding β -sarcoglycan wherein the polynucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO: 1.~~

~~2. The recombinant AAV vector of claim 1, wherein the polynucleotide sequence is operably linked to a muscle-specific control element.~~

~~4. The recombinant AAV vector of claim 1, wherein the polynucleotide sequence encodes β -sarcoglycan that comprises an amino acid sequence of SEQ ID NO: 2.~~

~~53. The recombinant AAV vector of any one of claims 1-4~~ claim 1 or 2, wherein the vector is of the serotype AAV1, -AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 ~~or~~, AAV rh.74 or a variant thereof.

~~64. The recombinant AAV vector of claim 2~~ any one of claims 1-5 ~~wherein the polynucleotide sequence is operably linked to a muscle-specific control element~~, wherein the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor (MEF) element, muscle creatine kinase (MCK) promoter, truncated MCK (tMCK) promoter, myosin heavy chain (MHC) promoter, MHCK7 promoter, C5-12 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 factor binding element, steroid-inducible element or glucocorticoid response element -(GRE).

~~75. The recombinant AAV vector of claim 64, wherein the muscle-specific control element is truncated MCK (tMCK).~~

~~86. The recombinant AAV vector of claim 64, wherein the muscle-specific control element is MHCK7.~~

97. The recombinant AAV vector of claim 1 or 2~~any of claims 1-5~~ comprising the nucleotide sequence set forth in SEQ ID NO: 3

108. The recombinant AAV vector of claim 1 or 2~~any of claims 1-5~~ comprising the nucleotide sequence set forth in SEQ ID NO: 5.

119. A composition comprising the recombinant AAV vector of any one of claims 1-8~~1-10~~.

1210. Use of the recombinant AAV vector of any one of claims 1-10~~8~~ or the composition of claim 11-9 in the manufacture of a medicament for treating muscular dystrophy in a subject.

1311. Use of claim 10~~12~~, wherein administration of the recombinant AAV vector or the composition results in an increase in muscular force and/or muscle mass in the subject.

1412. Use of claim 12~~10~~, wherein administration of the recombinant AAV vector or the composition results in a reduction in fibrosis in the subject suffering.

1513. Use of claim 12~~10~~, wherein administration of the recombinant AAV vector or the composition results in a reduction in contraction-induced injury in the subject.

1614. Use of the recombinant AAV vector of any one of claims 1-8~~1-10~~ or the composition of claim 11-9 in the manufacture of a medicament for treating β -sarcoglycanopathy in a subject.

1715. The use of ~~claim~~ any one of claims 10-14~~12-16~~, wherein the subject is suffering from limb-girdle muscular dystrophy.

1816. The use of any one of claims 10-15~~12-17~~, wherein the recombinant AAV vector or the composition is to be administered by intramuscular injection or intravenous injection.

1917. The use of any one of claims 10-15~~12-17~~, wherein the recombinant AAV vector or the composition is to be administered systemically.

2018. The use of claim 19~~17~~, where the recombinant AAV vector or the composition is to be parentally administered by injection, infusion or implantation.

2119. The use of any one of claims 10-18~~12-20~~, further comprising that a second recombinant AAV vector comprising a polynucleotide sequence comprising miR29C is to be administered.

~~22~~20. The use of claim ~~21~~19, wherein the second recombinant vector comprises the nucleotide sequence set forth in SEQ ID NO: 9 or the nucleotide sequence set forth in SEQ ID NO: 8.

~~23~~21. The use of claim ~~21~~or 22~~19~~ or 20, wherein the second recombinant AAV vector is to be administered by intramuscular injection or intravenous injection.

~~24~~22. A composition comprising the recombinant AAV vector of any one of claims 1-~~8~~10 for use in therapy for reducing fibrosis in the muscle of a subject in need thereof.

~~25~~23. A composition comprising the recombinant AAV vector of any one of claims 1-~~8~~10 for use in treating muscular dystrophy in a subject in need thereof.

~~26~~24. Use of the recombinant AAV vector of any one of claims 1-~~8~~10 or the composition of claim ~~9~~11 in combination with a second recombinant AAV vector comprising a polynucleotide sequence encoding miR29C in the preparation of a combination therapy for reducing fibrosis in the muscle of a -subject in need thereof.

~~27~~25. The composition of claim ~~25~~23, wherein the muscular dystrophy is β -sarcoglycanopathy or LGMD2E.