

Considering that a subset of patients can develop cardiomyopathy, (8, 9, 10, 13) this would have to be considered in the long-term care of these patients. In previous reports, the Sgcb-null mouse was well characterized. Araishi et al.³ developed the β -sarcoglycan-deficient 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 mammalian subject suffering from muscular dystrophy.

[0010a] In one aspect, the present disclosure provides a composition for treating muscular dystrophy in a subject in need thereof, wherein the composition is formulated for a systemic route of administration and where the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB at a dose of about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard. In another aspect, the present disclosure provides a composition for treating muscular dystrophy in a subject in need thereof, wherein the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein administration of the composition decreases the serum creatine kinase (CK) level in the subject as compared to the serum CK kinase before the administration of the rAAV.

[0010b] In another aspect, the present disclosure provides a composition for treating a muscular dystrophy in a subject comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the composition comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19, wherein the rAAV is at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard. In yet another aspect, the present disclosure provides a composition for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the composition comprises a rAAV comprising a scAAVrh74.MHCK7.hSGCB construct comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19. In yet another aspect, the present disclosure provides a composition for increasing sarcoglycan expression in muscle tissue of a subject, wherein the composition comprises a construct comprising a nucleotide sequence encoding a first sarcoglycan, wherein administration of the composition results in increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

[0010c] In a further aspect, the present disclosure provides a composition, comprising an rAAV vector, a buffer agent, an ionic strength agent, and a surfactant, wherein the surfactant comprises a poloxamer at a concentration of about 0.00001% to about 1%. In a further aspect, the present disclosure provides a polynucleotide sequence comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

[0010d] In a further aspect, the present disclosure provides a method of generating a recombinant AAV, comprising transferring to a cell the polynucleotide sequence as described herein. In a further aspect, the present disclosure provides a cell, comprising the polynucleotide sequence as described herein a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

[0010e] In a further aspect, the present disclosure provides use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the medicament is formulated for a systemic route of administration and the rAAV is at a dose of about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard. In a further aspect, the present disclosure provides use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB A for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof for the preparation

of a medicament for decreasing creatine kinase (CK) level in a subject in need thereof. In a further aspect, the present disclosure provides use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the rAAV comprise a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19, wherein the rAAV is at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.

[0011] In one example, described herein is a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB to a subject in need thereof, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard or 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard; wherein the serum creatine kinase (CK) level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV.

[0012] In another example, provided is a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV; wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV; or wherein motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV, and wherein the motor function is determined by a 100 meter timed walk test.

[0013] In another example, this disclosure provides a method of treating a limb-girdle muscular dystrophy in a subject in need, comprising administering to the subject an rAAV intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises a nucleotide sequence of SEQ ID

NO: 3 or SEQ ID NO: 19. In another example, the disclosure describes a method of expressing beta-sarcoglycan gene in a subject's cell comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19. In one example, the disclosure provides a method of increasing beta-sarcoglycan positive fibers and/or decreasing CK level in a subject's muscle tissue comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.

[0014] In one example, described herein is a method of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject an rAAV comprising a scAAVrh74.MHCK7.hSGCB construct with a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19. In another example, provided herein is a method of increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19. In another example, provided is a method of increasing sarcoglycan expression in muscle tissue or improving muscle function of a subject comprising administering to the subject an rAAV comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19. In another example, the disclosure provides a method of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a construct comprising a nucleotide sequence encoding a first sarcoglycan, and detecting increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

[0015] In another example, described is a composition, comprising an rAAV scAAVrh74.MHCK7.hSGCB vector, a buffer agent, an ionic strength agent, and a surfactant. In another example, described herein is a pharmaceutical composition comprising a recombinant AAV (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the scAAVrh74.MHCK7.hSGCB comprising a nucleotide sequence that is at least 90%, 95% or 99% identical to SEQ ID NO: 19.

[0016] In another example, provided is a method of generating a recombinant AAV scAAVrh74.MHCK7.hSGCB, comprising transferring a plasmid to a cell, wherein the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to

SEQ ID NO: 24. In particular, the plasmid comprises a nucleotide sequence of SEQ ID NO: 24. In another example, the plasmid comprises a nucleotide sequence of SEQ ID NO: 19.

[0017] In another example, described here in a recombinant AAV vector comprising a polynucleotide sequence encoding β -sarcoglycan. In some 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 examples, the polynucleotide sequence encoding β -sarcoglycan comprises the nucleotide sequence set forth in SEQ ID NO: 1. In some examples, the polynucleotide sequence encoding β -sarcoglycan consists of the nucleotide sequence set forth in SEQ ID NO: 1.

[0018] 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.

[0019] 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.

[0020] 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).

[0021] When ranges are used herein for physical properties, such as molecular weight, concentration, or dosage, all combinations and subcombinations of ranges and specific examples therein are intended to be included. The term “about” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range.

[0022] 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.

[0023] 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, hypoza-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

[0024] In some examples, 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. Within the nucleotide sequence of SEQ ID NO: 3, the MCHK7 promoter spans nucleotides 130-921, a SV40 chimeric intron (SEQ ID NO: 20) spans nucleotides 931-1078, the β -sarcoglycan sequence (SEQ ID NO: 1) spans nucleotides 1091-2047 and the poly A (SEQ ID NO: 21) spans nucleotides 2054-2106. In some examples, the rAAV pAAV.MHCK7.hSCGB comprises a nucleotide sequence of SEQ ID NO: 19. Within the nucleotide sequence of SEQ ID NO: 19, the MCHK7 promoter spans nucleotides 128-919, a SV40 chimeric intron spans nucleotides 929-1076, the β -sarcoglycan sequence spans nucleotides 1086-2042 and the poly A spans nucleotides 2049-2101.

[0025] In some examples, the rAAV pAAV.MHCK7.hSCGB comprises a nucleotide sequence that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 19, or a nucleotide sequence that encodes a polypeptide that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID NO: 2.

[0026] In one example, the polynucleotide sequence encodes a protein that retains sarcoglycan activity, including beta- and/or alpha-sarcoglycan activity. In another example, the polynucleotide sequence encodes a protein that retains beta-sarcoglycan activity.

[0027] In some examples, 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. Within the nucleotide sequence of SEQ ID NO: 5, 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. In some examples, the polynucleotide sequence encoding a pAAV.tMCK.hSCGB comprises a sequence e.g. at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about

87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% or more identical to the nucleotide sequence set forth in SEQ ID NO: 5, wherein the polynucleotide sequence encodes a protein that retains sarcoglycan activity, including but not limited to, beta- and/or alpha-sarcoglycan activity.

[0028] 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 AAVrh.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).

[0029] Compositions comprising any of the rAAV vectors described herein are also contemplated.

[0030] In some examples, the disclosure provides a composition or pharmaceutical composition that comprises an scAAVrh74.MHCK7.hSCGB rAAV vector comprising a nucleotide sequence that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 19, or comprising a nucleotide sequence that encodes a polypeptide that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID NO: 2. In addition, the disclosure provides a provides a composition or pharmaceutical composition that comprises an scAAVrh74.MHCK7.hSCGB rAAV vector comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19, or comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

[0031] Provided are methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV)

scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg.

[0032] Also provided are composition for treating muscular dystrophy, wherein the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGC at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg and the composition is formulated for systemic administration.

[0033] In addition, provided are uses of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGC for the preparation of a medicament for treating muscular dystrophy, wherein the medicament comprises scAAVrh74.MHCK7.hSGC at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg and the medicament is formulated for systemic administration.

[0034] In any of the provided methods, compositions and uses, the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV; wherein the serum creatine kinase (CK) level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV; and/or wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV.

[0035] In another example, in any of the provided methods, compositions and uses, motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV, and wherein the motor function is determined by a 100 meter timed walk test. For example, motor function is improved by at least 5% in 1 month or thirty days post-gene transfer, at least 10 % in 2 months or sixty days post-gene transfer, or at least 15% in 3 months or ninety days post gene transfer. In some examples, the motor function is improved by at least 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, or 50%.

[0036] For example, in any of the provided methods, compositions and uses, the systemic route of administration is an intravenous route. For example, the rAAV is administered using an intravenous route and the dose of the rAAV administered is about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the

quantification standard or the dose of the rAAV administered is about 5×10^{13} vg/kg or about 2×10^{14} vg/kg based on a supercoiled plasmid as the quantification standard.

[0037] In some examples, the dose of rAAV administered using an intravenous route and the dose is about about 1.0×10^{13} vg/kg to about 5×10^{14} based on a supercoiled plasmid as the quantitation standard or about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.

[0038] In addition, the dose of the rAAV administered is about 1.5×10^{13} vg to about 2×10^{16} vg, or 1.5×10^{13} vg to 1×10^{16} vg, or about 1.5×10^{13} vg to about 2×10^{15} vg, or about 1.5×10^{13} vg to about 1×10^{15} vg. In addition, in any of the methods, compositions and uses, the dose of rAAV is administered at a concentration of about 10 mL/kg. In any of the methods, compositions or uses provided, the muscular dystrophy is limb-girdle muscular dystrophy.

[0039] In addition, provided are methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg; wherein the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV; wherein the serum CK level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV; or wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV. For example, in any of the provided methods, the systemic route of administration is an intravenous route and the dose of the rAAV administered is about 5.0×10^{13} vg/kg based on a supercoiled plasmid as the quantitation standard. In another example, the dose of the rAAV administered is about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard. In another example, the dose of the rAAV administered is about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard. In another example, the dose of rAAV administered is about 1.85×10^{13} vg/kg based on a linearized plasmid as the quantitated standard. In addition, the dose of the rAAV administered is about 1.5×10^{13} vg to about 2×10^{16} vg, or 1.5×10^{13} vg to 1×10^{16} vg, or about 1.5×10^{13} vg to about 2×10^{15} vg, or about 1.5×10^{13} vg to about 1×10^{15} vg. In addition, in any of

the methods, the dose of rAAV is administered at a concentration of about 10 mL/kg. In any of the methods provided, the muscular dystrophy is limb-girdle muscular dystrophy.

[0040] In some examples, the disclosure includes a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein motor function is demonstrably improved in said subject as compared to motor function of said subject before administration of the rAAV, and wherein motor function is determined by a 100m timed walk test. In some examples, motor function is improved by at least 5% in 1 month or thirty days post-gene transfer, at least 10% in 2 months or sixty days post-gene transfer, or at least 15% in 3 months or ninety days post gene transfer. In some examples, motor function is improved by at least about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 45%, or about 50%.

[0041] Provided are methods of increasing the level of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19. In addition, provided are composition for increasing the level of alpha-sarcoglycan in a subject in need, wherein the composition comprises scAAVrh74.MHCK7.hSGCB construct comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19. Also provides are uses of scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19 for the preparation of a medicament for increasing the level of alpha-sarcoglycan in a subject in need thereof. In some examples, the alpha-sarcoglycan is colocalized to the membrane of a cell expressing a beta-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCB.

[0042] In some examples, the scAAVrh74.MHCK7.hSGCB construct comprises an intron sequence. In one example, the intron sequence comprise a nucleotide sequence of SEQ ID NO: 20. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a polyA sequence. In one example, the poly A sequence comprises a nucleotide sequence of SEQ ID NO: 21. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a 5' inverted terminal repeat (ITR) sequence. In one example, the 5' ITR sequence comprises a nucleotide sequence of SEQ ID NO: 22. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a 3' inverted terminal repeat (ITR) sequence. In one example, the 3' ITR sequence comprises a nucleotide sequence of SEQ ID NO: 23.

[0043] Also provided are methods of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a construct comprising a nucleotide sequence encoding a first sarcoglycan, and detecting increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan. In some examples, the first sarcoglycan is β -sarcoglycan (SGCB), and said second sarcoglycan is α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), or δ -sarcoglycan (SGCD).

[0044] In any of the methods, uses and compositions of treating muscular dystrophy provided, the subject is 4-15 years of age, has confirmed beta-sarcoglycan (SGCB) mutation in both alleles, was negative for AAVrh74 antibodies and/or had >40% or normal 100 meter walk test. In any of the methods, uses and compositions of treating muscular dystrophy provided, the subject is a pediatric subject. In some examples, the subject is a pediatric subject, such as a subject ranging in age from 1 to 10 years. In some examples, the subject is 4 to 15 years of age. The subject, in one example, is an adolescent subject, such as a subject ranging in age from 10 to 19 years. In addition, the subject, in one example, is a young adult subject such as a subject ranging in age from late teens or early twenties, such as the subject may range in age from 15 to 29 years of age. In some example, the subject is a middle-aged adult or an elderly subject, such that the middle-aged adult may range in age from 25-55 years of age and the elderly subject may range in age over 50 years of age.

[0045] In some examples, the rAAV is administered by injection, infusion or implantation. For example, the rAAV is administered by infusion over approximately 1 to 2 hours. In addition, the rAAV is administered by an intravenous route through a peripheral limb vein.

[0046] In the methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard and the rAAV comprises the human β -sarcoglycan nucleotide sequence of SEQ ID NO: 1. In addition, the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 4. In some examples, the rAAV is of the serotype AAVrh.74. In addition, the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0047] In another example, the scAAVrh74.MHCK7.hSGCB construct comprises an intron sequence. In one example, the intron sequence comprise a nucleotide sequence of SEQ ID NO: 20. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a polyA sequence. In one example, the poly A sequence comprises a nucleotide sequence of SEQ ID NO: 21. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a 5' inverted terminal repeat (ITR) sequence. In one example, the 5' ITR sequence comprises a nucleotide sequence of SEQ ID NO: 22. In another example, the scAAVrh74.MHCK7.hSGCB construct comprises a 3' inverted terminal repeat (ITR) sequence. In one example, the 3' ITR sequence comprises a nucleotide sequence of SEQ ID NO: 23.

[0048] In an exemplary example, methods of treating muscular dystrophy in a subject in need thereof comprise the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg, wherein the subject is suffering from limb-girdle muscular dystrophy, and the rAAV is administered by intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0049] In addition, the disclosure provides a composition for treating limb-girdle muscular dystrophy comprising a dose of recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19, and the composition is formulated to deliver a dose of about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard by intravenous infusion over approximately 1 to 2 hours.

[0050] The disclosure also provides for use of a dose of recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for the treatment of limb-girdle muscular dystrophy, wherein the dose of rAAV about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation

standard, or about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard and the medicament is formulated to deliver the dose by intravenous infusion over approximately 1 to 2 hours.

[0051] The disclosure further provides a method of improving muscle function of a subject comprising administering to the subject a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity, or 100% identity to SEQ ID NO: 1, 3, 5, or 19. In addition, provided are composition for improving muscle function of a subject, wherein the composition comprises a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity or 100% identity to SEQ ID NO: 1, 3, 5, or 19. Also provided are uses of a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity or 100% identity to SEQ ID NO: 1, 3, 5, or 19 for the preparation of a medicament for improving muscle function of a subject.

[0052] In any of the provided methods, uses or compositions, the subject suffers from a genetic mutation in a gene encoding a sarcoglycan or a muscular dystrophy. In some examples, the sarcoglycan is β -sarcoglycan (SGCB), α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), or δ -sarcoglycan (SGCD). In some examples, the sarcoglycan is β -sarcoglycan or α -sarcoglycan.

[0053] In any of the provided methods, uses or compositions, the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV.

[0054] In addition, in any of the provided methods, uses or compositions, the expression of the beta-sarcoglycan gene in the cell is detected by measuring the beta-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsied before and after administration of the rAAV.

[0055] In any of the provided methods, uses or compositions, the level of beta-sarcoglycan protein is increased by at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least 35% or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45% or at least 46%, or at least 47%, or at least 48%, or at least 49%, or

at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90% or at least 95%, or at least 98% after administration of rAAV. For example, the level of the level of beta-sarcoglycan protein is increased by at least 33% as detected by measuring the beta-sarcoglycan protein level on a Western blot in muscle biopsied before and after administration of the rAAV, or the level of beta-sarcoglycan protein is increased by at least 38% or at least 39% as detected by measuring the beta-sarcoglycan protein level by immunohistochemistry in muscle biopsies before and after administration of the rAAV

[0056] In any of the methods, uses or compositions provided herein, the serum CK level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV. For example, the serum level CK level in the subject is decreased by at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86 %or at least 87%, or at least 88%, or at least 89%, or at least 90% or at least 95%, or at least 98% by 60 to 90 days or 60 days or 90 days after administration of rAAV as compared to the serum CK level before administration of the rAAV.

[0057] In any of the methods, uses or compositions provided herein, the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV. For example, the number of beta-sarcoglycan positive fibers is detected by measuring the beta-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. For example, the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased by at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least 35% or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45% or at least 46%, or at least 47%, or at least 48%, or at least 49%, or at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%,

or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90% or at least 95%, or at least 98% after administration of rAAV.

[0058] In any of the methods, compositions and uses provided herein, the level of alpha-sarcoglycan in the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan before administration of the rAAV. The level of alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry or Western blot on muscle biopsies before and after administration of the rAAV.

[0059] Another example provides for methods expressing beta-sarcoglycan gene in a cell comprising administering to the subjects the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0060] Also provided are compositions for expressing beta-sarcoglycan gene in a cell, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0061] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for the expressing beta-sarcoglycan gene in a cell, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0062] In any of the provided methods, uses or compositions for expressing beta-sarcoglycan gene in a cell, expression of the beta-sarcoglycan gene in the cell is detected by measuring the beta-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsies before and after administration of the scAAVrh74.MHCK7.hSGCB construct. For example, the cell has more than one AAV viral copy number. In addition, the beta-sarcoglycan gene is measured in the subject by detecting greater than 1 rAAV vector genome copy per nucleus.

[0063] Also provided are compositions for decreasing serum CK levels in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3

or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0064] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for decreasing serum CK levels in a subject in need thereof, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0065] In any of these methods, uses, and compositions, the serum CK level in the subject is decreased by at least 82% by 60 days after administration of the rAAV as compared to the serum CK level before administration of the rAAV.

[0066] Methods of increasing beta-sarcoglycan positive fibers in a muscle tissue of a subject comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19 are provided.

[0067] Also provided are compositions for increasing beta-sarcoglycan positive fibers in muscle tissue of a subject, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0068] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing beta-sarcoglycan positive fibers in muscle tissue of a subject, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0069] In any of these methods, uses, and compositions, the number of beta-sarcoglycan positive fibers is detected by measuring the beta-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. In addition, in any of the methods, uses and compositions, the number of beta-sarcoglycan

positive fibers is measured by detecting greater than 1 rAAV vector genome copy per nucleus.

[0070] Another example provides for methods of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0071] Also provided are compositions for increasing the expression of alpha-sarcoglycan in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0072] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing the expression of alpha-sarcoglycan in a subject, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0073] Also provided are methods of increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0074] Also provided are compositions for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0075] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein

the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0076] In any of these methods, uses and compositions the level of alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. In addition, in any of the provided methods, uses and compositions, alpha-sarcoglycan is colocalized to the membrane of a cell expressing a beta-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCB.

[0077] Another example provides for methods of increasing sarcoglycan expression in muscle tissue of a subject in need thereof, comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0078] Also provided are compositions for increasing the expression of sarcoglycan expression in muscle tissue of a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0079] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing sarcoglycan expression in muscle tissue of a subject in need thereof, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0080] In any of these methods, uses and compositions for increasing sarcoglycan expression in muscle tissue, the subject suffers from a genetic mutation in a gene encoding a sarcoglycan or a muscular dystrophy. For example in any of these methods, uses or

compositions, the sarcoglycan is β -sarcoglycan (SGCB), α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), or δ -sarcoglycan (SGCD).

[0081] Methods of producing a recombinant AAV vector particle comprising culturing a cell that is transferred with a plasmid 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. In one example, the method of generating the rAAV comprising transferring an AAV vector plasmid to a host cell. In another example, the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24. In another example, the disclosure provides a cell that comprising an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. The cell described herein comprises an insect cell, e.g., a *Drosophila* cell (e.g., an S2 cell or Kc cell), a silkworm cell (e.g., a Bme21 cell), or a mosquito cell (e.g., a C6/36 cell); or a mammalian cell (preferably a human cell, e.g., a human primary cell or an established cell line). In one example, the mammalian cell comprises a 293 cell, a COS cell, a HeLa cells, or a KB cell.

[0082] In another example, the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, 3, 5, or 19. In some examples, the the vector plasmid comprises a nucleotide sequence of any one of SEQ ID NO: 1, 3, 5, or 19. In some examples, the AAV vector plasmid is stably expressed in the host cell. The host cell stably harboring the AAV vector plasmid can be used to generate rAAV. In one example, the AAV vector plasmid is a pAAV.MHCK7.hSGCB. KAN plasmid.

[0083] The method of producing recombinant AAV vector particles provided herein may further comprise a step of transferring a packaging plasmid and/or a helper virus to the host cell. For example, the methods further comprise a step wherein the packaging cell comprises a stably integrated AAV cap gene and/or wherein the packaging cell comprises a stably integrated AAV rep gene. The disclosure also provides for a cell comprising a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24 or an plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. Also provides is a cell comprising a nucleotide sequence of SEQ ID NO: 1, 3, 5, or 19.

[0084] 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 examples, the mammalian subject suffers from muscular dystrophy. In some examples, 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.

[0085] 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 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 examples, the subject is suffering from limb-girdle muscular dystrophy. In some examples, the subject is suffering from limb-girdle muscular dystrophy type 2E (LGMD2E).

[0086] 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.

[0087] 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. In one example, the subject is a human.

[0088] 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.

[0089] 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 examples, the muscular dystrophy is limb-girdle muscular dystrophy.

[0090] 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.

[0091] 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.

[0092] 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 examples, 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.

[0093] 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 examples, 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.

[0094] The disclosure also provides for a formulation or composition, comprising an rAAV virion that comprises an AAVrh74 derived capsid, a buffer agent, an ionic strength

agent, and a surfactant. In the provided formulation or composition, the rAAV is at a concentration of about 1.0×10^{12} vg/ml to about 5.0×10^{14} vg/ml or at a concentration of about 5.0×10^{12} vg/ml to about 1.0×10^{14} vg/ml. In addition, the rAAV is at a concentration of about 2.0×10^{13} vg/ml, 4×10^{13} vg/ml, or 5×10^{13} vg/ml. In the provided formations or compositions, the rAAV may be an scAAVrh74.MHCK7.hSGCB viral particle or an scAAVrh74.MHCK7.hSGCB vector. For example, in any of the provided formulations or compositions scAAVrh74.MHCK7.hSGCB comprises a nucleotide sequence of SEQ ID NO: 19.

[0095] In any of the provided formulations or compositions, the buffer agent comprises one or more of tris, tricine, Bis-tricine, HEPES, MOPS, TES, TAPS, PIPES, and CAPS. For example, the buffer agent comprises the tris with pH 8.0 at concentration of about 5 mM to about 40 mM or the buffer agent comprises the tris with pH 8.0 at about 20 mM.

[0096] In any of the provided formulations or compositions, the ionic strength agent comprises one or more of potassium chloride (KCl), potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride (NH_4Cl), ammonium acetate, magnesium chloride (MgCl_2), magnesium acetate, magnesium sulfate, manganese chloride (MnCl_2), manganese acetate, manganese sulfate, sodium chloride (NaCl), sodium acetate, lithium chloride (LiCl), and lithium acetate. For example, the ionic strength agent comprises MgCl_2 at a concentration of about 0.2 mM to about 4 mM or the ionic strength agent comprises NaCl at a concentration of about 50 mM to about 500 mM, or the ionic strength agent comprises MgCl_2 at a concentration of about 0.2 mM to about 4 mM and NaCl at a concentration of about 50 mM to about 500 mM, or the ionic strength agent comprises MgCl_2 at a concentration of about 1 mM and NaCl at a concentration of about 200 mM.

[0097] In any of the provided formulations or compositions, the surfactant comprises one or more of a sulfonate, a sulfate, a phosphonate, a phosphate, a Poloxamer, and a cationic surfactant. For example, the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407. The Poloxamer may be at a concentration of about 0.00001% to about 1%. An exemplary surfactant is Poloxamer 188 at a concentration of about 0.001%.

[0098] 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 examples 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

[0099] **Figure 1** provides a schematic of therapeutic β -sarcoglycan transgene cassette. Self-complementary AAV vector containing the codon-optimized human β -sarcoglycan

Claims

What is claimed is:

1. A composition for treating muscular dystrophy in a subject in need thereof, wherein the composition is formulated for a systemic route of administration and

where the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB

at a dose of about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard.
2. A composition for treating muscular dystrophy in a subject in need thereof, wherein the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB,

wherein administration of the composition decreases the serum creatine kinase (CK) level in the subject as compared to the serum CK kinase before the administration of the rAAV.
3. The composition of claim 2, wherein the composition is formulated for administration using an intravenous route.
4. The composition of claim 2 or 3, wherein the rAAV is at a dose of about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.
5. The composition of any one of claims 1-4, wherein the composition administers rAAV at a concentration of about 10 mL/kg.
6. The composition-of any one of claims 1-5 wherein the composition is formulated for administration by injection, infusion or implantation.
7. A composition for treating a muscular dystrophy in a subject comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the composition comprising a nucleotide sequence that is at least 90%, 95%, or 99%

identical to SEQ ID NO: 3 or SEQ ID NO: 19, wherein the rAAV is at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.

8. The composition of any one of claims 1-7, wherein the rAAV comprises the nucleotide sequence of SEQ ID NO: 1.

19. The composition of any one of claims 1-8, wherein the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 4.

10. The composition of any one of claims 1-9, wherein the rAAV comprises an intron sequence of SEQ ID NO: 20.

11. The composition of any one of claims 1-10, wherein the rAAV comprises a polyA sequence of SEQ ID NO: 21.

12. The composition of any one of claims 1-11, wherein the rAAV comprises a 5' inverted terminal repeat (ITR) sequence of SEQ ID NO: 22.

13. The composition of any one of claims 1-12, wherein the rAAV comprises a 3' inverted terminal repeat (ITR) sequence of SEQ ID NO: 23.

14. The composition of any one of claims 1-13, wherein the muscular dystrophy is limb-girdle muscular dystrophy.

15. The composition of any one of claims 1-14, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2E.

16. A composition for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the composition comprises a rAAV comprising a scAAVrh74.MHCK7.hSGCB construct comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19.

17. A composition for increasing sarcoglycan expression in muscle tissue of a subject, wherein the composition comprises a construct comprising a nucleotide

sequence encoding a first sarcoglycan, wherein administration of the composition results in-increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

18. The composition of claim 17, wherein said first sarcoglycan is β -sarcoglycan (SGCB), and said second sarcoglycan is α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), and/or γ -sarcoglycan (SGCD).

19. The composition of any one of claims 1-18, wherein the subject is a human subject that is 4 to 15 years of age.

20. The composition of any one of claims 1-18, wherein the subject is a pediatric subject, an adolescent subject or a young adult subject.

21. The composition of any one of claims 1-18, wherein the subject is a human subject that is 25 to 55 years of age.

22. The composition of any one of claims 1-18, wherein the subject is a human subject that is over 50 years of age.

23. A composition, comprising
an rAAV vector,
a buffer agent,
an ionic strength agent, and
a surfactant, wherein the surfactant comprises a poloxamer at a concentration of about 0.00001% to about 1%.

24. The composition of claim 23, wherein the rAAV is at a concentration of about 1.0×10^{12} vg/ml to about 5.0×10^{14} vg/ml, or about 5.0×10^{12} vg/ml to about 1.0×10^{14} vg/ml.

25. The composition of claim 23 or 24, wherein the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407.

26. A polynucleotide sequence comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

27. The polynucleotide sequence of claim 26, wherein the polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 24.
28. The polynucleotide sequence of claim 26 or 27, is a plasmid.
29. A method of generating a recombinant AAV, comprising transferring to a cell the polynucleotide sequence of any one of claims 26-28.
30. The method of claim 29, further comprising transferring a packaging plasmid and/or a helper virus to the cell.
31. The method of claim 29 or 30, wherein the cell comprises a stably integrated AAV cap gene.
32. The method of any one of claims 29-31, wherein the cell comprises a stably integrated AAV rep gene.
33. A cell, comprising the polynucleotide sequence of any one of claims 26-28 a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.
34. The cell of claim 33, wherein the cell is an insect cell, a silkworm cell, a mosquito cell, a bacterial cell or a mammalian cell.
35. The cell of claim 33, wherein the cell is a 293 cell, a COS cell, a HeLa cells, or a KB cell.
36. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the medicament is formulated for a systemic route of administration and
- the rAAV is at a dose of about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard.
37. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB A for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof for the preparation of a medicament for decreasing creatine kinase (CK) level in a subject in need thereof.

38. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the rAAV comprise a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19, wherein the rAAV is at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.