ADENO-ASSOCIATED VIRUS VECTOR DELIVERY OF ALPHA-SARCOGLYCAN AND THE TREATMENT OF MUSCULAR DYSTROPHY

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/889,749 filed August 21, 2019, U.S. Provisional Application No. 63/014,934, filed April 24, 2020 and U.S Provisional Application No. 63/022,843 filed May 11, 2020, all of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] Described herein are therapy vectors such as AAV vectors expressing alpha-sarcoglycan and method of using these vectors to treat limb girdle muscular dystrophies such as LGMD2D.

INCORPORATION BY REFERENCE OF THE SEQUENCE LISTING

[0003] This application contains, as a separate part of disclosure, a Sequence Listing in computer-readable form (filename: 54652_SeqListing.txt; 18,768 byte – ASCII text file; created August 17, 2020) which is incorporated by reference herein in its entirety.

BACKGROUND

[0004] Muscular dystrophies (MDs) are a group of genetic diseases. The group is characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Some forms of MD develop in infancy or childhood, while others may not appear until middle age or later. The disorders differ in terms of the distribution and extent of muscle weakness (some forms of MD also affect cardiac muscle), the age of onset, the rate of progression, and the pattern of inheritance.

[0005] One group of MDs is the limb girdle muscular dystrophies (LGMD). LGMDs are rare conditions and they present differently in different people with respect to age of onset, areas of muscle weakness, heart and respiratory involvement, rate of progression and severity. LGMDs can begin in childhood, adolescence, young adulthood or even later. Both genders are affected equally. LGMDs cause weakness in the shoulder and pelvic girdle, with nearby muscles in the upper legs and arms sometimes also weakening with time. Weakness of the legs often appears before that of the arms. Facial muscles are usually unaffected. As the condition progresses, people can have problems with walking and may need to use a wheelchair over time. The involvement of shoulder and arm muscles can lead to difficulty in raising arms over head and in lifting objects. In some types of LGMD, the heart and breathing muscles may be involved.

[0006] Specialized tests for LGMD are now available through a national scheme for diagnosis, the National Commissioning Group (NCG).

[0007] LGMD subtype 2D (LGMD2D), often referred to as α-sarcoglycanopathy, is an autosomal recessive disorder caused by mutations in the alpha-sarcoglycan gene (SGCA; α-sarcoglycan), leading to complete or reduced loss of functional protein with loss of other structural components of the dystrophin-associated protein complex. Notably, loss of the alpha-sarcoglycan protein leads to a progressive muscular dystrophy with deteriorating muscle function, with an onset from 3 to 8 years of age. Symptoms include: delayed ambulation, weakness in proximal muscles caused by fat replacement and fibrosis, elevated creatine kinase, scoliosis, and joint contractures. The debilitating disease often leads to wheelchair dependency and death due to respiratory failure. Thus, there remains a need for treatments for LGMD2D.

SUMMARY

[0008] Described herein are methods for treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA, 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^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. In one aspect, the disclosure relates to an rAAV expressing the alpha-sarcoglycan gene and methods of delivering alpha-sarcoglycan to the muscle to reduce and/or prevent fibrosis; and/or to increase muscular force, and/or to treat a α-sarcoglycanopathy in a subject suffering from muscular dystrophy.

[0009] In one aspect, described herein is a recombinant AAV (rAAV) comprising a polynucleotide sequence encoding alpha-sarcoglycan protein. In some embodiments, the polynucleotide sequence comprises a sequence at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identical to the nucleotide sequence set forth in SEQ ID NO: 1 and encodes a protein that retains alpha-sarcoglycan activity. In some embodiments, the polynucleotide sequence comprises the nucleotide sequence set forth in SEQ ID NO: 1. In another embodiment, the polynucleotide encodes a protein that is at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identical to the nucleotide sequence set forth in SEQ ID NO: 2. In another embodiment, the polynucleotide encodes a protein that comprises an amino acid sequence set forth in SEQ ID NO: 2.

[0010] In some embodiments, the rAAV comprises a nucleotide sequence comprising a tMCK promoter. For example, the tMCK promoter comprises a nucleotide sequence set forth in SEQ ID NO: 3. In addition, the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5 and/or a 3' inverted terminal repeat sequence of SEQ ID NO: 6. In some embodiments, the rAAV comprises a poly A sequence of SEQ ID NO: 7. In one embodiment, the disclosed rAAV is of the serotype AAVrh.74.

[0011] In one embodiment, the polynucleotide comprises a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identical to SEQ ID NO: 4. In one embodiment, the polynucleotide comprises a nucleotide sequence set forth in SEQ ID NO: 4.

[0012] The disclosure provides for methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering any of the rAAV disclosed herein, wherein the rAAV is administered by a systemic route. In particular, in any of the disclosed methods, the rAAV is AAVrh74.tMCK.hSCGA, wherein the rAAV is administered using a systemic route of administration.

[0013] In any of the disclosed methods, the rAAV is administered at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. For example, the rAAV is administered at a dose of about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg, or about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg, or the rAAV is administered at a dose of about $5x10^{13}$ vg/kg, about $6x10^{13}$ vg/kg, about $7x10^{13}$ vg/kg, about $8x10^{13}$ vg/kg, about $9x10^{13}$ vg/kg, about $1x10^{14}$ vg/kg, about $2x10^{14}$ vg/kg, about $3x10^{14}$ vg/kg, about $4x10^{14}$ vg/kg or about $5x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.

[0014] In another embodiment, in any of the disclosed methods, the rAAV is administered at a dose about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. For example, the rAAV is administered at a dose of about 1.0×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.5×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.6×10^{13}vg/kg to about 8.0×10^{13} vg/kg. about 1.8×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.2×10^{13}vg/kg to about 7.5×10^{13} vg/kg, about 1.9×10^{13}vg/kg to about 7.5×10^{13} vg/kg, about 1.4×10^{13}vg/kg to about 7.4×10^{13} vg/kg, about 1.9×10^{13}vg/kg to about 7.5×10^{13} vg/kg, or about 1.8×10^{13}vg/kg to about 8.0×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[0015] In addition, in any of the disclosed methods, the systemic route of administration is an intravenous route. For example, in any of the disclosed methods, the rAAV is administered by injection, infusion or implantation. In some embodiments, the rAAV is administered by an intravenous route through a peripheral limb vein.

[0016] In any of the disclosed methods, the muscular dystrophy is limb-girdle muscular dystrophy. For example, the muscular dystrophy is limb-girdle muscular dystrophy type 2D (LGMD2D).

[0017] In an exemplary embodiment, the methods of treating muscular dystrophy, comprise administering the rAAV to a subject is suffering from limb-girdle muscular dystrophy, and the rAAV is administered by intravenous infusion at a dose of about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0018] In exemplary embodiments, the disclosure provides for methods of treating muscular dystrophy in a subject in need thereof, the method comprising the step of administering a rAAV to the

subject wherein the subject is suffering from limb-girdle muscular dystrophy, and the rAAV is administered by intravenous infusion at a dose of about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. For example, in these methods, the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan gene expression before administration of the rAAV.

[0019] In any of the disclosed methods, the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan gene expression before administration of the rAAV; and/or 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; and/or wherein the locomotor activity and specific-force generation are increased; wherein fibrosis is reduced; wherein the resistance to contraction-induced injury in tibialis anterior muscle is increased; and/or wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of alpha-sarcoglycan positive fibers before administration of the rAAV; or wherein fibrosis is reduced in the subject after administration of the rAAV as compared to before administration of the rAAV; and/or wherein fibrosis is reduced in the subject after administration of the rAAV as compared to before administration of the rAAV; and/or wherein the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject are increased after administration of the rAAV as compared to before administration of the rAAV.

[0020] In some embodiments, the the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.

[0021] In another aspect, the disclosure provides for methods of expressing alpha-sarcoglycan gene in a cell comprising administering to a subject any of the disclosed rAAV. For example, the disclosure provides for method of expressing alpha-sarcoglycan gene in a cell comprising administering to a subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4 . In addition, in any of the methods, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, in any of the methods, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0022] The disclosure provides for methods of decreasing serum CK level in a subject in need thereof, the method comprising administering to the subject any of the disclosed rAAV. For example, the disclosure provides for methods of decreasing a serum CK level in a subject in need thereof, the method comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0023] In another aspect, the disclosure provide for methods of increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject comprising administering to the subject any of the disclosed rAAV. For example, the disclosure provides for method of increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject in a need thereof, the method comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0024] The disclosure also provides for methods of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject any of the disclosed rAAV. For example, the disclosure provides for methods of increasing the expression of alpha-sarcoglycan in a subject in need thereof, the method comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. In addition, in any of the disclosed methods, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, in any of the methods, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0025] The disclosure provides for compositions for treating muscular dystrophy in a subject in need thereof, wherein the composition comprises any of the rAAV disclosed herein, wherein the composition is formulated for administration by a systemic route. In particular, in any of the compositions, the rAAV is AAVrh74.tMCK.hSCGA.

[0026] Any of the disclosed compositions comprise rAAV at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. For example, the rAAV is at a dose of about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg, or about 5×10^{13} vg/kg to about 2×10^{14} vg/kg, or the rAAV is at a dose of about 5×10^{13} vg/kg, about 6×10^{13} vg/kg, about 7×10^{13} vg/kg, about 8×10^{13} vg/kg, about 9×10^{13} vg/kg, about 1×10^{14} vg/kg, about 2×10^{14} vg/kg, about 3×10^{14} vg/kg, about 4×10^{14} vg/kg or about 5×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.

[0027] In another embodiment, in any of the disclosed compositions, the rAAV is administered at a dose about 1.85×10^{13} vg/kg or about 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. For example, the rAAV is administered at a dose of about 1.0×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.5×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.6×10^{13}vg/kg to about 8.0×10^{13} vg/kg. about 1.8×10^{13}vg/kg to about 8.0×10^{13} vg/kg, about 1.2×10^{13}vg/kg to about 7.5×10^{13} vg/kg, about 1.9×10^{13}vg/kg to about 7.5×10^{13} vg/kg, about 1.4×10^{13}vg/kg to about 7.4×10^{13} vg/kg, about 1.9×10^{13}vg/kg to about 7.5×10^{13} vg/kg, or about 1.8×10^{13}vg/kg to about 8.0×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[0028] In addition, any of the disclosed compositions are formulated for administration by an intravenous route, such as compositions formulated for administration by injection, infusion or

implantation. In some embodiments, the disclosed compositions are formulated for administration by an intravenous route through a peripheral limb vein.

[0029] Any of the disclosed compositions are for the treatment for limb-girdle muscular dystrophy, such as limb-girdle muscular dystrophy type 2D (LGMD2D).

[0030] In an exemplary embodiment, the disclosure provides for compositions for treating a subject suffering from limb-girdle muscular dystrophy, wherein the composition comprises a dose of rAAV at about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the composition is formulated for administration by intravenous infusion, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0031] In addition, the disclosure provides for compositions for treating limb-girdle muscular dystrophy in a subject in need thereof, wherein the composition comprises a dose of rAAV of about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and the composition is formulated for administration by intravenous infusion and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. For example, administration of the composition increases the level of alpha-sarcoglycan gene expression in a cell of the subject as compared to the level of alpha-sarcoglycan gene expression before administration of the composition.

[0032] In addition, administration of any of the disclosed compositions increases the level of alpha-sarcoglycan gene expression in a cell of the subject as compared to the level of alpha-sarcoglycan gene expression before administration of the composition; and/or wherein administration of the disclosed composition decreased the serum CK level in the subject as compared to serum CK level before administration of the composition; and/or wherein the locomotor activity and specific-force generation are increased; wherein fibrosis is reduced; wherein the resistance to contraction-induced injury in tibialis anterior muscle is increased; and/or wherein administration of the composition increases the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject as compared to the number of alpha-sarcoglycan positive fibers before administration of the composition; and/or wherein administration of composition reduced fibrosis in the subject as compared to before administration of the rAAV; and/or wherein the composition reduced fibrosis as compared to before administration of the composition; or wherein administration of the composition increased the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject as compared to before administration of the composition. In some embodiments, the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.

[0033] In another aspect, the disclosure provides for compositions for expressing alpha-sarcoglycan gene in a cell, wherein composition comprises any of the disclosed rAAV. For example, the disclosure provides compositions for expressing alpha-sarcoglycan gene in a cell comprising the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. In addition, in any of the

compositions, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, in any of the methods, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0034] The disclosure provides for compositions for decreasing a serum CK level in a subject in need thereof, the composition comprising any of the disclosed rAAV. For example, the disclosure provides for compositions for decreasing a serum CK level in a subject in need thereof, the composition comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0035] In another aspect, the disclosure provides for composition for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject, wherein the composition comprises any of the disclosed rAAV. For example, the disclosure provides for compositions for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject, wherein the composition comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[0036] The disclosure also provides for composition for increasing the expression of alpha-sarcoglycan in a subject in need thereof, wherein the composition comprises any of the disclosed rAAV. For example, the disclosure provides compositions for increasing the expression of alpha-sarcoglycan in a subject in need thereof, wherein the composition comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. In addition, after administration of the any of the disclosed compositions, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, after administration of the any of the disclosed compositions, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, after administration of the any of the disclosed compositions, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0037] The disclosure provides for use of any of the disclosed rAAV for the preparation of a medicament for the treating muscular dystrophy in a subject in need thereof, wherein the medicament is formulated for administration by a systemic route. In particular, the disclosure provides for use of AAVrh74.tMCK.hSCGA for the preparation of a medicament for treating muscular dystrophy, wherein the medicament is formulated for administration by a systemic route of administration.

[0038] In any of the disclosed uses, the medicament comprises rAAV at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. For example, the rAAV is at a dose of about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg, or about 5×10^{13} vg/kg to about 2×10^{14} vg/kg, or the rAAV is at a dose of

about $5x10^{13}$ vg/kg, about $6x10^{13}$ vg/kg, about $7x10^{13}$ vg/kg, about $8x10^{13}$ vg/kg, about $9x10^{13}$ vg/kg, about $1x10^{14}$ vg/kg, about $2x10^{14}$ vg/kg, about $3x10^{14}$ vg/kg, about $4x10^{14}$ vg/kg or about $5x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.

[0039] In another embodiment, in any of the disclosed uses, the medicament comprises rAAV at a dose about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. For example, the medicament comprises rAAV at a dose of about 1.0 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.5 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.6 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg. about 1.8 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.2 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, about 1.9 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, about 1.4 x 10^{13}vg/kg to about 7.4 x 10^{13} vg/kg, about 1.9 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, or about 1.8 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[0040] In addition, in any of the disclosed uses, the medicament is formulated for administration by an intravenous route. For example, in any of the disclosed uses, the medicament is formulated for administration by injection, infusion or implantation. In some embodiments, the medicament is formulated for administration by an intravenous route through a peripheral limb vein.

[0041] In any of the disclosed uses, the medicament is for the treatment of limb-girdle muscular dystrophy, such as limb-girdle muscular dystrophy type 2D (LGMD2D).

[0042] In an exemplary embodiment, the disclosure provides for use of a rAAV for the preparation of a medicament for treating limb-girdle muscular dystrophy, wherein the medicament is formulated for administration by intravenous infusion and the rAAV is at a dose of about $5x10^{13}$ vg/kg to about $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard., and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. For example, administration of the medicament to a subject in need results in an increase in alpha-sarcoglycan gene expression in a cell of the subject as compared to the level of alpha-sarcoglycan gene expression before administration of the rAAV.

[0043] In any of the disclose uses, administration of the medicament to a subject in need results in an increase in the level of alpha-sarcoglycan gene expression in a cell of the subject as compared to the level of alpha-sarcoglycan gene expression before administration of the medicament; and/or administration of the medicament to a subject results in decrease in the the serum CK level in the subject as compared to serum CK level before administration of the medicament; and/or wherein the locomotor activity and specific-force generation are increased; wherein fibrosis is reduced; wherein the resistance to contraction-induced injury in tibialis anterior muscle is increased; and/or administration of the medicament to the subject results in an increase in the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased as compared to the number of alpha-sarcoglycan positive fibers before administration of the medicament, and/or administration of the medicament to the subject in need results in reduced fibrosis in the subject as compared to before administration of the medicament; and/or administration of the medicament results in an increase in

the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject as compared to before administration of the medicament. In some embodiments, the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.

[0044] In another aspect, the disclosure provides for use of any of the disclosed rAAV for the preparation of a medicament for expressing alpha-sarcoglycan gene in a cell in a subject in need. For example, the disclosure provides for use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for expressing alpha-sarcoglycan gene in a cell in a subject in need, wherien the scAAVrh74.tMCK.hSGCA construct comprises a nucleotide sequence of SEQ ID NO: 4. In addition, in any of the uses, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, in any of the uses, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0045] The disclosure provides for use of any of the disclosed rAAV for the preparation of medicament for decreasing a serum CK level in a subject in need thereof. For example, the disclosure provides for use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for decreasing a serum CK level in a subject in need thereof, wherein scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.

[0046] In another aspect, the disclosure provides for use of any of the disclosed rAAV for the preparation of a medicament for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject. For example, the disclosure provides for use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject, wherein scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.

[0047] The disclosure also provides for use any of the disclosed rAAV for the preparation of a medicament for increasing the expression of alpha-sarcoglycan in a subject in need thereof. For example, the disclosure provides for use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for increasing the expression of alpha-sarcoglycan in a subject in need thereof, wherein the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4. In addition, in any of the disclosed uses, the expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies. Alternatively, in any of the disclosed uses, expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies. In other embodiments, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[0048] In any of the disclosed methods, compositions or uses, the subject is a human subject that is 4 to 15 years of age, or a human subject that is 25 to 55 years of age or a human subject that is over 50 years of age.

[0049] In any of the disclosed methods, compositions or uses, the subject is a pediatric subject, an adolescent subject or a young adult subject. Alternatively, the subject is a middle aged adult or elderly subject.

[0050] For example, in any of the disclosed methods, compositions or uses, the subject is a human subject that is 4-15 years of age, has a confirmed alpha-sarcoglycan (SGCA) mutation in both alleles, was negative for AAVrh74 antibodies and/or had >40% or normal 100 meter walk test.

[0051] In another aspect, the disclosure provides for methods of generating a rAAV administered in a method of any of the disclosed methods, compositions or uses, the method comprising transferring an AAV vector plasmid to a host cell, wherein the AAV vector plasmid comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%identical to SEQ ID NO: 8. For example, the AAV vector plasmid comprises a nucleotide sequence of SEQ ID NO: 8. In some embodiments, the vector plasmid comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%identical to SEQ ID NO: 1, 4, or 8, or the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8.

[0052] In any of the disclosed methods of generating a rAAV, the method further comprises transferring a packaging plasmid and/or a helper virus to the host cell. In addition, in any of the disclosed methods of generating a rAAV, a packaging cell comprises a stably integrated AAV cap gene and/or comprises a packaging cell comprises a stably integrated AAV rep gene.

[0053] In another aspect, the disclosure provides for host cells comprising an AAV vector plasmid that comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%identical to SEQ ID NO: 1, 4, or 8. For example, the host cell comprises an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8.

BRIEF DESCRIPTION OF THE DRAWING

[0054] **Figure 1** shows the scAAVrh74.tMCK.hSGCA gene cassette.

[0055] **Figure 2** shows transgene expression in a dose-escalation study after systemic treatment with scAAVrh74.tMCK.hSGCA. (A) alpha-sarcoglycan immunofluorescence stain of multiple muscles from mice systemically (intravenous) treated with $1x10^{12}$ vg, $3x10^{12}$ vg, and $6x10^{12}$ vg (or 5x 10^{13} vg/kg, $1x10^{14}$ vg/kg, and $2x10^{14}$ vg/kg, respectively, based on a 20-g mouse) (n=6 per group). Muscle fibers expressing the alpha-sarcoglycan 12 weeks post-treatment ranged from 70%-93% compared to untreated controls. (B) Western blots of muscles from treated *sgca*$^{-/-}$ mice confirm hSGCA protein expression. Abbreviations: TA, tibialis anterior; GAS, gastrocnemius; QD, quadricep; TRI, tricep; GLUT, gluteus; PSO, psoas major; DIA, diaphragm; HRT, heart; WT, wild-type.

[0056] **Figure 3** shows improvement in muscle morphology by scAAVrh74.tMCK.hSGCA independent of dose in *sgca-/-*mice. (A) Hematoxylin & eosin images of various muscles from *sgca-/-* mice treated with $1x10^{12}$ vg, $3x10^{12}$ vg, and $6x10^{12}$ vg (or 5x 10^{13} vg/kg, $1x10^{14}$ vg/kg, and $2x10^{14}$ vg/kg, respectively, based on a 20-g mouse) of scAAVrh74.tMCK.hSGCA. Representative 20x images show a dramatic reduction in centralized nuclei and an overall normalization of fiber size independent of treatment dose. (B) Quantification confirming myofiber diameter normalization of various muscles in treated groups compared to vehicle-treated mice and wild-type controls (n=6 per group). (C) Quantification of centrally located nuclei in muscles of treated mice compared to untreated mice and wild-type controls (n=6 per group). Abbreviations: TA, tibialis anterior; GAS, gastrocnemius; QD, quadricep; GLUT, gluteus; PSO, psoas major; TRI, tricep; DIA, diaphragm; WT, wild-type.

[0057] **Figure 4** shows reduction fibrosis in *sgca*$^{-/-}$ mice treated with scAAVrh74.tMCK.hSGCA. (A) Picrosirius red staining shows reduced fibrosis in scAAVrh74.tMCK.hSGCA treated mice indicated by a decrease in collagen deposition compared to vehicle-treated *sgca*$^{-/-}$ mice in various muscles Representative 20x images shown. (B) Quantification of collagen levels in various muscles confirms reduction in collagen levels in all three treated groups compared to untreated mice and wild-type controls (n=6 per group). Abbreviations: PSO, psoas major; DIA, diaphragm; TRI, triceps; GLUT, gluteus.

[0058] **Figure 5** shows functional benefits to skeletal muscle after treatment with scAAVrh74.tMCK.hSGCA. (A) Following 3 months of treatment, tibialis anterior (TA) muscles were harvested (both left and right) to measure specific force and resistance to contraction-induced damage (normalized to TA weight). The quantification of specific force and eccentric contraction was increased in all treated groups (with minimal difference between doses) compared to untreated controls (n=6 per group). (B) Diaphragm muscle strips were harvested to measure specific force. Following 12 weeks of treatment, the force was significantly increased in treated mice compared to untreated *sgca*$^{-/-}$ mice. (C) Following 12 weeks of treatment, improvement was seen in ambulation and vertical activity through open-field analysis in treated mice compared to untreated *sgca*$^{-/-}$ controls (n=6 per group). (D) Creatine kinase levels in serum decreased in all treatment groups compared to untreated *sgca*$^{-/-}$ controls. Data were analyzed by one-way ANOVA followed by Tukey's post hoc analysis for multiple comparisons. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$ compared to vehicle-treated *sgca*$^{-/-}$ mice, unless noted. Abbreviations: TA, tibialis anterior; DIA, diaphragm; WT, wild-type.

[0059] **Figure 6** shows no evidence of toxicity through blood chemistries after treatment with scAAVrh74.tMCK.hSGCA. Liver enzymes (ALT, AST and ALP/K) and blood glucose (GLU) levels were analyzed for toxicity (n=6 per group). All chemistry values of treated mice were within the normal/healthy limits of mice as indicated by the dotted lines.

[0060] **Figure 7A** shows biodistribution analysis of systemic scAAVrh74.tMCK.hSGCA delivery—distribution histogram of mean vg copies of transcript per microgram DNA added to quantitative

polymerase chain reaction in various tissues from *sgca*$^{-/-}$ mice after intravenous delivery of scAAVrh74.tMCK.hSGCA at 3x10^{12} vg and 6x10^{12} vg (or 1x10^{14} vg/kg, and 2x10^{14} vg/kg, respectively, based on a 20-g mouse). **Figure 7B** shows Western blots of alpha-sarcoglycan protein expression in the liver of WT and *sgca*$^{-/-}$ mice treated with either vehicle (*sgca*$^{-/-}$ LR (lactate ringer)) or scAArh74.tMCK.hSGCA at 1.0 x10^{12} vg (Left Western blot) or 6 x 10^{12} vg (Right Western blot). Each lane represents an independent mouse (M1: Mouse 1; M2: Mouse 2; M3: Mouse 3). Bottom panel represents vinculin that was used as loading control. Abbreviations: DIA, diaphragm; TA, tibialis anterior; TRI, triceps.

[0061] **Figure 8** shows expression of scAAVrh74.tMCK.hSGCA in skeletal muscle of 12-month-old *sgca*$^{-/-}$ mice by immunofluorescence staining (Fig. 8A) and western blot (Fig. 8B). The graph in Figure 8C provides the percent positive muscle fibers in the scAAVrh74.tMCK.hSGCA *sgca*$^{-/-}$ mice. Abbreviations: TA, tibialis anterior; GAS, gastrocnemius; QD, quadricep; GLUT, gluteus; TRI, tricep; PSOAS, psoas major, diaphragm; WT, wild-type, LRS, lactated ringers solution

[0062] **Figure 9** shows histological results of administering scAAVrh74.tMCK.hSGCA in 12-month-old *SGCA*$^{-/-}$ mice. Figure 9a shows improved muscle pathology. Figure 9b shows reduction in central nucleation and increase in average fiber size in gastrocnemius (GAS) and triceps (TRI) muscles. Figure 9c shows a reduction in levels of fibrosis compared to untreated controls. Abbreviations: TA, tibialis anterior; GAS, gastrocnemius; QD, quadricep; GLUT, gluteus; PSO, psoas major, TRI, tricep; diaphragm; WT, wild-type.

[0063] Figure 10 shows functional improvement in aged mouse after administering scAAVrh74.tMCK.hSGCA.

DETAILED DESCRIPTION

[0064] The present disclosure is based on the discovery that administration of an rAAV comprising a polynucleotide expressing alpha-sarcoglycan results in a reduction or complete reversal of muscle fibrosis in a limb-girdle muscular dystrophy animal model. As demonstrated herein in the Examples, administration of the rAAV described herein resulted in the reversal of dystrophic features including reduced CK levels, increased muscle force, improved ambulation and vertical activity, and other motor functions.

[0065] The practice of the present invention will employ, unless otherwise indicated, conventional methods of virology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Sambrook et al. Molecular Cloning: A Laboratory Manual* (Current Edition); *DNA Cloning: A Practical Approach*, Vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., Current Edition); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., Current Edition); *Transcription and Translation* (B. Hames & S. Higgins, eds., Current Edition); *CRC Handbook of Parvoviruses*, vol. I & II (P. Tijssen, ed.); *Fundamental Virology*, 2nd Edition, vol. I & II (B. N. Fields and D. M. Knipe, eds.); *Freshney Culture of Animal Cells, A Manual of Basic Technique* (Wiley-Liss, Third Edition); and Ausubel et al. (1991) *Current Protocols in Molecular Biology* (Wiley Interscience, N.Y.).

[0066] All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Definitions

[0067] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to “the culture” includes reference to one or more cultures and equivalents thereof known to those skilled in the art, and so forth. Reference to “a recombinant AAV” includes a mixture of two or more rAAV virions, and the like. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[0068] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only, or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.”

[0069] Throughout this application, the term “about” is used to indicate that a value includes the statistical experimental error (standard deviation of error) for the device or method being employed to determine the value.

[0070] The term “vector” or “expression vector” is meant to be any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. In one embodiment, the vector is a viral vector. Expression vectors can contain a variety of control sequences, structural genes (e.g., genes of interest), and nucleic acid sequences that serve other functions as well.

[0071] As used herein, the term "AAV" is a standard abbreviation for adeno-associated virus. Adeno-associated virus is a single-stranded DNA parvovirus that grows only in cells in which certain functions are provided by a co-infecting helper virus. There are currently thirteen serotypes of AAV that have been characterized. General information and reviews of AAV can be found in, for example, Carter, 1989, Handbook of Parvoviruses, Vol. 1, pp. 169-228, and Berns, 1990, Virology, pp. 1743-1764, Raven Press, (New York). However, it is fully expected that these same principles will be applicable to additional AAV serotypes since it is well known that the various serotypes are quite closely related, both structurally and functionally, even at the genetic level. (See, for example, Blacklowe, 1988, pp. 165-174 of Parvoviruses and Human Disease, J. R. Pattison, ed.; and Rose, Comprehensive Virology 3:1-61 (1974)). For example, all AAV serotypes apparently exhibit very similar replication properties mediated by homologous rep genes; and all bear three related capsid proteins such as those expressed in AAV2. The degree of relatedness is further suggested by heteroduplex analysis which reveals extensive cross-hybridization between serotypes along the length of the genome; and the presence of analogous self-annealing segments at the termini that correspond to “inverted terminal repeat sequences” (ITRs). The similar infectivity patterns also suggest that the replication functions in each serotype are under similar regulatory control.

[0072] The term "AAV vector" refers to a vector comprising one or more polynucleotides of interest (or transgenes) that are flanked by AAV terminal repeat sequences (ITRs). Such AAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been transfected with a vector encoding and expressing rep and cap gene products. In one embodiment, the AAV vector is a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAV rh10, and AAV rh74. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences. Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV virion. Thus, an AAV vector is defined herein to include at least those sequences required in cis for replication and packaging (e.g., functional ITRs) of the virus. The ITRs need not be the wild-type nucleotide sequences, and may be altered, e.g., by the insertion, deletion or substitution of nucleotides, so long as the sequences provide for functional rescue, replication and packaging.

[0073] The term "AAV helper functions" refer to AAV-derived coding sequences that can be expressed to provide AAV gene products that, in turn, function in trans for productive AAV replication. Thus, AAV helper functions comprise the major AAV open reading frames (ORFs), reps and cap. The Rep expression products have been shown to possess many functions, including, among others: recognition, binding and nicking of the AAV origin of DNA replication; DNA helicase activity; and modulation of transcription from AAV (or other heterologous) promoters. The Cap expression products supply necessary packaging functions. AAV helper functions are used herein to complement AAV functions in trans that are missing from AAV vectors.

[0074] By "recombinant virus" is meant a virus that has been genetically altered, e.g., by the addition or insertion of a heterologous nucleic acid sequence into the viral particle.

[0075] By "AAV virion" "AAV viral particle" or "AAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. The AAV virion, in one embodiment, comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell). Production of AAV viral particles, in some embodiment, includes production of AAV vector, as such a vector is contained within an AAV vector particle. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector" or simply "rAAV particle." Thus, production of AAV vector particle necessarily includes production of rAAV, as such a rAAV genome is contained within an rAAV vector particle.

[0076] For example, a wild-type (wt) AAV virus particle comprising a linear, single-stranded AAV nucleic acid genome associated with an AAV capsid protein coat. The AAV virion can be either a single-stranded (ss) AAV or self-complementary (SC) AAV. In one embodiment, a single-stranded AAV nucleic acid molecules of either complementary sense, e.g., "sense" or "antisense" strands, can be packaged into a AAV virion and both strands are equally infectious.

[0077] The term "recombinant AAV," or "rAAV" is defined herein as an infectious, replication-defective virus composed of an AAV protein shell, encapsidating a heterologous nucleotide sequence of interest which is flanked on both sides by AAV ITRs. A rAAV, in one embodiment, is produced in a suitable host cell which has an AAV vector, AAV helper functions and accessory functions introduced therein. In this manner, the host cell is capable of encoding AAV polypeptides that are required for packaging the AAV vector (containing a recombinant nucleotide sequence of interest) into infectious recombinant virion particles for subsequent gene delivery.

[0078] The term "transfection" refers to the uptake of foreign DNA by a cell, and a cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) Virology, 52:456, Sambrook et al. (1989) Molecular Cloning, a laboratory manual, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier, and Chu et al. (1981) Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties, such as a nucleotide integration vector and other nucleic acid molecules, into suitable host cells.

[0079] The term "transduction" denotes the delivery of a DNA molecule to a recipient cell either in vivo or in vitro, via a replication-defective viral vector, such as via a recombinant AAV virion.

[0080] The term "host cell" denotes, for example, microorganisms, yeast cells, insect. cells, and mammalian cells, that can be, or have been, used as recipients of an AAV helper construct, an AAV vector plasmid, an accessory function vector, or other transfer DNA. The term includes the progeny of the original cell which has been transfected. Thus, a "host cell" as used herein generally refers to a cell which has been transfected with an exogenous DNA sequence. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0081] The term "heterologous" as it relates to nucleic acid sequences such as coding sequences and control sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct or a vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a cell transformed with a construct which is not normally present in the cell would be considered heterologous for purposes of this invention. Allelic variation or naturally occurring mutational events do not give rise to heterologous DNA, as used herein.

[0082] A "coding sequence" or a sequence which "encodes" a particular protein, is a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a

polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

[0083] A "nucleic acid" sequence refers to a DNA or RNA sequence. The nucleic acids include base analogues of DNA and RNA including, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl)uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5′-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, -uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[0084] The term DNA "control sequences" refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

[0085] The term "promoter" is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3′-direction) coding sequence. Transcription promoters can include "inducible promoters" (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), "repressible promoters" (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and "constitutive promoters." In one embodiment, the promoter is a muscle-specific promoter, which includes but is not limited to, a human skeletal actin gene element, a cardiac actin gene element, a desmin promoter, a skeletal alpha-actin (ASKA) promoter, a troponin I (TNNI2) promoter, a myocyte-specific enhancer binding factor mef binding element, a muscle creatine kinase (MCK) promoter, a truncated MCK (tMCK) promoter, a myosin heavy chain (MHC) promoter, a hybrid a-myosin heavy chain enhancer-/MCK enhancer-promoter (MHCK7) promoter, a C5-12 promoter, a murine creatine kinase enhancer element, a skeletal fast-twitch troponin c gene element, a slow-twitch cardiac troponin c gene element, a slow-twitch troponin i gene element, hypoxia- inducible nuclear factor

(HIF)-response element (HRE), a steroid-inducible element, and a glucocorticoid response element (gre). In another embodiment, the promoter is an MCK promoter, a tMCK promoter, or an MHCK7 promoter.

[0086] The term "operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0087] A promoter "directs the transcription" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

[0088] "Expression cassette" or "expression construct" refers to an assembly which is capable of directing the expression of the sequence(s) or gene(s) of interest. The expression cassette includes control elements, as described above, such as a promoter which is operably linked to (so as to direct transcription of) the sequence(s) or gene(s) of interest, and often includes a polyadenylation sequence as well. Within certain embodiments of the invention, the expression cassette described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA, at least one multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

[0089] By "isolated" when referring to a nucleotide sequence, is meant that the indicated molecule is present in the substantial absence of other biological macromolecules such as other nucleotide sequences, chromatin material, etc. Thus, an "isolated nucleic acid molecule which encodes a particular polypeptide" refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

[0090] For the purpose of describing the relative position of nucleotide sequences in a particular nucleic acid molecule throughout the instant application, such as when a particular nucleotide sequence is described as being situated "upstream," "downstream," "3," or "5" relative to another sequence, it is to be understood that it is the position of the sequences in the "sense" or "coding" strand of a DNA molecule that is being referred to as is conventional in the art.

[0091] The terms "sequence identity", "percent sequence identity", or "percent identical" in the context of nucleic acid or amino acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a

fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, e.g. of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. The percentage identity of the sequences can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs such as ALIGN, ClustalW2 and BLAST. In one embodiment, when BLAST is used as the alignment tool, the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+Swiss protein+Spupdate+PIR.

[0092] The term "subject" refers to any member of the animal kingdom, which includes, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. In some embodiments, the subject is a human ranging in age from birth to 2 years, from 1 to 10 years, or ranging from 4 to 15 years, or ranging from 10 to 19 years, or from 20 to 40 years of age, or from 15 to 29 years of age or from 25-55 years, or ranging from 40 to 60 years, or over 50 years or over 60 years or over 65 years or over 70 years. For example, the subject is a human child (2 to 12 years), a human adolescent (10 to 19 years). . In some embodiments, the subject is an adult human (18 years or older). In particular, the subject is a young adult human (15 to 29 years of age), middle aged adult human (25 to 55 year of age) or an older adult human (over 50 years of age) or elderly human subject (over 65 years of age) or a geriatric human subject (over 70 years of age).

[0093] By "therapeutic effect" is meant any therapeutic benefit conferred by the treatment described herein. For example, such an effect can be sustained expression, in an appropriate target tissue, of a protein or an enzyme which is deficient or missing in the muscular dystrophy of interest. Additionally, a therapeutic effect may be any reduction or elimination of one or more clinical or subclinical manifestations of the disease or disorder of interest. For example, a reduction in the CK level, reduction in fibrosis is reduced; an increase in the resistance to contraction-induced injury in tibialis anterior muscle; and/increased specific force in the muscle, and improved motor function provides a therapeutic benefit to the treated subject with LGMD-2D.

[0094] In another aspect, a recombinant AAV vector described herein comprises a polynucleotide sequence encoding alpha-sarcoglycan that is at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2, or a protein retains α -sarcoglycan activity. In another embodiment, the alpha-sarcoglycan comprises a polypeptide sequence set forth in SEQ ID NO: 2.

[0095] In another aspect, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding functional alpha-sarcoglycan that comprises a nucleotide

sequence that hybridizes under stringent conditions to the nucleic acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, or a complement thereof. In another embodiment, the rAAV comprises a nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4. In another embodiment, the rAAV comprises a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 4.

[0096] 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).

[0097] When ranges are used herein for physical properties, such as molecular weight, concentration, or dosage, all combinations and subcombinations of ranges and specific embodiments 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.

[0098] 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, $NaDodSO_4$, (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.

[0099] Limb-girdle muscular dystrophy type 2D (LGMD2D) is a progressive muscular dystrophy that manifests with muscle weakness, respiratory abnormalities, and in rare cases cardiomyopathy. LGMD2D is caused by mutations in the alpha-sarcoglycan gene resulting in loss of protein and concomitant loss of the sarcoglycan and dystrophin-associated glycoprotein complex. The Sgca-null

(*sgca*$^{-/-}$) mouse recapitulates the clinical phenotype of patients with LGMD2D, including dystrophic features such as muscle necrosis and fibrosis, elevated serum creatine kinase (CK), and reduction in generation of absolute muscle force and locomotor activity. Thus, sgca$^{-/-}$ mice provide a relevant model to test the safety and efficacy of gene replacement. Hereby, this disclosure provides a self-complementary AAVrh74 vector containing a codon-optimized full-length human SGCA (hSGCA) transgene driven by a muscle-specific promoter, truncated muscle creatine kinase (tMCK). The efficacy and safety of scAAVrh74.tMCK.hSGCA in *sgca*$^{-/-}$ mice were tested using a dose-escalation design to evaluate a single systemic injection of 1 x 10^{12}, 3 x 10^{12}, and 6 x 10^{12} vg compared to vehicle-treatment and wild-type mice. In sgca$^{-/-}$ mice treatment with scAAVrh74.tMCK.hSGCA resulted in robust protein expression of α-SG at the sarcolemma membrane in skeletal muscle at all doses tested. Additionally, scAAVrh74.tMCK.hSGCA was effective in improving the histopathology of limb and diaphragm muscle of sgca$^{-/-}$ mice, as indicated by reductions in fibrosis and central nucleation and normalization of myofiber size. These molecular changes were concomitant with significant increases in specific force generation in the diaphragm and tibialis anterior muscle, protection against eccentric force loss, and reduction in serum CK. Locomotor activity was improved at all doses of vector-treated compared to vehicle-treated sgca$^{-/-}$ mice. Lastly, a lack of vector-associated toxicity was detected in a serum chemistry panel and by gross necropsy. Collectively, the study provides support for a systemic delivery of scAAVrh74.tMCK.hSGCA in a clinical setting for the treatment of LGMD2D.

[00100] In another aspect, the recombinant AAV vectors described herein may be operably linked to a muscle-specific control element. For example, the muscle-specific promoter comprises one or more of a human skeletal actin gene element, a cardiac actin gene element, a desmin promoter, a skeletal alpha-actin (ASKA) promoter, a troponin I (TNNI2) promoter, a myocyte-specific enhancer binding factor mef binding element, a muscle creatine kinase (MCK) promoter, a truncated MCK (tMCK) promoter, a myosin heavy chain (MHC) promoter, a hybrid a-myosin heavy chain enhancer-/MCK enhancer-promoter (MHCK7) promoter, a C5-12 promoter, a murine creatine kinase enhancer element, a skeletal fast-twitch troponin c gene element, a slow-twitch cardiac troponin c gene element, a slow-twitch troponin i gene element, hypoxia- inducible nuclear factor (HIF)-response element (HRE), a steroid-inducible element, and a glucocorticoid response element (gre).

[00101] In one embodiment, the muscle-specific promoter is a tMCK promoter, which comprises a sequence of SEQ ID NO: 3. An exemplary rAAV described herein is AAVrh74.tMCK.hSCGA which comprises a nucleotide sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide sequence encoding a AAVrh74.tMCK.hSCGA comprises a sequence at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the nucleotide sequence set forth in SEQ ID NO: 4, or to a nucleotide sequence that is at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to SEQ ID NO: 1.

[00102] In another embodiment, the polynucleotide sequence encoding a AAVrh74.tMCK.hSCGA comprises a nucleotide sequence that encodes a polypeptide sequence at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the nucleotide sequence set forth in SEQ ID NO: 1. In some embodiments, the polynucleotide sequence encodes a protein that retains the alpha-sarcoglycan activity.

[00103] In one embodiment, the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5. In another embodiment, the rAAV comprises a 3' inverted terminal repeat sequence of SEQ ID NO: 6. In some embodiments, the rAAV comprises a poly A sequence of SEQ ID NO: 7.

[00104] 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, AAV rh.10, AAV rh.74, or variants and derivatives thereof. In one embodiment, the rAAV is of the serotype AAVrh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 2001/083692, which is incorporated by reference in its entirety. 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).

[00105] Compositions comprising any of the rAAV vectors described herein are also contemplated.

[00106] Provided are methods of treating muscular dystrophy in a human subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.tMCK.hSGCA, wherein the rAAV is administered using at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg. For example, in any of the provided methods, the dose of the rAAV administered is between about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 5×10^{13} vg/kg to about 2×10^{14} vg/kg, or about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg. In another embodiment, the dose is about 5.0×10^{13} vg/kg, 1.0×10^{14} vg/kg, or 2.0×10^{14} vg/kg. In one embodiment, the rAAV is administered by a systemic route, which comprises an intravenous route. In another embodiment, the rAAV is administered intravenously at a dose of about 5.0×10^{13} vg/kg, 1.0×10^{14} vg/kg, or 2.0×10^{14} vg/kg. In one embodiment, the muscular dystrophy is limb-girdle muscular dystrophy.

[00107] In addition, the dose of the rAAV administered is about 1.5×10^{13} vg to about 3.5×10^{16} vg, or 3×10^{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 one embodiment, the muscular dystrophy is limb-girdle muscular dystrophy. In one embodiment, the muscular dystrophy is limb-girdle muscular dystrophy, type 2D. The doses in this disclosure, expressed in either vg or vg/kg, are based on a titration qualification method by quantitative PCR (qPCR). The qPCR-based titration method is known in the art.

[00108] 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.tMCK.hSGCA, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0 x 10^{12} vg/kg to about 2.0 x 10^{15} vg/kg; wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of alpha-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; and/or wherein the locomotor activity and specific-force generation are increased; wherein fibrosis is reduced; wherein the resistance to contraction-induced injury in tibialis anterior muscle is increased; and/ wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of alpha-sarcoglycan positive fibers before administration of the rAAV; wherein the fiber diameter size in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of the fiber diameter before administration of the rAAV; or wherein the central nucleation in the muscle tissue of the subject is reduced after administration of the rAAV as compared to the central nucleation before administration of the rAAV. The muscle tissues include but are not limited to triceps, tibialis anterior, soleus, gastrocnemius, biceps, trapezius, gluteus, psoas major, deltoids, quadriceps, and diaphragm. In one embodiment, the muscle tissues comprise tibialis anterior, gastrocnemius, gluteus, psoas major, and triceps. The expression of alpha-sarcoglycan is determined by methods known to a person with ordinary skill in the art. In one embodiment, the expression is determined by Western blot, immunochemistry in muscle biopsies, and/or by detecting the number of vector genome per microgram of genomic DNA.

[00109] In some embodiments, 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.tMCK.hSGCA, wherein motor function is demonstrably improved in said human subject as compared to motor function of said human subject before administration of the rAAV.

[00110] Provided are methods of increasing alpha-sarcoglycan in a patient in need thereof comprising administering to the patient the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

[00111] In any of the methods, uses and compositions of treating muscular dystrophy provided, the subject is 4-15 years of age, has confirmed alpha-sarcoglycan (SGCA) mutation in both alleles, is 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 embodiments, the subject is a pediatric subject, such as a subject ranging in age from 1 to 21 years. In some embodiments, the subject is 1 to 10 years of age, or 2 to 12 years of age,4 to 15 years of age, or 10 to 19 years of age. The subject, in one embodiment, is an adolescent subject, such as a subject ranging in age from 12 to 21 years. In addition, the subject, in one embodiment, is a young adult subject such as a subject ranging in age from 15 to 29 years of age or 18-39 years of age. In some embodiment, 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, the older adult subject may range in age over 50 years of age, and the elderly subject may range in age over 65 years of

age. In some embodiments, 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.

[00112] In the methods of treating muscular dystrophy in a human subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.tMCK.hSGCA, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0 x 10^{12} vg/kg to about 5.0 x 10^{14} vg/kg and the rAAV comprises a nucleotide sequence at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In another embodiment, the rAAV comprises a nucleotide sequence set forth in SEQ ID NO: 1. In one embodiment, the rAAV encodes a protein comprising a polypeptide sequence that is at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 2. In another embodiment, the rAAV comprises a nucleotide sequence encoding a protein comprising a polypeptide sequence set forth in SEQ ID NO: 2. In addition, the any of the disclosed rAAV comprise a promoter such as the tMCK promoter sequence of SEQ ID NO: 3. In some embodiments, the rAAV is of the serotype AAVrh.74. In addition, the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 3. In on embodiment, the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5. In another embodiment, the rAAV comprises a 3' inverted terminal repeat sequence of SEQ ID NO: 6. In another embodiment, the rAAV comprises a poly A sequence of SEQ ID NO: 7.

[00113] AAV dosage can be determined by multiple methods, which include but are not limited to LISA, assessment of the reverse transcriptase activity, FACS, transduction assays northern blotting (e.g., semi-quantitative northern), dot blot analysis or PCR (e.g., qPCR). It is well known that the AAV doses can be determined by measuring AAV vector genomes with quantitative real-time PCR (qPCR). Such qPCR methods overcome the inconsistency or arbitrary results from conventional transduction assays. In one embodiment of PCR dosage determination, plasmid DNA is used as a calibration standard. The forms of the plasmids can impact the dosage results from the qPCR methods. In one embodiment, the circular or supercoiled DNA or plasmids are used as a quantification standard. In another embodiment, the linearized DNA or plasmids are used as the quantification standard.

[00114] The term "supercoiled DNA" or "supercoiled plasmid" refers to a DNA or plasmid that comprises no free end. The term "linearized DNA" or linearized plasmid" refer to a DNA or plasmid that comprises a free 5' end and a free 3' end, which are not linked to each other. In one embodiment, a linearized DNA or plasmid is obtained by a restriction digest of a circular DNA (e.g. plasmid DNA) or by a restriction digest of a dbDNA. In another embodiment, the restriction digest is performed using enzymes that generate at least one blunt end.

[00115] In an exemplary embodiment, methods of treating muscular dystrophy in a human subject in need thereof comprise the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74. tMCK7.hSGCA, wherein the rAAV is administered using a systemic route of

administration and at a dose of about 1.0 x 10^{12} vg/kg to about 5.0 x 10^{14} vg/kg, wherein the human subject is suffering from limb-girdle muscular dystrophy. In one embodiment, the rAAV is administered by intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0 x 10^{13} vg/kg, 1.0 x 10^{14} vg/kg, or 2.0 x 10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74. tMCK7.hSGCA construct nucleotide sequence of SEQ ID NO: 3. In another embodiment, the dose is about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[00116] The disclosure also provides a method of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a scAAVrh74.tMCK.hSGCA construct comprising a nucleotide sequence at least 90% identical, at least 95% identical, or 99% identical to SEQ ID NOs: 1, and/or 4.

[00117] The disclosure further provides a method of improving muscle function of a subject comprising administering to the subject a construct comprising a nucleotide sequence at least 90% identical, at least 95% identical, or 99% identical to SEQ ID NO: 1, and/or 4.

[00118] In some aspects, the subject suffers from a genetic mutation in a gene encoding a sarcoglycan protein or a muscular dystrophy. In some aspects, the subject suffers from a genetic mutation in a gene encoding alpha-sarcoglycan protein.

[00119] In any of the provided methods, the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of scAAVrh74.tMCK 7.hSGCA construct as compared to the level of alpha-sarcoglycan gene expression before administration of scAAVrh74.tMCK.hSGCA construct .

[00120] In addition, in any of the provided methods, the expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsied before and after administration of scAAVrh74.tMCK.hSGCA construct.

[00121] In any of the provided methods, the level of alpha-sarcoglycan protein is increased after administration of scAAVrh74.tMCK.hSGCA construct. For example, the level of the level of alpha-sarcoglycan protein is increased by at least 33% as detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsied before and after administration of scAAVrh74.tMCK.hSGCA construct, or the level of alpha -sarcoglycan protein is measured by immunohistochemistry in muscle biopsies and/or by detecting the number of vector genome per microgram of genomic DNA before and after administration ofscAAVrh74.tMCK.hSGCA construct .

[00122] In any of the methods provided herein, the serum CK level in the subject is decreased after administration of scAAVrh74.tMCK.hSGCA construct as compared to serum CK level before administration of scAAVrh74.tMCK.hSGCA construct .

[00123] In any of the methods provided herein, the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of scAAVrh74.tMCK.hSGCA

construct as compared to the number of alpha-sarcoglycan positive fibers before administration ofscAAVrh74.tMCK.hSGCA construct . For example, the number of alpha-sarcoglycan positive fibers is detected by measuring the alpha-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of scAAVrh74.tMCK.hSGCA construct. For example, the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of scAAVrh74.tMCK.hSGCA construct.

[00124] In any of the methods 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 scAAVrh74.tMCK.hSGCA construct . 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 scAAVrh74.tMCK.hSGCA construct .

[00125] Another embodiment provides for methods expressing alpha-sarcoglycan gene in a patient cell comprising administering to the patient the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. In any of the provided methods of expressing alpha-sarcoglycan gene in a patient cell, expression of the alpha-sarcoglycan gene in the patient cell is detected by measuring the alpha-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsies before and after administration of the scAAVrh74.tMCK.hSGCA construct. In one embodiment,, the alpha-sarcoglycan gene is measured in the patient by detecting greater than one rAAV vector genome copy per nucleus. In another embodiment, the expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

[00126] Methods of decreasing serum CK levels in a patient in need thereof, the method comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4 are also provided.

[00127] Methods of increasing alpha-sarcoglycan positive fibers in a patient muscle tissue comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4 are provided. In any of these methods, the number of alpha-sarcoglycan positive fibers is detected by measuring the alpha-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV.

[00128] Another embodiment provides for methods of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4. In any of these methods, 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.

[00129] Methods of producing a recombinant AAV vector particle comprising culturing a host cell that is transferred 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. In one embodiment, the method of generating the rAAV comprising transferring an AAV vector plasmid to a host cell. In another embodiment, the recombinant AAV vector particle and/or the AAV vector plasmid comprises a nucleotide sequence that is at least about 65%, about 70%, about 75%, about 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 SEQ ID NO: 8. In another aspect, the disclosure provides a host cell that comprising an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 8. In some embodiment, 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 embodiment, the AAV vector plasmid is a pAAV.tMCK.hSGCA. KAN plasmid (SEQ ID NO: 8).

[00130] 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 one embodiment, the muscular dystrophy is LGMD2D. In some embodiments, administration of an AAV vector described herein (or composition comprising an AAV vector described herein) reduces fibrosis in the muscle tissue of the subject. In one embodiment, the muscle tissue comprises psoas major, diaphragm, triceps, and/or gluteus.

[00131] 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 us suffering from limb-girdle muscular dystrophy type 2D (LGMD2D).

[00132] 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.

[00133] In another aspect, described herein is a method of increasing alpha-sarcoglycan positive fibers in a muscle tissue, fiber diameter size, eccentric contraction in the muscle, muscular force and/or expression of alpha-sarcoglycan 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. Also described herein is a method of reducing fibrosis, central nucleation, CK level, and/or collage deposition in a subject comprising administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to a subject.

[00134] In any of the methods of the invention, the subject may be suffering from muscular dystrophy such as limb-girdle muscular dystrophy or any other dystrophin-associated muscular dystrophy. In one embodiment, the muscular dystrophy is LGMD-2D.

[00135] 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.

[00136] In any of the methods of the invention, the rAAV is administered by intramuscular injection or intravenous injection. In addition, in any of the method of the invention, the rAAV is administered systemically, such as parental administration by injection, infusion or implantation.

[00137] The compositions of the invention are formulated for intramuscular injection or intravenous injection. In addition, the compositions of the invention are formulated for systemic administration, such as parental administration by injection, infusion or implantation.

[00138] 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 that expressed alpha-sarcoglycan or a second recombinant AAV vector comprising a polynucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 4.

[00139] In any of the uses of the invention, the medicament is formulated for intramuscular injection or intravenous injection. In addition, in any of the uses of the invention, 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 that expressed alpha-sarcoglycan or a second recombinant AAV vector comprising a polynucleotide sequence in SEQ ID NO: 1 or SEQ ID NO: 4.

[00140] The foregoing paragraphs are not intended to define every aspect of the invention, and additional aspects 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 invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by specific paragraphs above. For example, where certain aspects of the invention that are described as a genus, it should be understood that every member of a genus is, individually, an aspect of the invention.

AAV

[00141] Recombinant AAV genomes of the invention comprise nucleic acid molecule of the invention and one or more AAV ITRs flanking a nucleic acid molecule. AAV DNA in the rAAV genomes may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, 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.

[00142] DNA plasmids of the invention comprise rAAV genomes. The DNA plasmids are transferred to cells permissible for infection with a helper virus of AAV (e.g., adenovirus, E1-deleted adenovirus or herpesvirus) for assembly of the rAAV genome into infectious viral particles. Techniques to produce rAAV particles, in which an AAV genome to be packaged, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (*i.e.*, not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes may be from any AAV serotype for which recombinant virus can be derived and may be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAV rh10, and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 2001/083692, which is incorporated by reference herein in its entirety.

[00143] A method of generating a packaging cell is to create a cell line that stably expresses all the necessary components for AAV particle production. For example, a plasmid (or multiple plasmids) comprising a rAAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the rAAV genome, and a selectable marker, such as a neomycin resistance gene, are integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing (Samulski et al., 1982, Proc. Natl. Acad. S6. USA, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin et al., 1983, Gene, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, J. Biol. Chem., 259:4661-4666). The packaging cell line is then infected with a helper virus such as adenovirus. The advantages of

this method are that the cells are selectable and are suitable for large-scale production of rAAV. Other examples of suitable methods employ adenovirus or baculovirus rather than plasmids to introduce rAAV genomes and/or rep and cap genes into packaging cells.

[00144] General principles of rAAV production are reviewed in, for example, Carter, 1992, Current Opinions in Biotechnology, 1533-539; and Muzyczka, 1992, Curr. Topics in Microbial. and Immunol., 158:97-129). Various approaches are described in Ratschin et al., Mol. Cell. Biol. 4:2072 (1984); Hermonat et al., Proc. Natl. Acad. Sci. USA, 81:6466 (1984); Tratschin et al., Mo1. Cell. Biol. 5:3251 (1985); McLaughlin et al., J. Virol., 62:1963 (1988); and Lebkowski et al., 1988 Mol. Cell. Biol., 7:349 (1988). Samulski et al. (1989, J. Virol., 63:3822-3828); U.S. Patent No. 5,173,414; WO 95/13365 and corresponding U.S. Patent No. 5,658.776 ; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin et al. (1995) Vaccine 13:1244-1250; Paul et al. (1993) Human Gene Therapy 4:609-615; Clark et al. (1996) Gene Therapy 3:1124-1132; U.S. Patent. No. 5,786,211; U.S. Patent No. 5,871,982; and U.S. Patent. No. 6,258,595. The foregoing documents are hereby incorporated by reference in their entirety herein, with particular emphasis on those sections of the documents relating to rAAV production.

[00145] The invention thus provides packaging cells that produce infectious rAAV. In one embodiment packaging cells may be stably transformed cancer cells such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another embodiment, packaging cells are cells that are not transformed cancer cells, such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[00146] Recombinant AAV (*i.e.*, infectious encapsidated rAAV particles) of the invention comprise a rAAV genome. Embodiments include, but are not limited to, the rAAV named pAAV.tMCK.hSCGA which comprises the polynucleotide sequence set forth in SEQ ID NO: 3..

[00147] The rAAV may be purified by methods standard in the art such as by column chromatography or cesium chloride gradients. Methods for purifying rAAV vectors from helper virus are known in the art and include methods disclosed in, for example, Clark *et al., Hum. Gene Ther., 10*(6): 1031-1039 (1999); Schenpp and Clark, *Methods Mol. Med., 69* 427-443 (2002); U.S. Patent No. 6,566,118 and WO 98/09657.

[00148] In another embodiment, the invention contemplates compositions comprising rAAV of the present invention. Compositions described herein comprise rAAV in a pharmaceutically acceptable carrier. The compositions may also comprise other ingredients such as diluents and adjuvants. Acceptable carriers, diluents and adjuvants are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides,

disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-formig counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

[00149] Titers of rAAV to be administered in methods of the invention will vary depending, for example, on the particular rAAV, the mode of administration, the treatment goal, the individual, and the cell type(s) being targeted, and may be determined by methods standard in the art. Titers of rAAV may range from about $1x10^6$, about $1x10^7$, about $1x10^8$, about $1x10^9$, about $1x10^{10}$, about $1x10^{11}$, about $1x10^{12}$, about $1x10^{13}$ to about $1x10^{14}$ or more DNase resistant particles (DRP) per ml. Dosages may also be expressed in units of viral genomes (vg) as measured by qPCR.

[00150] Methods of transducing a target cell with rAAV, *in vivo or in vitro*, are contemplated by the invention. The *in vivo* methods comprise the step of administering an effective dose, or effective multiple doses, of a composition comprising a rAAV of the invention to an animal (including a human being) in need thereof. If the dose is administered prior to development of a disorder/disease, the administration is prophylactic. If the dose is administered after the development of a disorder/disease, the administration is therapeutic. In embodiments of the invention, an effective dose is a dose that alleviates (eliminates or reduces) at least one symptom associated with the disorder/disease state being treated, that slows or prevents progression to a disorder/disease state, that slows or prevents progression of a disorder/disease state, that diminishes the extent of disease, that results in remission (partial or total) of disease, and/or that prolongs survival. An example of a disease contemplated for prevention or treatment with methods of the invention is muscular dystrophy, such as limb-girdle muscular dystrophy. Thus, provided is a method of transducing a target cell with an rAAV scAAVrh74.tMCK.hSGCA, which comprises a nucleotide sequence of SEQ ID NO: 4.

[00151] In another embodiment, the disclosure provides a method of generating the rAAV scAAVrh74.tMCK.hSGCA which comprises transferring an AAV vector plasmid to a host cell. The methods of transferring a DNA to a host cell are well known in the art, which include but are not limited to transfection, infection, transformation, electroporation, and transduction. In one embodiment, the vector plasmid comprises a nucleotide sequence that is at least about 65%, about 70%, about 75%, about 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 SEQ ID NO: 8. In another embodiment, the vector plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 8. In another embodiment, the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 8. In another aspect, the disclosure provides a host cell that comprising an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 8. In some embodiment, 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 embodiment, the AAV vector plasmid is a pAAV.tMCK.hSGCA. KAN plasmid.

[00152] In one embodiment, the vector plasmid comprises a nucleotide sequence that is at least about 65%, about 70%, about 75%, about 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 SEQ ID NO: 1, 4, or 8.

[00153] In one embodiment, the vector plasmid comprises a nucleotide sequence that is at least about 90%, 95%, or 99% identical to SEQ ID NO: 1, 4, or 8. In one embodiment, the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8. The method of generating rAAV, in one embodiment, further comprises transferring a packaging plasmid and/or a helper virus to the host cell. In the packaging plasmid, in some embodiments, comprises an AAV rep and/or cap gene that is operably linked to a promoter. The promoter, in one embodiment, is an AAV transcription promoter. In one embodiment, the host cell is a packaging cell. In one embodiment, the packaging cell comprises a stably integrated AAV cap gene. In another embodiment, the packaging cell comprises a stably integrated AAV rep gene.

[00154] As used herein, the term "host cell" refers to a cell that can be used to express an exogenous DNA sequence. Non-limiting examples of a host cell comprise a microorganism, a yeast cell, an insect cell, and/or a mammalian cell. The host cell can be used as a recipient for an AAV helper construct, a packaging plasmid, an AAV vector plasmid, an accessary function vector, or other DNA. The term as used here encompasses the progeny of the original cell after expressing the exogenous DNA sequence in the original host cell. Non-limiting examples of host cells for AAV production include Sf9 insect cells and HEK 293T cells. The AAV vector plasmid can be introduced to the host cells, e.g., Sf9 or 293T, by infection (virus or baculovirus), transient transfection using reagents (e.g., liposomal, calcium phosphate) or physical means (e.g., electroporation), or other means know in the art. In another embodiment, the host cell lines are stably integrated with the rAAV plasmids into their genomes. Such stable cell lines can be established by incorporating a selection marker into the vector plasmid.

[0098] In one embodiment, the host cell is a packaging cell for production of AAV viral particles. Thus, in another aspect, the disclosure provides a host cell that comprises an AAV vector plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 8. In one embodiment, the AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 8. In another embodiment, the host cell comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8.

[00155] Combination therapies are also contemplated by the invention. Combination as used herein includes both simultaneous treatment or sequential treatments. Combinations of methods of the invention with standard medical treatments (*e.g.,* steroids, corticosteroids, and/or glucocorticoids including but not limited to one or more of prednisone, prednisolone; and deflazacort) are specifically contemplated, as are combinations with novel therapies. In this regard, the combinations include administering to a subject one or more steroids, corticosteroids, and/or glucocorticoids including but not limited to one or more of prednisone, prednisolone; and deflazacort before administering an rAAV

of the inventive methods to the subject, simultaneously with administering the rAAV to the subject, or after administering the rAAV to the subject.

[00156] In related embodiments of a combination therapy contemplated by the invention, the glucocorticoid includes, but is not limited to beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, or triamcinolone.

[00157] It is recognized that an antigen specific T-cell response may occur in a subject administered with the rAAV vector. This is an expected response between 2-4 weeks following gene transfer. One possible consequence to such antigen specific T-cell responses is clearance of the transduced cells and loss of transgene expression. To dampen the host immune response to the rAAV based therapy, before the therapy, for example, twenty-four hours prior to the therapy procedure, subjects can be started on approximately 1mg/kg/day prophylactic prednisone or comparable glucocorticoid by mouth with a maximum dose of 60 mg/day. IV administration of a comparable glucocorticoid at the approximate dose of 1 mg/kg/day would also be allowable if needed. Treatment will continue for approximately one month. A tapering protocol for prednisone or comparable glucocorticoid can be implemented based on individual subjects' immune response to the gene transfer, assessed by ELISpot assay and also by liver function monitoring with GGT.

[00158] A therapeutically effective amount of the rAAV vector is a dose of rAAV ranging from between about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 5×10^{13} vg/kg to about 2×10^{14} vg/kg, or about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg. In another embodiment, the dose is about 5.0×10^{13} vg/kg, about 1.0×10^{14} vg/kg, or about 2.0×10^{14} vg/kg. In another embodiment, the dose is 5.0×10^{13} vg/kg, 1.0×10^{14} vg/kg, or 2.0×10^{14} vg/kg. The invention is also contemplated to include compositions comprising these ranges of rAAV vector.

[00159] Dosages may also be expressed in units of viral genomes (vg). The titers of rAAV may be determined by the supercoiled DNA or plasmid quantitation standard or the linearized DNA or plasmid quantitation standard. The titer or dosage of AAV vectors can vary based on the physical forms of plasmid or DNA as a quantitation standard. For example, the value of titer or dosage may vary based off of a supercoiled standard qPCR titering method or a linear standard qPCR titering method. In one embodiment, the dosage in this disclosure is based on a supercoiled DNA or plasmid as the quantitation standard. In another embodiment, the dosage in this disclosure is based on a linearized DNA or plasmid as the quantitation standard. Therefore, in one embodiment, the therapeutically effective amount of the rAAV vector is a dose of rAAV ranging from between about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 5×10^{13} vg/kg to about 2×10^{14} vg/kg, or about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. In another embodiment, the dose is about 5.0×10^{13} vg/kg, about 1.0×10^{14} vg/kg, or about 2.0×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard. In another embodiment,

the dose is 5.0 x 10^{13} vg/kg, 1.0 x 10^{14} vg/kg, or 2.0 x 10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.

[00160] In another embodiment, the therapeutically effective amount of the rAAV vector is a dose of rAAV ranging from between about 1.0 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.5 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.6 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg. about 1.8 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg, about 1.2 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, about 1.9 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, about 1.4 x 10^{13}vg/kg to about 7.4 x 10^{13} vg/kg, about 1.9 x 10^{13}vg/kg to about 7.5 x 10^{13} vg/kg, or about 1.8 x 10^{13}vg/kg to about 8.0 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. For example, the therapeutically effective amount of the rAAV vector is a dose of about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[00161] In one embodiment, the dose of 5.0 x 10^{13} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard is equivalent to the dose of 1.85 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. In another embodiment, the dose of 2.0 x 10^{14} vg/kg based on a supercoiled DNA or plasmid is equivalent to 7.41 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard. Therefore, in another embodiment, about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

[00162] Administration of an effective dose of the compositions may be by routes standard in the art including, but not limited to, intramuscular, parenteral, intravenous, oral, buccal, nasal, pulmonary, intracranial, intraosseous, intraocular, rectal, or vaginal. Route(s) of administration and serotype(s) of AAV components of the rAAV (in particular, the AAV ITRs and capsid protein) of the invention may be chosen and/or matched by those skilled in the art taking into account the infection and/or disease state being treated and the target cells/tissue(s) that are to express the α-sarcoglycan.

[00163] The invention provides for local administration and systemic administration of an effective dose of rAAV and compositions of the invention. For example, systemic administration is administration into the circulatory system so that the entire body is affected. Systemic administration includes enteral administration such as absorption through the gastrointestinal tract and parental administration through injection, infusion or implantation.

[00164] In particular, actual administration of rAAV of the present invention may be accomplished by using any physical method that will transport the rAAV recombinant vector into the target tissue of an animal. Administration according to the invention includes, but is not limited to, injection into muscle, the bloodstream and/or directly into the liver. Simply resuspending a rAAV in phosphate buffered saline has been demonstrated to be sufficient to provide a vehicle useful for muscle tissue expression, and there are no known restrictions on the carriers or other components that can be co-administered with the rAAV (although compositions that degrade DNA should be avoided in the normal manner with rAAV). Capsid proteins of a rAAV may be modified so that the rAAV is targeted to a particular target tissue of interest such as muscle. See, for example, WO 02/053703, the disclosure of which is incorporated by reference herein.

[00165] Pharmaceutical compositions can be prepared as injectable formulations or as topical formulations to be delivered to the muscles by transdermal transport. Numerous formulations for both intramuscular injection and transdermal transport have been previously developed and can be used in the practice of the invention. The rAAV can be used with any pharmaceutically acceptable carrier for ease of administration and handling. Thus, in another aspect, the application is directed to a formulation that comprises an rAAV that comprises an AAVrh74 derived capsid, a buffer agent, an ionic strength agent, and a surfactant. In one embodiment, the rAAV is at a concentration of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg. In another embodiment, the rAAV is at a concentration of about 5.0×10^{12} vg/kg to about 1.0×10^{14} vg/kg. In another embodiment, the rAAV is at a concentration of about $5x10^{13}$ vg/kg, about $1x10^{14}$ vg/kg, and/or about $2x10^{14}$ vg/kg. In one embodiment, the dosage is based on a supercoiled DNA or plasmid as the quantitation standard. In one embodiment, the rAAV is an scAAVrh74. tMCK.hSGCA vector. In one embodiment, the buffer agent comprises one or more of tris, tricine, Bis-tricine, HEPES, MOPS, TES, TAPS, PIPES, and CAPS. In another embodiment, the buffer agent comprises tris with pH 8.0 at concentration of about 5 mM to about 40 mM. In one embodiment, the buffer agent comprises tris with pH 8.0 at about 20 mM. In one embodiment, the ionic strength agent comprises one of 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. In one embodiment, the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM. In another embodiment, the ionic strength agent comprises NaCl at a concentration of about 50 mM to about 500 mM. In another embodiment, 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. In another embodiment, the ionic strength agent comprises $MgCl_2$ at a concentration of about 1 mM and NaCl at a concentration of about 200 mM. In one embodiment, the surfactant comprises one or more of a sulfonate, a sulfate, a phosphonate, a phosphate, a Poloxamer, and a cationic surfactant. In one embodiment, the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407. In one embodiment, the surfactant comprises the Poloxamer at a concentration of about 0.00001% to about 1%. In another embodiment, the surfactant comprises Poloxamer 188 at a concentration of about 0.001%. For purposes of intramuscular injection, solutions in an adjuvant such as sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of rAAV as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxpropylcellulose. A dispersion of rAAV can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In this connection, the

sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

[00166] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00167] Sterile injectable solutions are prepared by incorporating rAAV in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[00168] Transduction with rAAV may also be carried out *in vitro*. In one embodiment, desired target muscle cells are removed from the subject, transduced with rAAV and reintroduced into the subject. Alternatively, syngeneic or xenogeneic muscle cells can be used where those cells will not generate an inappropriate immune response in the subject.

[00169] Suitable methods for the transduction and reintroduction of transduced cells into a subject are known in the art. In one embodiment, cells can be transduced *in vitro* by combining rAAV with muscle cells, *e.g.,* in appropriate media, and screening for those cells harboring the DNA of interest using conventional techniques such as Southern blots and/or PCR, or by using selectable markers. Transduced cells can then be formulated into pharmaceutical compositions, and the composition introduced into the subject by various techniques, such as by intramuscular, intravenous, subcutaneous and intraperitoneal injection, or by injection into smooth and cardiac muscle, using *e.g.*, a catheter.

[00170] Transduction of cells with rAAV of the invention results in sustained expression of α-sarcoglycan. The present invention thus provides methods of administering/delivering rAAV which

express alpha-sarcoglycan to a mammalian subject, preferably a human being. These methods include transducing tissues (including, but not limited to, tissues such as muscle, organs such as liver and brain, and glands such as salivary glands) with one or more rAAV of the present invention. Transduction may be carried out with gene cassettes comprising tissue specific control elements. For example, one embodiment of the invention provides methods of transducing muscle cells and muscle tissues directed by muscle specific control elements, including, but not limited to, those derived from the actin and myosin gene families, such as from the myoD gene family [See Weintraub *et al., Science, 251*: 761-766 (1991)], the myocyte-specific enhancer binding factor MEF-2 [Cserjesi and Olson, *Mol Cell Biol* 11: 4854-4862 (1991)], control elements derived from the human skeletal actin gene [Muscat *et al., Mol Cell Biol, 7*: 4089-4099 (1987)], the cardiac actin gene, muscle creatine kinase sequence elements [See Johnson *et al., Mol Cell Biol, 9*:3393-3399 (1989)] and the murine creatine kinase enhancer (mCK) element, control elements derived from the skeletal fast-twitch troponin C gene, the slow-twitch cardiac troponin C gene and the slow-twitch troponin I gene: hypoxia-inducible nuclear factors (Semenza *et al., Proc Natl Acad Sci USA, 88*: 5680-5684 (1991)), steroid-inducible elements and promoters including the glucocorticoid response element (GRE) (See Mader and White, *Proc. Natl. Acad. Sci. USA* 90: 5603-5607 (1993)), and other control elements.

[00171] Muscle tissue is an attractive target for *in vivo* DNA delivery, because it is not a vital organ and is easy to access. The invention contemplates sustained expression of miRNAs from transduced myofibers.

[00172] By “muscle cell” or “muscle tissue” is meant a cell or group of cells derived from muscle of any kind (for example, skeletal muscle and smooth muscle, *e.g.* from the digestive tract, urinary bladder, blood vessels or cardiac tissue). Such muscle cells may be differentiated or undifferentiated, such as myoblasts, myocytes, myotubes, cardiomyocytes and cardiomyoblasts.

[00173] The term “transduction” is used to refer to the administration/delivery of a polynucleotide of interest (e.g., a polynucleotide sequence encoding α-sarcoglycan) to a recipient cell either *in vivo* or *in vitro*, via a replication-deficient rAAV described resulting in expression of alpha-sarcoglycan by the recipient cell.

[00174] Thus, also described herein are methods of administering an effective dose (or doses, administered essentially simultaneously or doses given at intervals) of rAAV that encode alpha-sarcoglycan to a mammalian subject in need thereof.

[00175] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. Described numerical ranges are inclusive of each integer value within each range and inclusive of the lowest and highest stated integers.

[00176] The invention is further described in the following Examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

[00177] Preclinical studies using AAVrh74.tMCK.hSCGA are described in International Patent Publication No. WO 2013/078316 and U.S. Patent Nos. 9,434,928 and 10,105,453, which areincorporated by reference herein in its entirety.

Example 1
Materials and Methods

Animal Models

[00178] All procedures were approved by The Research Institute at the Nationwide Children's Hospital Institutional Animal Care and Use Committee. Knockout (sgca$^{-/-}$) mice were bred and maintained as homozygous animals under standardized conditions in the Animal Resources Core at the Research Institute at Nationwide Children's Hospital. Mice were maintained on Teklad Global Rodent Diet (3.8% fiber, 18.8% protein, 5% fat chow) with a 12:12-hour dark:light cycle. All animals were housed in standard mouse cages with food and water ad libitum.

Genotyping

[00179] DNA genotyping was used to identify sgca$^{-/-}$ mice. DNA from tail clippings was isolated and analyzed by polymerase chain reaction (PCR) using OneTaq DNA Polymerase (New England Biolabs, Ipswich, MA). A series of primers was used in the PCR analysis to determine the α-SG knockout status. The following primers and conditions were used: Intron1 (CAGGGCTGGGAGCTGGGTTCTG; SEQ ID NO: 9); mutant primer-intron 3 (CCCAGGGCCTTGATGCCT; SEQ ID NO: 10); and NEOTR (GCTATCAGGACATAGCGTTGGCTA; SEQ ID NO: 11). Reactions were carried out on genomic DNA for 30 cycles under the following conditions: 94°C, 5 min; 94°C, 1 min; 64°C, 1 min; 72°C, 2.5 min; and 72°C, 7 min.

α-SG Gene Construction

[00180] The scAAVrh74.tMCK.hSGCA transgene cassette was made using an adeno-associated virus (AAV) vector DNA plasmid pAAV.tMCK.aSG-neo, by inserting the tMCK expression cassette driving a codon-optimized human α-SG cDNA sequence (human cDNA, Genbank Accession # U08895) into the self-complementary vector backbone pHpa7. The only viral sequences included in this vector are the inverted terminal repeats of AAV2, which are required for both viral DNA replication and packaging of the rAAV vector genome. One of the inverted terminal repeats (ITRs) has a targeted deletion of the terminal resolution site (TRS) to restrict replication from this ITR facilitating generation of the dimeric replicative form for self-complementary vector packaging. The AAVrh74 virus has been proven in mice, non-human primates (NHPs), and humans to be safe and highly efficient in transducing muscle across the vascular barrier.

Vector production

[00181] The recombinant AAV, (sc)rAAVrh74.tMCK.hSGCA, was made in by triple transfection. A qPCR-based titration method was used to determine an encapsulated vg titer utilizing a Prism 7500

Fast Taqman detector system (PE Applied Biosystems). The construct comprises a chimeric intron to promote high-level expression. The chimeric intron is composed of the 5' donor site from the first intron of the human β-globin gene and the branchpoint and 3' splice acceptor site from the intron that is between the leader and the body of an immunoglobulin gene heavy chain variable region. The rAAV also comprises a synthetic SV40 polyadenylation signal is used for efficient transcription termination. A schematic of the expression cassette is shown below in Figure 1. The vector was produced using the human alpha-sacroglycan (α-SG) gene flanked by AAV2 ITR sequences and encapsidated into AAVrh74 virions. The construct contains the tMCK immediate early promoter/enhancer (GenBank Accession No. M21390) and uses the ß-globin intron for high-level expression.

Gene delivery

[00182] Systemic delivery in mice was achieved through injection of vector into the tail vein of sgca-/- mice. Mice were injected with a $1x10^{12}$ vg, $3x10^{12}$ vg, or $6x10^{12}$ vg total dose (mice ranging from 13–20 g; $5x10^{13}$ vg/kg, $1x10^{14}$ vg/kg, and $2x10^{14}$ vg/kg—the dosages based on a supercoiled DNA or plasmid as the quantitation standard--respectively, based on a 20-g mouse) of scAAVrh74.tMCK.hSGCA diluted in lactated Ringer's solution in a 200-250 µL volume using a 30-gauge ultra-fine insulin syringe. All treated mice were injected at 4-5 weeks of age and euthanized 12 weeks post-injection. In another embodiment, the dose is about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.

Serum creatine kinase measurement

[00183] Levels of creatine kinase were measured in the sera of wild-type C57BL/6 mice (n = 6), vehicle (lactated Ringers solution)-treated *sgca*$^{-/-}$ mice (n = 6), and scAAVrh74.tMCK.hSGCA-treated *sgca*$^{-/-}$ mice (n = 6 per dose) using the Creatine Kinase SL Assay and the corresponding manufacturer's protocol (Sekisui Diagnostics; Charlottetown, PE, Canada) (catalog no. 326-10). Briefly, 25 µL of serum was mixed with 1 mL of the working reagents and added to a cuvette. A kinetic assay was set on the spectrophotometer to measure the absorbance at 340 nm every 30 sec for 180 sec. Creatine kinase levels were calculated using the absorbance readings and the equation listed below:

$$U/L= [\Delta Abs./min) * 1.025 * 1000] / [1 * 6.22 * 0.025] = (\Delta Abs./min) * 6592.$$

Diaphragm tetanic contraction for functional assessment

[00184] Mice were euthanized and the diaphragm (DIA) was dissected with rib attachments and central tendon intact, and placed in K-H buffer. A 2-4 mm wide section of DIA was isolated. DIA strips were tied firmly with braided surgical silk (6/0; Surgical Specialties, Reading, PA) at the central tendon, and sutured through a portion of rib bone affixed to the distal end of the strip. Each muscle was transferred to a water bath filled with oxygenated K-H solution that was maintained at 37°C. The muscles were aligned horizontally and tied directly between a fixed pin and a dual-mode force transducer-servomotor (305C; Aurora Scientific, Aurora, Ontario, Canada). Two platinum plate electrodes were positioned in the organ bath so as to flank the length of the muscle. The muscle was

stretched to optimal length for measurement of twitch contractions, and then allowed to rest for 10 minutes before initiation of the tetanic protocol. Once the muscle was stabilized, it was set to an optimal length of 1 g and subjected to a warm-up, which consisted of three 1-Hz twitches every 30 sec followed by three 150-Hz twitches every minute. After a 3-min rest period, the DIA was stimulated at 20, 50, 80, 120, 150, 180 Hz, allowing a 2-min rest period between each stimulus, each with a duration of 250 ms to determine maximum tetanic force. Muscle length and weight were measured. The force was normalized for muscle weight and length.

Tibialis anterior (TA) tetanic contraction for functional assessment

[00185] The TA assessment procedure followed the protocol listed in Hakim et al., *Methods Mol Biol*; 709:75–89 (2011). Mice were anesthetized via intraperitoneal cavity using ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively). Under a dissecting scope, the hind limb skin was removed to expose the TA muscle and patella. A double square was tied around the patella tendon with a 4-0 suture. The TA distal tendon was then dissected out and a double square knot was tied around the tendon with 4-0 suture as close to the muscle as possible and then the tendon was cut. The exposed muscle was constantly dampened with saline. Mice were then transferred to a thermal controlled platform and maintained at 37°C. The knee was secured to the metal pin with the patella tendon suture and the distal TA tendon suture to the level arm of the force transducer (Aurora Scientific, Aurora, Canada). An electrode was placed near the sciatic nerve to stimulate it. Once the muscle was stabilized, the resting tension was set to a length (optimal length) where twitch contractions were maximal. After a 3-min rest period, the TA was stimulated at 50, 100, 150 and 200 Hz, allowing a 1-min rest between each stimulus. Following a 5-min rest, the muscles were then subjected to a series of 10 isometric contractions, occurring at 1-min intervals with a 10% stretch-re-lengthening procedure. After the eccentric contractions, the mice were then euthanized and the TA muscle was dissected and frozen for histology.

Immunofluorescence

[00186] Cryosections (12-µm thick) from the TA, gastrocnemius (GAS), quadricep (QD), psoas major (PSOAS), gluteus (GLUT), tricep (TRI), DIA, and heart (HRT) muscles were subjected to immunofluorescence staining for the hSGCA transgene. Sections were incubated with a rabbit monoclonal α-SG primary antibody (Abcam; Cambridge, UK; catalog no.ab189254) at a dilution of 1:100. Four random 20x images covering the four different quadrants of the muscle section were taken using a Zeiss (Germany) AxioCam MRC5 camera. The percentage of fibers positive for α-SG staining compared to controls was determined for each image and averaged for each muscle. Positive α-SG fiber expression was defined as having at least 30% of the fiber staining brighter than the vehicle-treated sgca-/- controls.

Western blot analysis

[00187] Samples from wild-type C57BL/6 mice, vehicle-treated sgca-/- mice, and vector-dosed sgca-/- mice were used for each Western blot. A 1:10,000 dilution of a rabbit monoclonal α-SG antibody (Abcam, catalog no.ab189254) and a 1:5,000 dilution of a mouse monoclonal α-actinin

antibody (Sigma-Aldrich, catalog no. A7811) were used for hSGCA blots. A 1:1,000 dilution of a rabbit monoclonal mouse vinculin antibody (Invitrogen, catalog no. 70062) was also used. Anti-mouse (Millipore, catalog no. AP308P) and anti-rabbit (Life Technologies, catalog no. 656120) secondary horseradish peroxidase antibodies were used for enhanced chemiluminescence immunodetection. Western blot quantification was performed by densitometry using ImageQuantTL 1D 8.1.0 (GE Healthcare Life Sciences).

Morphometric analysis

[00188] Hematoxylin & eosin (H&E) staining was performed on 12-µm thick cryosections of muscle from 16-17-week-old wild-type C57BL/6 mice (n=6), vehicle-treated sgca-/- mice (n=6), and scAAVrh74.tMCK.hSGCA 16-17-week-old treated sgca-/- mice (n=6 per dose) for analysis. The percentage of myofibers with central nuclei was determined in the TA, GAS, QD, GLUT, PSOAS, and TRI muscles. Additionally, muscle fiber diameters were measured in the TA, GAS, QD, TRI, and PSOAS muscles. Four random 20x images per muscle per animal were taken with a Zeiss AxioCam MRC5 camera. Centrally nucleated fibers were quantified using the National Institutes of Health's ImageJ software, and fiber diameters were measured using Zeiss Axiovision LE4 software.

Biodistribution quantitative polymerase chain reaction (PCR) analysis

[00189] Taqman quantitative PCR was performed to quantify the number of vector genome copies present in targeted and untargeted contralateral muscle as well as non-targeted organs as previously described. A vector-specific primer probe set was used to amplify a sequence of the intronic region directly downstream from the tMCK promoter that is unique and located within the scAAVrh.74.tMCK.hSGCA transgene cassette. The following primers and probe were used in this study: tMCK intron Forward Primer 5′-ACC CGA GAT GCC TGG TTA TAA TT-3′; tMCK intron Reverse Primer 5′-TCC ATG GTG TAC AGA GCC TAA GAC-3′; and tMCK intron probe 5′-FAM-CTG CTG CCT GAG CCT GAG CGG TTA C- IABkFQ-3' (Integrated DNA Technologies). Copy number was reported as vector genomes per microgram of genomic DNA.

Picrosirius red stain and collagen quantification

[00190] Picrosirius red staining was performed to determine the levels of collagen deposition in muscle tissue. Staining was performed on 12-µm cryosections from 16-17-week-old wild-type C57BL/6 (n=6), vehicle-treated sgca-/- (n=6), and scAAVrh74.tMCK.hSGCA 16-17-week-old treated sgca-/- (n=6 per dose) GLUT, PSOAS, TRI, and DIA muscles. Four 20x images were taken per muscle per mouse, and the amount of collagen deposition was determined with the ImageJ software program. The mean percent collagen for each muscle was calculated for all groups.

Laser monitoring of open-field cage activity

[00191] An open-field activity chamber was used to determine the overall activity of the experimental mice. Mice at 16-17 weeks of age from the wild-type C57BL/6 (n=6) and untreated $sgca^{-/-}$ (n=6) control groups, along with scAAVrh74.tMCK.hSGCA 16-17-week-old treated $sgca^{-/-}$ mice (n=6 per dose) were subjected to analysis. All mice were tested at the same time of day, in the early

morning near the end of the night cycle, when mice are most active. All mice were tested in an isolated room under dim light and with the same handler each time. Also, to reduce anxiety and keep behavioral variables at a minimum that could potentially affect normal activity of the mice and consequently the results of the assay, we tested mice that were not individually housed. Mouse activity was monitored using the Photobeam activity system (San Diego Instruments, San Diego, CA). This system uses a grid of invisible infrared light beams that traverse the animal chamber front to back and left to right to monitor the position and movement of the mouse within an x-y-z plane. Activity was recorded for 1-hour cycles at 5-min intervals. Mice were acclimatized to the activity test room for an initial 1-hour session several days prior to beginning data acquisition. Mice were tested in individual chambers in sets of four. The testing equipment was cleaned between each use to reduce mouse reactionary behavioral variables that could alter results. The data were converted to a Microsoft Excel worksheet, and all calculations were done within the Excel program. Individual beam breaks for movement in the x and y planes were added up for each mouse to represent total ambulation, and beam breaks in the z plane were added up to obtain vertical activity within the 1-hour time interval.

Safety studies

Hematology

[00192] Whole blood was retrieved from cardiac puncture for blood chemistries. Blood was collected in a serum separating tube and centrifuged for 10 min at 15,000 rpm. Serum was collected, frozen, and sent to Charles River Laboratories for chemistry testing. Liver enzymes and glucose chemistries were prioritized in the hematology analysis.

Histopathology

[00193] At necropsy, muscles were fresh frozen in liquid nitrogen-cooled methyl-butane; all other organs were harvested and fixed in formalin and embedded in paraffin. After processing, tissues were stained with H&E, and slides and all tissues were sent to GEMPath, Inc, for formal review by a veterinary pathologist.

Statistical analysis

[00194] Data were expressed as the mean ± SEM (error bars) and analyzed using a one-way ANOVA with multiple comparisons between groups assessed by Tukey's post-hoc analysis test using GraphPad Prism 5 (GraphPad Software, La Jolla, CA) unless otherwise specified.

Example 2

Efficiency of systemic delivery of scAAVrh74.tMCK.hSGCA

[00195] A small pilot study was initiated to observe efficacy of gene delivery by intravenous injection into the lateral tail vein of *sgca*$^{-/-}$ mice at a dose of $1x10^{12}$ vg total dose ($5x10^{13}$ vg/kg based on 20-g mouse, n=4). Immunofluorescence analysis was performed on harvested muscles 4 weeks post-gene transfer. The amount of hSGCA transgene expression in seven different limb skeletal muscles: TA, GAS, GLUT, QD, PSOAS, and TRI and DIA. Mice deficient for α-SG showed a

complete absence of the protein when analyzed by immunofluorescence (**Fig. 2A**; representative images of TA, GAS, TRI and DIA). The therapeutic dose of $1x10^{12}$ vg total dose resulted in a mean 54±23.81% vector transduction across all skeletal muscles including the DIA 4 weeks post-gene delivery.

Dose-escalation study of scAAVrh74.tMCK.hSGCA delivered systemically to sgca$^{-/-}$ mice

[00196] To determine the safest and most efficacious dose, the delivery of three separate doses of vector was studied in a dose-escalation study, where the lateral tail vein of 4-week-old *sgca*$^{-/-}$ mice were treated with $1x10^{12}$ vg total dose ($5x10^{13}$ vg/kg), $3x10^{12}$ vg total dose ($1x10^{14}$ vg/kg), or $6x10^{12}$ vg total dose ($2x10^{14}$ vg/kg) of scAAVrh74.tMCK.hSGCA. Mice were euthanized 12 weeks post-gene delivery to assess hSGCA transgene expression in the TA, GAS, QD, GLUT, PSOAS, TRI, DIA, and HRT muscles using immunofluorescence. Mean hSGCA expression in mice treated with the lowest dose of $1x10^{12}$ vg total dose ($5x10^{13}$ vg/kg) was 70.07±3.71% overall expression in the skeletal muscles, including the DIA. Mean hSGCA expression in mice treated with the intermediate dose of $3x10^{12}$ vg total dose ($1x10^{14}$ vg/kg) was 85.35±2.36% in all skeletal muscles. Mean hSGCA expression in mice treated with the highest dose of $6x10^{12}$ vg total dose ($2x10^{14}$ vg/kg) was 93.86±2.02% in all skeletal muscles. For clarity, the doses were calculated based on a supercoiled DNA or plasmid as the quantitation standard. The hSGCA expression in the HRT muscle remained at 75% independent of dose. Representative images of tissues are shown in **Fig. 2A**. The robust hSGCA expression shows efficacy of gene delivery at all three doses. The gene delivery targeted multiple muscles in both forelimbs and hind limbs, showing exceptional α-SG expression in the mice at all three doses. Most importantly, the vital diaphragm muscle also showed α-SG gene expression after delivery. Western blots shown in **Fig. 2B** confirm protein expression in all muscles of all three dosing cohorts of the treated mice. The cardiac muscle in mice also showed α-SG expression after treatment.

[00197] Histopathological characteristics of both humans and mice devoid of α-SG protein include central nucleation, irregularities in fiber size distribution, necrosis, and fibrosis. H&E staining was used to visualize muscle morphology, including fiber size and central nucleation (**Fig. 3**). As shown in **Fig. 3A** and **3B**, a normalization of fiber size distribution, similar to that observed in wild-type controls, was observed in the TA, QD, and TRI of scAAVrh74.tMCK.hSGCA-treated *sgca*$^{-/-}$ mice compared to vehicle-treated *sgca*$^{-/-}$ controls. The average diameter size of fibers was significantly increased at all doses in the TA, QD, and TRI muscles compared to vehicle-treated *sgca-/-* control mice (**Table 1**).

TABLE 1

	Fiber Diameter (μm)			
Tissue	Untreated	Lowest Dose	Intermediate Dose	Highest Dose
TA	40.23±19.33	46.24±18.06****	43.16±15.88****	41.37±13.54*
QD	30.87±15.56	48.10±19.96****	43.00±16.87****	45.65±14.51****
TRI	25.05±12.09	36.47±16.50****	37.01±14.14****	36.63±11.58****

*=p<0.05, ****=p<0,0. Abbreviation: TA, tibialis anterior, AD: quadricep; and TRI, tricep. Lowest dose: $1.0X10^{12}$ vg; intermediate dose: $3.0X10^{12}$ vg; highest dose: $6.0X10^{12}$ vg.

[00198] In *sgca*$^{-/-}$ mice treated with scAAVrh74.tMCK.hSGCA, reduction in central nucleation was also observed. Skeletal muscles of vehicle-treated *sgca*$^{-/-}$ mice had 68.72±3.01% fibers with centrally located nuclei. After treatment with scAAVrh74.tMCK.hSGCA, the overall value of central nucleation across all muscle tissues was reduced with the lowest dose of scAAVrh74.tMCK.hSGCA, resulting in 55.60±3.25% of skeletal muscle fibers showing centrally located nuclei (**Table 2**).

TABLE 2

	Central nucleation (%)			
Tissue	Untreated	Lowest Dose	Intermediate Dose	Highest Dose
TA	82.08	61.6****	64.61	51.29****
GAS	78.01	44.98****	48.43	16.19****
QD	78.28	63.82	70.35	42.79****
GLUT	60.61	51.73	58.28	31.18
PSO	43.42	50.99	58.78	44.42
TRI	68.34	59.57	70.37	41.72
AVG	68.72±3.01	55.60±3.25	61.85±4.00	37.93±12.46

****=p<0.0001. Abbreviation: TA, tibialis anterior; GAS, gastrocnemius; QD, quadricep; GLUT, gluteus; PSO, psoas majorl, AVG, average; and TRI, tricept

[00199] Mice treated with the intermediate dose had 61.85±4.00% of muscle fibers with centralized nuclei, while the nucleation of muscle fibers treated with the highest dose was reduced to 37.93±12.46% (**Fig. 3C**).

[00200] Fibrosis, where the tissue is overcome by collagen, often occurs in the muscles of LGMD patients, leading to the formation of scar tissue. Fibrosis was assessed using a picrosirius red stain to detect collagen I and III content, as a marker of fibrosis. As shown in **Fig. 4**, a robust reduction in red staining was observed in *sgca-/-* mice after treatment with scAAVrh74.tMCK.hSGCA. Quantification revealed a significant reduction in collagen content across all muscles in scAAVrh74.tMCK.hSGCA treated *sgca-/-* mice compared to vehicle-treated *sgca*$^{-/-}$ control mice (**Fig. 4B**). Together, these data demonstrate successful systemic delivery of the hSGCA transgene as indicated by robust expression in muscle tissues and improvement in histopathological hallmarks associated with the lack of α-SG protein in *sgca*$^{-/-}$ mice.

Example 3

scAAVrh74.tMCK.hSGCA rAAV improved diaphragm and tibialis anterior muscle function and increases locomotor ability

[00201] As weakness and loss of function of proximal muscles are major symptoms of LGMD2D and respiratory failure is the leading cause of death in LGMD2D, improving the functionality and strength of the TA and DIA is imperative to increasing the length and quality of life in subjects with LGMD2D. Strips of the DIA and whole TA muscles were used to confirm the correlation between hSGCA expression and muscle strength. As shown in Figures 5A and 5B, a deficit in specific force

and resistance to contraction-induced injury was identified in the TA and specific force in the DIA muscles of *sgca*$^{-/-}$ untreated mice compared to wild-type mice.

[00202] TA muscles of *sgca*$^{-/-}$ mice exhibited a significant functional deficit of 44% in the reduction of specific force output compared to wild-type mice (161.6±8.20 mN/mm^2 vs. 291.7±6.17 mN/mm^2, respectively; $p<0.0001$), as well as a greater loss of force from that produced following a rigorous eccentric contraction protocol (44.0±6.0% loss in *sgca*$^{-/-}$ mice; 18.0±1.0% loss in wild-type mice; $p<0.0001$) (**Fig. 5A**). Twelve weeks following tail vein delivery, Applicant noted a dramatic improvement after treatment with low, intermediate, and high doses of scAAVrh74.tMCK.hSGCA in specific force output, which increased to 218±11.94 mN/mm^2, 227±11.7 mN/mm^2, and 255±11.7 mN/mm^2, respectively. Resistance to injury following an eccentric contraction protocol also improved compared to vehicle-treated *sgca*$^{-/-}$ mice, in which the low-, intermediate-, and high-dose treated mice only lost 22.0±4.0%, 22.0±3.0%, and 12.0±1.0%, respectively ($p<0.0001$ compared to vehicle-treated *sgca*$^{-/-}$ mice) (**Fig. 5A**).

[00203] In the DIA of vehicle-treated *sgca*$^{-/-}$ mice, the specific force generated showed a 41% reduction in strength compared to wild-type mice (131.5±12.07 mN/mm^2 vs. 223.8±15.85 mN/mm^2). An improvement in force was observed following treatment with scAAVrh74.tMCK.hSGCA at all three doses, where the specific force of the DIA in low-dosed mice increased to 179.2±21.03 mN/mm^2, in intermediate-dosed mice increased to 201.2±22.94 mN/mm^2 and in high-dosed mice increased to 261.46±9.73 mN/mm^2 (**Fig. 65B**). These data show that the TA and DIA muscles in *sgca*$^{-/-}$ mice have a deficit in force and are faster to exhaust than wild-type mice. However, after the delivery of scAAVrh74.tMCK.hSGCA, functional recovery was achieved.

[00204] Additional symptoms of LGMD2D include exercise intolerance and reduced activity and ambulation, possibly due to muscle damage, resulting in pain and muscle fatigue. To assess the level of physical activity, *sgca*$^{-/-}$ and wild-type C57BL/6 mice were subjected to an open-field activity protocol similar to that used in previous reports. The ambulation-related activities of mice were monitored to determine if the lack of α-SG in the *sgca*$^{-/-}$ mouse leads to a decrease in ambulation compared to wild-type mice. The graphs in **Fig. 5C** depict a reduction in ambulation and vertical rearing in the *sgca*$^{-/-}$ mouse model compared to wild-type controls. The mean horizontal ambulatory beam breaks recorded in the *sgca*$^{-/-}$ mice was 2000±159 beam breaks/hr, a 77.5% decrease in ambulation compared to 8911±1193 beam breaks/ hr in wild-type controls. The mean vertical rearing beam breaks recorded in the *sgca*$^{-/-}$ mice was 24.75±11.47 beam breaks/hr, a 97% decrease in vertical rearing compared to 803.3 ± 55.03 beam break/hr in wild-type mice. After treatment with scAAVrh74.tMCK.hSGCA, the ambulation and vertical rearing activities of mice increased 12 weeks post-gene delivery. The mean horizontal ambulation increased to 3595±55.03 beam breaks/hr in mice treated with $1x10^{12}$ vg total dose, 5238±861.9 beam breaks/hr in mice treated with $3x10^{12}$ vg total dose, and 6487±467.9 beam breaks/hr in mice treated with $6x10^{12}$ vg total dose. The mean vertical rearing activity increased to 377±146.1 beam breaks/hr in mice treated with $1x10^{12}$ vg total dose, 321±126.1 beam breaks/hr in mice treated with $3x10^{12}$ vg total dose, and 448.8±53.43 beam breaks/hr in mice treated with $6x10^{12}$ vg total dose (**Fig. 5C**). The physical activities of the treated

mice showed improvement from 44%-69% in ambulation and 92%-94% in vertical rearing compared to vehicle-treated *sgca*$^{-/-}$ mice. Additionally, serum creatine kinase levels were significantly reduced in all treated groups compared to untreated mice (**Fig. 5D**). Together, these data show that the delivery of α-SG restores the physical activity and protects against the breakdown of muscle in *sgca*$^{-/-}$ mice.

Safety and biodistribution analysis of scAAVrh74.tMCK.hSGCA

[00205] As a safety provision, blood chemistries and hematology studies were performed on vector-dosed *sgca*$^{-/-}$ and wild-type mice. All values were within the normal reference ranges for mice (**Fig. 6**). Furthermore, tissue sections of all muscles and organs stained with H&E from scAAVrh74.tMCK.hSGCA-dosed *sgca*$^{-/-}$ and wild-type mice were sent to a veterinary pathologist for formal review. No adverse effects were noted in any sample from any of the scAAVrh74.tMCK.hSGCA-dosed *sgca*$^{-/-}$ and wild-type mice. In addition to efficacy, these data demonstrated that the systemic delivery of all three doses of scAAVrh74.tMCK.hSGCA was well-tolerated, safe, and non-toxic to *sgca*$^{-/-}$ and wild-type mice.

[00206] To test for potential toxicity or safety concerns from the delivery of scAAVrh74.tMCK.hSGCA, vector biodistribution quantitative PCR was performed to quantify vector genome presence (**Fig. 7A**). Vector-specific tMCK.hSGCA primer probe sets were used to detect vector genomes in all muscles and organs tested from the scAAVrh74.tMCK/hSGCA-dosed *sgca*$^{-/-}$ mice. As expected, vector genomes were present in the tissues tested, with the highest copy number in the liver, followed by muscles. Western blots of alpha-sarcoglycan protein in the liver of WT and sgca-/- mice treated with either vehicle (*sgca*$^{-/-}$ LR) or scAArh74.tMCK.hSGCA are shown in Fig. 7B.

[00207] To improve efficiency of α-SG expression, in one embodiment, the hSGCA cDNA sequence is packaged into a self-complementary vector. Self-complementary AAV vectors contain an inverted repeat genome that promotes the formation of dsDNA, thus allowing replication and transcription to occur without the need for multiple vector genomes to promote these processes. As such, use of self-complementary vectors eliminates the rate-limiting step to allow more rapid expression of the transgene. Applicant has shown that intravascular delivery of scAAVrh74.tMCK.hSGCA in patients with LGMD2D was associated with increased α-SG expression 180 days post-gene transfer at doses of 1×10^{12} and 3×10^{12}.

[00208] Intravenous delivery of scAAVrh74.tMCK.hSGCA provides muscles with increased strength and resistance against contraction-induced damage in the tibialis anterior and diaphragm muscles in all three vector-treated cohorts compared to vehicle-treated controls. In addition, treatment with scAAVrh74.tMCK.hSGCA resulted in a significant reduction in CK. Moreover, after treatment with scAAVrh74.tMCK.hSGCA, mice were able to ambulate and rear onto hind limbs more frequently than vehicle-treated mice.

[00209] Prominent histopathology, which includes centrally located nuclei, wide variability in fiber size, inflammation, necrosis, and fibrosis, is typically observed through muscle biopsies of patients with LGMD2D After hSGCA delivery, mice had a reduction in CN, a more even distribution of myofiber size, and a reduction in collagen content, with muscles having an overall healthier appearance

compared to vehicle-treated mice. The total reduction in histopathology and scar tissue was concomitantly associated with improvement in the overall normal function and physiology of the muscles in vector-treated mice.

[00210] Finally, safety studies conducted through quantitative PCR, serum chemistry analysis, and histopathology did not review signs of toxicity. The tMCK promoter was detected only in targeted tissues (all muscles) and was absent in other (non-muscle) organs, except for the liver. tMCK detection in the liver is not uncommon or concerning as it is a clearance organ. Histopathology review of all tissues (including liver) by a certified veterinarian pathologist concluded that the systemic delivery of scAAVrh74.tMCK.hSGCA was not only safe in all tissues but also that gene delivery dramatically reduced the amount of dystrophic pathology found in skeletal muscles of vehicle-treated $sgca^{-/-}$ mice. Chemistries performed on blood samples of vector-treated mice also support the lack of toxicity.

[00211] The dose-escalation study, as in this disclosure, provides preclinical data to support that the lowest dose systemically tested here, *i.e* 1×10^{12} vg total (5×10^{13} vg/kg), is ample to reduce the signs and symptoms associated with loss of α-SG protein. At the lowest dose tested functional improvement in all muscles, as demonstrated by increase in strength and locomotor behavior (ambulation and rearing) was observed in vector-treated mice. Safety studies show no signs of toxicity, even at the highest delivered dose of 6×10^{12} vg total (2×10^{14} vg/kg).

Example 4

Elder patients and durability

[00212] The rAAVrh74.tMCK.hSGCA -mediated gene replacement has shown positive results in treating LGMD-2D and other associated diseases. This study was designed to test the ability of rAAVrh74.tMCK.hSGCA to treat older, more severely affected muscle, and to determine the long-term durability of the AAV viral vector.

[00213] All procedures were conducted in accordance with approval by the Research Institute at the Nationwide Children's Hospital Institutional Animal Care and Use Committee. Mice were maintained under standardized conditions on a 12:12-hour light:dark cycle, with food and water provided ad libitum. First, rAAVrh74.tMCK.hSGCA was systemically administered by tail vein injection to 12-month-old *sgca*$^{-/-}$ mice (n=5) presenting with severe muscle histopathology at three doses (1.0×10^{12}, 3.0×10^{12}, and 6.0×10^{12} vg. The controls included lactated ringers solution (LRS) injected *sgca*$^{-/-}$ mice (n=5) and LRS injected BL6 wild type mice (n=4). At the 6-month endpoint post treatment, muscle from the treated mice were evaluated for SCGA protein expression, histological rescue, and functional improvement. All three doses showed robust protein expression of α-SG at the sarcolemma, improved histopathology, increased locomotor activity and specific-force generation, protection against eccentric force loss, and reduced serum CK compared with controls. No vector toxicity was detected. In aged mice, treatment resulted in widespread, high-level protein expression in muscles analyzed, reduced fibrosis, and increased resistance to contraction-induced injury in tibialis anterior muscle.

[00214] Particularly, IV administration of rAAVrh74.tMCK.hSGCA to 12-month-old *sgca*$^{-/-}$ mice resulted in widespread high-level protein expression in muscles throughout the lower limb, upper limb, and proximal torso muscles, including the diaphragm and heart (Fig. 8).

[00215] Overall improvement in muscle pathology (**Fig 9a**) and reduction in central nucleation was observed after administration of scAAVrh74.tMCK.hSGCA. In addition, average fiber size increased to levels similar to levels in WT fibers in gastrocnemius (GAS) and triceps (TRI) muscles (Fig. 9b) after administration.

[00216] The level of collagen deposition was quantitated as a measure of fibrosis. Administration of scAAVrh74.tMCK.hSGCA resulted in a reduction in the level of fibrosis compared to untreated controls (Fig. 9c). Functional improvement after administration of scAAVrh74.tMCK.hSGCA was evidenced by improved force output (specific force) in the tibialis anterior (TA) and diaphragm (DIA) muscle and increased resistance to contraction-induced injury in the TA muscle (Fig. 10).

[00217] To further investigate the long-term durability of the gene therapy, *sgca*$^{-/-}$mice at 4 weeks of age are systemically administered rAAVrh74.tMCK.hSGCA. More than 24 months post-treatment, the vector genome copy numbers are detected with qPCR across all transduced muscles tested (TA, TRI, DIA, GLUT, PSOAS, GAS and QUAD). Protein expression and localization is studied by immunofluorescence staining of treated muscle.

[00218] While the present disclosure has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. Accordingly, only such limitations as appear in the claims should be placed on the disclosure.

[00219] All documents referred to in this application are hereby incorporated by reference in their entirety.

SEQ ID NO: 1

SGCA cDNA Codon Optimized sequence:

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SEQ ID NO: 2

Human SGCA Protein Sequence:

MAETLFWTPLLVVLLAGLGDTEAQQTTLHPLVGRVFVHTLDHETFLSLPEHVAVPPAVHITYHAHLQGHPDLPRW
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SEQ ID NO: 3

tMCK Promoter Sequence:

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SEQ ID NO: 4

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TCTAGGCTGC CCATGTAAGG AGGCAAGGCC TGGGGACACC CGAGATGCCT

GGTTATAATT AACCCCAACA CCTGCTGCCC CCCCCCCCCC AACACCTGCT

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CCGAGATGCC TGGTTATAAT TAACCCCAAC ACCTGCTGCC CCCCCCCCCC

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SEQ ID NO: 5

5'ITR

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SEQ ID NO: 6

3'ITR

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GCCCGGGCTT TGCCCGGGCG GCCTCAGTGA GCGAGCGAGC GCGC

SEQ ID NO: 7

PolyA

GGCCGCAAT AAAAGATCTT TATTTTCATT AGATCTGTGT GTTGGTTTTT TGTG

SEQ ID NO: 8

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Claims

1. Use of a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA in the manufacture of a medicament for treating limb-girdle muscular dystrophy type 2D (LGMD2D) in a subject in need thereof, wherein the rAAV is at a dose between $5x10^{13}$ vg/kg and $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the medicament is formulated for a systemic route of administration, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4.

2. The use of claim 1, wherein the rAAV is at a dose selected from $5x10^{13}$ vg/kg, $1x10^{14}$ vg/kg, or $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.

3. The use of claim 1, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct with a nucleotide sequence of SEQ ID NO: 4.

4. The use of any one of claims 1-3, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the medicament as compared to the level of alpha-sarcoglycan gene expression before administration of the medicament; or wherein the serum CK level in the subject is decreased after administration of the medicament as compared to serum CK level before administration of the medicament; or wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the medicament as compared to the number of alpha-sarcoglycan positive fibers before administration of the medicament.

5. The use of any one of claims 1-4, wherein the systemic route of administration is an intravenous route.

6. The use of any one of claims 1-5, wherein the medicament is formulated for administration by injection, infusion or implantation.

7. The use of any one of claims 1-6, wherein the medicament is formulated for administration by infusion.

8. The use of any one of claims 1-6, wherein the medicament is formulated for administration by an intravenous route through a peripheral limb vein.

9. The use of any one of claims 1-8, wherein the medicament is formulated for intravenous infusion and the rAAV is at a dose between $5x10^{13}$ vg/kg and $2x10^{14}$ vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

10. Use of a scAAVrh74.tMCK.hSGCA construct in the manufacture of a medicament for the treatment of limb-girdle muscular dystrophy type 2D (LGMD2D), wherein the medicament is for decreasing a serum CK level in a subject in need thereof, and wherein the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.