

Considering that a subset of patients can develop cardiomyopathy, (8, 9, 10, 13) this would have to be considered in the long-term care of these patients. In previous reports, the Sgcb-null mouse was well characterized. Araishi et al.³ developed the β -sarcoglycan-deficient mouse with accompanying loss of all of the sarcoglycans as well as sarcospan, with at least minor preservation of merosin, the dystroglycans and dystrophin, reproducing the clinical picture seen in LGMD2E. The histological changes in this animal model were also a prototype for the clinical counterpart, including the prominence of skeletal muscle fibrosis.⁽¹⁴⁾ Dressman et al. ⁽²⁵⁾ injected the transverse abdominal muscle using rAAV2.CMV.SGCB. Expression persisted for 21 months and muscle fibers were protected from recurrent necrosis. The use of self-complementary AAV to enhance transgene expression,¹⁶ a muscle-specific promoter to better target skeletal muscle ^(20, 26) and the optimization of a human β -sarcoglycan gene (hSGCB) has also been described.

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

SUMMARY

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

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

[0010b] In a further aspect, the present disclosure provides a composition, comprising an rAAV vector suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard, a buffer agent, an ionic strength agent, and a surfactant, wherein the surfactant comprises a poloxamer at a concentration of about 0.00001% to about 1%. In a further aspect, the present disclosure provides a polynucleotide sequence comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

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

[0010d] In a further aspect, the present disclosure provides use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB A for the preparation of a medicament

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

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

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

[0013] In another example, this disclosure provides a method of treating a limb-girdle muscular dystrophy in a subject in need, comprising administering to the subject an rAAV intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0×10^{13} vg/kg or

about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises a nucleotide sequence of SEQ ID

Claims

What is claimed is:

1. A composition for treating a muscular dystrophy in a subject comprising a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19, wherein the rAAV is suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.
2. The composition of claim 1, wherein the muscular dystrophy is limb-girdle muscular dystrophy, and wherein the dose is 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard.
3. The composition of claim 1 or 2, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2E.
4. A composition for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.
5. A composition for increasing sarcoglycan expression in muscle tissue of a subject, wherein the composition comprises a rAAV comprising a nucleotide sequence encoding a first sarcoglycan, wherein the rAAV comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19, wherein the rAAV is suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard, wherein administration of the composition results in increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

6. The composition of claim 5, wherein said first sarcoglycan is β -sarcoglycan (SGCB), and said second sarcoglycan is α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), and/or γ -sarcoglycan (SGCD).
7. The composition of any one of claims 1 to 6, wherein the subject is a human subject that is 4 to 15 years of age.
8. The composition of any one of claims 1 to 6, wherein the subject is a pediatric subject, an adolescent subject or a young adult subject.
9. The composition of any one of claims 1 to 6, wherein the subject is a human subject that is 25 to 55 years of age.
10. The composition of any one of claims 1 to 6, wherein the subject is a human subject that is over 50 years of age.
11. A composition, comprising
an rAAV vector suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard,
a buffer agent,
an ionic strength agent, and
a surfactant, wherein the surfactant comprises a poloxamer at a concentration of about 0.00001% to about 1%.
12. The composition of claim 11, wherein the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407.
13. A polynucleotide sequence comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.
14. The polynucleotide sequence of claim 13, wherein the polynucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 24.
15. The polynucleotide sequence of claim 13 or 14, is a plasmid.
16. A method of generating a recombinant AAV, comprising transferring to a cell the polynucleotide sequence of any one of claims 13 to 16.

17. The method of claim 16, further comprising transferring a packaging plasmid and/or a helper virus to the cell.
18. The method of claim 16 or 17, wherein the cell comprises a stably integrated AAV cap gene.
19. The method of any one of claims 16 to 18, wherein the cell comprises a stably integrated AAV rep gene.
20. A cell, comprising the polynucleotide sequence of any one of claims 13 to 15, a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.
21. The cell of claim 20, wherein the cell is an insect cell, a silkworm cell, a mosquito cell, a bacterial cell or a mammalian cell.
22. The cell of claim 21, wherein the cell is a 293 cell, a COS cell, a HeLa cells, or a KB cell.
23. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB A for the preparation of a medicament for decreasing creatine kinase (CK) level in a subject in need thereof, wherein the rAAV comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19 and is suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.
24. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the rAAV comprise a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19, wherein the rAAV is suitable for administration at a dose of about 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.