Claims

~~We claim:~~

1. A method of treating limb girdle muscular dystrophy 2A in a subject comprising administering to the subject a therapeutically effective amount of:

i) A recombinant adeno-associated virus (rAAV) comprises a polynucleotide which comprises a first AAV inverted terminal repeat (ITR), a promoter, a nucleotide sequence encoding a protein with calpain 3 (CAPN3) activity and a second AAV ITR~~.~~;

~~2.~~ii) The rAAV of ~~claim 1~~ (i), wherein the nucleotide sequence encoding the protein with CAPN3 activity is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 2~~.~~;

~~3.~~iii) The rAAV of ~~claim 1 or 2~~ (i) or (ii), wherein the nucleotide sequence encoding the protein with CAPN3 activity is at least 95% identical to SEQ ID NO: 2~~.~~;

~~4.~~iv) The rAAV of any one of ~~claims 1-3~~ (i) to (iii), wherein the nucleotide sequence encoding the protein with CAPN3 activity comprises the sequence of SEQ ID NO: 2~~.~~;

~~5.~~v) The rAAV of any one of ~~claims 1-4~~ (i) to (iv), wherein the protein with CAPN3 activity comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 7~~.~~;

~~6.~~vi) The rAAV of any one of ~~claims 1-4~~ (i) to (iv), wherein the protein with CAPN3 activity comprises an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 7~~.~~;

~~7.~~vii) The rAAV of any one of ~~claims 1-4~~ (i) to (iv), wherein the protein with CAPN3 activity comprises the amino acid sequence of SEQ ID NO: 7~~.~~;

~~8.~~viii) The rAAV of any one of ~~claims 1-7~~ (i) to (vii), wherein the polynucleotide comprises a sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1~~.~~;

~~9.~~ix) The rAAV of any one of ~~claims 1-7~~ (i) to (vii), wherein the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 1~~.~~;

~~10.~~x) The rAAV of any one of ~~claims 1-7~~ (i) to (vii), wherein the polynucleotide comprises the sequence of SEQ ID NO: 1~~.~~;

~~11.~~xi) The rAAV of any one of ~~claims 1-10~~ (i) to (x) wherein the promoter is a muscle-specific promoter~~.~~;

~~12.~~xii) The rAAV of ~~claim 11~~ (xi), wherein 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)~~.~~;

~~13.~~xiii) The rAAV of ~~claim 11~~ (xi), wherein the muscle-specific promoter is an MCK promoter, a tMCK promoter, or an MHCK7 promoter~~.~~;

~~14.~~xiv) The rAAV of ~~claim 11~~ (xi), wherein the muscle-specific promoter is a truncated MCK promoter comprising the nucleotide sequence of SEQ ID NO: 3~~.~~;

~~15.~~xv) The rAAV of any one of ~~claims 1-14~~ (i) to (xiv), wherein the first and second AAV inverted terminal repeats are AAV2 inverted terminal repeats~~.~~;

~~16.~~xvi) The rAAV of any one of ~~claims 1-15~~ (i) to (xv), wherein the rAAV comprises one or more of 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 rh.74 and AAV rh.10 capsid proteins~~.~~;

~~17.~~xvii) The rAAV of ~~any one of claims 16~~ (xvi), wherein the rAAV comprises an rh.74 capsid protein or an AAV9 capsid protein~~.~~; or

~~18.~~xviii) A composition comprising the rAAV of any one of ~~claims 1-17~~ (i) to (xvii).

~~19. A method of treating limb girdle muscular dystrophy 2A in a subject comprising administering to the subject a therapeutically effective amount of the rAAV of any one of claims 1-17 or the composition of claim 18.~~

2~~0~~. The method of claim 1~~9~~, wherein the treatment results in one or more of:

(a) an increased muscle fiber diameter,

(b) a decreased number of small lobulated muscle fibers,

(c) a decreased number of fibers with internal nuclei,

(d) a decreased endomysial connective tissue content,

(e) correction of muscle atrophy, and

(f) an increased muscle force generation.

~~21~~3. The method of claim 2~~0~~, wherein the muscle fiber comprises one or more of slow twitch oxidative (STO) muscle fiber, fast twitch oxidative (FTO) muscle fiber, and fast twitch glycolytic (FTG) fiber.

~~22~~4. The method of any one of claims ~~19~~1-~~21~~3, wherein the treatment results in one or more of:

(a) at least a 5%, 10%, 15%, 20%, 25%, 30%, or 35%, or 40% decrease of total muscle fiber number per mm^2 by 4 weeks after administration;

(b) at least a 5%, 10%, 15%, 20%, or 25% increase of muscle fiber diameter by 4 weeks after administration;

(c) at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 42% decrease of STO muscle fiber number per mm^2 by 4 weeks after administration;

(d) at least a 5%, 10%, 15%, 20%, or 25% increase of STO muscle fiber diameter by 4 weeks after administration;

(e) at least a 5%, 10%, 15%, or 20% decrease of FTO muscle fiber number per mm^2 by 4 weeks after administration;

(f) at least a 5%, 10%, 15%, or 20% increase of FTO muscle fiber diameter by 4 weeks after administration;

(g) at least a 5%, 10%, 15%, 20%, 25%, 30%, or 35% decrease of FTG muscle fiber number per mm^2 by 4 weeks after administration; and

(h) at least a 5%, 10%, 15%, 20%, or 25% increase of FTG muscle fiber diameter by 4 weeks after administration.

~~23~~5. The method of any one of claims ~~19~~1-~~22~~4, wherein the administration is by intramuscular injection or intravenous injection.

~~24~~6. The method of any one of claims ~~19~~1-~~23~~5, wherein the heart muscle of the subject shows minimum or low calpain 3 protein expressed from the rAAV of any one of ~~claims 1-17~~ (i) to (xvii) or the composition of ~~claim 18~~ (xviii).

~~25. A composition for treating limb girdle muscular dystrophy 2A comprising a therapeutically effective amount of the rAAV of any one of claims 1-17 or the composition of claim 18.~~

~~26. The composition of claim 25, wherein the treatment with the composition results in one or more of:~~

~~(a) an increased muscle fiber diameter,~~

~~(b) a decreased number of small lobulated muscle fibers,~~

~~(c) a decreased number of fibers with internal nuclei,~~

~~(d) a decreased endomysial connective tissue content,~~

~~(e) correction of muscle atrophy, and~~

~~(f) a increased muscle force generation.~~

~~27. The composition of claim 26, wherein the muscle fiber comprises one or more of slow twitch oxidative (STO) muscle fiber, fast twitch oxidative (FTO) muscle fiber, and fast twitch glycolytic (FTG) fiber.~~

~~28. The composition of any one of claims 25-27, wherein the treatment with the composition results in one or more of:~~

~~(a) at least a 5% , 10%, 15%, 20%, 25%, 30%, or 35%, or 40% decrease of total muscle fiber number per mm^2 by 4 weeks after administration;~~

~~(b) at least a 5%, 10%, 15%, 20%, or 25% increase of muscle fiber diameter by 4 weeks after administration;~~

~~(c) at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 42% decrease of STO muscle fiber number per mm^2 by 4 weeks after administration;~~

~~(d) at least a 5%, 10%, 15%, 20%, or 25% increase of STO muscle fiber diameter by 4 weeks after administration;~~

~~(e) at least a 5%, 10%, 15%, or 20% decrease of FTO muscle fiber number per mm^2 by 4 weeks after administration;~~

(f) at least a 5%, 10%, 15%, or 20% increase of FTO muscle fiber diameter by 4 weeks after administration;

(g) at least a 5%, 10%, 15%, 20%, 25%, 30%, or 35% decrease of FTG muscle fiber number per mm^2 by 4 weeks after administration; and

(h) at least a 5%, 10%, 15%, 20%, or 25% increase of FTG muscle fiber diameter by 4 weeks after administration.

29. The composition of any one of claims 25-28, wherein the composition is formulated for administration by intramuscular injection or intravenous injection.

30. The composition of any one of claims 25-28, wherein after treatment with the composition, the heart muscle of the subject shows minimum or low calpain 3 protein expressed from the rAAV of any one of claims 1-17 or the composition of claim 18.

31. Use of a therapeutically effective amount of the rAAV of any one of claims 1-17 or the composition of claim 18 for the preparation of a medicament for the treatment of limb girdle muscular dystrophy 2A.

32. The use of claim 31, wherein the treatment with the medicament results in one or more of:

(a) an increased muscle fiber diameter,

(b) a decreased number of small lobulated muscle fibers,

(c) a decreased number of fibers with internal nuclei,

(d) a decreased endomysial connective tissue content,

(e) correction of muscle atrophy, and

(f) an increased muscle force generation.

33. The use of claim 32, wherein the muscle fiber comprises one or more of slow twitch oxidative (STO) muscle fiber, fast twitch oxidative (FTO) muscle fiber, and fast twitch glycolytic (FTG) fiber.

34. The use of any one of claims 31-33, wherein the treatment with the medicament results in one or more of:

(a) at least a 5% , 10%, 15%, 20%, 25%, 30%, or 35%, or 40% decrease of total muscle fiber number per mm^2 by 4 weeks after administration;

(b) at least a 5%, 10%, 15%, 20%, or 25% increase of muscle fiber diameter

~~by 4 weeks after administration;~~

~~(c) at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 42% decrease of STO muscle fiber number per mm^2 by 4 weeks after administration;~~

~~(d) at least a 5%, 10%, 15%, 20%, or 25% increase of STO muscle fiber diameter by 4 weeks after administration;~~

~~(e) at least a 5%, 10%, 15%, or 20% decrease of FTO muscle fiber number per mm^2 by 4 weeks after administration;~~

~~(f) at least a 5%, 10%, 15%, or 20% increase of FTO muscle fiber diameter by 4 weeks after administration;~~

~~(g) at least a 5%, 10%, 15%, 20%, 25%, 30%, or 35% decrease of FTG muscle fiber number per mm^2 by 4 weeks after administration; and~~

~~(h) at least a 5%, 10%, 15%, 20%, or 25% increase of FTG muscle fiber diameter by 4 weeks after administration.~~

~~35. The use of any one of claims 31-34, wherein the medicament is formulated for administration by intramuscular injection or intravenous injection.~~

~~36. The use of any one of claims 31-35, wherein after treatment with the medicament, the heart muscle of the subject shows minimum or low calpain 3 protein expressed from the rAAV of any one of claims 1-17 or the composition of claim 18.~~