Claims

1. A recombinant adeno-associated virus rh.74 (rAAVrh.74) ~~comprises~~ comprising a polynucleotide which comprises a first AAV inverted terminal repeat (ITR), a promoter, a nucleotide sequence ~~encoding a protein with calpain 3 (CAPN3) activity~~ comprising the nucleotide sequence of SEQ ID NO: 2 and a second AAV ITR.

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

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

~~5. The rAAV of any one of claims 1-4, 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. The rAAV of any one of claims 1-4, 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. The rAAV of any one of claims 1-4, wherein the protein with CAPN3 activity comprises the amino acid sequence of SEQ ID NO: 7.~~

~~8. The rAAV of any one of claims 1-7, 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. The rAAV of any one of claims 1-7, wherein the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 1.~~

~~10. The rAAV of any one of claims 1-7, wherein the polynucleotide comprises the sequence of SEQ ID NO: 1.~~

~~11~~2. The rAAVrh.74 of ~~any one of claims 1-10~~ claim 1 wherein the promoter is a muscle-specific promoter.

~~12~~3. The rAAVrh.74 of claim ~~11~~2, 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~~ (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~~ C gene element, a slow-twitch cardiac troponin ~~c~~ C gene element, a slow-twitch troponin ~~i~~ I gene element, hypoxia- inducible nuclear factor (HIF)-response element (HRE), a steroid-inducible element, and a glucocorticoid response element ~~(gre)~~ (GRE).

~~13~~4. The rAAVrh.74 of claim ~~11~~3, wherein the muscle-specific promoter is an MCK promoter, a tMCK promoter, or an MHCK7 promoter.

~~14~~5. The rAAVrh.74 of claim ~~11~~3, wherein the muscle-specific promoter is a truncated MCK promoter comprising the nucleotide sequence of SEQ ID NO: 3.

~~15~~6. The rAAVrh.74 of any one of claims 1-~~14~~5, wherein the first and second AAV inverted terminal repeats are AAV2 inverted terminal repeats.

7. A recombinant adeno-associated virus rh.74 (rAAVrh.74) comprising a polynucleotide which comprises a first AAV inverted terminal repeat (ITR), a promoter comprising the nucleotide sequence of SEQ ID NO: 3, a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 2 encoding a protein with calpain 3 (CAPN3) activity and a second AAV ITR.

~~16. The rAAV of any one of claims 1-15, 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. The rAAV of any one of claims 16, wherein the rAAV comprises an rh.74 capsid protein or an AAV9 capsid protein.~~

~~1~~8. A composition comprising the rAAVrh.74 of any one of claims 1-~~1~~7.

9. A polynucleotide for making an rAAV comprising a sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1.

10. The polynucleotide of claim 9, wherein the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 1.

11. The polynucleotide of claim 9 or 10, wherein the polynucleotide comprises the sequence of SEQ ID NO: 1.

~~19~~12. A composition for use in treating limb girdle muscular dystrophy 2A, ~~comprising~~ wherein the composition comprises a therapeutically effective amount of the rAAVrh.74 of any one of claims 1-~~1~~7 or the composition of claim ~~1~~8.

~~20~~13. The composition ~~for use~~ of claim ~~19~~12, 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) an increased muscle force generation.

~~21~~14. The composition ~~for use~~ of claim ~~20~~13, 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~~15. The composition ~~for use~~ of any one of claims ~~19-21~~ 12-14, 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.

~~23~~16. The composition ~~for use~~ of any one of claims ~~19-22~~ 12-15, wherein the composition is formulated for administration by intramuscular injection or intravenous injection.

~~24~~17. The composition ~~for use~~ of any one of claims ~~19-22~~ 12-15, wherein after treatment with the composition, the heart muscle of the subject shows minimum or low calpain 3 protein expressed from the rAAVrh.74 ~~of any one of claims 1-17 or the composition of claim 18~~.

~~25~~18. Use of a therapeutically effective amount of the rAAV of any one of claims 1-~~1~~7 or the composition of claim ~~18~~ ~~for~~ in the preparation of a medicament for the treatment of limb girdle muscular dystrophy 2A.

~~26~~19. The use of claim ~~25~~18, 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.

~~27~~20. The use of claim ~~26~~19, 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~~21. The use of ~~any one of claims 25-27~~ claim 19, 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.

~~29~~22. The use of ~~any one of claims 25-28~~ claim 18, wherein the medicament is formulated for administration by intramuscular injection or intravenous injection.

~~30~~23. The use of ~~any one of claims 25-29~~ claim 18, 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~~-1~~7 or the composition of claim ~~1~~8.