

several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

[0008] Multiple studies have demonstrated long-term (> 1.5 years) recombinant AAV-mediated protein expression in muscle. See, Clark *et al.*, *Hum Gene Ther*, 8: 659-669 (1997); Kessler *et al.*, *Proc Natl Acad Sci USA*, 93: 14082-14087 (1996); and Xiao *et al.*, *J Virol*, 70: 8098-8108 (1996). See also, Chao *et al.*, *Mol Ther*, 2:619-623 (2000) and Chao *et al.*, *Mol Ther*, 4:217-222 (2001). Moreover, because muscle is highly vascularized, recombinant AAV transduction has resulted in the appearance of transgene products in the systemic circulation following intramuscular injection as described in Herzog *et al.*, *Proc Natl Acad Sci USA*, 94: 5804-5809 (1997) and Murphy *et al.*, *Proc Natl Acad Sci USA*, 94: 13921-13926 (1997). Moreover, Lewis *et al.*, *J Virol*, 76: 8769-8775 (2002) demonstrated that skeletal myofibers possess the necessary cellular factors for correct antibody glycosylation, folding, and secretion, indicating that muscle is capable of stable expression of secreted protein therapeutics.

[0009] As shown herein, disruption of the *Ano5* gene in mice produces several dystrophic muscle histopathogenic features, exercise intolerance, dysfunction in sarcolemmal repair, and myoblast fusion defects. In addition, the data provided demonstrates that these defects are related to changes in subcellular membrane and/or membrane and/or sarcolemmal membrane dynamics mediated by ANO5. The present invention relates to using AAV vectors expressing the *Ano5* gene in methods of inducing muscle repair and/or treating MD.

SUMMARY

[0010] The present invention is directed to AAV vectors comprising a nucleotide sequence encoding the ANO5 protein or a functionally active fragment thereof. The experiments described in the Examples 1-4 demonstrate an essential role for *Ano5* in muscle regeneration and repair. Loss of ANO5 causes a dystrophic phenotype in mice, reminiscent of LGMD2L patients, with mild histopathology that varies among muscles, exercise intolerance, impaired regeneration, and elevated creatine kinase levels. Mitochondrial abnormalities were observed in both young and aged *Ano5*^{-/-} mice. In addition to the structural defect of the mitochondria displayed through electron microscopic imaging, citrate synthase quantification assays also demonstrated that the mitochondria have a functional deficit. Quantitative enzyme

analysis of citrate synthase suggests the presence of damaged mitochondria. These results suggest that mitochondrial abnormalities may be a secondary effect caused by the loss of the Anoctamin 5 protein. The experiments described herein demonstrate an attenuation of sarcolemmal patch repair in *Ano5*^{-/-} fibers that is rescued by viral
5 expression of human ANO5 and find that the disruption of Ano5 perturbs the fusogenic quality of primary myoblasts.

[0010a] In one aspect, the present invention refers to the use of a recombinant AAV vector comprising 1) a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent
10 condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity in the manufacture of a medicament for treating muscular dystrophy in a subject in need thereof.

[0010b] In another aspect, the present invention refers to the use of a recombinant
15 AAV vector comprising 1) a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1, or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity in the manufacture of a medicament for regenerating muscle in a subject.

[0010c] In yet another aspect, the present invention refers to the use of a recombinant
20 AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity for treating chronic muscle
25 wasting in a subject in need thereof.

[0010d] In yet another aspect, the present invention refers to the use of an antioxidant composition in the manufacture of a medicament for treating muscular dystrophy in a subject in need thereof.

[0010e] In yet another aspect, the present invention refers to the use of an antioxidant
30 composition in the manufacture of a medicament for treating chronic muscle wasting in a subject in need thereof.

[0010f] In yet another aspect, the present invention refers to the use of an antioxidant composition in the manufacture of a medicament for slowing the progression of muscular dystrophy in a subject in need thereof.

5 **[0010g]** In yet another aspect, the present invention refers to the use of an antioxidant composition in the manufacture of a medicament for slowing the progression of chronic muscle wasting in a subject in need thereof.

[0010h] In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the nucleic acid
10 sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity, for the treatment of muscular dystrophy in a subject in need.

15 **[0010i]** In yet another aspect, the present invention refers to a composition comprising a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity to a subject in need thereof, for the regeneration of muscle in a subject in need.

20 **[0010j]** In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1, or 3) a fragment of nucleic acid of SEQ ID
25 NO: 1 encoding a protein that exhibits ANO5 activity for the treatment of chronic muscle wasting in a subject in need.

[0010k] In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of an antioxidant composition, for the treatment of muscular dystrophy in a subject in need.

[0010l] In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of an antioxidant composition, for the treatment of chronic muscle wasting in a subject in need.

- 5 **[0010m]** In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of an antioxidant composition, for slowing the progression of muscular dystrophy in a subject in need.

[0010n] In yet another aspect, the present invention refers to a composition comprising a therapeutically effective amount of an antioxidant composition, for slowing the progression of chronic muscle wasting in a subject in need.

[0011] In one example, the disclosure provides for a recombinant AAV vector comprising a polynucleotide comprising an ANO5 nucleic acid sequence. The ANO5 nucleic acid sequence is set out as Genbank Accession No. NM_213599.2 (SEQ ID NO: 13). An exemplary nucleic acid sequence encoding the ANO5 protein
5 in an AAV vector is set out as SEQ ID NO: 1. The recombinant vectors of the present disclosure comprise a polynucleotide sequence that is at least is at least 85% identical to nucleic acid of SEQ ID NO:1 or SEQ ID NO: 13 or comprise a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 13 or comprises a fragment of nucleic acid of SEQ ID NO: 1 or SEQ ID
10 NO: 13 encoding a protein that exhibits ANO5 activity. In one example, the recombinant AAV vectors of the disclosure are AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or AAV rh.74.

[0012] The recombinant AAV vectors of the disclosure comprise a fragment of SEQ ID NO: 1 that encodes a protein that comprise a domain that is involved in PLS
15 activity and said protein retains PLS activity. In another example, the AAV vector comprises a fragment of SEQ ID NO: 1 or SEQ ID NO: 13 that encodes a protein that retains ANO5 activity.

[0013] The recombinant AAV vectors of the disclosure comprise a polynucleotide sequence that is, e.g., at least 65%, at least 70%, at least 75%, at least 80%, 81%,
20 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least 90%, 91%, 92%, 93%, or 94% and even more typically at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 13, wherein the polynucleotide encodes a protein that retains ANO5 activity.

[0014] The recombinant AAV vectors of the disclosure comprise a polynucleotide
25 sequence encoding a ANO5 protein that is, e.g., at least at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least 90%, 91%, 92%, 93%, or 94% and even more typically at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 2, wherein the polynucleotide encodes a protein that retains ANO5 activity

[0015] The recombinant AAV vectors of the disclosure comprise a polynucleotide
30 sequence that hybridizes under stringent conditions to the nucleic acid sequence of SEQ ID NOS: 1 or SEQ ID NO: 13, or compliments thereof, which fragment is

greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, e.g., 15, 17, or 20 nucleotides or more that are selective for (i.e., specifically hybridize to any one of the polynucleotides of the disclosure) are contemplated and these

5 fragment retain ANO5activity. Probes capable of specifically hybridizing to a polynucleotide can differentiate NTHi polynucleotide sequences of the disclosure from other polynucleotide sequences in the same family of genes or can differentiate NTHi genes from other bacterial genes, and are preferably based on unique nucleotide sequences.

10 **[0016]** 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 65-68oC or 0.015 M sodium chloride,
15 0.0015M sodium citrate, and 50% formamide at 42oC. 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
20 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).

[0017] Other agents may be included in the hybridization and washing buffers for
25 the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO₄, (SDS), ficoll, Denhardt’s solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration
30 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
5 form hybrids.

[0018] In another example, the recombinant AAV vectors of the disclosure may be operably linked to a muscle-specific control element. For example the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, murine creatine kinase
10 enhancer element (MCK), triple copies of the MCK (tMCK), skeletal fast-twitch troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypoxia-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

[0019] In another example, the disclosure provides for methods of producing a
15 recombinant AAV vector particle comprising culturing a cell that has been transfected with any recombinant AAV vector of the disclosure and recovering recombinant AAV particles from the supernatant of the transfected cells. The disclosure also provides for viral particles comprising any of the recombinant AAV vectors of the disclosure.

20 [0020] In another example, the disclosure provides for methods of treating muscular dystrophy comprising administering a therapeutically effective amount of any recombinant AAV vector of the disclosure to a subject in need thereof. In one example, the subject in need has a recessive mutation in the ANO5 gene. The methods may treat dysferlin-associated muscular dystrophy, limb girdle muscular
25 dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy. In addition, any of the foregoing methods to treat muscular dystrophy further comprise the step of administering a therapeutically effective amount of an antioxidant composition to a subject in need thereof. For example, the
30 antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In particular, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0021] In a further example, the disclosure provides for methods of regenerating muscle in a subject in need comprising administering a therapeutically effective amount of any recombinant AAV vector of the disclosure to subject in need thereof. In one example, the subject in need has a recessive mutation in the ANO5 gene. In another example, the subject in need is suffering from muscular dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathy or ZASP-related myopathy. In addition, any of the foregoing methods to regenerate muscle further comprise the step of administering a therapeutically effective amount of an antioxidant composition to a subject in need thereof. For example, the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In particular, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0022] In another example, the disclosure provides for methods of treating chronic muscle wasting comprising administering a therapeutically effective amount of any of the recombinant AAV vectors of the disclosure to subject in need thereof. In one example, the subject in need has a recessive mutation in the ANO5 gene. In another example, the subject in need is suffering from muscular dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L or Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathy or ZASP-related myopathy. In addition, any of the foregoing methods to treat chronic muscle wasting further comprise the step of administering a therapeutically effective amount of an antioxidant composition to a subject in need thereof. For example, the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In particular, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0023] The disclosure also provides for a composition comprising any recombinant AAV vector of the disclosure for the treatment of muscular dystrophy or chronic muscle wasting syndrome in a subject in need. The disclosure also provides for composition comprising any recombinant AAV vector of the disclosure for the

regeneration of muscle in a subject in need. In one example, the composition of the disclosure is administered to a subject having recessive mutation in the ANO5 gene. In another example, the composition of the disclosure is administered to a subject in suffering from muscular dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy. Any of the compositions of the disclosure are formulated for intramuscular or intravenous injection. Any of the compositions of the disclosure further comprise a therapeutically effective amount of an antioxidant composition. For example, the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In particular, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0024] In another example, the disclosure provides for use of any recombinant AAV vector of the disclosure for the preparation of a medicament for treatment of muscular dystrophy or chronic muscle wasting syndrome in a subject in need thereof. In addition, the disclosure provides for use of any of the recombinant AAV vectors of the disclosure for the preparation of a medicament for regenerating muscle in subject need thereof. In one example, the medicament is administered to a subject having recessive mutation in the ANO5 gene. In another example, the medicament of the disclosure is administered to a subject in suffering from muscular dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy. Any of the medicaments of the disclosure are formulated for intramuscular or intravenous injection. Any of the medicaments of the disclosure further comprise a therapeutically effective amount of an antioxidant composition. For example, the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In particular, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0025] In another example, the disclosure provides for methods of treating muscular dystrophy or chronic muscle wasting comprising administering a therapeutically effective amount of antioxidant composition to a subject in need thereof. The antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In some examples, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E. In one example, the subject in need has a recessive mutation in the ANO5 gene. In another example, oxidative stress is reduced in skeletal muscle of the subject. In another example, the skeletal muscle function of the subject is improved.

10 The In another example, the subject is suffering from dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy.

[0026] The disclosure also provides for methods for slowing the progression of muscular dystrophy or chronic muscle wasting comprising administering a therapeutically effective amount of an antioxidant composition to a subject in need thereof. The antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In some examples, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E. In one example, the subject in need has a recessive mutation in the ANO5 gene. In another example, oxidative stress is reduced in skeletal muscle of the subject. In another example, the skeletal muscle function of the subject is improved. In another example, the subject is suffering from dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy.

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[0027] In any of the methods of the disclosure, the antioxidant composition is administered orally. In any of the methods of the disclosure, the antioxidants are administered in the same composition or the antioxidants are administered in separate compositions. If the antioxidants are administered separately but concurrently. In addition, in any of the methods of the disclosure, the antioxidants are administered at separate times or consecutively. In any of the methods of the disclosure, the

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antioxidant composition is administered once daily, once weekly, twice weekly, once every two weeks, once every three weeks, monthly or once every two months.

[0028] The disclosure also provides for a composition comprising a therapeutically effective amount of an antioxidant composition. The antioxidant composition
5 comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid for the treatment of muscular dystrophy or chronic muscle wasting syndrome in a subject in need.

[0029] The disclosure also provides for composition comprising a therapeutically effective amount of an antioxidant composition. The antioxidant compositions
10 comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid for slowing the progression of muscular dystrophy or chronic muscle wasting in a subject in need. In some examples, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0030] In one example, any of the compositions of the disclosure is administered to
15 a subject having recessive mutation in the ANO5 gene. In another example, oxidative stress is reduced in skeletal muscle of the subject after administration of the composition. In another example, the skeletal muscle function of the subject is improved after administration of the composition. In another example, any of the compositions of the disclosure is administered to a subject suffering from muscular
20 dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy. Any of the compositions of the disclosure are formulated for intramuscular or intravenous injection.

[0031] In another example, the disclosure provides for use of an antioxidant
25 composition for the preparation of a medicament for the treatment of muscular dystrophy or chronic muscle wasting syndrome in a subject in need thereof. The antioxidant composition in any of the medicaments of the disclosure comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor,
30 mineral, polyphenol, or flavonoid.

[0032] In addition, the disclosure provides for use of an antioxidant composition for the preparation of a medicament for regenerating muscle in a subject in need thereof. The antioxidant composition in any of the medicaments of the disclosure comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin
5 cofactor, mineral, polyphenol, or flavonoid. In some examples, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0033] In addition, the disclosure provides for use of an antioxidant composition for the preparation of a medicament for slowing the progression of muscular dystrophy or muscle wasting syndrome in a subject in need thereof. The antioxidant
10 composition in any of the medicaments fo the disclosure comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid. In some examples, the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

[0034] Any of the medicaments of the disclosure are administered to a subject
15 having recessive mutation in the ANO5 gene. In another example, oxidative stress is reduced in skeletal muscle of the subject. In another example, the skeletal muscle function of the subject is improved. In another example, any of the medicaments of the disclosure is administered to a subject in suffering from muscular dystrophy such as dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L,
20 Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy. In addition, any of the medicaments of the disclosure are formulated for intramuscular or intravenous injection.

[0035] In some examples, the antioxidant composition is administered orally. In
25 some examples, the antioxidants are in the same composition. In other examples, the antioxidants are in separate compositions. In some examples, the antioxidants of the antioxidant composition are administered concurrently. In some examples, the antioxidants of the antioxidant composition are administered at separate times or consecutively. In some examples, the antioxidant composition is administered

CLAIMS

What is claimed:

1. Use of a recombinant AAV vector comprising 1) a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide
5 that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity in the manufacture of a medicament for treating muscular dystrophy in a subject in need thereof.
2. Use of a recombinant AAV vector comprising 1) a nucleic acid sequence that
10 is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1, or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity in the manufacture of a medicament for regenerating muscle in a subject.
- 15 3. Use of a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity for treating chronic muscle wasting in a subject in need thereof.
- 20 4. The use of any one of claims 1-3 wherein the recombinant AAV comprises a polynucleotide sequence of SEQ ID NO: 1.
5. The use of any one of claims 1-4 wherein the recombinant AAV vector of is AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or AAV rh.74.
- 25 6. The use of any one of claims 1-5 wherein the recombinant AAV vector comprises a polynucleotide operably linked to a muscle-specific control element.
7. The use of claim 6 wherein the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, murine creatine kinase enhancer element, skeletal fast-twitch

troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypoza-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

8. The use of any one of claims 2-7 wherein the subject is suffering from
5 muscular dystrophy.

9. The use of any one of claims 1-8 wherein the muscular dystrophy is dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, or Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related
10 myopathy.

10. The use of any one of claims 1-9 wherein the subject has a recessive mutation in the ANO5 gene.

11. The use of any one of claims 1-10 wherein the recombinant AAV vector is to be administered by intramuscular or intravenous injection.

15 12. The use of any one of claims 1-11 further comprises a therapeutically effective amount of an antioxidant composition to be administered to a subject in need thereof.

13. The use of claim 12 wherein the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid.

20 14. The use of claim 12 or 13 wherein the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

15. Use of an antioxidant composition in the manufacture of a medicament for treating muscular dystrophy in a subject in need thereof.

25 16. Use of an antioxidant composition in the manufacture of a medicament for treating chronic muscle wasting in a subject in need thereof.

17. Use of an antioxidant composition in the manufacture of a medicament for slowing the progression of muscular dystrophy in a subject in need thereof.

18. Use of an antioxidant composition in the manufacture of a medicament for slowing the progression of chronic muscle wasting in a subject in need thereof.
19. The use of any one of claims 15-18 wherein the antioxidant composition comprises at least one of a coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin
5 cofactor, mineral, polyphenol, or flavonoid.
20. The use of any one of claims 15-19 wherein the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.
21. The use of any one of claims 15-20 wherein muscular dystrophy is dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, or Miyoshi
10 myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy.
22. The use of any one of claims 15-21 wherein the oxidative stress is reduced in skeletal muscle of the subject.
- 15 23. The use of any one of claims 15-21 wherein the skeletal muscle function of the subject is improved.
24. The use of any one of claims 15-21 wherein the subject has a recessive mutation in the ANO5 gene.
25. The use of any one of claims 12-25 wherein the antioxidant composition is to
20 be administered orally.
26. The use of any one of claims 12-25 wherein the antioxidants are in the same composition.
27. The use of any one of claims 12-25 wherein the antioxidants are in separate compositions.
- 25 28. The use of any one of claims 12-25 wherein the antioxidants of the antioxidant composition are to be administered concurrently.

29. The use of any one of claims 12-25 wherein the antioxidants of the antioxidant composition are to be administered at separate times or consecutively.
30. The use of any one of claims 12-29 wherein the antioxidant composition is to be administered once daily, once weekly, twice weekly, once every two weeks, once
5 every three weeks, monthly or once every two months.
31. A composition comprising a therapeutically effective amount of a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or 3) a fragment of nucleic
10 acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity, for the treatment of muscular dystrophy in a subject in need.
32. A composition comprising a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1 or
15 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity to a subject in need thereof, for the regeneration of muscle in a subject in need.
33. A composition comprising a therapeutically effective amount of a recombinant AAV vector comprising 1) a nucleic acid sequence is at least 85% identical to the
20 nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO: 1, or 3) a fragment of nucleic acid of SEQ ID NO: 1 encoding a protein that exhibits ANO5 activity for the treatment of chronic muscle wasting in a subject in need.
34. The composition of any one of claims 31-33 wherein the recombinant AAV
25 comprises a polynucleotide sequence of SEQ ID NO: 1.
35. The composition of any one of claims 31-34, wherein the recombinant AAV vector of is AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or AAV rh.74.

36. The composition of any one of claims 31-35 wherein the recombinant AAV vector comprises a polynucleotide operably linked to a muscle-specific control element.

37. The composition of claim 36 wherein the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, murine creatine kinase enhancer element, skeletal fast-twitch troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypoxia-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

38. The composition of any one of claims 31-37 wherein the subject is suffering from muscular dystrophy.

39. The composition of any one of claims 31-39 wherein the muscular dystrophy is dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathy or ZASP-related myopathy.

40. The composition of any one of claims 31-39 wherein the subject has a recessive mutation in the ANO5 gene.

41. The composition of any one of claims 31-40 that is formulated for intramuscular or intravenous injection.

42. The composition of any one of claims 31-41 further comprising a therapeutically effective amount of an antioxidant composition.

43. The composition of claim 42, wherein the antioxidant composition comprises at least one of coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid,

44. The composition of claim 42 or 43, wherein the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.

45. A composition comprising a therapeutically effective amount of an antioxidant composition, for the treatment of muscular dystrophy in a subject in need.
46. A composition comprising a therapeutically effective amount of an antioxidant composition, for the treatment of chronic muscle wasting in a subject in need.
- 5 47. A composition comprising a therapeutically effective amount of an antioxidant composition, for slowing the progression of muscular dystrophy in a subject in need.
48. A composition comprising a therapeutically effective amount of an antioxidant composition, for slowing the progression of chronic muscle wasting in a subject in need.
- 10 49. The composition of any one of claims 45-48 wherein the antioxidant composition comprises at least one of coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid,
50. The composition of any one of claims 45-49. wherein the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.
- 15 51. The composition of any one of claims 45-50 wherein muscular dystrophy is dysferlin-associated muscular dystrophy, limb girdle muscular dystrophy type 2L, or Miyoshi myopathy type 3, Bethlem myopathy, calpainopathy, desmin myopathy, dysferlinopathy, myofibrillar myopathy, sarcoglycanopathie or ZASP-related myopathy.
- 20 52. The composition of any one of claims 45-51 wherein oxidative stress is reduced in skeletal muscle of the subject.
53. The composition of any one of claims 45-51 wherein skeletal muscle function of the subject is improved.
54. The composition of any one of claims 45-51 wherein the subject has a
25 recessive mutation in the ANO5 gene.
55. The composition of any one of claims 42-54 that is formulated for oral administration.

56. The composition of any one of claims 42-55 wherein the agents are in the same composition.
57. The composition of any one of claims 42-55 wherein the agents are in separate compositions.
- 5 58. The composition of any one of claims 42-55 wherein the antioxidants of the antioxidant composition are to be administered concurrently.
59. The composition of any one of claims 42-55 wherein the antioxidants of the antioxidant composition are to be administered at separate times or consecutively.
60. The composition of any one of claims 45-59 wherein the antioxidant
10 composition is to be administered once daily, once weekly, twice weekly, once every two weeks, once every three weeks, monthly or once every two months.
61. The use of any of claims 15-30 further comprising a therapeutically effective amount of recombinant AAV vector comprising 1) a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO:1, 2) a nucleotide that
15 hybridizes under stringent condition to the nucleotide sequence of SEQ ID NO:1 or 3) a fragment of nucleic acid of SEQ ID NO:1 encoding a protein that exhibits ANO5 activity, to be administered in combination with a therapeutically effective amount of an antioxidant composition, to a subject in need thereof.
62. The use of claim 61, wherein the antioxidant composition comprises at least
20 one of coenzyme Q10, lipoic acid, vitamin, carotenoid, vitamin cofactor, mineral, polyphenol, or flavonoid,
63. The use of claim 61 or 62, wherein the antioxidant composition comprises coenzyme Q10, lipoic acid and vitamin E.
64. The use of any one of claims 61-63, wherein the recombinant AAV vector
25 comprises a polynucleotide sequence of SEQ ID NO:1.
65. The use of any one of claims 61-64 wherein the recombinant AAV vector is AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or AAV rh.74.

66. The use of any one of claims 61-64 wherein the recombinant AAV vector is to be administered by intramuscular or intravenous injection.
67. The use of any one of claims 61-66 wherein the antioxidant composition is to be administered orally.
- 5 68. The use of any one of claims 61-66, wherein the said recombinant AAV vector and antioxidant composition are in the same composition.
69. The use of any one of claims 61-67, wherein the said recombinant AAV vector and antioxidant composition are in separate compositions.
70. The use of any one of claims 61-67, wherein the said recombinant AAV vector
10 and antioxidant composition are to be administered concurrently.
71. The use of any one of claims 61-67 wherein the said recombinant AAV vector and antioxidant composition are to be administered at separate times or consecutively.
72. The use of any one of claims 61-71 wherein the said recombinant AAV vector and antioxidant composition are to be administered once daily, once weekly, twice
15 weekly, once every two weeks, once every three weeks, monthly or once every two months.