(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 May 2017 (11.05.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/077451 A1

(51) International Patent Classification:
A61K 48/00 (2006.01)

(21) International Application Number:
PCT/IB2016/056572

(22) International Filing Date:
1 November 2016 (01.11.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/251,288 5 November 2015 (05.11.2015) US
62/411,980 24 October 2016 (24.10.2016) US

(71) Applicant: **BAMBOO THERAPEUTICS, INC.** [US/US]; 870 Martin Luther King Jr. Blvd., Chapel Hill, North Carolina 27514 (US).

(72) Inventor: **SAMULSKI, Richard J.**; 102 Darlin Circle, Chapel Hill, North Carolina 27514 (US).

(74) Agent: **WALDRON, Roy F.**; Pfizer INC., 235 East 42nd Street, MS 235/9/S20, New York, New York 10017 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

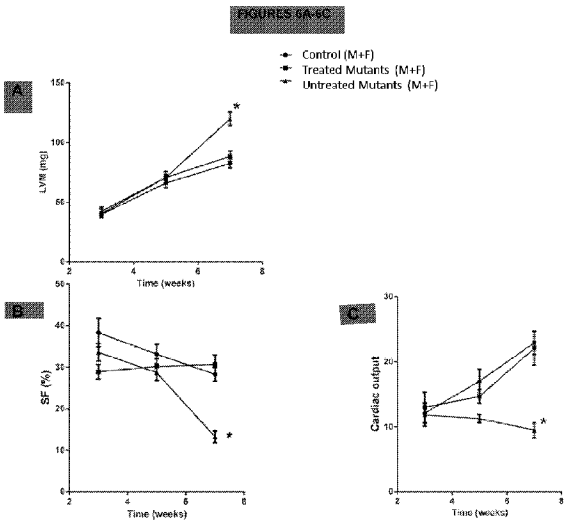
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: MODIFIED FRIEDREICH ATAXIA GENES AND VECTORS FOR GENE THERAPY



(57) Abstract: The present invention relates to a modified FXN gene providing for increased expression of the encoded protein frataxin that can be used for treatment of Friedreich ataxia.

WO 2017/077451 A1

MODIFIED FRIEDREICH ATAXIA GENES AND VECTORS FOR GENE THERAPY

FIELD OF THE INVENTION

[1] The invention relates to modified frataxin (FXN) genes, vectors comprising the modified FXN genes, methods of using the modified FXN genes, and vectors containing them in the treatment of Friedreich ataxia, including cardiomyopathy and/or neurodegenerative disease associated therewith, by providing increased expression levels of non-mutated (wild type) mitochondrial protein frataxin.

BACKGROUND OF THE INVENTION

Friedreich ataxia (FRDA) is associated with reduction of expression of and/or mutation in the FXN gene that encodes for the mitochondria protein frataxin. FRDA is an autosomal recessive disease, meaning individuals only develop this disease if they inherit a defective gene from both parents. FRDA is caused by mutations in the FXN gene that results in reduction of mRNA and protein levels of frataxin. Defective frataxin expression causes critical metabolic changes, including redox imbalance and ATP deficiency.

[2] FRDA is a neurodegenerative disease that affects children and young adults and leads to progressive disability and premature death. Neurological signs are associated with degeneration of sensory neurons and the flow of sensory information through the peripheral nerves and the spinal cord is severely affected. There is also some impairment of muscle-controlling signals from the cerebellum and spinal cord. These problems lead to the progressive loss of balance, coordination and muscle strength that characterize FRDA. Further, patients often develop a hypertrophic cardiomyopathy that is likely the cause of premature death. Enlargement of the heart, irregular heartbeat and other symptoms of heart trouble are evident.

[3] It is believed that the frataxin protein regulates the levels of iron inside the mitochondria which is necessary for using oxygen to produce energy. Frataxin appears to act as a storage depot for iron, releasing it only when it's needed for synthesis of enzymes in the mitochondrial. Therefore, a deficiency of frataxin results in a deficiency of these enzymes and further reduces mitochondrial function which likely explains why Friedreich ataxia affects cells of the nervous system and heart.

[4] To date, no treatment exists for stopping or slowing down the negative effects of FRDA. Current therapeutic approaches in clinical use or under evaluation are directed at alleviating symptoms and maximizing quality of life. Physical therapy and speech therapy have been used to improve movement. Further, some medications have been used to treat heart disease.

Thus, there is an important need for a novel therapeutic approach to treat the symptoms associated with FRDA.

SUMMARY OF THE INVENTION

[5] Disclosed and exemplified herein are modified nucleic acids encoding frataxin (FXN) and vectors comprising the modified nucleic acid and methods of treating a disease mediated by decreased level of FXN by administering the modified nucleic acid or the vector comprising the nucleic acid to a patient in need thereof.

[6] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following embodiments (E).

E1. A modified FXN gene for treating FRDA in a human subject wherein the modified FXN gene has been modified to alter the content of GC nucleotides and/or to have a reduced number of CpG dinucleotides.

E2. The modified FXN gene of embodiment 1 wherein the reduced number of CpG dinucleotides is in an amount sufficient to suppress the silencing of gene expression due to the methylation of CpG motifs.

E3. The modified FXN gene of embodiment 1 wherein the content of GC nucleotides is greater than 10%, 20%, 30%, 40%, 50%, 60% or 70% relative to the wild-type gene.

E4. The modified FXN gene of embodiment 3, having a codon adaptation index that is >0.75, >0.80, >0.85, >0.90, or >0.95.

E5. The modified FXN gene of embodiment 3, comprising a sequence selected from any one of SEQ ID NOs: 3 to 9.

E6. The modified FXN gene of embodiment 1 wherein the content of GC nucleotides is less than 10%, 20%, 30%, 40%, 50%, 60% or 70% relative to the wild-type gene.

E7. The modified FXN gene of embodiment 1 included in a viral vector or plasmid.

E8. The modified FXN gene of embodiment 7, wherein the viral vector is a self-complementary AAV sequence.

E9. The modified FXN gene of embodiment 8, wherein the viral vector is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 AAV8, AAV9, AAV10, AAV11, AAV12, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, AAV Hu.26, AAV2i8, AAV2G9, rhAAV10, rhAAV74, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAV-LK03, and combinations and variants thereof.

E10. The modified FXN gene of embodiment 8, wherein the viral vector is an ancestral AAV vector.

E11. The modified FXN gene of embodiment 8, wherein the viral vector is a chimeric AAV including a combination of AAV backbones from AAV2, AAV3B, AAV6 or AAV8 and further comprising a galactose (Gal) binding footprint from AAV9.

E12. The modified FXN gene of embodiment 1, wherein frataxin protein has an amino acid sequence of SEQ ID NO. 1 or a functional fragment thereof.

E13. A method for treating a disease associated with frataxin deficiency in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a modified FXN gene wherein the modified FXN gene has been modified to increase or decrease content of GC nucleotides and/or to reduce the number of CpG dinucleotides.

E14. The method of embodiment 13, wherein the modified FXN gene encodes the frataxin protein having the amino acid sequence of SEQ ID NO. 1.

E15. The method of embodiment 13, wherein the modified FXN gene is expressed in target cells, wherein the target cells are cardiac or neuron cells

E16. The method of embodiment 13, wherein the modified FXN gene is delivered in a viral or non-viral vector to the target cells.

E17. The method of embodiment 16, wherein the vector is delivered by systemic injection or by direct cardiac or intracranial injection.

E18. A method of treating Friedreich ataxia (FRDA) in a subject in need thereof, the method comprising: providing at least one recombinant virus vector comprising a modified FXN gene, wherein the modified FXN gene has been modified to increase or decrease the content of GC nucleotides and/or to reduce the amount of CpG dinucleotides; and
administering the recombinant virus vector to the subject under conditions such that the modified FXN gene is expressed at a level which produces a therapeutically effective amount of frataxin in cardiac and/or neuron tissue of the subject.

E19. The method of embodiment 18, wherein the recombinant virus vector is administered to neurons or heart muscles cells of the subject.

E20. A host cell transfected with a modified FXN gene that encodes a frataxin peptide or a functional fragment thereof wherein the modified FXN gene has been modified to increase or decrease content of GC nucleotides and/or to reduce number of CpG dinucleotides.

E21. A process of preparing a frataxin peptide or fragment thereof comprising:
transfecting a host cell with a modified FXN gene that encodes the frataxin peptide or functional fragment thereof; and maintaining the host cell under biological conditions sufficient for expression of the frataxin peptide.

E22. The process of embodiment 21, wherein the modified FXN gene has increased levels of GC nucleotides and/or reduced levels of CpG dinucleotides compared with the nucleic acid sequence of wild type frataxin as set forth in SEQ ID NO:2.

E23. A pharmaceutical composition comprising a modified FXN gene, wherein the modified FXN gene has an increased or decreased content of GC nucleotides and/or a reduced number of CpG dinucleotides, and a pharmaceutically acceptable carrier.

E24. A method for treating FRDA comprising delivering to a subject in need of treatment, a vector comprising a modified polynucleotide sequence encoding a FXN gene, wherein the FXN gene is expressed in the target cells, thereby treating FRDA in the subject. The target cells are preferably cardiac or neuron cells and the vector is preferably delivered to the target cells via direct cardiac or intracranial injection.

E25. The modified nucleic acid of embodiment 1, wherein the modified nucleic acid has a reduced GC content, relative to the wild type gene, that being 20%, 30%, 40%, 50%, or 60% less than the wild type gene while still having the same expression level as the wild type. Silent mutations can be introduced into the coding sequence in order to reduce the GC content of the gene.

E26. A modified nucleic acid encoding FXN with a reduced level of CpG dinucleotides.

E27. A modified nucleic acid encoding FXN (also referred to as a “modified FXN gene”) for treating FRDA in a human subject in need thereof, wherein the modified FXN gene had been modified to increase GC content and reduce certain cis motifs relative to the wild type nucleic acid sequence encoding FXN set forth as SEQ ID NO:2.

E28. A modified FXN gene having a reduced number of CpG dinucleotides in an amount to suppress the silencing of gene expression due to the methylation of CpG motifs compared with the number of CpG dinucleotides present in the wild type nucleic acid sequence encoding FXN set forth as SEQ ID NO:2.

E29. A method of treating FRDA in a subject, the method comprising:
providing at least one recombinant virus vector comprising a modified FXN gene of any one of embodiments 1-12, 23, and 25-28, and administering the recombinant virus vector to the subject under conditions such that the modified FXN gene is expressed at a level which produces a therapeutically effective amount of frataxin in cardiac and or neuron tissue of the subject.

E30. A method for reducing the effects of or treating Friedreich ataxia in neurons and heart muscles cells of a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a recombinant virus vector which comprises a modified FXN nucleic acid encoding the protein frataxin.

E31. A method for treating Friedreich ataxia in a subject in need thereof, including gene therapy based on administration of a nucleic acid comprising a nucleotide sequence selected from the group consisting of a sequence of SEQ ID NOs:3-9.

E32. A composition comprising an adeno-associated virus (AAV) vector comprising a modified FXN gene, or functional fragment thereof, wherein the AAV vector comprises a single stranded AAV vector genome, a double-stranded AAV vector genome or a self-complementary (sc) AAV vector genome.

E33. An expression vector comprising a polynucleotide that includes a modified FXN gene or fragment thereof.

E34. The vector of embodiment 33, wherein the AAV comprises a capsid of a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rhAAV10, rhAAV74, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV Hu.26, AAV1.1 (SEQ ID NO:15), AAV2.5 (SEQ ID NO. 13), AAV6.1 (SEQ ID NO:17), AAV6.3.1 (SEQ ID NO:18), AAV9.45, AAV2i8 (SEQ ID NO:29), AAV2G9, AAV2-TT (SEQ ID NO:31), AAV2-TT-S312N (SEQ ID NO:33), AAV3B-S312N, and AAV-LK03.

E35. The vector of embodiment 34, further comprising a AAV1.1 capsid wherein amino acid residue 265 is deleted (SEQ ID NO: 15), an AAV 6.1 capsid wherein amino acid residue 265 is deleted (SEQ ID NO: 17), an AAV 6.3.1 capsid wherein amino acid residue 265 is deleted and amino acid residue 531 is changed from a Lys to a Glu (SEQ ID NO: 18). The nucleotide sequence of wildtype AAV 1 capsid is shown in (SEQ ID NO: 14) and the nucleotide sequence of wildtype AAV 6 capsid is set forth in (SEQ ID NO: 16).

E36. A chimeric AAV virus vector comprising the modified FXN gene of any one of embodiments 1-12, 23, and 25-28, further comprising a capsid that includes the combination of AAV backbones from AAV2, AAV3, AAV6, AAV8, with a galactose (Gal) binding footprint from AAV9. Specifically, the galactose (Gal) binding footprint from AAV9 is grafted onto the heparin sulfate-binding AAV serotype 2 to improve transduction efficiency.

E37. A chimeric AAV virus vector comprising the modified FXN gene of any one of embodiments 1-12, 23, and 25-28, further comprising wherein the vector capsid includes tyrosine mutants in combination with 265 deletion mutations of AAV1 and or AAV6 as well as addition of a galactose binding footprint to the capsid protein.

E38. A chimeric AAV virus vector comprising the modified FXN gene of any one of embodiments 1-12, 23, and 25-28, further comprising a targeting peptides inserted in the HI structure loop of AAV or in position of 585 aa in AAV 2 backbone. Additionally, ancestral AAV vectors may be used for therapeutic in vivo gene therapy. Notably, the use of the virus particles assembled from ancestral viral sequences exhibit reduced susceptibility to pre-existing immunity in current day human population than do contemporary viruses or portions thereof.

E39. A host cell comprising the modified FXN gene of any one of embodiments 1-12, 23, and 25-28.

E40. A process of preparing a frataxin peptide or fragment thereof comprising: transfecting a host cell with the modified FXN gene of any one of embodiments 1-12, 23, and 25-28, and maintaining the host cell under biological conditions sufficient for expression of the frataxin peptide.

E41. Use of a modified FXN gene of any one of embodiments 1-12, 23, and 25-28, in the treatment of Friedreich ataxia.

E42. A pharmaceutical composition comprising a modified FXN gene for treating Friedreich ataxia that causes degeneration of neurons and cells in the cardiac tissues of a human subject wherein the modified FXN gene has an increased amount of GC nucleotides, decreased amount of GC nucleotides and/or has a reduced number of CpG dinucleotides; and a pharmaceutically acceptable carrier.

E43. An expression optimized nucleic acid encoding frataxin comprising a nucleic acid sequence selected from any one of SEQ ID NOs:3-9.

E44. A modified nucleic acid encoding frataxin comprising the amino acid set forth in SEQ ID NO:1, wherein the nucleic acid has a GC content of at least 55%,a decreased number of CpG dinucleotides compared with the nucleic acid sequence of SEQ ID NO:2, a codon adaptation index (CAI) of at least 0.8, and wherein it is expressed at a greater level compared with the level of expression of wild type frataxin comprising the nucleic acid sequence of SEQ ID NO:2.

E45. The modified nucleic acid of embodiment 44, wherein the CAI is at least 0.86.

E46. The modified nucleic acid of embodiment 44, wherein the CAI is at least 0.95.

E47. The modified nucleic acid of embodiment 44, wherein the CAI is at least 0.98.

E48. The modified nucleic acid of any one of embodiments 44-47, wherein the GC content is at least 61%.

E49. The modified nucleic acid of any one of embodiments 44-47, wherein the GC content is at least 69%.

E50. The modified nucleic acid of any one of embodiments 44-49, wherein the number of CpG dinucleotides is from about 114 to 124.

E51. A modified nucleic acid encoding frataxin (FXN) comprising the amino acid sequence set forth in SEQ ID NO:1, wherein said nucleic acid is expressed at a greater level compared with the expression level of the wild type FXN nucleic acid sequence of SEQ ID NO:2, and wherein said modified nucleic acid comprises at least one characteristic selected from the group consisting of: a GC content of at least 55%, a number of CpG dinucleotides not greater than 124, and a codon adaptation index (CAI) of at least 0.76.

E52. The modified nucleic acid of embodiment 51, said nucleic acid comprising at least one characteristic selected from the group consisting of: a CAI of at least 0.86, at least 0.95, or at least 0.98; a GC content is at least 57%, at least 61%, or at least 69%; a number of CpG

dinucleotides is less than 124; and a nucleic acid sequence selected from the group consisting of a sequence as set forth in SEQ ID NOs:3-9.

E53. A modified nucleic acid encoding FXN, wherein said nucleic acid is expressed at a greater level compared with the level of expression of the wild type FXN nucleic acid sequence of SEQ ID NO:2, and wherein the nucleic acid comprises at least one of: a nucleic acid sequence selected from the group consisting of SEQ ID NOs:3-9; a GC content of at least 55%; a number of CpG dinucleotides not greater than 117; and a CAI of at least 0.86.

E54. The modified nucleic acid of embodiment 53, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:7.

E55. The modified nucleic acid of claim any one of embodiments 43-54, comprising the nucleic acid sequence of SEQ ID NO:7.

E56. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28 and 43-55, further comprising a nucleic acid sequence encoding at least one AAV terminal repeat (TR).

E57. The modified nucleic acid of embodiment 55 wherein the nucleic acid single stranded, double stranded, and/or self complementary.

E58. The modified nucleic acid of embodiment 57, wherein the nucleic acid is self complementary.

E59. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-58, further comprising an enhancer.

E60. The modified nucleic acid of embodiment 59, wherein the enhancer is a cytomegalovirus (CMV) immediate-early enhancer.

E61. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-60, further comprising a promoter.

E62. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-61, wherein the promoter is constitutive or regulated.

E63. The modified nucleic acid of embodiment 62, wherein the promoter is regulated.

E64. The modified nucleic acid of embodiment 63, wherein the promoter inducible or repressible.

E65. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-64, further comprising a nucleic acid sequence encoding a collagen stabilization sequence (CSS).

E66. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-65, further comprising a stop codon.

E67. The modified nucleic acid of any one of embodiments 1-12, 23, 25-28, and 43-66, further comprising a poly-adenylation (polyA) signal sequence.

E68. The modified nucleic acid of embodiment 67, wherein the promoter is selected from the group consisting of a chicken beta-actin (CBA) promoter, a cytomegalovirus (CMV) promoter, a CMV enhancer/CBA promoter (CBh), and a synthetic CAG promoter.

E69. The modified nucleic acid of embodiment 68, wherein the promoter is a CBh promoter.

E70. The modified nucleic acid of any one of embodiments 1-6, 12, 25-28, and 44-69, further comprising a nucleic acid sequence encoding a collagen stabilization sequence (CSS).

E71. A recombinant AAV vector (rAAV) comprising the modified nucleic acid encoding FXN of any one of embodiments 1-12, 23, 25-28, and 43-70.

E72. The rAAV of embodiment 71, wherein the rAAV comprises a capsid selected from the group consisting of a capsid from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh10, AAVrh74, AAV2.5 (SEQ ID NO. 13), AAV hu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV2i8, AAV2G9, AAV9.45, AAV2i8G9, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, and AAV-LK03.

E73. The rAAV of embodiment 72, wherein the capsid is selected from the group consisting of AAV2-TT, AAV2-TT-S312N, and AAV2i8 capsid.

E74. The rAAV of embodiment 73, wherein the modified nucleic acid comprises the sequence of SEQ ID NO:7 and wherein the capsid is selected from an AAV2i8 capsid and an AAV2-TT-S312N capsid.

E75. The rAAV of embodiment 74, wherein the nucleic acid further comprises two AAV terminal repeat sequences flanking the sequence encoding FXN, and further comprises a CBh promoter upstream of the sequence encoding FXN.

E76. The rAAV of embodiment 75, said nucleic acid further comprising a collagen stabilization sequence (CSS; SEQ ID NO:25) 3' from the sequence encoding FXN.

E77. The rAAV of any one of embodiments 71-76, wherein the nucleic acid comprises a bovine growth hormone polyA (bGHpolyA) signal sequence.

E78. A rAAV vector comprising an AAV2i8 capsid wherein VP1 comprises the amino acid of SEQ ID NO:29, and further comprising a nucleic acid comprising, from 5' to 3':

(a) an AAV2 terminal repeat (TR);

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:26;

(c) a modified nucleic acid encoding FXN comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:3-9;

(d) a CSS having the sequence of SEQ ID NO:25;

(e) a bGHpolyA signal sequence having the sequence of SEQ ID NO:27; and

(f) an AAV2 TR.

E79. A rAAV vector comprising an AAV2-TT capsid wherein VP1 comprises the amino acid of SEQ ID NO:31, and further comprising a nucleic acid comprising, from 5' to 3':

(a) an AAV2 TR;

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:26;

(c) a modified nucleic acid encoding FXN comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:3-9;

(d) a CSS having the sequence of SEQ ID NO:25;

(e) a bGHpolyA signal sequence having the sequence of SEQ ID NO:27; and

(f) an AAV2 TR.

E80. A rAAV vector comprising an AAV2-TT-S312N capsid wherein VP1 comprises the amino acid of SEQ ID NO:33, and further comprising a nucleic acid comprising, from 5' to 3':

(a) an AAV2 TR;

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:26;

(c) a modified nucleic acid encoding FXN comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:3-9;

(d) a CSS having the sequence of SEQ ID NO:25;

(e) a bGHpolyA signal sequence having the sequence of SEQ ID NO:27; and

(f) an AAV2 TR.

E81. The rAAV vector of any one of embodiments 71-80, wherein the modified nucleic acid encoding FXN comprises the nucleic acid sequence of SEQ ID NO:7.

E82. A rAAV vector for treating Friedreich ataxia in a subject in need thereof, wherein said vector comprises the modified nucleic acid encoding frataxin of any one of embodiments 1-6, 12, 25-28 and 71-81.

E83. A pharmaceutical composition comprising the rAAV vector of any one of embodiments 7-11, 33-39, and 71-82, and a pharmaceutically acceptable carrier.

E84. A method of treating FRDA in a subject, the method comprising administering at least one of: a modified nucleic acid encoding frataxin of any one of embodiments 1-12, 23, 25-28 and 43-70; a rAAV vector of any one of embodiments 7-11, 33-39 and 71-82; and the pharmaceutical composition of embodiment 83.

E85. The method of embodiment 84, wherein the rAAV vector of any one of embodiments 7-11, 33-39, and 71-82, is administered systemically, or by direct cardiac or intracranial administration.

E86. The method of embodiment 85, wherein the rAAV vector of any one of embodiments 71-82 is administered intracranially.

E87. The method of embodiment 85, wherein the rAAV vector of any one of embodiments 71-82 is directly administered into the heart.

E88. The method of embodiment 84, wherein the modified nucleic acid encoding FXN comprises the nucleic acid sequence of SEQ ID NO:6.

E89. The method of embodiment 84, wherein the modified nucleic acid encoding FXN comprises the nucleic acid sequence of SEQ ID NO:7.

E90. A method of treating a disease, disorder or condition mediated by a decreased level of FTX, the method comprising administering at least one of: the modified nucleic acid encoding frataxin of any one of embodiments 1-6, 12, 25-28 and 43-70; the rAAV vector of any one of embodiments 7-11, 33-39 and 71-82; and the pharmaceutical composition of embodiment 83.

E91. A host cell comprising a modified nucleic acid encoding FXN of any one of embodiments 1-6, 12, 25-28 and 43-70.

E92. The host cell of embodiment 91, wherein the cell is selected from the group consisting of VERO, WI38, MRC5, A549, HEK293 cells, B-50 or any other HeLa cells, HepG2, Saos-2, HuH7, and HT1080.

E93. The host cell of embodiment 92, wherein the host cell is a HEK293 adapted to growth in suspension culture.

E94. The host cell of any one of embodiments 91-93, wherein the cell is a HEK293 cell having ATCC No. PTA 13274.

E95. A packaging cell comprising a rAAV vector of any one of embodiments 7-11, 33-39, and 70-82, wherein said cell further comprises at least one nucleic acid encoding an AAV Rep protein, at least one nucleic acid encoding an AAV Cap protein, and at least one nucleic acid encoding a helper function.

E96. A method for producing a rAAV vector, the method comprising culturing the cell of any one of embodiments 91-95 under conditions where rAAV is produced.

E97. The method of embodiment 96, further comprising isolating the rAAV produced.

E98. Use of at least one of: the modified nucleic acid encoding frataxin of any one of embodiments 1-6, 12, 25-28 and 43-70; the rAAV vector of any one of embodiments 7-11, 33-39 and 71-82; and the pharmaceutical composition of embodiment 83 to increase the level of frataxin in a cell.

E99. The modified nucleic acid encoding frataxin of any one of embodiments 1-6, 12, 25-28 and 43-70; the rAAV vector of any one of embodiments 7-11, 33-39 and 71-82; and the pharmaceutical composition of embodiment 83 for use in increasing the level of frataxin in a subject.

E100. The modified nucleic acid encoding frataxin of any one of embodiments 1-6, 12, 25-28 and 43-70; the rAAV vector of any one of embodiments 7-11, 33-39 and 71-82; and the pharmaceutical composition of embodiment 83 for use in treating Friedreich ataxia in a subject.

[7] Other features and advantages of the invention will be apparent from the following detailed description, drawings, exemplary embodiments and claims

BRIEF DESCRIPTION OF THE DRAWINGS

[8] Figures 1A and 1B. Figures 1A and 1B both show the results of expression in HeLa cells of frataxin from selected modified nucleic acids encoding FXN compared to a wild type nucleic acid (lane 1). Extracts from HeLa cells comprising the following modified nucleic acids were examined to detect FXN produced in the cells. Frataxin was detected by Western blotting using an anti-frataxin antibody detected using a secondary antibody conjugated with HRP (horse radish peroxidase) for chemiluminescence detection by exposure of the Western blot to light sensitive film. The lanes were loaded with extracts from HeLa cells transfected with the following modified nucleic acids encoding frataxin: lane 1: wild type control nucleic acid; lane 2: IDT2; lane 3: IDT5; lane 4: JCAT; lane 5: GeneArt; lane 6: Genscript (control); and lane 7: Genscript (low CpG).

[9] Figures 2A-2F show the sequence of various modified FXN gene constructs for cloning into the self-complementary rAAV vector pTRs-KS-CBh-EGFP-bGHpolyA – where the EGFP marker gene was replaced with either wild type FXN gene (SEQ ID NO:2) or a modified version thereof (*e.g.*, SEQ ID NOs:3-9). Each figure shows WT FXN (Fig. 2A) or a modified FXN gene (Figs. 2B-2F). Each construct comprises (from 5' to 3') an AgeI cut site, the FXN/modified FXN gene, AvrII cut site, a collagen stability sequence (CSS), a SpeI cut site, a bGHpolyA signal sequence, and a MluI cut site. Figure 2A shows the pTRs-KS-CBh-WT FXN-bGHpolyA construct (SEQ ID NO:19); Figure 2B shows the Integrated DNA Technologies IDT 1 (IDT1) modified FXN gene construct pTRs-KS-CBh-IDT1 FXN-bGHpolyA (SEQ ID NO:20); Figure 2C shows IDT3 modified FXN gene construct pTRs-KS-CBh-IDT3 FXN-bGHpolyA (SEQ ID NO:21); Figure 2D shows the IDT4 modified FXN gene construct pTRs-KS-CBh-IDT4 FXN-bGHpolyA (SEQ ID NO:22); Figure 2E shows the GenScript modified FXN gene construct pTRs-KS-CBh-GenScript FXN-bGHpolyA (SEQ ID NO:23); and Figure 2F shows the GenScript (low CpG) modified FXN gene construct pTRs-KS-CBh-Genscript (low CpG) FXN-bGHpolyA (SEQ ID NO:24), each sequence includes the elements (*e.g.*, AgeI, AvrII, CSS, SpeI, bGHpolyA, and MluI) which are indicated as follows, from 5' to 3', in Figs. 2A-2F: an AgeI cut site (**ACCGGT**) indicated in **bold**; the FXN gene in lower case letters, an AvrII cut site (CCTAGG) indicated by underlining; a sequence encoding a collagen stabilization sequence (CSS) indicated by double underlining; an SpeI cut site (**ACTAGT**) indicated in **bold underlined**; a bovine growth hormone poly-adenylation signal sequence (bGHpolyA) indicated in *italics*; and a MluI cut site (***ACGCGT***) indicated in ***bold italics***. The FXN gene in the construct is under the control of the CBh promoter upstream from the AgeI cut site. The

sequence of the CBh promoter is not shown in Figures 2A-2F, but is set forth in SEQ ID NO:25.

[10] Figure 3 shows a vector (plasmid) map for the pTRs-KS-CBh-eGFP cloning construct depicting the various restriction (cut) sites and elements of the vector including the CBh promoter upstream from the AgeI cut site.

[11] Figures 4A and 4B show graphs illustrating the baseline cardiac phenotype in control, treated mutant and untreated mutant male (Fig. 4A) and female (Fig. 4B) mice. Figure 4A shows the cardiac phenotype for, from left to right within each grouping: control males, treated mutants and untreated mutants, where the groupings are: EF (ejection fraction), FS (fractional shortening); LV Vol_d (left ventricle volume diastolic); and LV Vol_s (left ventricle volume systolic). Figure 4B shows the baseline cardiac phenotype for female mice groups: control (circles); treated mutants (squares); and untreated mutants (triangles).

[12] Figures 5A and 5B show graphs illustrating the reversal of FRDA cardiac phenotype in treated Mck mutant mice compared with the cardiac phenotype in untreated Mck mutant mice at 5 weeks of age (and 14 days post-treatment in treated mutants). Figure 5A shows the cardiac phenotype of control (circles), treated mutant (squares) and untreated mutant (triangles) male mice 14 days after rAAV-FXN injection. The abbreviations are as follows: AoV SV (aortic valve stroke volume); AoV CO (aortic valve cardiac output); FS (fractional shortening); and LV Mass AW (left ventricle mass anterior wall). Figure 5B shows the cardiac phenotype of control (circles), treated mutant (squares) and untreated mutant (triangles) female mice 14 days after rAAV-FXN injection. The abbreviations are as follows: ES (ejection fraction): FS (fractional shortening); AoV SV (aortic valve stroke volume); AoV CO (aortic valve cardiac output).

[13] Figures 6A-6C show graphs illustrating cardiac function in male and female control mice (circles), treated mutants male and female mice (squares), and untreated mutant male and female mice (triangles) twenty-eight (28) days post-rAAV-FXN treatment in the treated Mck mutant group. Figure 6A shows the left ventricle mass (LVM) echocardiography assessment for all three mouse groups over successive weeks, i.e., at 3 weeks of age (time of rAAV administration), 5 weeks of age (14 days post-rAAV administration) and 7 weeks of age (28 days post-rAAV administration) where treatment was administered at the age of 5 weeks. Figure 6B shows the shortening factor (SF) echocardiography assessment for all three mouse groups over successive weeks. Figure 6C shows the cardiac output echocardiography assessment for all three mouse groups over successive weeks. The data are mean ± S.E.M of 8 mice per group. The data of Mck mutant mice were compared to the Mck positive control group using multiple t-tests comparisons (Sidak-Bonferroni method). * $p<0.05$.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[14] Unless otherwise defined, all technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. The following terms have the meanings given:

[15] The term “about,” as used herein, when referring to a measurable value such as an amount of the biological activity, length of a polynucleotide or polypeptide sequence, content of G and C nucleotides, codon adaptation index, number of CpG dinucleotides, dose, time, temperature, and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5% or even 0.1% of the specified amount.

[16] As used herein, the term “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[17] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV "packaging genes."

[18] The present disclosure provides a recombinant adeno-associated virus (rAAV) vector. "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector") or simply, an “AAV vector.” The term "AAV" includes, for example, AAVs of various serotypes, *e.g.*, AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), AAV type 9 (AAV-9), AAV type 10 (AAV-10, including AAVrh10), AAVrh74, AAV type 12 (AAV-12), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. "Primate AAV" refers to AAV that infect primates, "non-primate AAV" refers to AAV that infect non- primate mammals, "bovine AAV" refers to AAV that infect bovine mammals, and so on.

[19] The various serotypes of AAV are attractive for several reasons, most prominently that AAV is believed to be non-pathogenic and that the wildtype virus can integrate its genome site-specifically into human chromosome 19 (Linden et al., 1996, Proc Natl Acad Sci USA 93:11288-

11294). The insertion site of AAV into the human genome is called AAVS1. Site-specific integration, as opposed to random integration, is believed to likely result in a predictable long-term expression profile.

[20] The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC-002077 (AAV-1), AF063497 (AAV-1), NC-001401 (AAV-2), AF043303 (AAV-2), NC-001729 (AAV-3), NC-001829 (AAV- 4), U89790 (AAV-4), NC-006152 (AAV-5), AF513851 (AAV-7), AF513852 (AAV-8), and NC-006261 (AAV-8); the disclosures of which are incorporated by reference herein. See also, e.g., Srivistava et al., 1983, J. Virology 45:555; Chiorini et al., 1998, J. Virology 71:6823; Chiorini et al., 1999, J. Virology 73: 1309; Bantel-Schaal et al., 1999, J. Virology 73:939; Xiao et al., 1999, J. Virology 73:3994; Muramatsu et al., 1996, Virology 221:208; Shade et al., 1986, J. Virol. 58:921; Gao et al., 2002, Proc. Nat. Acad. Sci. USA 99: 11854; Moris et al., 2004, Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; WO 2013/063379; WO 2014/194132; WO 2015/121501, and U. S. Pat. Nos. 6,156,303 and 7,906,111.

[21] An "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In some embodiments, the heterologous polynucleotide may be flanked by at least one, and sometimes by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. A rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV). An "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide rAAV vector. 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 a "rAAV vector particle" or simply an "rAAV vector". Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[22] "Recombinant," as used herein means that the vector, polynucleotide, polypeptide or cell is the product of various combinations of cloning, restriction or ligation steps (e.g. relating to a polynucleotide or polypeptide comprised therein), and/or other procedures that result in a construct that is distinct from a product found in nature. A recombinant virus or vector is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[23] “AAV Rep” means AAV replication proteins and analogs thereof.

[24] “AAV Cap” means AAV capsid proteins, VP1, VP2 and VP3 and analogs thereof. In wild type AAV virus, three capsid genes vp1, vp2 and vp3 overlap each other. *See*, Grieger and Samulski, 2005, J. Virol. 79(15):9933-9944. A single P40 promoter allows all three capsid proteins to be expressed at a ratio of about 1:1:10, vp1, vp2, vp3, respectively, which complement with rAAV production. For the production of recombinant AAV vectors, desired ratio of VP1:VP2:VP3 is in the range of about 1:1:1 to about 1:1:100, preferably in the range of about 1:1:2 to about 1:1:50, more preferably in the range of about 1:1:5 to about 1:1:20. Although the desired ratio of VP1:VP2 is 1:1, the ratio range of VP1:VP2 could vary from 1:50 to 50:1.

[25] A comprehensive list and alignment of amino acid sequences of capsids of known AAV serotypes is provided by Marsic et al., 2014, Molecular Therapy 22(11):1900-1909, especially at supplementary Figure 1.

[26] For illustrative purposes only, wild type AAV2 comprises a small (20-25 nm) icosahedral virus capsid of AAV composed of three proteins (VP1, VP2, and VP3; a total of 60 capsid proteins compose the AAV capsid) with overlapping sequences. The proteins VP1 (735 aa; Genbank Accession No. AAC03780), VP2 (598 aa; Genbank Accession No. AAC03778) and VP3 (533 aa; Genbank Accession No. AAC03779) exist in a 1:1:10 ratio in the capsid. That is, for AAVs, VP1 is the full length protein and VP2 and VP3 are progressively shorter versions of VP1, with increasing truncation of the N-terminus relative to VP1.

[27] “AAV TR” means a palindromic terminal repeat sequence at or near the ends of the AAV genome, comprising mostly complementary, symmetrically arranged sequences, and includes analogs of native AAV TRs and analogs thereof.

[28] “Cis-motifs” includes conserved sequences such as found at or close to the termini of the genomic sequence and recognized for initiation of replication; cryptic promoters or sequences at internal positions likely used for transcription initiation, splicing or termination.

[29] "Treating" or "treatment" means reversing, alleviating, or inhibiting the progress of the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition.

[30] "Therapeutically effective amount" means a minimal amount of active agent which is necessary to impart therapeutic benefit to a subject. For example, a "therapeutically effective amount" to a patient is such an amount which induces, ameliorates, stabilizes, slows down the progression or otherwise causes an improvement in the pathological symptoms, disease progression or physiological conditions associated with or resistance to succumbing to a disorder.

[31] "Gene" means a polynucleotide containing at least one open reading frame that is capable of encoding a particular polypeptide or protein after being transcribed and translated.

[32] "Coding sequence" means a sequence which encodes a particular protein" or "encoding nucleic acid", denotes 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 (operably linked to) 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.

[33] "Chimeric" means, with respect to a viral capsid or particle, that the capsid or particle includes sequences from different parvoviruses, preferably different AAV serotypes, as described in Rabinowitz et al., U.S. Patent 6,491,907, the disclosure of which is incorporated in its entirety herein by reference. *See also* Rabinowitz et al., 2004, J. Virol. 78(9):4421-4432. A particularly preferred chimeric viral capsid is the AAV2.5 capsid, which has the sequence of the AAV2 capsid with the following mutations: 263 Q to A; 265 insertion T; 705 N to A; 708 V to A; and 716 T to N. wherein the nucleotide sequence encoding such capsid is defined as SEQ ID NO: 15 as described in WO 2006/066066. Other preferred chimeric AAVs include, but are not limited to, AAV2i8 described in WO 2010/093784, AAV2G9 and AAV8G9 described in WO 2014/144229, and AAV9.45 (Pulicherla et al., 2011, Molecular Therapy 19(6):1070-1078).

[34] "Flanked," with respect to a sequence that is flanked by other elements, indicates the presence of one or more the flanking elements upstream and/or downstream, i.e., 5' and/or 3', relative to the sequence. The term "flanked" is not intended to indicate that the sequences are necessarily contiguous. For example, there may be intervening sequences between the nucleic acid encoding the transgene and a flanking element. A sequence (e.g., a transgene) that is "flanked" by two other elements (e.g., TRs), indicates that one element is located 5' to the sequence and the other is located 3' to the sequence; however, there may be intervening sequences there between.

[35] "Polynucleotide" means a sequence of nucleotides connected by phosphodiester linkages. Polynucleotides are presented herein in the direction from the 5' to the 3' direction. A polynucleotide of the present invention can be a deoxyribonucleic acid (DNA) molecule or ribonucleic acid (RNA) molecule. Where a polynucleotide is a DNA molecule, that molecule can be a gene or a cDNA molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U). A polynucleotide of the present invention can be prepared using standard techniques well known to one of skill in the art.

[36] "Transduction" of a cell by a virus means that there is transfer of a nucleic acid from the virus particle to the cell.

[37] “Modified FXN gene” means a modified nucleic acid encoding FXN (e.g., the amino acid sequence of SEQ ID NO:1) with at least one modification compared with a wild type nucleic acid encoding FXN (e.g., SEQ ID NO:2), wherein the modification includes, but is not limited to, increased GC content, decreased GC content or a FXN gene with a reduced CpG content. Preferably, the modified FXN gene exhibits improved protein expression, e.g., the protein encoded thereby is expressed at a detectably greater level in a cell compared with the level of expression of the protein provided by the wild type gene in an otherwise identical cell.

[38] “Transfection” of a cell means that genetic material is introduced into a cell for the purpose of genetically modifying the cell. Transfection can be accomplished by a variety of means known in the art, such as calcium phosphate, polyethyleneimine, electroporation, and the like.

[39] “Polypeptide” encompasses both peptides and proteins, unless indicated otherwise.

[40] "Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g. episomes), or integration of transferred genetic material into the genomic DNA of host cells.

[41] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants, "transformed cells," and “transduced cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages.

[42] “Transgene” is used to mean any heterologous nucleotide sequence incorporated in a vector, including a viral vector, for delivery to and including expression in a target cell (also referred to herein as a “host cell”), and associated expression control sequences, such as promoters. It is appreciated by those of skill in the art that expression control sequences will be selected based on ability to promote expression of the transgene in the target cell. An example of a transgene is a nucleic acid encoding a therapeutic polypeptide.

[43] “Vector," means a recombinant plasmid or virus that comprises a polynucleotide to be delivered into a host cell, either in vitro or in vivo.

[44] "Substantial homology" or "substantial similarity," means, when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the sequence.

[45] "Recombinant viral vector" means a recombinant polynucleotide vector comprising one or more heterologous sequences (i.e., polynucleotide sequence not of viral origin). In the case of

recombinant parvovirus vectors, the recombinant polynucleotide is flanked by at least one, preferably two, inverted terminal repeat sequences (ITRs).

[46] "Homologous" used in reference to peptides, refers to amino acid sequence similarity between two peptides. When an amino acid position in both of the peptides is occupied by identical amino acids, they are homologous at that position. Thus by "substantially homologous" means an amino acid sequence that is largely, but not entirely, homologous, and which retains most or all of the activity as the sequence to which it is homologous. As used herein, "substantially homologous" as used herein means that a sequence is at least 50% identical, and preferably at least 75% and more preferably 95% homology to the reference peptide. Additional peptide sequence modification are included, such as minor variations, deletions, substitutions or derivatizations of the amino acid sequence of the sequences disclosed herein, so long as the peptide has substantially the same activity or function as the unmodified peptides. Derivatives of an amino acid may include but not limited to trifluoroleucine, hexafluoroleucine, 5,5,5-trifluoroisoleucine, 4,4,4-trifluorovaline, p-fluorophenylaline, o-fluorotyrosine, m-fluorotyrosine, 2,3-difluorotyrosine, 4-fluorohistidine, 2-fluorohistidine, 2,4-difluorohistidine, fluoroproline, difluoroproline, 4-hydroxyproline, selenomethionine, telluromethionine, selenocysteine, selenatryptophans, 4-aminotryptophan, 5-aminotryptophan, 5-hydroxytryptophan, 7-azatryptophan, 4-fluorotryptophan, 5-fluorotryptophan, 6-fluorotryptophan, homoallylglycine, homopropargylglycine, 2-butynylglycine, cis-crotylglycine, allylglycine, dehydroleucine, dehydroproline, 2-amino-3-methyl-4-pentenoic acid, azidohomoalanine, asidoalanine, azidonorleucine, p-ethynylphenylalanine, p-azidophenylalanine, p-bromophenylalanine, p-acetylphenylalanine and benzofuranylalanine. Notably, a modified peptide will retain activity or function associated with the unmodified peptide, the modified peptide will generally have an amino acid sequence "substantially homologous" with the amino acid sequence of the unmodified sequence.

[0042] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187

(1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See J. Mol. Biol. 48: 443-453 (1970).

[0043] Of interest is the BestFit program using the local homology algorithm of Smith and Waterman (1981, Advances in Applied Mathematics 2: 482-489) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, WI, USA.

[0044] Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc.

[0045] Percent sequence identity is calculated by FastDB based upon the following parameters: Mismatch Penalty: 1.00; Gap Penalty: 1.00; Gap Size Penalty: 0.33; and Joining Penalty: 30.0.

[47] The present invention provides for modified FXN genes. The invention also provides nucleic acid constructs, such as vectors, which include as part of their sequence a modified FXN gene, e.g., GC content optimized FXN gene sequence comprising a greater or lesser amount of GC nucleotides compared with the wild type FXN gene sequence and/or a FXN gene sequence having reduced levels of CpG dinucleotides compared with the level of CpG dinucleotides present in the wild type FXN gene. For example, the invention includes plasmids and/or other vectors that include the modified FXN sequence along with other elements, such as regulatory elements. Further, the invention provides packaged gene delivery vehicle, such as a viral capsid, including the modified FXN sequence. The invention also includes methods of delivery and, preferably, expressing the modified FXN gene by delivering the modified sequence into a cell along with elements required to promote expression in the cell. The invention also provides gene therapy methods in which the modified FXN gene sequence is administered to a subject, e.g., as a component of a vector and/or packaged as a component of a viral gene delivery vehicle. Treatment may, for example, be effected to increase levels of frataxin in a subject and treat a frataxin deficiency in the subject. Each of these aspects of the invention is discussed further in the ensuing sections.

Modified Nucleic Acid for Expression of Frataxin

[48] The invention provides a modified nucleotide sequence encoding frataxin. The modified nucleotide sequence includes the wild type or native FXN gene sequence including one or more modifications.

[49] In one aspect, the modified nucleic acid sequence provides a detectably greater level of expression of frataxin in a cell compared with the expression of frataxin from the wild type nucleic acid sequence of SEQ ID NO:2 in an otherwise identical cell. This can be referred to as an "expression optimized" or "enhanced expression" nucleic acid, or simply, as a "modified nucleic acid."

[50] "Optimized" or "codon-optimized" as referred to interchangeably herein, refers to a coding sequence that has been optimized relative to a wild type coding sequence (*e.g.*, a coding sequence for frataxin) to increase expression of the coding sequence, *e.g.,* by minimizing usage of rare codons, decreasing the number of CpG dinucleotides, removing cryptic splice donor or acceptor sites, removing Kozak sequences, removing ribosomal entry sites, and the like.

[51] Examples of modifications include elimination of one or more cis-acting motifs and introduction of one or more Kozak sequences. In one embodiment, one or more cis-acting motifs are eliminated and one or more Kozak sequences are introduced.

[52] Examples of cis acting motifs that may be eliminated include internal TATA-boxes; chi-sites; ribosomal entry sites; ARE, INS, and/or CRS sequence elements; repeat sequences and/or RNA secondary structures; (cryptic) splice donor and/or acceptor sites, branch points; and SalI.

[53] In one embodiment, the GC content (*e.g.*, the number of G and C nucleotides present in a nucleic acid sequence) is enhanced relative to wild-type FXN gene sequence of SEQ ID NO:2. The GC content is preferably at least 5%, more preferably, at least 6%, yet more preferably, at least 7%, even more preferably, at least 8%, more preferably, at least 9%, even more preferably, at least 10%, yet more preferably, at least 12%, even more preferably, at least 14%, yet more preferably, at least 15%, more preferably, at least 17%, even more preferably, at least 20%, even further preferably, at least 30%, yet more preferably, at least 40%, more preferably, at least 50%, even more preferably, at least 60%, and most preferably, at least 70% greater than the wild type gene (SEQ ID NO:2).

[54] In another embodiment, the GC content is expressed as a percentage of G (guanine) and C (cytosine) nucleotides in the sequence. That is, the GC content of the wild type nucleic acid encoding frataxin (SEQ ID NO:1) is about 55% whereas the GC content of representative modified FXN genes of the invention ranges from about 57% for IDT-3 (SEQ ID NO:8), 57% for Genescript (SEQ ID NO:6); 61% for GeneArt (SEQ ID NO:5), and 69% for JCAT (SEQ ID NO:4). Thus, the modified nucleic acid of the invention comprises a of at least 57%, more preferably, a GC content of at least 61%, even more preferably, a GC content of least 69%,

compared with the GC content of about 55% of the wild type nucleic acid sequence encoding frataxin as set forth in SEQ ID NO:2.

[55] In one embodiment, the GC content of a modified nucleic acid of the invention is greater than the GC content of the wild type nucleic acid encoding frataxin comprising the nucleic acid sequence of SEQ ID NO:2. One skilled in the art would appreciate, knowing the degeneracy of the nucleic acid code, that irrespective of the sequence of the nucleic acid encoding the protein, the amino acid sequence of frataxin expressed therefrom is, preferably, the amino acid sequence of SEQ ID NO:1.

[56] In one embodiment, the GC content of a modified nucleic acid encoding FXN of the invention is about the same, i.e., 55%, as the GC content of wild type FNX gene (SEQ ID NO:2).

[57] Additionally, the codon adaptation index of the modified nucleic acid encoding frataxin (i.e., the modified FXN gene) is preferably at least 0.74, preferably, at least 0.76, even more preferably, at least 0.77, yet more preferably, at least 0.80, preferably, at least0.85, more preferably, at least 0.86, yet more preferably, at least 0.87, even more preferably, at least 0.90, yet more preferably, at least 0.95, and most preferably, at least 0.98.

[58] In another embodiment the modified FXN sequence has a reduced level of CpG dinucleotides that being a reduction of about 10%, 20%, 30%, 50% or more, compared with the wild type nucleic acid sequence encoding FXN (e.g., SEQ ID NO:2).

[59] It is known that methylation of CpG dinucleotides plays an important role in the regulation of gene expression in eukaryotes. Specifically, methylation of CpG dinucleotides in eukaryotes essentially serves to silence gene expression through interfering with the transcriptional machinery. As such, because of the gene silencing evoked by methylation of CpG motifs, the nucleic acids and vectors of the invention having a reduced number of CpG dinucleotides will provide for high and long lasting transgene expression level.

[60] In one embodiment, the modified FXN gene comprises fewer potential CpG island regions than wild type FXN gene, *i.e.,* 128. Preferably, the modified FXN gene comprises about 124 potential CpG island regions, more preferably, about 123, even more preferably, about 117, and more preferably, about 114 potential CpG island regions.

[61] The modified FXN gene sequence may also include flanking restriction sites to facilitate subcloning into expression vector. Many such restriction sites are well known in the art, and include, but are not limited to, those shown in Figures 2A-2F, and Figure 3 (plasmid map of scAAV plasmid vector pTRs-KS-CBh-EGFP-BGH) and Table 8 (SEQ ID NOs:19-23), such as, AgeI, AvrII, SpeI and MluI.

[62] The invention also includes fragments of any one of sequences SEQ ID NOs:3 through 9 which encode a functionally active fragment frataxin. “Functionally active” or “functional frataxin”

indicates that the fragment provides the same or similar biological activity as a full-length frataxin. That is, the fragment provides the same activity including, but not limited to, correcting primary Fe-S cluster deficit, decreasing mitochondrial iron accumulation (Puccio et al., 2001, Nature Genetics 27:181-186; Seznec et al., 2004, Human Mol. Genet. 13:1017-1024) and other deficiencies as discussed in Perdomini et al., 2014, Nature Med. 20(5):542-547. The biological activity of FXN, or a functional fragment thereof, also encompasses reversing or preventing the cardiac phenotype associated with FRDA as demonstrated elsewhere herein in Mck mice.

[63] The invention includes a nucleic acid vector including the modified FXN gene sequence and various regulatory or control elements. The precise nature of regulatory elements useful for gene expression will vary from organism to organism and from cell type to cell type. In general, they include a promoter which directs the initiation of RNA transcription in the cell of interest. The promoter may be constitutive or regulated. Constitutive promoters are those which cause an operably linked gene to be expressed essentially at all times. Regulated promoters are those which can be activated or deactivated. Regulated promoters include inducible promoters, which are usually “off” but which may be induced to turn “on,” and “repressible” promoters, which are usually “on” but may be turned “off.” Many different regulators are known, including temperature, hormones, cytokines, heavy metals and regulatory proteins. The distinctions are not absolute; a constitutive promoter may often be regulated to some degree. In some cases an endogenous pathway may be utilized to provide regulation of the transgene expression, e.g., using a promoter that is naturally downregulated when the pathological condition improves.

[64] Examples of suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus promoter; the Rous Sarcoma Virus (RSV) promoter; the albumin promoter; inducible promoters, such as the Mouse Mammary Tumor Virus (MMTV) promoter; the metallothionein promoter; heat shock promoters; the α-1-antitrypsin promoter; the hepatitis B surface antigen promoter; the transferrin promoter; the apolipoprotein A-1 promoter; chicken beta-actin CBA) promoter, the CBh promoter (SEQ ID NO:25), and the CAG promoter (cytomegalovirus early enhancer element and the promoter, the first exon, and the first intron of chicken beta-actin gene and the splice acceptor of the rabbit beta-globin gene) (Alexopoulou et al., 2008, BioMed. Central Cell Biol. 9:2), and human FXN promoters. The promoter may be a tissue-specific promoter, such as the mouse albumin promoter, which is active in liver cells as well as the transthyretin promoter (TTR).

[65] In another aspect, the modified nucleic acid encoding FXN further comprises an enhancer to increase expression of the FXN protein. Many enhancers are known in the art, including, but not limited to, the cytomegalovirus major immediate-early enhancer. More specifically, the CMV MIE promoter comprises three regions: the modulator, the unique region and the enhancer

(Isomura and Stinski, 2003, J. Virol. 77(6):3602-3614). The CMV enhancer region can be combined with other promoters, or a portion thereof, to form hybrid promoters to further increase expression of a nucleic acid operably linked thereto. For example, a chicken beta-actin (CBA) promoter, or a portion thereof, can be combined with the CMV promoter/enhancer, or a portion thereof, to make a version of CBA termed the "CBh" promoter, which stands for chicken beta-actin hybrid promoter, as described in Gray et al. (2011, Human Gene Therapy 22:1143-1153).

[66] Further, the control elements can include a collagen stabilization sequence (CSS), a stop codon, a termination sequence, and a poly-adenylation signal sequence, such as, but not limited to a bovine growth hormone poly A signal sequence (bGHpolyA), to drive efficient addition of a poly-adenosine "tail" at the 3' end of a eukaryotic mRNA (*see*, *e.g.,* Goodwin and Rottman, 1992, J. Biol. Chem. 267(23):16330-16334).

Non-Viral Vectors

[67] In a particular embodiment, the vector used according to the invention is a non-viral vector. Typically, the non-viral vector may be a plasmid which includes nucleic acid sequences reciting the modified FXN gene, or variants thereof.

Packaged Modified FXN Sequence

[68] The modified FXN gene sequence may also be provided as a component of a packaged viral vector. In general, packaged viral vectors include a viral vector packaged in a capsid. Viral vectors and viral capsids are discussed in the ensuing sections. The nucleic acid packaged in the rAAV vector can be single-stranded (ss), self-complementary (sc), or double-stranded (ds).

Viral Vector

[69] Typically, viral vectors carrying transgenes are assembled from polynucleotides encoding the transgene, suitable regulatory elements and elements necessary for production of viral proteins which mediate cell transduction. Examples of a viral vector include but are not limited to adenoviral, retroviral, lentiviral, herpesvirus and adeno-associated virus (AAV) vectors.

[70] The viral vector component of the packaged viral vectors produced according to the methods of the invention includes at least one transgene, *e.g.,* a modified FXN gene sequence and associated expression control sequences for controlling expression of the modified FXN gene sequence.

[71] In a preferred embodiment, the viral vector includes a portion of a parvovirus genome, such as an AAV genome with rep and cap deleted and/or replaced by the modified FXN gene

sequence and its associated expression control sequences. The modified FXN gene sequence is typically inserted adjacent to one or two (i.e., is flanked by) AAV TRs or TR elements adequate for viral replication (Xiao et al., 1997, J. Virol. 71(2): 941-948), in place of the nucleic acid encoding viral rep and cap proteins. Other regulatory sequences suitable for use in facilitating tissue-specific expression of the modified FXN gene sequence in the target cell may also be included.

[72] One skilled in the art would appreciate that an AAV vector comprising a transgene and lacking virus proteins needed for viral replication (e.g., cap and rep), cannot replicate since such proteins are necessary for virus replication and packaging. Further, AAV is a Dependovirus in that it cannot replicate in a cell without co-infection of the cell by a helper virus. Helper viruses include, typically, adenovirus or herpes simplex virus. Alternatively, as discussed below, the helper functions (E1a, E1b, E2a, E4, and VA RNA) can be provided to a packaging cell including by transfecting the cell with one or more nucleic acids encoding the various helper elements and/or the cell can comprise the nucleic acid encoding the helper protein. For instance, HEK 293 were generated by transforming human cells with adenovirus 5 DNA and now express a number of adenoviral genes, including, but not limited to E1 and E3 (*see*, *e.g.*, Graham et al., 1977, J. Gen. Virol. 36:59-72). Thus, those helper functions can be provided by the HEK 293 packaging cell without the need of supplying them to the cell by, e.g., a plasmid encoding them.

[73] The viral vector may be any suitable nucleic acid construct, such as a DNA or RNA construct and may be single stranded, double stranded, or duplexed (i.e., self complementary as described in WO 2001/92551).

[74] One skilled in the art would appreciate that a rAAV vector can further include a “stuffer” or “filler” sequence (filler/stuffer) where the nucleic acid comprising the transgene is less than the approximately 4.1 to 4.9 kb size for optimal packaging of the nucleic acid into the AAV capsid. *See*, Grieger and Samulski, 2005, J. Virol. 79(15):9933-9944. That is, AAV vectors typically accept inserts of DNA having a defined size range which is generally about 4 kb to about 5.2 kb, or slightly more. Thus, for shorter sequences, inclusion of a filler/stuffer in the insert fragment in order to adjust the length to near or at the normal size of the virus genomic sequence acceptable for AAV vector packaging into virus particle. In various embodiments, a filler/stuffer nucleic acid sequence is an untranslated (non-protein encoding) segment of nucleic acid. In particular embodiments of a rAAV vector, a heterologous polynucleotide sequence has a length less than 4.7 Kb and the filler/stuffer polynucleotide sequence has a length that when combined (e.g., inserted into a vector) with the heterologous polynucleotide sequence has a total length between about 3.0-5.5Kb, or between about 4.0-5.0Kb, or between about 4.3-4.8Kb.

[75] An intron can also function as a filler/stuffer polynucleotide sequence in order to achieve a length for AAV vector packaging into a virus particle. Introns and intron fragments that function as a filler/stuffer polynucleotide sequence also can enhance expression. For example, inclusion of an intron element may enhance expression compared with expression in the absence of the intron element (Kurachi et al., 1995, J. Biol. Chem. 270(10):5276-5281). Furthermore, filler/stuffer polynucleotide sequences are well known in the art and include, but are not limited to, those described in WO 2014/144486.

Viral Capsid

[76] The viral capsid component of the packaged viral vectors may be a parvovirus capsid. AAV Cap and chimeric capsids are preferred. Examples of suitable parvovirus viral capsid components are capsid components from the family Parvoviridae, such as an autonomous parvovirus or a Dependovirus. For example, the viral capsid may be an AAV capsid (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 AAV8, AAV9, AAV10, AAV11, AAV12, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, AAVrh10, AAVrh74, RHM4-1 (SEQ ID NO:5 of WO 2015/013313), AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAV-LK03, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, and any other AAV now known or later discovered. *see*, *e.g.,* Fields et al., VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). Capsids may be derived from a number of AAV serotypes disclosed in U.S. Patent No. 7,906,111; Gao et al., 2004, J. Virol. 78:6381; Moris et al., 2004, Virol. 33:375; WO 2013/063379; WO 2014/194132; and include true type AAV (AAV-TT) variants disclosed in WO 2015/121501, and RHM4-1, RHM15-1 through RHM15-6, and variants thereof, disclosed in WO 2015/013313, and one skilled in the art would know there are likely other variants not yet identified that perform the same or similar function, or may include components from two or more AAV capsids. A full complement of AAV Cap proteins includes VP1, VP2, and VP3. The ORF comprising nucleotide sequences encoding AAV VP capsid proteins may comprise less than a full complement AAV Cap proteins or the full complement of AAV Cap proteins may be provided.

[77] One or more of the AAV Cap proteins may be a chimeric protein, including amino acid sequences of AAV Caps from two or more viruses, preferably two or more AAVs, as described in Rabinowitz et al., U.S. Patent 6,491,907, the entire disclosure of which is incorporated herein by reference. For example, the chimeric virus capsid can include an AAV1 Cap protein or subunit and at least one AAV2 Cap or subunit. The chimeric capsid can, for example, include an AAV capsid with one or more B19 Cap subunits, e.g., an AAV Cap protein or subunit can be replaced by a B19 Cap protein or subunit. For example, in a preferred embodiment, the Vp3 subunit of the AAV capsid can be replaced by the Vp2 subunit of B19.

[78] Another embodiment includes chimeric viral strains synthesized include the combination of AAV backbones from AAV2, AAV3, AAV6, AAV8, etc., with a galactose (Gal) binding footprint from AAV9. Adeno-associated viruses (AAVs) are helper-dependent parvoviruses that exploit heparan sulfate (HS), galactose (Gal), or sialic acids (Sia) as primary receptors for cell surface binding. For instance, AAV serotypes 2 and 3b utilize HS. AAV1, 4, and 5 bind Sia with different linkage specificities, AAV serotype 6, which recognizes both Sia and HS, whereas AAV9 exploits Gal for host cell attachment. Specifically, the galactose (Gal) binding footprint from AAV9 was grafted onto the heparin sulfate-binding AAV serotype 2 and just grafting of orthogonal glycan binding footprints improves transduction efficiency. A new dual glycan-binding strain (AAV2G9) and a chimeric, muscle-tropic strain (AAV2i8G9) were generated by incorporating the Gal binding footprint from AAV9 into the AAV2 VP3 backbone or the chimeric AAV2i8 capsid template using structural alignment and site-directed mutagenesis. In vitro binding and transduction assays confirmed the exploitation of both HS and Gal receptors by AAV2G9 for cell entry. Subsequent in vivo characterization of the kinetics of transgene expression and vector genome biodistribution profiles indicate fast, sustained, and enhanced transgene expression by this rationally engineered chimeric AAV strain. A similar, improved transduction profile was observed with the liver-detargeted, muscle-specific AAV2i8G9 chimera (Shen, et al., 2013, J. Biol. Chem. 288(4):28814-28823). Such new grafting combination is fully described in WO2014/144229 the contents of which are incorporated by reference herein. Additional liver de-targeted AAVs, such as AAV9.45, are described in Pulicherla et al., 2011, Molecular Therapy 19(6):1070-1078, the contents of which are incorporated by reference as if set forth in their entirety herein.

[79] In yet another embodiment the present invention provides for the use of ancestral AAV vectors for use in therapeutic in vivo gene therapy. Specifically, in silico-derived sequences were synthesized de novo and characterized for biological activities. This effort led to the generation of nine functional putative ancestral AAVs and the identification of Anc80, the predicted ancestor of AAV serotypes 1, 2, 8 and 9 (Zinn et al., 2015, Cell Reports 12:1056-1068). Predicting and synthesis of such ancestral sequences in addition to assembling into a virus particle may be accomplished by using the methods described in WO 2015/054653, the contents of which are incorporated by reference herein. Notably, the use of the virus particles assembled from ancestral viral sequences exhibit reduced susceptibility to pre-existing immunity in current day human population than do contemporary viruses or portions thereof.

Production of Packaged Viral Vector

[80] The invention includes packaging cells, which are encompassed by “host cells,” which may be cultured to produce packaged viral vectors of the invention. The packaging cells of the

invention generally include cells with heterologous (1) viral vector function(s), (2) packaging function(s), and (3) helper function(s). Each of these component functions is discussed in the ensuing sections.

[81] Initially, the vectors can be made by several methods known to skilled artisans (*see*, *e.g.,* WO 2013/063379). A preferred method is described in Grieger, et al. 2015, Molecular Therapy 24(2):287-297, the contents of which are incorporated by reference herein for all purposes. Briefly, efficient transfection of HEK293 cells is used as a starting point, wherein an adherent HEK293 cell line from a qualified clinical master cell bank is used to grow in animal component-free suspension conditions in shaker flasks and WAVE bioreactors that allow for rapid and scalable rAAV production. Using the triple transfection method (e.g., WO 96/40240), the suspension HEK293 cell line generates greater than $1x10^5$ vector genome containing particles (vg)/cell or greater than $1x10^{14}$ vg/L of cell culture when harvested 48 hours post-transfection. More specifically, triple transfection refers to the fact that the packaging cell is transfected with three plasmids: one plasmid encodes the AAV rep and cap genes, another plasmid encodes various helper functions (e.g., adenovirus or HSV proteins such as E1a, E1b, E2a, E4, and VA RNA, and another plasmid encodes the transgene and its various control elements (e.g., modified FXN gene and CBh promoter).

[82] To achieve the desired yields, a number of variables are optimized such as selection of a compatible serum-free suspension media that supports both growth and transfection, selection of a transfection reagent, transfection conditions and cell density. A universal purification strategy, based on ion exchange chromatography methods, was also developed that resulted in high purity vector preps of AAV serotypes 1-6, 8, 9 and various chimeric capsids. This user-friendly process can be completed within one week, results in high full to empty particle ratios (>90% full particles), provides post-purification yields ($>1x10^{13}$ vg/L) and purity suitable for clinical applications and is universal with respect to all serotypes and chimeric particles. This scalable manufacturing technology has been utilized to manufacture GMP Phase I clinical AAV vectors for retinal neovascularization (AAV2), Hemophilia B (scAAV8), Giant Axonal Neuropathy (scAAV9) and Retinitis Pigmentosa (AAV2), which have been administered into patients. In addition, a minimum of a 5-fold increase in overall vector production by implementing a perfusion method that entails harvesting rAAV from the culture media at numerous time-points post-transfection.

Viral Vector Functions

[83] The packaging cells of the invention include viral vector functions, along with packaging and vector functions. The viral vector functions typically include a portion of a parvovirus genome, such as an AAV genome, with rep and cap deleted and replaced by the modified FXN

sequence and its associated expression control sequences. The viral vector functions include sufficient expression control sequences to result in replication of the viral vector for packaging. Typically, the viral vector includes a portion of a parvovirus genome, such as an AAV genome with rep and cap deleted and replaced by the transgene and its associated expression control sequences. The transgene is typically flanked by two AAV TRs, in place of the deleted viral rep and cap ORFs. Appropriate expression control sequences are included, such as a tissue-specific promoter and other regulatory sequences suitable for use in facilitating tissue-specific expression of the transgene in the target cell. The transgene is typically a nucleic acid sequence that can be expressed to produce a therapeutic polypeptide or a marker polypeptide.

[84] "Duplexed vectors" may interchangeably be referred to herein as "dimeric" or "self-complementary" vectors. The duplexed parvovirus particles may, for example, comprise a parvovirus capsid containing a virion DNA (vDNA). The vDNA is self-complementary so that it may form a hairpin structure upon release from the viral capsid. The duplexed vDNA appears to provide to the host cell a double-stranded DNA that may be expressed (i.e., transcribed and, optionally, translated) by the host cell without the need for second-strand synthesis, as required with conventional parvovirus vectors. Duplexed/self-complementary rAAV vectors are well-known in the art and described, *e.g.*, in WO 2001/92551, WO 2015/006743, and many others.

[85] The viral vector functions may suitably be provided as duplexed vector templates, as described in U.S. Patent No. 7,465,583 to Samulski et al. (the entire disclosure of which is incorporated herein by reference for its teaching regarding duplexed vectors). Duplexed vectors are dimeric self-complementary (sc) polynucleotides (typically, DNA). The duplexed vector genome preferably contains sufficient packaging sequences for encapsidation within the selected parvovirus capsid (e.g., AAV capsid). Those skilled in the art will appreciate that the duplexed vDNA may not exist in a double-stranded form under all conditions, but has the ability to do so under conditions that favor annealing of complementary nucleotide bases. "Duplexed parvovirus particle" encompasses hybrid, chimeric and targeted virus particles. Preferably, the duplexed parvovirus particle has an AAV capsid, which may further be a chimeric or targeted capsid, as described above.

[86] The viral vector functions may suitably be provided as duplexed vector templates, as described in U.S. Patent No. 7,465,583 to Samulski et al. (the entire disclosure of which is incorporated herein by reference for its teaching regarding duplexed vectors). Duplexed vectors are dimeric self-complementary (sc) polynucleotides (typically, DNA). For example, the DNA of the duplexed vectors can be selected so as to form a double-stranded hairpin structure due to intrastrand base pairing. Both strands of the duplexed DNA vectors may be packaged within a viral capsid. The duplexed vector provides a function comparable to double-stranded

DNA virus vectors and can alleviate the need of the target cell to synthesize complementary DNA to the single-stranded genome normally encapsulated by the virus.

[87] The TR(s) (resolvable and non-resolvable) selected for use in the viral vectors are preferably AAV sequences, with serotypes 1, 2, 3, 4, 5 and 6 being preferred. Resolvable AAV TRs need not have a wild-type TR sequence (e.g., a wild-type sequence may be altered by insertion, deletion, truncation or missense mutations), as long as the TR mediates the desired functions, e.g., virus packaging, integration, and/or provirus rescue, and the like. The TRs may be synthetic sequences that function as AAV inverted terminal repeats, such as the "double-D sequence" as described in U.S. Pat. No. 5,478,745 to Samulski et al., the entire disclosure of which is incorporated in its entirety herein by reference. Typically, but not necessarily, the TRs are from the same parvovirus, e.g., both TR sequences are from AAV2

[88] The packaging functions include capsid components. The capsid components are preferably from a parvoviral capsid, such as an AAV capsid or a chimeric AAV capsid function. Examples of suitable parvovirus viral capsid components are capsid components from the family Parvoviridae, such as an autonomous parvovirus or a Dependovirus. For example, the capsid components may be selected from AAV capsids, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh10, AAVrh74, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV Hu.26, AAV1.1 (SEQ ID NO:15), AAV2.5 (SEQ ID NO. 13), AAV6.1 (SEQ ID NO:17), AAV6.3.1 (SEQ ID NO:18), AAV9.45, AAV2i8 (SEQ ID NO:29), AAV2G9, AAV2i8G9, AAV2-TT (SEQ ID NO:31), AAV2-TT-S312N (SEQ ID NO:33), AAV3B-S312N, and AAV-LK03, and other novel capsids as yet unidentified or from non-human primate sources. Capsid components may include components from two or more AAV capsids.

[89] In a more preferred embodiment, one or more of the VP capsid proteins is a chimeric protein, comprising amino acid sequences from two or more viruses, preferably two or more AAVs, as described in Rabinowitz et al., U.S. Patent 6,491,907. A chimeric capsid is described herein as having at least one amino acid residue from one serotype combined with another serotype that is sufficient to modify a) viral yield, b) immune response, c) targeting, d) de-targeting, etc.

[90] Further chimeric proteins can be made by instruction set forth in Li, et al., 2008, Mol. Ther. 16(7):1252-1260, the contents of which are incorporated by reference herein. Specifically, a DNA shuffling-based approach was used for developing cell type-specific vectors through directed evolution. Capsid genomes of adeno-associated virus (AAV) serotypes 1-9 were randomly fragmented and reassembled using PCR to generate a chimeric capsid library. A single infectious clone (chimeric-1829) containing genome fragments from AAV1, 2, 8, and 9 was isolated from an integrin minus hamster melanoma cell line previously shown to have low

permissiveness to AAV. Molecular modeling studies suggest that AAV2 contributes to surface loops at the icosahedral threefold axis of symmetry, while AAV1 and 9 contribute to two- and five-fold symmetry interactions, respectively. The C-terminal domain (AAV9) was identified as a critical structural determinant of melanoma tropism through rational mutagenesis. Chimeric-1829 utilizes heparan sulfate as a primary receptor and transduces melanoma cells more efficiently than all serotypes. Application of this technology to alternative cell/tissue types using AAV or other viral capsid sequences is likely to yield a new class of biological nanoparticles as vectors for human gene transfer.

[91] The packaged viral vector generally includes the modified FXN gene sequence and expression control sequences flanked by TR elements, referred to herein as the "transgene" or "transgene expression cassette," sufficient to result in packaging of the vector DNA and subsequent expression of the modified FXN gene sequence in the transduced cell. The viral vector functions may, for example, be supplied to the cell as a component of a plasmid or an amplicon. The viral vector functions may exist extrachromosomally within the cell line and/or may be integrated into the cell's chromosomal DNA.

[92] Any method of introducing the nucleotide sequence carrying the viral vector functions into a cellular host for replication and packaging may be employed, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal. In embodiments wherein the viral vector functions are provided by transfection using a virus vector; standard methods for producing viral infection may be used.

Packaging Functions

[93] The packaging functions include genes for viral vector replication and packaging. Thus, for example, the packaging functions may include, as needed, functions necessary for viral gene expression, viral vector replication, rescue of the viral vector from the integrated state, viral gene expression, and packaging of the viral vector into a viral particle. The packaging functions may be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon, a Baculovirus, or HSV helper construct. The packaging functions may exist extrachromosomally within the packaging cell, but are preferably integrated into the cell's chromosomal DNA. Examples include genes encoding AAV Rep and Cap proteins.

Helper Functions

[94] The helper functions include helper virus elements needed for establishing active infection of the packaging cell, which is required to initiate packaging of the viral vector. Examples

include functions derived from adenovirus, baculovirus and/or herpes virus sufficient to result in packaging of the viral vector. For example, adenovirus helper functions will typically include adenovirus components E1a, E1b, E2a, E4, and VA RNA. The packaging functions may be supplied by infection of the packaging cell with the required virus. The packaging functions may be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon. *See*, *e.g.*, pXR helper plasmids as described in Rabinowitz et al., 2002, J. Virol. 76:791, and pDG plasmids described in Grimm et al., 1998, Human Gene Therapy 9:2745-2760. The packaging functions may exist extrachromosomally within the packaging cell, but are preferably integrated into the cell's chromosomal DNA (*e.g.*, E1 or E3 in HEK 293 cells).

[95] Any suitable helper virus functions may be employed. For example, where the packaging cells are insect cells, baculovirus may serve as a helper virus. Herpes virus may also be used as a helper virus in AAV packaging methods. Hybrid herpes viruses encoding the AAV Rep protein(s) may advantageously facilitate for more scalable AAV vector production schemes.

[96] Any method of introducing the nucleotide sequence carrying the helper functions into a cellular host for replication and packaging may be employed, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal. In embodiments wherein the helper functions are provided by transfection using a virus vector or infection using a helper virus; standard methods for producing viral infection may be used.

Packaging Cell

[97] Any suitable permissive or packaging cell known in the art may be employed in the production of the packaged viral vector. Mammalian cells or insect cells are preferred. Examples of cells useful for the production of packaging cells in the practice of the invention include, for example, human cell lines, such as VERO, WI38, MRC5, A549, HEK 293 cells (which express functional adenoviral E1 under the control of a constitutive promoter), B-50 or any other HeLa cells, HepG2, Saos-2, HuH7, and HT1080 cell lines. In one aspect, the packaging cell is capable of growing in suspension culture, more preferably, the cell is capable of growing in serum-free culture. In one embodiment, the packaging cell is a HEK293 that grows in suspension in serum free medium. In another embodiment, the packaging cell is the HEK293 cell described in US Patent No. 9,441,206 and deposited as ATCC No. PTA 13274. Numerous rAAV packaging cell lines are known in the art, including, but not limited to, those disclosed in WO 2002/46359.

[98] Cell lines for use as packaging cells include insect cell lines. Any insect cell which allows for replication of AAV and which can be maintained in culture can be used in accordance with the present invention. Examples include Spodoptera frugiperda, such as the Sf9 or Sf21 cell

lines, Drosophila spp. cell lines, or mosquito cell lines, e.g., Aedes albopictus derived cell lines. A preferred cell line is the Spodoptera frugiperda Sf9 cell line. The following references are incorporated herein for their teachings concerning use of insect cells for expression of heterologous polypeptides, methods of introducing nucleic acids into such cells, and methods of maintaining such cells in culture: Methods in Molecular Biology, ed. Richard, Humana Press, NJ (1995); O'Reilly et al., Baculovirus Expression Vectors: A Laboratory Manual, Oxford Univ. Press (1994); Samulski et al., 1989, J. Virol. 63:3822-3828; Kajigaya et al., 1991, Proc. Nat'l. Acad. Sci. USA 88: 4646-4650; Ruffing et al., 1992, J. Virol. 66:6922-6930; Kimbauer et al., 1996, Virol. 219:37-44; Zhao et al., 2000, Virol. 272:382-393; and Samulski et al., U.S. Pat. No. 6,204,059.

[99] Virus capsids according to the invention can be produced using any method known in the art, e.g., by expression from a baculovirus (Brown et al., (1994) Virology 198:477-488). As a further alternative, the virus vectors of the invention can be produced in insect cells using baculovirus vectors to deliver the rep/cap genes and rAAV template as described, for example, by Urabe et al., 2002, Human Gene Therapy 13:1935-1943.

[100] In another aspect, the present invention provide for a method of rAAV production in insect cells wherein a baculovirus packaging system or vectors may be constructed to carry the AAV Rep and Cap coding region by engineering these genes into the polyhedrin coding region of a baculovirus vector and producing viral recombinants by transfection into a host cell. Notably when using Baculavirus production for AAV, preferably the AAV DNA vector product is a self-complementary AAV like molecule without using mutation to the AAV ITR. This appears to be a by-product of inefficient AAV rep nicking in insect cells which results in a self-complementary DNA molecule by virtue of lack of functional Rep enzyme activity. The host cell is a baculovirus-infected cell or has introduced therein additional nucleic acid encoding baculovirus helper functions or includes these baculovirus helper functions therein. These baculovirus viruses can express the AAV components and subsequently facilitate the production of the capsids.

[101] During production, the packaging cells generally include one or more viral vector functions along with helper functions and packaging functions sufficient to result in replication and packaging of the viral vector. These various functions may be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon, and they may exist extrachromosomally within the cell line or integrated into the cell's chromosomes.

[102] The cells may be supplied with any one or more of the stated functions already incorporated, e.g., a cell line with one or more vector functions incorporated extrachromosomally or integrated into the cell's chromosomal DNA, a cell line with one or more packaging functions incorporated extrachromosomally or integrated into the cell's chromosomal

DNA, or a cell line with helper functions incorporated extrachromosomally or integrated into the cell's chromosomal DNA

rAAV Purification

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

Treatment Methods

[104] The modified FXN gene may be used for gene therapy of Friedreich ataxia associated disorders, such as, degenerative neuro-muscular disorders and/or cardiomyopathy associated with Friedreich ataxia. An individual may be in need of gene therapy because, as a result of one or more mutations in the coding sequence of the FXN gene, FXN is expressed inappropriately, e.g., has an incorrect amino acid sequence, or is expressed in the wrong tissues or at the wrong times or is underexpressed. The modified FXN gene of the present invention may be used as gene therapy to enhance production of the protein frataxin and thereby increasing energy production in the mitochondria. *See., e.g.,* U.S. Patent No. 9,066,966.

[105] The target cells of the vectors of the instant invention are cells capable of expressing frataxin, such as those of the cardiac system of a mammal, neuron cells, muscle cells, and other cells with the proper cellular machinery to process the precursor to yield protein with frataxin activity.

Pharmaceutical Composition

[106] In particular embodiments, the present invention provides a pharmaceutical composition for preventing or treating a disease or condition mediated by or associated with decreased expression of frataxin, e.g., Friedreich ataxia. The composition comprises a therapeutically effective amount of a vector which comprises a modified FXN gene which can increase the level of expression of FXN in a call. The composition comprises the vector comprising the modified, e.g., optimized, nucleic acid encoding FXN wherein the composition further comprises a pharmaceutically-acceptable carrier and/or other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. As an injection medium, it is preferred to use water that contains the additives usual for injection solutions, such as stabilizing agents, salts or saline, and/or buffers.

[107] Exemplary pharmaceutically acceptable carriers include sterile, pyrogen-free water and sterile, pyrogen-free, phosphate buffered saline. Physiologically-acceptable carriers include pharmaceutically-acceptable carriers. Pharmaceutically acceptable carriers are those which are that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject without causing undesirable biological effects which outweigh the advantageous biological effects of the material.

[108] A pharmaceutical composition may be used, for example, in transfection of a cell ex vivo or in administering a viral vector or cell directly to a subject.

[109] Recombinant virus vectors comprising the modified FXN gene are preferably administered to the cell in a biologically-effective amount. If the virus vector is administered to a cell in vivo (e.g., the virus is administered to a subject as described below), a biologically-effective amount of the virus vector is an amount that is sufficient to result in transduction and expression of the transgene in a target cell.

[110] In one embodiment, the invention includes a method of increasing the level of frataxin in a cell by administering to the cell a nucleic acid, either alone or in a vector (including a plasmid, a virus, a nanoparticle, a liposome, or any known method for providing a nucleic acid to a cell) comprising a modified nucleic acid encoding frataxin. The method comprises a method wherein the level of mRNA encoding frataxin and/or the level of frataxin protein expressed is detectably greater than the level of frataxin (mRNA and/or protein) in an otherwise identical cell that is not administered the nucleic acid. The skilled artisan would understand that the cell can be cultured or grown *in vitro* or can be present in an organism (*i.e.*, *in vivo*). Further, the cell may express endogenous frataxin such that the level of frataxin in the cell can be increased, and/or the cell can express an endogenous frataxin that is a mutant or variant of wild type frataxin, *e.g.*, frataxin having the sequence of SEQ ID NO:2, especially as there may be more than one wild type alleles for human frataxin. Thus, the level of frataxin is increased compared with the level of frataxin compared with the level of frataxin expressed in an otherwise identical but untreated cell.

[111] A further aspect of the invention is a method of treating subjects *in vivo* with the vector containing modified genes. Administration of the vector to a human subject or an animal in need thereof can be by any means known in the art for administering virus vectors.

[112] The vector can be administered in addition, and as an adjunct to, the standard of care. That is, the vector can be co-administered with another therapeutic agent or compound, either simultaneously, contemporaneously, or at a determined dosing interval as would be determined by one skilled in the art using routine methods.

[113] In one aspect, the rAAV of the invention can be co-administered with empty capsids (i.e., a virus capsid that does not contain a nucleic acid molecule) comprising the same, or a

different, capsid protein as the rAAV-FXN vector. This is because one skilled in the art would understand that co-administration of empty capsids may decrease an immune response, e.g., a neutralizing response, the rAAV of the invention. That is, the empty capsid may serve as a decoy allowing the rAAV-FXN vector to avoid a neutralizing antibody (Nab) immune response as discussed in, *e.g.,* WO 2015/013313.

[114] Exemplary modes of administration systemic administration, including, but not limited to, intravenous, subcutaneous, intradermal, intramuscular, and intraarticular administration, and the like, as well as direct tissue or organ injection.

[115] In one embodiment, the vector is administered systemically. One skilled in the art would appreciate that systemic administration can deliver the therapeutic gene encoding FXN to all tissues, including all muscles, affected by the reduced level of FXN therein.

[116] Nonetheless, the skilled artisan would appreciate that the vector can be delivered directly to areas affected by the FXN deficiency, i.e., the brain and the heart.

[117] Accordingly, in other preferred embodiments, the inventive vector comprising the modified FXN gene is administered by direct injection into cardiac or central nervous system (CNS) tissue.

[118] In one embodiment, modified nucleic acid encoding FNX, the vector, or composition comprising the vector, is delivered intracranially including, intrathecal, intraneural, intra-cerebral, intra-ventricular administration.

[119] In one embodiment, modified nucleic acid encoding FNX, the vector, or composition comprising the vector, is delivered to the heart by direct administration into the myocardium by epicardiac injection followed by minithoracotomy, by intracoronary injection, by endomyocardic injection or by another type of injection useful in the heart.

[120] Additional routes of administration may also comprise local application of the vector under direct visualization, e.g., superficial cortical application, or other nonstereotactic application. The vector may also be delivered, for example, intrathecally, into the ventricles or by intravenous injection.

[121] The target cells of the vectors of the present invention are cells of the myocardium of a subject afflicted with a cardiomyopathy associated with Friedreich ataxia. Preferably the subject is a human being, adult or child. However, veterinary applications are also contemplated.

[122] The target cells of the vectors of the present invention also include cells of the CNS, preferably neurons. Delivery to the brain to treat neurodegenerative aspects of Friedreich ataxia may be by intrathecal administration.

[123] In one aspect, modified nucleic acid encoding FNX, the vector, or composition comprising the vector, is delivery systemically, e.g., intravenously, to treat the FA associated cardiomyopathy and/or the neurodegenerative aspect of the disease.

[124] In another embodiment, the vector is administered by at least two routes. That is, the vector can be administered systemically and also directly into the brain and/or heart, or any combination thereof.

[125] If performed via at least two routes, the administration of the vector can be, but need not be, simultaneous or contemporaneous. Instead, the administrations via different routes can be performed separately with an interval of time between each administration. Appropriate dosing regimens are routinely determined by those skilled in the art to achieve maximum therapeutic benefit for each individual patient.

[126] In one aspect, the invention includes at least one modified nucleic acid encoding frataxin of the invention, including, but not limited to, the nucleic acid in a vector or a pharmaceutical composition, for use in increasing the level of frataxin in a subject.

[127] In one aspect, the invention includes at least one modified nucleic acid, rAAV vector comprising the nucleic acid, and a pharmaceutical composition comprising either the nucleic acid or the vector, for use in treating Friedreich ataxia in a subject.

[128] The use encompasses administering the modified nucleic acid, or vector comprising the same, in addition to and/or concurrent with, the standard of care for FRDA as known in the art.

[129] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

[130] Dosages of the virus vector with the modified FXN gene will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular viral vector, and the gene to be delivered, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are virus titers of at least about 10^5, 106, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} transducing units or more, preferably about 10^8-1013 transducing units, yet more preferably 10^{12} transducing units/kg body weight.

[131] The modified FXN gene may be administered as components of a DNA molecule having regulatory elements appropriate for expression in the target cells. The modified FXN gene may be administered as components of viral plasmids, such as rAAV vectors. Viral particles may be administered as viral particles alone, whether as an in vivo direct delivery to the portal vasculature or as an ex vivo treatment comprising administering the vector viral particles in vitro to cells from the animal receiving treatment followed by introduction of the transduced cells back into the donor.

Equivalents

[132] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the disclosure. The foregoing description and Examples detail certain

exemplary embodiments of the disclosure. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the disclosure may be practiced in many ways and the disclosure should be construed in accordance with the appended claims and any equivalents thereof.

[133] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

Exemplary Embodiments

[134] The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLES

Example 1: Generation of a self-complimentary rAAV-FXN construct

Materials and Methods

[135] Vector construction

[136] The pTRs-KS-CBh-EGFP-bGHpolyA construct (shown diagrammatically in Figure 3) encoding a self-complementary AAV genome was used as the backbone of the transgene expression construct (Gray et al., 2011, Human Gene Therapy 22:1143-1153). Two codon optimized FXN gene inserts were ordered from GenScript in pUC57, *i.e.*, Genscript and Genscript (low CpG), and used to replace the EGFP in the backbone vector. The Genscript (SEQ ID NO:6) and Genscript (low CpG) (SEQ ID NO:7) modified FXN genes were each operably linked to the CBh promoter as illustrated in Figures 3. The GenScript FXN (SEQ ID NO: 6 and 7) constructs included an N-terminal AgeI site, a collagen stability sequence (CSS) (5'-CCCAGCCCACTTTTCCCCAA-3') downstream of the FXN stop codon, a bovine growth hormone (BGH) polyA sequence downstream of the CSS, and a MluI site downstream of the BGH polyA all as shown in Figures 2E (Genscript) and 2F (Genscript (low CpG)). Exemplary inserts for insertion into the pTRs-KS-CBh-**FXN**-bGHpolyA constructs are shown in Figures 2A-2F and are set forth in Table 8. More specifically, wild type frataxin gene (WT FXN; SEQ ID NO:2) was cloned into pTRs-KS-CBh-**WT FXN**-bGHpolyA (Fig. 2A); an IDT1 modified FXN gene (SEQ ID NO:11) was cloned into pTRs-KS-CBh-**IDT1**-bGHpolyA (Fig. 2B); a nucleic acid encoding IDT3 low expresser modified FXN gene (SEQ ID NO:8) was cloned into pTRs-KS-CBh-**IDT3**-bGHpolyA (Fig. 2C); an IDT4 modified FXN gene (SEQ ID NO:12) was cloned into

pTRs-KS-CBh-**IDT4**-bGHpolyA (Fig. 2D); a Genscript (control) modified FXN gene (SEQ ID NO:6) was cloned into pTRs-KS-CBh-**Genescript**-bGHpolyA (Fig. 2E); and a Genscript (low CpG) modified FXN gene (SEQ ID NO:7) was cloned into pTRs-KS-CBh-**Genescript (low CpG)**-bGHpolyA (Fig. 2F). Each insert encoding a FXN gene was cloned into the vector and the gene was flanked by an AgeI site on the 5' side and by an AvrII cut site on the 3' side, followed by a CSS sequence after the AvrII site, a SpeI cut site after the CSS, a bGHpolyA signal sequence after the SpeI cut site, and a MluI cut site after the polyA signal sequence.

[137] The backbone pTRs-KS-CBh-**EGFP**-bGHpolyA and the FXN gene constructs were digested with AgeI and MluI (New England Biolabs, R0552S and R0198S, respectively), gel extracted, and ligated using ExTaq polymerase (Clontech, RR001A). The ligation reaction was transformed into SURE cells (Agilent, 200227), placed in SOC recovery media (Cat. No. 15544-034, Invitrogen) for one hour at 37°C, then plated on LB plates with ampicillin (10 mg/ml). Colonies were sequenced and chosen for amplification for virus production. Recombinant AAV (rAAV) vectors with the AAV serotype 2 capsid were produced the UNC Vector Core by a triple-transfection method in human embryonic kidney 293 (HEK293) cells as described (Grieger et al., 2006, Nature Protocols 1:1412-1428). Alternatively, rAAV vector with the serotype 2i8 capsid (amino acid sequence of SEQ ID NO:28) was similarly produced. Highly pure recombinant virus containing self-complementary genomes was recovered by passage through a non-ionic iodixanol gradient followed by ion exchange chromatography. Peak fractions were determined by qPCR then dialyzed in phosphate-buffered saline (PBS) containing 5% d-sorbitol. Viral titers were determined by qPCR (Gray et al., 2010, J. Amer. Soc. Gene Therapy 18:570-578). Following preliminary testing in vitro (below), GenScript (low CpG) was used to generate a construct with an HA tag TACCCATACGATGTTCCAGATTACGCT inserted prior to the FXN stop codon in pTRs-KS-CBh-**Genescript (low CpG)**-bGHpolyA.

[138] The University of North Carolina (UNC) Vector Core generated viruses with the FXN-HA construct with rAAV TK serotypes.

[139] <u>In vitro testing of sc rAAV-FXN</u>.

[140] HEK293 (ATCC: CRL-1573) and HeLa (ATCC: CCL-2) cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco). Cell growth media was supplemented with 9% fetal bovine serum (FBS, Gibco), 3.4 mM l-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco). Cells were kept in a 5% CO2 atmosphere at 37 °C. Dipstick assay: Cells were seeded in 24-well plates so that they reached approximately 60% confluence at 24 hours (h), then mock treated or infected in triplicate with scAAV-FXN (interchangeably referred to herein as "rAAV-FXN" or "rAAV-FXN-HA") at MOI 10,000 (VG/cell). At 60 h post transduction (h p.t.) cells were according to the manufacturer protocol for the Frataxin Protein Quantity Dipstick Assay (Abcam, ab109881). Data was processed using ImageJ.

[141] Western blotting:

[142] Cells were seeded in 6-well plates so that they reached approximately 60% confluence at 24 h, then mock treated or infected with scAAV-FXN at MOI 10,000 (VG/cell). At 60 h post transduction cells were lysed with cellular lysis buffer (0.0625 M Tris-HCl pH 6.8, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, 0.02% (w/v) Bromophenol blue). Fifteen (15) µl of HeLa protein lysate was separated by gel electrophoresis on a 15-4% TGX gel and the proteins were electroblotted to a nitrocellulose membrane (NCM). NCMs were blocked using 5% non-fat powdered milk in PBS-T. The anti-frataxin antibody (Abcam, 18A5DB1) was used in PBS-T with 5% milk. A horseradish peroxidase (HRP)-conjugated secondary antibody in PBS-T with 5% milk antibodies was used to detect the presence of anti-frataxin. The WesternBright ECL Western Blotting Detection kit (Advansta, K-12045-D50) was used for detection per manufacturer's instructions.

[143] FIG. 1A-1B shows the results for expression of various optimized sequences compared with expression of the unoptimized, *i.e.*, wild type, sequence encoding FXN (SEQ ID NO:1) in HeLa cells. More specifically, both Figure 1A and 1B show a photograph of a Western blot showing expression of frataxin (FXN) in HeLa cells transfected with an expression vector comprising an insert encoding frataxin . Figure 1A shows expression of FXN in HeLa cells in a photograph of a WesternBright blot film exposed for 1 second. Figure 1B shows a repeat of the experiment shown in Fig. 1A demonstrating expression of FXN in HeLa cells as shown in a photograph of a WesternBright blot film exposed for 1 second. Each gel lane in Figs. 1A and 1B shows the expression of FXN from a modified FXN gene of the invention compared with expression from a wild type nucleic acid sequence encoding FXN. That is, lane 1 shows expression driven by wild type non-modified nucleic acid encoding FXN (SEQ ID NO:2); lane 2 shows expression driven by IDT2 modified FXN gene (SEQ ID NO:3); lane 3 shows expression driven by IDT5 modified FXN gene (SEQ ID NO:9); lane 4 shows expression driven by JCAT modified FXN gene (SEQ ID NO:4); lane 5 shows expression driven by GeneArt modified FXN gene (SEQ ID NO:5); lane 6 shows expression driven by GenScript (control) modified FXN gene (SEQ ID NO:6); lane 7 shows expression driven by Genscript (low CpG) modified FXN gene (SEQ ID NO:7); and lane GFP shows expression transgene encoding green fluorescent protein, a detectable marker, which is encoded by the insert instead of a nucleic acid encoding FXN.

[144] The data shown demonstrate that several modified FXN nucleic acid sequences – especially lanes 4 (JCAT), 5 (GeneArt), 6 (Genscript) and 7 (Genscript low CpG) - provided greater expression of frataxin in HeLa cells relative to the wild type nucleic acid sequence (lane 1). An actin loading control in each lane is as a protein loading control.

[145] The GC nucleotide content in a nucleic acid sequence, typically expressed as a percentage of the total number of nucleotides in the sequence, can have multiple influences, including, but not limited to, the stability of the mRNA is increased, and the secondary structure and transgenes which are typically negatively impacted by increased GC content. Thus, the skilled artisan would appreciate that the GC content of a modified nucleic acid reflects a balance between increased stability of the nucleic acid, and mRNA transcribed therefrom, against the negative effect, e.g., on secondary structure mediated by increased GC content.

[146] The CAI (codon adaptation index) is a measure of synonymous codon usage bias. The index uses a reference set of highly expressed genes from a species to assess the relative values of each codon, and a score for a gene is calculated from the frequency of use of all codons in that gene. The index assesses the extent to which selection has been effective in selecting the pattern of codon usage. It can be utilized for predicting the level of expression of a gene and for making comparisons of codon usage in different organisms/species. Human codon optimization was carried out on the frataxin gene to achieve a balance of the below factors:

[147] Transctiption Efficiency – GC content, CpG dinucleotides content, Cryptic splicing sites, etc.;

[148] Translation Efficiency – Codon usage bias, GC content, mRNA secondary structure, premature polyA sites, RNA instability motifs, internal ribosomal binding sites; and

[149] Protein refolding – codon usage bias, interaction of codon and anti-codon, RNA secondary structures.

[150] Basically, codon optimization balances these variables to, preferably, achieve a higher expressing frataxin gene sequence, increase stability of the message (GC content, secondary structure in both DNA and RNA), and the like, as well-known in the art.

[151] CpG islands can be recognized by Tol-like receptor nine (TLR9) in a transduced cell and can elicit an immune response to the foreign (exogenous) DNA. Accordingly, in one embodiment, the invention encompasses a modified nucleic acid encoding frataxin wherein the number of CpG islands has been reduced compared with the number of CpG island motifs in a wild type nucleic acid sequence (e.g., SEQ ID NO:2) encoding frataxin.

[152] The CAI, percent GC content, and number of potential CpG island regions for each modified FXN gene exemplified herein is shown in Table 1 below.

TABLE 1

Figure 1A and 1B gel lane number	FXN gene name	Codon adaptation index (CAI)	%GC content	Number of potential CpG island regions	SEQ ID NO:
1	WT-FXN	0.71	55	128	2
	Nucleotide sequence 22	0.71	55	--	10
	IDT-1	0.73	52	114	11
2	IDT-2	0.76	56	124	3
	IDT-3	0.80	57	123	8
	IDT-4	0.74	54	123	12
3	IDT-5	0.77	55	124	9
4	JCAT	0.98	69	144	4
5	GeneART	0.95	61	117	5
6	Genescript (Control)	0.87	57	257	6
7	Genescript (low CpG)	0.86	55	117	7

[153] That is, for wild type nucleic acid encoding FXN (WT-FXN; SEQ ID NO:2), the nucleic acid sequence demonstrates a CAI of 0.71 and a % GC content of 55%. In contrast, the JCAT modified FXN gene demonstrates a CAI of 0.98 and a GC content of 69%, both of which are substantially higher than the values for WT-FXN.

[154] Potential CpG Islands were identified using publicly available software found at http://www.bioinformatics.org/sms2/cpg_islands.html. The CpG Islands software reported potential CpG island regions using the method described by Gardiner-Garden and Frommer, 1987, J. Mol. Biol. 196(2):261-282. The calculation was performed using a 200 basepair (bp) window moving across the sequence at 1 bp intervals. CpG islands are defined as sequence ranges where the Obs/Exp value is greater than 0.6 and the GC content is greater than 50%. The expected number of CpG dimers in a window was calculated as the number of 'C's in the window multiplied by the number of 'G's in the window, divided by the window length. Thus, the potential CpG islands present in a nucleic acid sequence can be readily determined by inputting the sequence at issue into the window provided by software (indicated by the instructions to "Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 100000 characters."). CpG islands are often found in the 5' regions of vertebrate genes, therefore this program can be used to highlight potential genes in genomic sequences.

[155] Because of the high level of expression and the high GC content (55%), high CAI (0.86) and low number of CpG dinucleotides (117), the Genscript (low CpG) modified FXN gene was selected for production of a scAAV-2i8 vector used in the animal experiments set forth below.

Example 2: In vivo treatment in a mouse model of Friedreich ataxia

[156] An art-recognized mouse model of FRDA (Perdomini et al., 2014, Nature Med. 20(5):542) was used to assess the potential efficacy of rAAV mediated FXN gene therapy. That is, three groups of mice were examined: untreated Mck positive control mice (*Mck*-Cre x FXN L3/WT), untreated Mck mutant mice (*Mck*-Cre x FXN L3/L-), and treated Mck mutant mice that received a dose of rAAV comprising a FXN gene wherein the modified FXN gene comprised the nucleic acid sequence of SEQ ID NO:7 (GenScript (low CpG)) and the FXN gene was cloned into the pTRs-KS-CBh-EGFP-BGH construct as described above to provide pTRs-KS-CBh-**Genscript (low CpG)**-bGHpolyA.

[157] The rAAV-FXN vector used in the mouse studies further comprised a AAV2i8 capsid. Moreover, the pTRs-KS-CBh-**Genscript (low CpG)**-bGHpolyA construct further comprised a nucleic acid sequence encoding a detectable hemagglutinin tag (rAAV-FXN-HA) wherein the sequence encoding the HA tag was located 3' of the modified FXN gene such that expression of frataxin could be readily detected and localized by detecting the presence of HA, e.g., using an anti-HA antibody such as anti-HA mouse mAb (HA.11 clone 16B12, Covance Research Products, Inc., Princeton, NJ). The vector was designated rAAV-FXN-HA.

[158] The three animal groups of the study are listed and described in Table 2.

TABLE 2

Groups label	Group No.	Dose Level vg/kg	No. of Animals Mixed gender	Termination Weeks of age
Mck positive control	*Mck*-Cre x FXN L3/WT	0	8	Week 8
Untreated Mck mutant mice	Untreated *Mck*-Cre x FXN L3/L-	0	8	Week 8
Treated Mck mutant mice	rAAV-FXN-HA treated *Mck*-Cre x FXN L3/L-	$1x10^{13}$	8	Week 8

A. Biomarker study

Methods

Measurement of Galectin-3 and H-FABP in plasma

[159] Blood was collected by retro orbital puncture after isoflurane anaesthesia at the age of 5 weeks (2 weeks after treatment) and 8 weeks (5 weeks after treatment).

[160] Galectin-3 was measured in plasma using the Mouse Galectin-3 Elisa Kit from RayBiotech according to manufacturer's instructions.

[161] H-FABP was measured in plasma using the Mouse H-FABP Elisa Kit from HycultBiotech according to manufacturer's instructions.

Measurement of succinate dehydrogenase activity in heart homogenate

[162] Upon sacrifice, the heart was collected and half of the heart of 4 mice of each group was snap frozen for the measurement of SDH activity.

[163] SDH activity measurement in heart homogenate was performed following the instruction of the Succinate Dehydrogenase Activity Colorimetric Assay Kit (Catalog # K660-100) from Biovision.

Measurement of human frataxin in tissues

[164] Upon sacrifice, heart (half), skeletal muscle (gastrocnemius) and liver tissues were collected and snap frozen for the measurement of frataxin.

[165] The measurement in tissue homogenates was performed following the instructions of the Human Frataxin Elisa Kit (Abcam; ab176112).

Histology

[166] Cerebellum (including dentate nucleus), gonads, heart, kidney, liver, lung, pancreas, skeletal muscle (gastrocnemius and soleus), spleen and cervical, thoracic and lumbar vertebras were formol-fixed. Vertebras were then decalcified using EDTA solution. All organs were paraffin embedded to obtain 5 µm-thick sections; transversal sections for vertebras (including both spinal cord and dorsal root ganglia) and heart. All organs were hematoxylin and eosin stained; cardiac fibrosis was evaluated using Masson's trichrome staining.

Echocardiography:

[167] Transthoracic Echocardiographic images were captured by the mean of a 30 MHz linear probe (MS 400) on a Vevo-2100 Visual Sonics echograph in anesthetized mice (Isoflurane 1-2%).

[168] The following parameters are measured to assess:

a) The cardiac morphology and ventricular systolic function (Short axis, SAX): left ventricular end-diastolic (LVEDD) and end systolic diameters (LVESD), septal (SW) and posterior wall thicknesses (PW), left ventricular mass (LVM= 1.055x [(EDD+SW+PW) 3-EDD3)]), Ejection and shortening Fraction and cardiac output;
b) Hemodynamic profiles: pulmonary and aortic artery velocity and pressures to detect intra-cardiac pressures changes (AoV and RV function).

Mice

[169] Mice were maintained in a temperature- and humidity-controlled animal facility, with a 12-h light-dark cycle and free access to water and a standard rodent chow (D03, SAFE, Villemoisson-sur-Orge, France). All animal procedures and experiments were approved by the local ethical committee (Comité d'Ethique en Expérimentation Animale IGBMC-ICS) for Animal Care and Use (Com'Eth 2011-007).

[170] Bi-daily clinical observation of mice was performed, body weight was recorded weekly and food intake every 2 days until the end of the protocol.

[171] For bio-distribution and gene therapy studies, 3-weeks-old mice were anesthetized with isoflurane (1-2%) and injected intravenously into the retro-orbital vein with a rAAV-FXN-HA vector at a dose of $1x10^{13}$ vg/kg for the treated group and with an equivalent volume of saline water for Untreated MCK Mutant mice and Control.

[172] Mouse cardiac function was evaluated under isoflurane anesthesia (1-2%) by echocardiography 2 days before starting the treatment (baseline phenotype), at 5 weeks of age (14 days after treatment) and 7 weeks of age (28 days after treatment). At 5 and 8 weeks of age, blood collection was performed to measure the concentration of the heart type fatty acid binding protein (H-FABP), galectin-3 and Succinate dehydrogenase (SDH) as detailed elsewhere herein.

[173] Upon sacrifice, body weight, body length, heart, spleen, kidney, adrenals, and liver weights were recorded from all animals. Adrenals, cerebellum, cervical, thoracic and lumbar vertebras, gonads (testes and ovaries), heart, kidney, liver, lungs, pancreas, prostate in males, skeletal muscle (gastrocnemius and soleus), spleen and thymus were collected from 4 animals per group for pathological evaluation and ELISA assays.

[174] Cerebellum (including dentate nucleus), cervical, thoracic and lumbar dorsal root ganglia, heart, kidneys, liver, lungs, gonads, pancreas, skeletal muscle (gastrocnemius and soleus), and spleen of 4 other animals per group were collected and immediately snap frozen for molecular biology.

Results

Identification of potential biomarkers

[175] The levels of various biomarkers were determined in three groups of mice: untreated Mck positive control, untreated Mck mutant mice and treated Mck mutant mice that received a dose of rAAV2i8 comprising a FXN gene and further comprising a nucleic acid encoding an HA tag peptide (AAV-FXN-HA).

Measurement of Galectin-3 and H-FABP in plasma

[176] Blood was collected by retro orbital puncture after isoflurane anaesthesia at the age of 5 weeks (2 weeks of AAV treatment for the treated Mck mutant mice) and 8 weeks (5 weeks of rAAV treatment for the treated Mck mutant mice group) and the levels of galectin-3 and H-FABP were measured using standard methods.

Galectin-3:

[177] At the age of 5 weeks, galectin-3 levels were comparable between the 3 groups, even if galectin-3 levels tended to be higher in the untreated Mck positive control group and in the treated Mck mutant mice group compared to the untreated Mck mutant mice group.

[178] As show in Table 3, at the age of 8 weeks, galectin-3 levels were significantly lower in the untreated Mck mutant group than in the negative control group. Galectin-3 levels tended to be lower in the experimental group than in the negative control group, while Galectin-3 levels were comparable between the experimental group and the positive control group.

TABLE 3

	Plasma Galectin-3 level (ng/ml)	
	week 5	**week 8**
	mean +/- sem	**mean +/- sem**
Untreated Mck positive control mice (n=8)	41.2 +/- 3.5	68 +/- 7.8
Untreated Mck mutant mice (n=8)	49.7 +/- 3.6	42 +/- 2.1
Treated Mck mutant mice (n=8)	49.7 +/- 4.4	48.9 +/- 4.5

[179] Surprisingly, mice of the untreated Mck positive control group displayed higher levels of Galectin-3 at the age of 8 weeks than at the age of 5 weeks, while the levels of Galectin-3 at the age of 8 weeks were comparable to the levels at the age of 5 weeks for the untreated Mck mutant mice group and for the treated Mck mutant mice group.

[180] In conclusion, it appears that Galectin-3 is not an appropriate heart biomarker for this study on Mck mice. The mice of the untreated Mck mutant mice group did not show an expected, if galectin-3 was an appropriate biomarker, increase in this parameter.

H-FABP:

[181] Great variability was observed in H-FABP levels between mice within the same sample group using standard methods of detection.

[182] As shown in Table 4, H-FABP blood levels were comparable between the 3 groups of mice both at the age of 5 weeks and 8 weeks.

TABLE 4

	Plasma H-FABP (ng/ml)	
	week 5	**week 8**
	mean +/- sem	**mean +/- sem**
Untreated Mck positive control (n=8)	177.8 +/- 33.9	98.8 +/- 25.0
Untreated Mck mutant mice (n=8)	187.1 +/- 38.8	139.8 +/- 41.5
Treated Mck mutant mice (n=8)	232.2 +/- 53.3	129.6 +/- 25.8

[183] No significant change was observed in H-FABP levels between the age of 5 and 8 weeks in each group.

[184] In conclusion, it seems that H-FABP is not the appropriate heart biomarker for this study on Mck mice; the expected increase in this parameter was not observed in the untreated Mck mutant group and an important variability was observed between mice in a same group.

SDH activity in heart homogenates

[185] SDH activity was measured in heart homogenate from heart collected at the end of the study (8 weeks of age, 5 weeks of AAV treatment in the treated mutant mice group) using standard methods. Each group was comprised of four (4) mice.

[186] The results shown in Table 5 show that SDH activity was comparable between the 3 groups of mice. No decrease was observed in SDH activity in the untreated Mck positive control group compared to the untreated Mck mutant group.

TABLE 5

	SDH activity in heart homogenate (U/g proteins)
Untreated Mck positive control (n=4)	4.14 +/- 0.52
Untreated Mck mutant mice (n=4)	3.64 +/- 0.77
Treated Mck mutant mice (n=4)	4.24 +/- 0.62

[187] An important variability in SDH activity was observed between mice in a same group. Further, the expected decrease in SDH activity in the untreated Mck positive control group was not observed.

Frataxin levels in heart, skeletal muscle and liver homogenates

[188] The level human frataxin protein was measured from heart, skeletal muscle and liver collected at sacrifice using standard methods as shown in Table 6.

[189] Human frataxin was not detectable in any of the tissues examined (heart, skeletal muscle and liver) of the untreated Mck positive control and the untreated Mck mutant groups (i.e., the level was below the lowest limit of detection [LLD] of the assay).

[190] In treated Mck mutant mice receiving rAAV-FXN, human frataxin protein was detected in heart homogenate at the level of 38.35 +/- 1.99 ng/mg, and in skeletal muscle at a lower concentration: 4.57 +/- 0.39 ng/mg. Furthermore, traces of human frataxin were detected in the liver (0.07 +/- 0.01 ng/mg of proteins).

TABLE 6

	Frataxin in tissue (ng/mg proteins)		
	heart	**skeletal muscle**	**liver**
	mean +/- sem	**mean +/- sem**	**mean +/- sem**
Negative control (n=4)	<LLD	<LLD	<LLD
Positive control (n=4)	<LLD	<LLD	<LLD
Experimental group (n=4)	38.35 +/- 1.99	4.57 +/- 0.39	0.07 +/- 0.01

[191] These data demonstrate that treatment with rAAV vector comprising a FXN gene can increase frataxin levels in a mouse model of Friedreich ataxia (FRDA) Additionally, these data show that FXN levels can be increased *in vivo* by rAAV-FXN systemic administration such that FXN levels are increased in heart and, to a lesser extent, skeletal muscle, with much lower level in the liver. Thus, these data demonstrate that *in vivo* FXN levels can be selectively increased in affected tissues, e.g., heart and skeletal muscle, while minimizing delivery of FXN where it is not needed and/or desired – i.e., to the liver.

Gross pathology

[192] Untreated Mck positive Control male mice were significantly longer compared to untreated and AAV-treated Mck mutant animals (9.39 cm vs 8.89 cm [+5.62%]. P = 0.011 [t-test]). No other significant macroscopic lesion was observed, especially no macroscopic lesion or significant change was observed in heart weight in both males and females.

Histology

Heart

[193] Minimal interstitial fibrosis was observed in one untreated Mck positive Control animal (#58). All other 3 untreated Mck positive Control group hearts were normal.

[194] However, minimal (mouse #38) and moderate (mice #41, #49, and 81) interstitial fibrosis was observed in all 4 untreated Mck mutant animals analyzed. This lesion was associated to endocardiac focus of cardiomyocytes swelling in mice #38 (minimal) and #81 (slight). Fibrosis was associated to moderate macrophagic inflammation, minimal disseminated swelling and slight vacuolization of cardiomyocyte, in mice #41 and #49. Anitschkow (Howl eye-shaped) nuclei were observed in mice #41 and #81.

[195] In stark contrast to the untreated Mck mutant cohort, on overall assessment, hearts of rAAV-FXN-treated Mck mutant mice appeared normal except that few Anitschkow nuclei were observed in mice #47 and #13.

Kidneys

[196] In all groups, significant mineralization was frequently observed in lumen of multiple medullar tubules. Frequency was 4/4 for untreated Mck positive control animals (although they express the *Cre* transgene), 3/4 for untreated Mck mutant animals and 2/4 for treated Mck mutant animals receiving a dose of rAAV-FXN. Severity of the mineralization appeared decreased in the rAAV-treated Mck Mutant group compared to untreated Mck positive Control and untreated Mck mutant groups. Tubular basophilia (regeneration) was observed in 2/4 animals in the untreated Mck positive control group, in 3/4 animals in the untreated Mck mutant group and in 1 animal in the AAV-treated Mck mutant group.

Liver: minimal periportal inflammation was observed in one untreated Mck positive control animal (#38).

Lung: slight peribronchial inflammation was observed in one untreated Mck mutant animal (#41).

[197] No other significant microscopic lesion was observed. Especially, spinal cord, dorsal root ganglia, and cerebellum were all normal.

Echocardiography

Basal phenotype before treatment

[198] Echocardiography measurements showed a reduced left-ventricular function in untreated Mck Mutant males mice compared to untreated Mck positive Control. This cardiac insufficiency is characterized by a decrease of the left ventricular (LV) contractility (shortening fraction and ejection fraction) and an increase of the LV volume, both systolic and diastolic. At that stage, no cardiac phenotype was observed in untreated Mck Mutant female mice.

[199] Figure 4A shows graphs evaluation of the systolic function and LV volumes by echocardiography at 3 weeks of age (A) males and (B) females. Data are mean ± S.E.M of 8 mice per groups. The data of Mck Mutant (both treated and untreated) mice were compared to

the untreated Mck positive Control group using multiple t-tests comparisons (Sidak-Bonferroni method). * $p<0.05$

Results 14 days after rAAV treatment (5 weeks of age):

[200] To investigate the potential of a gene therapy approach for the treatment of the FRDA cardiomyopathy, a single intravenous injection of AAV.FXN-HA at a dose of $1x10^{13}$ vg/kg was administered to 3-weeks-old Mutant mice (treated Mck mutant group). 14 days after injection (5 weeks of age), echocardiography measurements showed an improvement of the cardiac hemodynamics (cardiac output) and a near normal morphological development in Treated Mck Mutant males (contractility, LV mass). In contrast, cardiac insufficiency was still observed in untreated Mck Mutant mice. Surprisingly, in females, the cardiac contractility defect was observed in all Mck Mutant mice whether treated or untreated.

[201] Figures 5A-5B show the evaluation of the systolic function and LV volumes by echocardiography at 5 weeks of age in males (Fig. 5A) and females (Fig. 5B). Data are mean ± S.E.M of 8 mice per groups. The data of Mutant mice were compared to the Control groups using multiple t-tests comparisons (Sidak-Bonferroni method). * $p<0.05$.

Results 28 days after rAAV treatment (7 weeks of age):

[202] Twenty-eight (28) days rAAV treatment (7 weeks of age), treated Mck mutant males and females were fully normalized and became indistinguishable between them and from untreated Mck positive control mice. This, a complete correction (males) and prevention (females) of the cardiac disease was demonstrated in treated Mck mutant mice. In contrast, untreated Mck Mutant mice developed a rapidly progressing cardiac insufficiency, with a marked decrease in left ventricle shortening fraction and cardiac output, as well as left ventricle hypertrophy.

[203] Figures 6A-6C show graphs depicting data obtained using echocardiography assessment of the left ventricle mass (LVm), shortening fraction (SF) and cardiac output for untreated Mck positive control, and Treated and Untreated Mck mutant mice over successive weeks. Data are mean ± S.E.M of 8 mice per groups. The data of Mck Mutant mice were compared to the untreated Mck positive control group using multiple t-tests comparisons (Sidak-Bonferroni method). * $p<0.05$.

Conclusions

Results of Echocardiography

[204] In this study, the efficacy of an rAAV-FXN optionally further comprising a detectable hemagglutinin tag (HA) vector (referred to herein as rAAV-FXN-HA) at a dose of $1x10^{13}$ vg/kg was assessed. This dose was approximately 5-fold less than the dose previously described in

the same Mck mouse cardiac-specific Friedreich ataxia mouse model using an rrhAAV10 vector encoding wild type FXN (Perdomini et al., 2014, Nature Medicine 20(5):542).

[205] As shown in Figure 5A, at 3 weeks of age, the untreated Mck Mutant male mice started to develop a left ventricular (LV) dysfunction, not observed in females of the same group at the same age (Figure 5B). Fourteen (14) days after the AAV.FXN-HA injection (at 5 weeks of age), a progressive correction of the cardiac phenotype was observed in Mck mutant males, but it was less in Mck mutant females. Without wishing to be bound by any particular theory, this difference may be due to a later start of the cardiac phenotype in females compared to males or to the reduced number of mice used so far in this protocol.

[206] These results suggest that systemic administration of rAAV-modified FXN reverses the cardiac disease phenotype in Mck mutant mouse model of FRDA. These results further suggest that rAAV-modified FXN administration can prevent and/or reverse FRDA in a subject in need thereof.

[207] Twenty-eight (28) days post-rAAV-FXN injection, a complete recovery of the cardiac function was observed in treated Mck mutant males and females, suggesting a robust correction of the pathology by the injected FXN transgene. That is, the data shown in Figures 6A-6C demonstrate the correction in the FRDA cardiac phenotype by twenty-eight (28) days after rAAV-FXN administration. More specifically, Figure 6A shows the left ventricle mass (LVm) of both treated Mck mutant mice and control (WT wild type Mck-Cre mice) is indistinguishable while the untreated (triangles) Mck mutant mice exhibit significantly greater LVm (*$p<0.05$). Figure 6B shows data demonstrating that by 28 days after rAAV-FXN treatment, both positive control (WT L3 Mck-Cre mice; circles) and treated Mck mutant (L-) mice (squares) demonstrated substantially identical shortening fraction (SF) measurements. In contrast, Figure 6B demonstrates that untreated Mck mutant mice (triangles) demonstrated greatly decreased SF (*$p<0.05$). In addition, Figure 6C shows data demonstrating that by 28 days following treatment with rAAV, treated Mck mutant mice (squares) exhibited cardiac output that was indistinguishable from control mice (circles) compared with untreated Mck mutant mice (triangles) which showed markedly decreased cardiac output (triangles; *$p<0.05$). All (treated and untreated) Mck mutant mice were compared with the control untreated mice using multiple t-test comparisons (Sidak-Bonferroni method). For each graph shown in Figures 6A-6C, *<0.05 is indicated.

[208] These data amply demonstrate that administration of rAAV comprising modified nucleic acid encoding frataxin can reverse, and/or prevent, the Mck phenotype in an art-recognized mouse model of FRDA. Thus, these data support that rAAV-modified FXN mediated treatment may be a potential useful therapeutic to treat, or prevent, FRDA, or a disease, disorder or condition mediated by decreased level of wild type (e.g., functional) frataxin, in a subject in

need thereof. Although these data demonstrate that rAAV-modified-FXN administered systemically can treat or prevent FRDA, these results further support that therapy also includes other, *e.g.,* more direct, routes of rAAV-FXN administration, such as, but not limited to, intracranial or direct cardiac administration. That is, because systemic administration was demonstrated to be therapeutic, one skilled in the art would understand based upon the disclosure provided herein that more direct administration routes can also provide a therapeutic benefit.

[209] These results strongly support that gene therapy using rAAV vector delivery of a modified FXN gene is a potential therapeutic approach for patients with FRDA and to treat or prevent kidney stones in a subject demonstrating a decreased level of wild type/functional frataxin.

Example 3: Histology Study of in vivo administration of rAAV-FXN in a mouse model of Friedreich ataxia

Study design

[210] Twenty four (24) 8-weeks old C57BL6/N male and female mice were assessed for histopathological analyses.

[211] Eight (8) mice harbored a Mck-Cre transgene (MCK: Muscular Creatine Kinase) associated to a functional engineered human Frataxin allele (*Mck*-Cre x FXN L3/WT; hereinafter referred as "Mck positive control mice"). Sixteen (16) mice harbored the same transgene now associated to an inactive engineered frataxin allele (*Mck*-Cre x FXN L3/L-; hereinafter referred to as "Mck mutant mice"). Among the Mck mutant mice, eight (8) were injected with a frataxin-encoding rAAV2i8 (rAAV-FXN; 10^{13} vg/kg) (hereinafter "treated Mck mutant mice"). The remaining eight (8) Mck mutant mice received an equivalent volume of saline water (hereinafter "untreated Mck mutant mice"). The positive control mice group (*Mck*-Cre x FXN L3/WT) were administered saline water. See Table 7 below.

TABLE 7

Groups label	Group No.	Dose Level vg/kg	No. of Animals (Mixed gender)	Termination Weeks of age
Mck positive control mice	*Mck*-Cre x FXN L3/WT	0	8	Week 8
Untreated Mck mutant mice	Untreated *Mck*-Cre x FXN L3/L-	0	8	Week 8
Treated Mck mutant mice	rAAV-FXN-HA treated	$1x10^{13}$	8	Week 8

	Mck-Cre x FXN L3/L-			

Methods

[212] Upon sacrifice, body weight, body length and heart, spleen, kidney, adrenals, and liver weight weights were recorded from all animals. Adrenals, cerebellum, cervical, thoracic and lumbar vertebras, Gonads (testes and ovaries), heart, kidney, liver, lungs, pancreas, prostate in males, skeletal muscle (gastrocnemius and soleus), spleen, and thymus, were collected from four (4) animals per group for pathological evaluation and ELISA assays.

[213] Cerebellum (including dentate nucleus), cervical, thoracic and lumbar dorsal root ganglia, heart, kidneys, liver, lungs, gonads, pancreas, skeletal muscle (gastrocnemius and soleus), and spleen of 4 other animals per group were collected and immediately snap frozen for molecular biology.

Histology

[214] Cerebellum (including dentate nucleus), gonads, heart, kidney, liver, lung, pancreas, skeletal muscle (gastrocnemius and soleus), spleen and cervical, thoracic and lumbar vertebras were formol-fixed. Vertebras were then decalcified, using EDTA solution. All organs were paraffin embedded to obtain 5 µm-thick sections, transversal sections for vertebras (including both spinal cord and dorsal root ganglia) and heart. All organs were hematoxylin and eosin stained. Cardiac fibrosis was evaluated using Masson's trichrome staining.

ELISA assays

[215] Half of the heart, right lobe of the liver, and soleus and gastrocnemius muscles were snap frozen immediately after collection.

Results

[216] **Gross pathology**

[217] Mck positive control male mice were significantly longer compared to untreated Mck mutant mice and treated Mck mutant mice (9.39 cm vs 8.89 cm [+5.62%]. P = 0.011 [t-test]). No other significant macroscopic lesion was observed, especially no macroscopic lesion or significant change was observed in heart weight in both males and females.

Histology

Heart

[218] *Mck*-Cre x FXN L3/WT: Minimal interstitial fibrosis was observed in one Mck positive control animal (#58). All other 3 positive control mouse hearts were normal.

[219] Untreated *Mck*-Cre x FXN L3/L-: In contrast, minimal (mouse #38) and moderate (mice #41, #49, and 81) interstitial fibrosis was observed in all 4 untreated Mck mutant mice analyzed. This lesion was associated to endocardiac focus of cardiomyocytes swelling in mice #38

(minimal) and #81 (slight). Fibrosis was associated to moderate macrophagic inflammation, minimal disseminated swelling and slight vacuolization of cardiomyocyte, in mice #41 and #49. Anitschkow (owl eye-shaped) nuclei were observed in mouse #41 and #81.

[220] Treated *Mck*-Cre x FXN L3/L-: In contrast to untreated Mck mutant mice, hearts of rAAV-FXN treated Mck mutant mice appeared normal except that a few Anitschkow nuclei were observed in mice #47 and #13.

Kidneys

[221] In all three groups, significant mineralization was frequently observed in the lumen of multiple medullar tubules. Frequency of mineralization was 4/4 for Mck positive control animals, 3/4 for untreated Mck mutant animals and 2/4 for rAAV-FXN treated Mck mutant animals. The severity of mineralization appeared decreased in rAAV-FXN treated Mck mutant group compared to Mck positive control and untreated Mck mutant groups. Tubular basophilia (regeneration) was observed in 2/4 animals in Mck positive control mice, in 3/4 animals in the untreated Mck mutant group and in 1 animal in the rAAV-FXN treated Mck mutant group.

Liver: minimal periportal inflammation was observed in one Mck positive control animal (#38).

Lung: slight peribronchial inflammation was observed in one untreated Mck mutant animal (#41).

[222] No other significant microscopic lesion was observed. Especially, spinal cord, dorsal root ganglia, and cerebellum were all normal for all groups.

Results of Histology

[223] With respect to histology, owl eye-nuclei, swelling and vacuolization observed in cardiomyocytes of untreated Mck mutant mice are all landmarks for cardiac degeneration. Interstitial fibrosis associated to macrophage inflammation may correspond to cardiomyocyte cell death. Thus, cardiomyocytes of untreated Mck mutant mice degenerate, meaning cells undergo decreased function and pathology evolve to cell death and subsequent heart failure.

[224] Strikingly, rAAV-FXN systemic delivery reverses this phenotype and rAAV-treated Mck mutant mice (both males and females) appeared normal and showed no significant sign of cardiomyocytes degeneration. These data demonstrate that the rAAV-huFXN transduction is sufficiently efficient to reverse the endogenous mouse Fxn gene inactivation effects. Thus, these data further support that rAAV-modified FXN mediated systemic administration can reverse and/or prevent a disease, disorder or condition mediated by a decreased level of wild type (functional) frataxin, such as, but not limited to, Friedreich ataxia, in a subject in need thereof. As noted previously, the skilled artisan, armed with the teachings of the instant disclosure, would appreciate that other routes of administration, e.g., more direct routes

including intracranial and into the heart, could be used to provide a therapeutic benefit to the subject in need thereof.

[225] Analysis of the kidneys identified the presence of kidney stones in medulla of both untreated Mck positive control and untreated Mck mutant animals which is an uncommon lesion. Since no specific diet was proposed to these animals and considering their age, the frequency and the severity of the lesions strongly suggest that this is not an incidental lesion, but a lesion related to genotype. Interestingly, this lesion severity is partially reduced by rAAV-FXN treatment, suggesting that the kidney stones development is related to an alteration in the Fxn function and or in the level of the protein. Hence, the so-called L3 allele, in which the mouse frataxin gene is flanked by loxP sequences (although in the intronic regions), could be a hypomorph allele. This would be consistent with the critical role of mitochondria in these cells to maintain trans-epithelial electrolyte active transports. To the best of applicants' knowledge and belief, these lesions have not been reported in Friedreich ataxia clinical observations, or in FRDA mouse models, to date.

[226] Therefore, the invention encompasses a method of treating or preventing kidney disease, disorder or condition, including, but not limited to, development or growth of kidney stones, in a subject in need thereof, wherein the kidney disease, disorder or condition is mediated by a decreased level of frataxin (e.g., functional and/or wild type frataxin) in the subject.

[227] In one embodiment, the invention includes assessing the level of frataxin in a subject, comparing the level of frataxin in the subject with the level of frataxin in a subject known not to be afflicted with kidney stones and/or comparing the level of frataxin with a "standard level of frataxin" determined for otherwise healthy individuals or known in the art, and administering a rAAV-modified frataxin to the subject if the level of frataxin in the subject is less than the level of frataxin in an otherwise healthy individual and/or below the standard level of frataxin, thereby treating and/or preventing a kidney stone in the subject.

[228] Finally, HA staining can be used to detect rAAV-FXN-HA within cells. First, this would be important to quantitate how many cells should express FXN to restore or maintain the cardiac function. Second, the Mck gene (and thus the Cre recombinase driven by the Mck promoter) is not expected to be expressed in kidney. Detecting, or not, rAAV-FXN-HA in kidney may help to elucidate whether the kidney stone formation is an unexpected direct effect of HA-FXN on medulla homeostasis, or not.

Conclusion

[229] In sum, the data presented herein demonstrate that administration, even systemically, of rAAV encoding a modified FXN gene can treat (prevent and/or reverse) the effects associated with a decrease or absence of frataxin. Thus, the data support that rAAV mediated expression

of frataxin in cells and subjects in need thereof can be a useful therapeutic to treat a disease or disorder associated with or mediated by a lack or deficit of frataxin such as, but not limited to, Friedreich ataxia.

[230] Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

[231] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

[232] The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

TABLE 8

SEQUENCES

SEQ ID NO:1	Amino acid sequence of human wild type frataxin	MWTLGRRAVA TGLLASPSPA QAQTLTRVPR PAELAPLCGR RGLRTDIDAT CPRRASSNQR GLNQIWNVKK QSVYLMNLRK SGTLGHPGSL DETTYERLAE ETLDSLAEFF EDLADKPYTF EDYDVSFGSG VLTVKLGGDL GTYVINKQTP NKQIWLSSPS SGPKRYDWTG KNWVYSHDGV SLHELLAAEL TKALKTKLDL SSLAYSGKDA
SEQ ID NO:2	Nucleotide sequence encoding wild type frataxin (Figs. 1A-1B; lane 1)	ATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGC GTCACCCAGCCCAGCCCAGGCCCAGACCCTCACCCGGGTCC CGCGGCCGGCAGAGTTGGCCCCACTCTGCGGCCGCCGTGGC CTGCGCACCGACATCGATGCGACCTGCACGCCCCGCCGCGC AAGTTCGAACCAACGTGGCCTCAACCAGATTTGGAATGTCA AAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAATCTGGA ACTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGA AAGACTAGCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTT TTGAAGACCTTGCAGACAAGCCATACACGTTTGAGGACTAT

		GATGTCTCCTTTGGGAGTGGTGTCTTAACTGTCAAACTGGG TGGAGATCTAGGAACCTATGTGATCAACAAGCAGACGCCAA ACAAGCAAATCTGGCTATCTTCTCCATCCAGTGGACCTAAG CGTTATGACTGGACTGGGAAAAACTGGGTGTACTCCCACGA CGGCGTGTCCCTCCATGAGCTGCTGGCCGCAGAGCTCACTA AAGCCTTAAAAACCAAACTGGACTTGTCTTCCTTGGCCTAT TCCGGAAAAGATGCT
SEQ ID NO:3	IDT2 optimized nucleotide sequence encoding frataxin (Figs. 1A-1B; lane 2)	ATGTGGACACTGGGCAGAAGGGCGGTGGCCGGACTGTTGGC GAGTCCCAGTCCCGCGCAGGCGCAGACCCTTACTAGGGTGC CGCGGCCCGCGGAGCTGGCGCCACTCTGCGGTCGCCGCGGT CTGAGAACGGACATTGATGCCACTTGTACACCTCGGAGG GCCAGCTCCAACCAAAGGGGCCTTAATCAAATTTGGAACG TGAAGAAGCAGTCCGTCTACCTGATGAACCTTCGGAAGTCA GGGACCCTGGGCCACCCGGGAAGCTTGGATGAAACAACTT ACGAAAGGTTGGCGGAGGAGACCTTGGATTCTCTTGCAGA GTTCTTCGAAGACCTGGCTGATAAGCCTTACACCTTTGAGG ACTACGATGTGTCTTTTGGATCTGGAGTGCTGACCGTTAAA CTGGGCGGGGATCTGGGCACCTACGTGATTAACAAGCAAAC TCCAAACAAGCAGATCTGGCTTTCAAGCCCCAGTAGCGG GCCAAAACGCTACGATTGGACCGGAAAGAATTGGGTTTACA GCCACGATGGCGTTTCACTGCACGAGCTTCTGGCAGCAGAA CTGACAAAAGCACTCAAGACGAAGCTCGACTTGTCATCCTT GGCATACTCCGGAAAGGATGCC
SEQ ID NO: 4	JCAT Optimized Nucleotide sequence encoding frataxin (Figs. 1A-1B; Lane 4)	ATGTGGACCCTGGGCCGCCGCGCCGTGGCCGGCCTGCTGGC CAGCCCCAGCCCCGCCCAGGCCCAGACCCTGACCCGCGTGC CCCGCCCCGCCGAGCTGGCCCCCCTGTGCGGCCGCCGCGGCC TGCGCACCGACATCGACGCCACCTGCACCCCCCGCCGCGCC AGCAGCAACCAGCGCGGCCTGAACCAGATCTGGAACGTGA AGAAGCAGAGCGTGTACCTGATGAACCTGCGCAAGAGCGG CACCCTGGGCCACCCCGGCAGCCTGGACGAGACCACCTAC GAGCGCCTGGCCGAGGAGACCCTGGACAGCCTGGCCGAGTT CTTCGAGGACCTGGCCGACAAGCCCTACACCTTCGAGGACT ACGACGTGAGCTTCGGCAGCGGCGTGCTGACCGTGAAGCTG GGCGGCGACCTGGGCACCTACGTGATCAACAAGCAGACCCC CAACAAGCAGATCTGGCTATCTAGCCCCAGCAGCGGCCCC AAGCGCTACGACTGGACCGGCAAGAACTGGGTGTACAGCCA CGACGGCGTGAGCCTGCACGAGCTGCTGGCCGCCGAGCTG ACCAAGGCCCTGAAGACCAAGCTGGACCTGAGCAGCCTG GCCTACAGCGGCAAGGACGCC
SEQ ID NO: 5	GeneArt optimized nucleotide sequence encoding frataxin (Figs. 1A-1B; Lane 5)	ATGTGGACACTGGGGAGAAGGGCTGTGGCCGGACTGCTGGC TTCTCCATCTCCAGCCCAGGCCCAGACCCTGACCAGAGTGC CTAGACCTGCCGAACTGGCCCCTCTGTGTGGCAGAAGAGGC CTGAGAACCGACATCGACGCCACCTGTACCCCCAGAAGGGC CAGCAGCAATCAGCGGGGCCTGAATCAGATCTGGAACGTGA AGAAACAGAGCGTGTACCTGATGAACCTGAGAAAGAGCGGC ACCCTGGGCCACCCTGGAAGCCTGGATGAGACAACCTACGA GCGGCTGGCCGAGGAAACCCTGGATTCCCTGGCCGAGTTCT TCGAGGACCTGGCCGACAAGCCCTACACCTTCGAGGATTAC GACGTGTCCTTCGGCAGCGGCGTGCTGACAGTGAAGCTGGG CGGAGATCTGGGCACCTACGTGATCAACAAGCAGACCCCCA ACAAACAGATCTGGCTATCTAGCCCCAGCAGCGGCCCCAAG AGATACGATTGGACCGGCAAGAACTGGGTGTACAGCCACGA

		CGGCGTGTCCCTGCATGAGCTGCTGGCTGCCGAGCTGACCA AGGCCCTGAAAACAAAGCTGGACCTGTCCAGCCTGGCCTAC AGCGGCAAGGATGCC
SEQ ID NO:6	Genscript (control) optimized Nucleotide sequence encoding frataxin Figs. 1A-1B; lane 6)	ATGTGGACACTGGGCCGGAGAGCCGTCGCTGGGCTGCTGGC ATCACCATCCCCCGCACAGGCACAGACCCTGACAAGAGTCC CTCGGCCAGCAGAGCTGGCCCCACTGTGCGGGCGGAGAGGA CTGCGAACCGACATCGATGCTACTTGTACCCCAAGGCGAG CAAGCTCCAACCAGCGAGGGCTGAACCAGATTTGGAATGTG AAGAAACAGTCTGTCTACCTGATGAATCTGAGAAAGAGCGG CACTCTGGGACACCCTGGCAGCCTGGACGAGACCACCTAC GAGCGGCTGGCCGAGGAAACCCTGGATTCCCTGGCCGAGTT CTTTGAAGACCTGGCTGATAAGCCATACACCTTCGAAGACTA TGACGTGAGCTTCGGCAGCGGCGTGCTGACAGTCAAACT GGGCGGGGACCTGGGAACATACGTGATCAACAAGCAGACT CCTAACAAGCAGATTTGGCTGTCTAGTCCCTCAAGCGGCCC TAAGAGGTACGACTGGACAGGGAAAAACTGGGTGTATAGT CACGATGGCGTCTCACTGCATGAGCTGCTGGCCGCTGAACT GACTAAAGCCCTGAAAACTAAACTGGACCTGTCTTCCCTG GCATACTCTGGCAAGGACGCC
SEQ ID NO:7	Genscript (low CpG) nucleotide sequence encoding frataxin (Figs. 1A-1B; Lane 7)	ATGTGGACTCTGGGCCGGAGAGCAGTGGCAGGACTGCTGGC AAGTCCATCACCTGCTCAGGCACAGACTCTGACAAGAGTCC CAAGACCTGCAGAGCTGGCTCCACTGTGCGGGAGGCGCGGA CTGAGAACAGACATCGATGCTACATGTACTCCTCGACGGGC AAGCTCCAACCAGCGAGGGCTGAACCAGATTTGGAATGTG AAGAAACAGTCCGTCTACCTGATGAATCTGAGGAAGTCA GGCACCCTGGGGCACCCAGGAAGTCTGGACGAGACCACAT ATGAACGGCTGGCTGAGGAAACACTGGATTCTCTGGCCGAG TTCTTTGAAGACCTGGCTGATAAGCCCTACACATTCGAAGAC TATGATGTGAGCTTTGGATCCGGCGTGCTGACTGTCAAACTG GGCGGGGACCTGGGCACTTACGTGATCAACAAGCAGACCCC TAACAAGCAGATTTGGCTGTCTAGTCCTTCAAGCGGACCAA AGCGGTACGACTGGACCGGCAAAAACTGGGTGTATTCTC ACGATGGGGTCAGTCTGCATGAGCTGCTGGCCGCTGAACT GACCAAGGCCCTGAAGACAAAACTGGACCTGTCCTCTCTG GCATATAGCGGAAAAGATGCC
SEQ ID NO:8	IDT3 optimized Nucleotide sequence encoding frataxin	ATGTGGACACTGGGAAGGCGCGCCGTGGCCGGTCTGTTGGC ATCACCATCCCCAGCCCAGGCTCAGACACTCACCCGAGTC CCAAGACCCGCAGAGCTGGCCCCTCTGTGCGGGCGCCGA GGCCTTCGCACCGATATCGATGCTACATGCACGCCACGCAG AGCTAGCTCAAATCAGAGGGGACTCAACCAGATATGGAATG TCAAGAAGCAAAGCGTGTATCTCATGAACCTCCGGAAAAGC GGCACCCTGGGACATCCCGGGTCTCTCGACGAGACCACT TATGAAAGACTGGCAGAGGAGACTCTTGACAGTCTGGCGG AGTTCTTCGAAGACCTCGCTGACAAGCCATATACCTTCGAAG ATTACGACGTCTCCTTCGGCTCTGGGGTGCTGACTGTCAA GCTTGGCGGCGACCTGGGGACCTACGTGATCAACAAGCAG ACTCCAAACAAGCAAATCTGGCTATCTAGTCCAAGCTCCGG ACCCAAGAGATACGATTGGACAGGCAAGAATTGGGTTTACT CCCACGACGGGGTGTCCCTCCATGAGCTGCTGGCCGCAGAG CTGACGAAGGCCCTGAAGACCAAGCTGGATCTCTCCTCCCT GGCATACAGTGGTAAGGACGCT
SEQ ID NO:9	IDT5 optimized Nucleotide sequence encoding frataxin	ATGTGGACACTGGGCCGGCGCGCCGTCGCTGGGCTGCTCGC AAGCCCCAGCCCAGCCCAAGCGCAGACTCTGACTAGGGTGC CGCGGCCTGCCGAGTTGGCCCCCCTGTGCGGTAGGAGAGGC

	(Figs. 1A-1B; Lane 3)	CTGCGCACAGACATCGATGCCACTTGCACACCCCGGCGGGC CAGCTCTAACCAAAGGGGCCTGAATCAAATTTGGAACGTCA AAAAACAGTCTGTATATCTGATGAATCTCCGGAAATCTGGA ACGCTCGGGCATCCCGGATCTCTTGACGAGACCACCTACGA GCGACTGGCCGAGGAAACCCTTGACAGCCTGGCAGAATTCT TTGAGGATCTGGCTGATAAACCCTATACCTTTGAAGATTAC GATGTGAGTTTTGGTAGCGGAGTACTGACTGTTAAGCTGGG CGGTGATCTCGGTACGTATGTTATCAATAAACAAACCCCCA ATAAACAGATTTGGCTCTCCTCCCCATCCTCTGGGCCTAAG CGCTATGACTGGACAGGAAAGAATTGGGTCTATTCACACGA CGGAGTCAGTTTGCACGAGCTCCTCGCCGGCAGAGTTACCA AGGCCCTTAAGACTAAGCTCGACCTGTCAAGCCTCGCTTAC TCTGGTAAGGACGCT
SEQ ID NO:10	Nucleotide sequence encoding frataxin (nucleic acid 22)	ATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGC GTCACCCAGCCCGGCCCAGGCCCAGACCCTCACCCGGGTCC CGCGGCCGGCAGAGTTGGCCCCACTCTGCGGCCGCCGTGGC CTGCGCACCGACATCGATGCGACCTGCACGCCCCGCCGCGC AAGTTCGAACCAACGTGGCCTCAACCAGATTTGGAATGTCA AAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAATCTGGAA CTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAA AGACTAGCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTT TTGAAGACCTTGCAGACAAGCCATACACGTTTGAGGACTA TGATGTCTCCTTTGGGAGTGGTGTCTTAACTGTCAAACTG GGTGGAGATCTAGGAACCTATGTGATCAACAAGCAGACGC CAAACAAGCAAATCTGGCTATCTTCTCCATCCAGTGGACCT AAGCGTTATGACTGGACTGGGAAAAACTGGGTGTACTCCC ACGACGGCGTGTCCCTCCATGAGCTGCTGGCCGCAGAGCT CACTAAAGCCTTAAAAACCAAACTGGACTTGTCTTCCTTGGC CTATTCCGGAAAAGATGCT
SEQ ID NO:11	IDT-1 optimized Nucleotide sequence encoding frataxin (nucleic acid 23)	ATGTGGACTCTGGGTAGGCGAGCGGTGGCCGGCCTGTTGGC ATCTCCTAGTCCTGCACAAGCTCAAACGCTGACTAGAGTCC CTCGGCCAGCAGAACTGGCGCCACTTTGCGGCCGGCGCGGT CTTCGCACTGATATTGATGCCACTTGCACACCCCGGCGCGC CTCCAGTAATCAGCGGGGACTTAATCAAATTTGGAATGTG AAGAAGCAGTCTGTGTATCTTATGAATCTGCGGAAGAGC GGGACCCTGGGCCACCCTGGTAGCCTTGATGAAACCACCTA TGAGCGCCTGGCCGAAGAGACACTGGACAGTCTTGCCGAGT TTTTTGAGGATCTGGCCGACAAACCTTATACTTTTGAGGA CTATGACGTGTCCTTTGGATCTGGTGTATTGACCGTAAAA CTCGGGGGGAGACCTTGGGACGTATGTAATAAATAAGCAGA CCCCAAACAAGCAGATCTGGCTATCTTCTCCAAGTAGTGGT CCTAAGAGATATGATTGGACGGGCAAGAACTGGGTCTATTCC CATGATGGCGTCTCTTTGCATGAACTCCTTGCAGCAGAGCT GACCAAGGCCTTGAAGACCAAATTGGATCTCAGCAGCCT CGCCTATAGTGGCAAAGATGCA
SEQ ID NO:12	IDT-4 optimized Nucleotide sequence encoding frataxin (nucleic acid 26)	ATGTGGACTCTGGGCCGGCGGGCCGTAGCTGGCTTGCTGGC TAGCCCAAGTCCCGCCCAGGCTCAGACTCTCACCAGGGTAC CCAGGCCCGCAGAGCTTGCTCCACTCTGCGGACGCAGGGGT CTGCGAACCGATATCGACGCAACTTGCACGCCGCGGAGGGC CTCTTCAAACCAGAGAGGACTCAATCAAATTTGGAATGTAA AGAAACAGAGCGTGTATCTCATGAACCTCCGAAAGAGTGGG ACTCTTGGGCACCCCGGCTCCCTGGACGAGACTACTTACGAG CGCCTGGCCGAAGAAACCTTGGATTCCCTGGCGGAGTTTTT TGAAGACTTGGCAGACAAGCCTTATACCTTCGAGGATTAC

		GACGTGAGTTTTGGCTCTGGTGTTCTTACAGTCAAGCTCGGT GGCGACCTTGGCACTTATGTAATTAACAAGCAGACACCTAAC AAGCAGATCTGGCTTTCTAGTCCGTCTTCCGGTCCCAAAAG GTACGATTGGACTGGAAAGAACTGGGTCTACAGTCACGACG GTGTCTCCCTGCACGAATTGCTTGCGGCAGAGCTGACTAAG GCGCTCAAAACAAAACTGGATCTGTCCAGCCTTGCCTATAG CGGGAAGGACGCA
SEQ ID NO:13	Nucleotide sequence encoding chimeric AAV2.5 Vector Capsid VP1	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAC TCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTG GCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGACGAC AGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGGCAG ACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCAG CTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGC CGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTT TTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAG AGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAA GACGGCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCTG TGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCCAG CAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGA CGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCAC CAGCAGCCCCCTCTGGTCTGGGAACTAATACGATGGCTACA GGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGA CGGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCA CATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACC TGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAAT TTCCAGCGCTTCAACGGGAGCCTCGAACGACAATCACTACT TTGGCTACAGCACCCCTTGGGGGTATTTTGACTTCAACAGA TTCCACTGCCACTTTTCACCACGTGACTGGCAAAGACTCAT CAACAACAACTGGGGATTCCGACCCAAGAGACTCAACTTCA AGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGAC GGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCA GGTGTTTACTGACTCGGAGTACCAGCTCCCGTACGTCCTCG GCTCGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGCAGAC GTCTTCATGGTGCCACAGTATGGATACCTCACCCTGAACAA CGGGAGTCAGGCAGTAGGACGCTCTTCATTTTACTGCCTGG AGTACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACTTT ACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAG CTACGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTC TCATCGACCAGTACCTGTATTACTTGAGCAGAACAAACACT CCAAGTGGAACCACCACGCAGTCAAGGCTTCAGTTTTCTCA GGCCGGAGCGAGTGACATTCGGGACCAGTCTAGGAACTGGC TTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACA TCTGCGGATAACAACAACAGTGAATACTCGTGGACTGGAGC TACCAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATC CGGGCCCGGCCATGGCAAGCCACAAGGACGATGAAGAAAAG TTTTTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGG CTCAGAGAAAACAAATGTGGACATTGAAAAGGTCATGATTA CAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGCTACG GAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGGCAA CAGACAAGCAGCTACCGCAGATGTCAACACACAAGGCGTTC TTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAG GGGCCCATCTGGGCAAAGATTCCACACACGGACGGACATTT TCACCCCTCTCCCCTCATGGGTGGATTCGGACTTAAACACC

		CTCCTCCACAGATTCTCATCAAGAACACCCCGGTACCTGCG AATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTT CATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCG AGTGGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCC GAAATTCAGTACACTTCCAACTACGCCAAGTCTGTCAATGT GGACTTTACTGTGGACAATAATGGCGTGTATTCAGAGCCTC GCCCCATTGGCACCAGATACCTGACTCGTAATCTGTAA
SEQ ID NO:14	Nucleotide sequence encoding wild type AAV1 capsid (VP1)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAA CCTCTCTGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTG GAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGAC GGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGG ACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAG CTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGC CGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTT TTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAG CGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAA GACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCAC AAGAGCCAGACTCCTCCTCGGGCATCGGCAAGACAGGCCAG CAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGA CTCAGAGTCAGTCCCCGATCCACAACCTCTCGGAGAACCTC CAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCA GGCGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGA CGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCA CATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGCACC TGGGCCTTGCCCACCTACAATAACCACCTCTACAAGCAAAT CTCCAGTGCTTCAACGGGGGCCAGCAACGACAACCACTACT TCGGCTACAGCACCCCCTGGGGGTATTTTGATTTCAACAGA TTCCACTGCCACTTTTCACCACGTGACTGGCAGCGACTCAT CAACAACAATTGGGGATTCCGGCCCAAGAGACTCAACTTCA AACTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGAT GGCGTCACAACCATCGCTAATAACCTTACCAGCACGGTTCA AGTCTTCTCGGACTCGGAGTACCAGCTTCCGTACGTCCTCG GCTCTGCGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGAC GTGTTCATGATTCCGCAATACGGCTACCTGACGCTCAACAA TGGCAGCCAAGCCGTGGGACGTTCATCCTTTTACTGCCTGG AATATTTCCCTTCTCAGATGCTGAGAACGGGCAACAACTTT ACCTTCAGCTACACCTTTGAGGAAGTGCCTTTCCACAGCAG CTACGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTC TCATCGACCAATACCTGTATTACCTGAACAGAACTCAAAAT CAGTCCGGAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCG TGGGTCTCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGGC TACCTGGACCCTGTTATCGGCAGCAGCGCGTTTCTAAAACA AAAACAGACAACAACAACAGCAATTTTACCTGGACTGGTGC TTCAAAATATAACCTCAATGGGCGTGAATCCATCATCAACC CTGGCACTGCTATGGCCTCACACAAAGACGACGAAGACAAG TTCTTTCCCATGAGCGGTGTCATGATTTTTGGAAAAGAGAG CGCCGGAGCTTCAAACACTGCATTGGACAATGTCATGATTA CAGACGAAGAGGAAATTAAAGCCACTAACCCTGTGGCCACC GAAAGATTTGGGACCGTGGCAGTCAATTTCCAGAGCAGCAG CACAGACCCTGCGACCGGAGATGTGCATGCTATGGGAGCAT TACCTGGCATGGTGTGGCAAGATAGAGACGTGTACCTGCAG GGTCCCATTTGGGCCAAAATTCCTCACACAGATGGACACTT TCACCCGTCTCCTCTTATGGGCGGCTTTGGACTCAAGAACC

		CGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTGCG AATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATT CATCACCCAATACTCCACAGGACAAGTGAGTGTGGAAATTG AATGGGAGCTGCAGAAAGAAAACAGCAAGCGCTGGAATCCC GAAGTGCAGTACACATCCAATTATGCAAAATCTGCCAACGT TGATTTTACTGTGGACAACAATGGACTTTATACTGAGCCTC GCCCCATTGGCACCCGTTACCTTACCCGTCCCCTGTAA
SEQ ID NO:15	Nucleotide sequence encoding modified AAV1.1 capsid VP1 (amino acid residue number 265 is deleted)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAA CCTCTCTGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTG GAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGAC GGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGG ACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAG CTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGC CGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTT TTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAG CGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAA GACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCAC AAGAGCCAGACTCCTCCTCGGGCATCGGCAAGACAGGCCAG CAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGA CTCAGAGTCAGTCCCCGATCCACAACCTCTCGGAGAACCTC CAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCA GGCGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGA CGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCA CATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGCACC TGGGCCTTGCCCACCTACAATAACCACCTCTACAAGCAAAT CTCCAGTGCTTCAGGGGCCAGCAACGACAACCACTACTTCG GCTACAGCACCCCCTGGGGGTATTTTGATTTCAACAGATTC CACTGCCACTTTTCACCACGTGACTGGCAGCGACTCATCAA CAACAATTGGGGATTCCGGCCCAAGAGACTCAACTTCAAAC TCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGATGGC GTCACAACCATCGCTAATAACCTTACCAGCACGGTTCAAGT CTTCTCGGACTCGGAGTACCAGCTTCCGTACGTCCTCGGCT CTGCGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGACGTG TTCATGATTCCGCAATACGGCTACCTGACGCTCAACAATGG CAGCCAAGCCGTGGGACGTTCATCCTTTTACTGCCTGGAAT ATTTCCCTTCTCAGATGCTGAGAACGGGCAACAACTTTACC TTCAGCTACACCTTTGAGGAAGTGCCTTTCCACAGCAGCTA CGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCA TCGACCAATACCTGTATTACCTGAACAGAACTCAAAATCAG TCCGGAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCGTGG GTCTCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGGCTAC CTGGACCCTGTTATCGGCAGCAGCGCGTTTCTAAAACAAAA ACAGACAACAACAACAGCAATTTTACCTGGACTGGTGCTTC AAAATATAACCTCAATGGGCGTGAATCCATCATCAACCCTG GCACTGCTATGGCCTCACACAAAGACGACGAAGACAAGTTC TTTCCCATGAGCGGTGTCATGATTTTTGGAAAAGAGAGCGC CGGAGCTTCAAACACTGCATTGGACAATGTCATGATTACAG ACGAAGAGGAAATTAAAGCCACTAACCCTGTGGCCACCGAA AGATTTGGGACCGTGGCAGTCAATTTCCAGAGCAGCAGCAC AGACCCTGCGACCGGAGATGTGCATGCTATGGGAGCATTAC CTGGCATGGTGTGGCAAGATAGAGACGTGTACCTGCAGGGT CCCATTTGGGCCAAAATTCCTCACACAGATGGACACTTTCA CCCGTCTCCTCTTATGGGCGGCTTTGGACTCAAGAACCCGC

		CTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTGCGAAT CCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATTCAT CACCCAATACTCCACAGGACAAGTGAGTGTGGAAATTGAAT GGGAGCTGCAGAAAGAAAACAGCAAGCGCTGGAATCCCGAA GTGCAGTACACATCCAATTATGCAAAATCTGCCAACGTTGA TTTTACTGTGGACAACAATGGACTTTATACTGAGCCTCGCC CCATTGGCACCCGTTACCTTACCCGTCCCCTGTAA
SEQ ID NO:16	Nucleotide sequence encoding wildtype AAV6 capsid (VP1)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAA CCTCTCTGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTG GAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGAC GGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGG ATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAG CTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGC CGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTT TTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAG AGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGCTAA GACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCAC AAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCCAG CAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGA CTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTC CAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCA GGCGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGA CGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCA CATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGAACA TGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAAT CTCCAGTGCTTCAACGGGGGCCAGCAACGACAACCACTACT TCGGCTACAGCACCCCCTGGGGGTATTTTGATTTCAACAGA TTCCACTGCCATTTCTCACCACGTGACTGGCAGCGACTCAT CAACAACAATTGGGGATTCCGGCCCAAGAGACTCAACTTCA AGCTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGAT GGCGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCA AGTCTTCTCGGACTCGGAGTACCAGTTGCCGTACGTCCTCG GCTCTGCGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGAC GTGTTCATGATTCCGCAGTACGGCTACCTAACGCTCAACAA TGGCAGCCAGGCAGTGGGACGGTCATCCTTTTACTGCCTGG AATATTTCCCATCGCAGATGCTGAGAACGGGCAATAACTTT ACCTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAG CTACGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTC TCATCGACCAGTACCTGTATTACCTGAACAGAACTCAGAAT CAGTCCGGAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCG GGGGTCTCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGGC TACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCTAAAACA AAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGC TTCAAAATATAACCTTAATGGGCGTGAATCTATAATCAACC CTGGCACTGCTATGGCCTCACACAAAGACGACAAAGACAAG TTCTTTCCCATGAGCGGTGTCATGATTTTTGGAAAGGAGAG CGCCGGAGCTTCAAACACTGCATTGGACAATGTCATGATCA CAGACGAAGAGGAAATCAAAGCCACTAACCCCGTGGCCACC GAAAGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAG CACAGACCCTGCGACCGGAGATGTGCATGTTATGGGAGCCT TACCTGGAATGGTGTGGCAAGACAGAGACGTATACCTGCAG GGTCCTATTTGGGCCAAAATTCCTCACACGGATGGACACTT TCACCCGTCTCCTCTCATGGGCGGCTTTGGACTTAAGCACC

		CGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTGCG AATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATT CATCACCCAGTATTCCACAGGACAAGTGAGCGTGGAGATTG AATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAATCCC GAAGTGCAGTATACATCTAACTATGCAAAATCTGCCAACGT TGATTTCACTGTGGACAACAATGGACTTTATACTGAGCCTC GCCCCATTGGCACCCGTTACCTCACCCGTCCCCTGTAA
SEQ ID NO:17	Nucleotide sequence encoding modified AAV6.1 capsid VP1 (aa residue number 265 is deleted)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAA CCTCTDGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTGG AGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACG GCCGGGGTDGGTGCTTCCTGGCTACAAGTACCTCGGACCCT TCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGAT GCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCT CAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCG ACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTT GGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGAG GGTTCTCGAACCTTTTGGTDGGTTGAGGAAGGTGCTAAGAC GGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCACAAG AGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCCAGCAG CCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTC AGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAG CAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCAGGC GGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGACGG AGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCACAT GGCTGGGCGACAGAGTCATCACCACCAGCACCCGAACATGG GCCTTGCCCACCTATAACAACCACCTCTACAAGCAAATCTC CAGTGCTTCAGGGGCCAGCAACGACAACCACTACTTCGGCT ACAGCACCCCCTGGGGGTATTTTGATTTCAACAGATTCCAC TGCCATTTCTCACCACGTGACTGGCAGCGACTCATCAACAA CAATTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGCTCT TCAACATCCAAGTCAAGGAGGTCACGACGAATGATGGCGTC ACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTT CTCGGACTCGGAGTACCAGTTGCCGTACGTCCTCGGCTCTG CGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGACGTGTTC ATGATTCCGCAGTACGGCTACCTAACGCTCAACAATGGCAG CCAGGCAGTGGGACGGTCATCCTTTTACTGCCTGGAATATT TCCCATCGCAGATGCTGAGAACGGGCAATAACTTTACCTTC AGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTACGC GCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCG ACCAGTACCTGTATTACCTGAACAGAACTCAGAATCAGTCC GGAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCGGGGGTC TCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGGCTACCTG GACCCTGTTACCGGCAGCAGCGCGTTTCTAAAACAAAAACA GACAACAACAACAGCAACTTTACCTGGACTGGTGCTTCAAA ATATAACCTTAATGGGCGTGAATCTATAATCAACCCTGGCA CTGCTATGGCCTCACACAAAGACGACAAAGACAAGTTCTTT CCCATGAGCGGTGTCATGATTTTTGGAAAGGAGAGCGCCGG AGCTTCAAACACTGCATTGGACAATGTCATGATCACAGACG AAGAGGAAATCAAAGCCACTAACCCCGTGGCCACCGAAAGA TTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAGCACAGA CCCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTACCTG GAATGGTGTGGCAAGACAGAGACGTATACCTGCAGGGTCCT ATTTGGGCCAAAATTCCTCACACGGATGGACACTTTCACCC GTCTCCTCTCATGGGCGGCTTTGGACTTAAGCACCCGCCTC

		CTCAGATCCTCATCAAAAACACGCCTGTTCCTGCGAATCCT CCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATTCATCAC CCAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAATGGG AGCTGCAGAAAGAAAACAGCAAACGCTGGAATCCCGAAGTG CAGTATACATCTAACTATGCAAAATCTGCCAACGTTGATTT CACTGTGGACAACAATGGACTTTATACTGAGCCTCGCCCCA TTGGCACCCGTTACCTCACCCGTCCCCTGTAA
SEQ ID NO:18	Nucleotide sequence encoding modified AAV6.3.1 capsid VP1 (aa residue 265 deleted, Lys 531 changed to Glu)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAA CCTCTCTGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTG GAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGAC GGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGG ATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAG CTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGC CGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTT TTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAG AGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGCTAA GACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCAC AAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCCAG CAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGA CTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTC CAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCA GGCGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGA CGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCA CATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGAACA TGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAAT CTCCAGTGCTTCAGGGGCCAGCAACGACAACCACTACTTCG GCTACAGCACCCCCTGGGGGTATTTTGATTTCAACAGATTC CACTGCCATTTCTCACCACGTGACTGGCAGCGACTCATCAA CAACAATTGGGGATTCCGGCCCAAGAGACTCAACTTCAAGC TCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGATGGC GTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGT CTTCTCGGACTCGGAGTACCAGTTGCCGTACGTCCTCGGCT CTGCGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGACGTG TTCATGATTCCGCAGTACGGCTACCTAACGCTCAACAATGG CAGCCAGGCAGTGGGACGGTCATCCTTTTACTGCCTGGAAT ATTTCCCATCGCAGATGCTGAGAACGGGCAATAACTTTACC TTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTA CGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCA TCGACCAGTACCTGTATTACCTGAACAGAACTCAGAATCAG TCCGGAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCGGGG GTCTCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGGCTAC CTGGACCCTGTTACCGGCAGCAGCGCGTTTCTAAAACAAAA ACAGACAACAACAACAGCAACTTTACCTGGACTGGTGCTTC AAAATATAACCTTAATGGGCGTGAATCTATAATCAACCCTG GCACTGCTATGGCCTCACACAAAGACGACGAAGACAAGTTC TTTCCCATGAGCGGTGTCATGATTTTTGGAAAGGAGAGCGC CGGAGCTTCAAACACTGCATTGGACAATGTCATGATCACAG ACGAAGAGGAAATCAAAGCCACTAACCCCGTGGCCACCGAA AGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAGCAC AGACCCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTAC CTGGAATGGTGTGGCAAGACAGAGACGTATACCTGCAGGGT CCTATTTGGGCCAAAATTCCTCACACGGATGGACACTTTCA CCCGTCTCCTCTCATGGGCGGCTTTGGACTTAAGCACCCGC

		CTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTGCGAAT CCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATTCAT CACCCAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAAT GGGAGCTGCAGAAAGAAAACAGCAAACGCTGGAATCCCGAA GTGCAGTATACATCTAACTATGCAAAATCTGCCAACGTTGA TTTCACTGTGGACAACAATGGACTTTATACTGAGCCTCGCC CCATTGGCACCCGTTACCTCACCCGTCCCCTGTAA
SEQ ID NO:19	Nucleotide sequence encoding human wild type frataxin (WT FXN) for cloning into pTRs-KS-CBh-EGFP-BGH scAAV vector AgeI site **in bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See figure 2A)	TAGAAG**ACCGGT**CGCCACCatgtggactctcgggcgccgcg cagtagccggcctcctggcgtcacccagcccagcccaggcc cagaccctcacccgggtcccgcggccggcagagttggcccc actctgcggccgccgtggcctgcgcaccgacatcgatgcga cctgcacgccccgccgcgcaagttcgaaccaacgtggcctc aaccagatttggaatgtcaaaaagcagagtgtctatttgat gaatttgaggaaatctggaactttgggccacccaggctctc tagatgagaccacctatgaaagactagcagaggaaacgctg gactctttagcagagtttttgaagaccttgcagacaagcc atacacgtttgaggactatgatgtctcctttgggagtggtg tcttaactgtcaaactgggtggagatctaggaacctatgtg atcaacaagcagacgccaaacaagcaaatctggctatcttc tccatccagtggacctaagcgttatgactggactgggaaaa actgggtgtactcccacgacggcgtgtccctccatgagctg ctggccgcagagctcactaaagccttaaaaaccaaactgga cttgtcttccttggcctattccggaaaagatgcttgaCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG* *CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:20	IDT1 Codon optimized nucleotide sequence encoding FXN for cloning into pTRs-KS-CBh-EGFP-BGH scAAV vector AgeI site **in bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See Figure 2B)	TAGAAG**ACCGGT**CGCCACCatgtggactctgggtaggcgag cggtggccggcctgttggcatctcctagtcctgcacaagct caaacgctgactagagtccctcggccagcagaactggcgcc actttgcggccggcgcggtcttcgcactgatattgatgcca cttgcacaccccggcgcgcctccagtaatcagcggggactt aatcaaatttggaatgtgaagaagcagtctgtgtatcttat gaatctgcggaagagcgggaccctgggccaccctggtagcc ttgatgaaaccacctatgagcgcctggccgaagagacactg gacagtcttgccgagtttttgaggatctggccgacaaacc ttatacttttgaggactatgacgtgtcctttggatctggtg tattgaccgtaaaactcgggggagaccttgggacgtatgta ataaataagcagaccccaaacaagcagatctggctcagctc tccaagtagtggtcctaagagatatgattggacgggcaaga actgggtctattcccatgatggcgtctctttgcatgaactc cttgcagcagagctgaccaaggccttgaagaccaaattgga tctcagcagcctcgcctatagtggcaaagatgcatagCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG*

		*CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:21	Codon optimized nucleotide sequence encoding FXN IDT3 (low expresser) for cloning into pTRs-KS-CBh-EGFP-BGH scAAV vector AgeI site **in bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See figure 2C)	TAGAAG**ACCGGT**CGCCACCatgtggacactgggaaggcgcg ccgtggccggtctgttggcatcaccatccccagcccaggct cagacactcacccgagtcccaagacccgcagagctggcccc tctgtgcgggcgccgaggccttcgcaccgatatcgatgcta catgcacgccacgcagagctagctcaaatcagaggggactc aaccagatatggaatgtcaagaagcaaagcgtgtatctcat gaacctccggaaaagcggcaccctgggacatcccgggtctc tcgacgagaccacttatgaaagactggcagaggagactctt gacagtctggcggagttcttcgaagacctcgctgacaagcc atataccttcgaagattacgacgtctccttcggctctgggg tgctgactgtcaagcttggcggcgacctggggacctacgtg atcaacaagcagactccaaacaagcaaatctggctcagcag tccaagctccggacccaagagatacgattggacaggcaaga attgggtttactcccacgacggggtgtccctccatgagctg ctggccgctgagctgacgaaggccctgaagaccaagctgga tctctcctccctggcatacagtggtaaggacgcttgaCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG* *CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:22	Codon-optimized nucleotide sequence encoding FXN IDT4 for cloning into pTRs-KS-CBh-EGFP-BGH scAAV vector AgeI site **in bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See Figure 2D)	TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggcggg ccgtagctggcttgctggctagcccaagtcccgcccaggct cagactctcaccagggtacccaggcccgcagagcttgctcc actctgcggacgcaggggtctgcgaaccgatatcgacgcaa cttgcacgccgcggagggcctcttcaaaccagagaggactc aatcaaatttggaatgtaaagaaacagagcgtgtatctcat gaacctccgaaagagtgggactcttgggcaccccggctccc tggacgagactacttacgagcgcctggccgaagaaaccttg gattccctggcggagttttttgaagacttggcagacaagcc ttataccttcgaggattacgacgtgagttttggctctggtg ttcttacagtcaagctcggtggcgaccttggcacttatgta attaacaagcagacacctaacaagcagatctggctttctag tccgtcttccggtcccaaaaggtacgattggactggaaaga actgggtctacagtcacgacggtgtctccctgcacgaattg cttgcggctgagctgactaaggcgctcaaaacaaaactgga tctgtccagccttgcctatagcgggaaggacgcatgaCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG* *CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:23	Codon-optimized nucleotide sequence encoding FXN GenScript for cloning into pTRs-KS-CBh-	TAGAAG**ACCGGT**CGCCACCatgtggacactgggccggagag ccgtcgctgggctgctggcatcaccatcccccgcacaggca cagaccctgacaagagtccctcggccagcagagctggcccc actgtgcgggcggagaggactgcgaaccgacatcgatgcta cttgtaccccaaggcgagcaagctccaaccagcgagggctg

	EGFP-BGH scAAV vector AgeI site **in bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See Figure 2E)	aaccagatttggaatgtgaagaaacagtctgtctacctgat gaatctgagaaagagcggcactctgggacaccctggcagcc tggacgagaccacctacgagcggctggccgaggaaaccctg gattccctggccgagttctttgaagacctggctgataagcc atacaccttcgaagactatgacgtgagcttcggcagcggcg tgctgacagtcaaactgggcggggacctgggaacatacgtg atcaacaagcagactcctaacaagcagatttggctgtctag tccctcaagcggccctaagaggtacgactggacagggaaaa actgggtgtatagtcacgatggcgtctcactgcatgagctg ctggccgctgaactgactaaagccctgaaaactaaactgga cctgtcttccctggcatactctggcaaggacgcctgaCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG* *CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:24	Codon-optimized nucleotide sequence encoding FXN GenScript (low CpG) for cloning into pTRs-KS-CBh-EGFP-BGH scAAV vector AgeI site in **bold**; AvrII underlined; CSS double underlined; SpeI in **bold underlined**; bGHpolyA in *italics*; MluI site in ***bold italics*** (See Figure 2F)	TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggagag cagtggcaggactgctggcaagtccatcacctgctcaggca cagactctgacaagagtcccaagacctgcagagctggctcc actgtgcgggaggcgcggactgagaacagacatcgatgcta catgtactcctcgacgggcaagctccaaccagcgagggctg aaccagatttggaatgtgaagaaacagtccgtctacctgat gaatctgaggaagtcaggcaccctggggcacccaggaagtc tggacgagaccacatatgaacggctggctgaggaaacactg gattctctggccgagttctttgaagacctggctgataagcc ctacacattcgaagactatgatgtgagctttggatccggcg tgctgactgtcaaactgggcggggacctgggcacttacgtg atcaacaagcagacccctaacaagcagatttggctgtctag tccttcaagcggaccaaagcggtacgactggaccggcaaaa actgggtgtattctcacgatggggtcagtctgcatgagctg ctggccgctgaactgaccaaggccctgaagacaaaactgga cctgtcctctctggcatatagcggaaaagatgcctgaCGAG CGGCCGCTCCTAGGAGCAGTATCGATCCCAGCCCACTTTTC CCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAG* *CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT* *GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGG* *AAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG* *GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA* *AGACAACAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG* *CTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG
SEQ ID NO:25	Nucleic acid sequence encoding collagen stabilizing sequence (CSS)	CCCAGCCCACTTTTCCCCAA
SEQ ID NO:26	Nucleic acid sequence of CBh promoter	tacataacttacggtaaatggcccgcctggctgaccgccca acgacccccgcccattgacgtcaatagtaacgccaataggg actttccattgacgtcaatgggtggagtatttacggtaaac tgcccacttggcagtacatcaagtgtatcatatgccaagta cgccccctattgacgtcaatgacggtaaatggcccgcctgg cattgtgcccagtacatgaccttatgggactttcctacttg gcagtacatctacgtattagtcatcgctattaccatggtcg

		aggtgagccccacgttctgcttcactctccccatctccccc ccctccccacccccaattttgtatttatttatttttaatt attttgtgcagcgatgggggcgggggggggggggggcgcg cgccaggcggggcggggcggggcgaggggcggggcggggcg aggcggagaggtgcggcggcagccaatcagagcggcgcgct ccgaaagtttccttttatggcgaggcggcggcggcggcggc cctataaaaagcgaagcgcgcggcgggcgggagtcgctgcg acgctgccttcgccccgtgccccgctccgccgccgcctcgc gccgcccgccccggctctgactgaccgcgttactcccacag gtgagcgggcgggacggcccttctcctccgggctgtaatta gctgagcaagaggtaagggtttaagggatggttggttggtg gggtattaatgtttaattacctggagcacctgcctgaaatc actttttttcaggttgga
SEQ ID NO:27	Nucleic acid sequence of bGHpoly A signal sequence	CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCC CCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCC ACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTG TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC AGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCAT GCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAG AACCAGCT
SEQ ID NO:28	Nucleotide sequence encoding AAV2i8 capsid (VP1)	atggctgccgatggttatcttccagattggctcgaggacac tctctctgaaggaataagacagtggtggaagctcaaacctg gcccaccaccaccaaagcccgcagagcggcataaggacgac agcaggggtcttgtgcttcctgggtacaagtacctcggacc cttcaacggactcgacaagggagagccggtcaacgaggcag acgccgcggccctcgagcacgacaaagcctacgaccggcag ctcgacagcggagacaacccgtacctcaagtacaaccacgc cgacgcggagtttcaggagcgccttaaagaagatacgtctt ttgggggcaacctcggacgagcagtcttccaggcgaaaaag agggttcttgaacctctgggcctggttgaggaacctgttaa gacggctccgggaaaaaagaggccggtagagcactctcctg tggagccagactcctcctcgggaaccggaaaggcgggccag cagcctgcaagaaaaagattgaattttggtcagactggaga cgcagactcagtacctgacccccagcctctcggacagccac cagcagccccctctggtctgggaactaatacgatggctaca ggcagtggcgcaccaatggcagacaataacgagggcgccga cggagtgggtaattcctcgggaaattggcattgcgattcca catggatgggcgacagagtcatcaccaccagcacccgaacc tgggccctgcccacctacaacaaccacctctacaaacaaat ttccagccaatcaggagcctcgaacgacaatcactactttg gctacagcacccctttgggggtattttgacttcaacagattc cactgccacttttcaccacgtgactggcaaagactcatcaa caacaactggggattccgacccaagagactcaacttcaagc tctttaacattcaagtcaaagaggtcacgcagaatgacggt acgacgacgattgccaataaccttaccagcacggttcaggt gtttactgactcggagtaccagctcccgtacgtcctcggct cggcgcatcaaggatgcctcccgccgttcccagcagacgtc ttcatggtgccacagtatggatacctcaccctgaacaacgg gagtcaggcagtaggacgctcttcattttactgcctggagt actttccttctcagatgctgcgtaccggaaacaactttacc ttcagctacacttttgaggacgttcctttccacagcagcta cgctcacagccagagtctggaccgtctcatgaatcctctca tcgaccagtacctgtattacttgagcagaacaaacactcca agtggaaccaccacgcagtcaaggcttcagttttctgtggc

		cggacccagtaacatggctgtccagggaaggaactggcttc ctggaccctgttaccgccagcagcgagtatcaaagacatct gcggataacaacaacagtgaatttgcttggactggagctac caagtaccacctcaatggcagagactctctggtgaatccgg gcccggccatggcaagccacaaggacgatgaagaaaagttt ttcctcagagcggggttctcatctttgggaagcaaggctc agagaaaacaaatgtggacattgaaaaggtcatgattacag acgaagaggaaatcaggacaaccaatcccgtggctacggag cagtatggttctgtatctaccaacctccagcaacagaacac agcaccagctaccgcagatgtcaacacacaaggcgttcttc caggcatggtctggcaggacagagatgtgtaccttcagggg cccatctgggcaaagattccacacacggacggacattttca cccctctcccctcatgggtggattcggacttaaacaccctc ctccacagattctcatcaagaacaccccggtacctgcgaat ccttcgaccaccttcagtgcggcaaagtttgcttccttcat cacacagtactccacgggacaggtcagcgtggagatcgagt gggagctgcagaaggaaaacagcaaacgctggaatcccgaa attcagtacacttccaactacaacaagtctgttaatgtgga ctttactgtggacactaatggcgtgtattcagagcctcgcc ccattggcaccagatacctgactcgtaatctgtaa
SEQ ID NO:29	Amino acid sequence of AAV2i8 capsid (VP1)	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDD SRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQ LDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKK RVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQ QPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMAT GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRT WALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRF HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDG TTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADV FMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFT FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTP SGTTTQSRLQFSVAGPSNMAVQGRNWLPGPCYRQQRVSKTS ADNNNSEFAWTGATKYHLNGRDSLVNPGPAMASHKDDEEKF FPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATE QYGSVSTNLQQQNTAPATADVNTQGVLPGMVWQDRDVYLQG PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPAN PSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPE IQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
SEQ ID NO:30	Nucleic acid encoding AAV2-TT capsid (VP1) (nucleotides that differ from WT AAV2 are underlined)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAC TCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTG GCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGACGAC AGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGGCAG ACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCAG CTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGC CGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTT TTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAG AGGATTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAA GACGGCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCTG CGGAGCCAGACTCCTCCTCGGGAACCGGAAAGTCGGGCCAG CAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGA CGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCAC CAGCAGCCCCCTCTGGTCTGGGAACTAATACGATGGCTTCA GGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGA

		CGGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCA CATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACC TGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAAT TTCCAGCCAATCAGGAGCCTCGAACGACAATCACTACTTTG GCTACAGCACCCCTTGGGGGTATTTTGACTTCAACAGATTC CACTGCCACTTTTCACCACGTGACTGGCAAAGACTCATCAA CAACAACTGGGGATTCCGACCCAAGAGACTCAGCTTCAAGC TCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGACGGT ACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGT GTTTACTGACTCGGAGTACCAGCTCCCGTACGTCCTCGGCT CGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGCAGACGTC TTCATGGTGCCACAGTATGGATACCTCACCCTGAACAACGG GAGTCAGGCAGTAGGACGCTCTTCATTTTACTGCCTGGAGT ACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACTTTACC TTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTA CGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCA TCGACCAGTACCTGTATTACTTGAGCAGAACAAACACTCCA AGTGGAACCACCACGATGTCAAGGCTTCAGTTTTCTCAGGC CGGAGCGAGTGACATTCGGGACCAGTCTAGGAACTGGCTTC CTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACAGCT GCGGATAACAACAACAGTGATTACTCGTGGACTGGAGCTAC CAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGG GCCCGGCCATGGCAAGCCACAAGGACGATGAAGAAAAGTAT TTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGACTC AGGAAAAACAAATGTGGACATTGAAAAGGTCATGATTACAG ACGAAGAGGAAATCAGGACAACCAATCCCGTGGCTACGGAG CAGTATGGTTCTGTATCTACCAACCTCCAGAGCGGCAACAC ACAAGCAGCTACCTCAGATGTCAACACACAAGGCGTTCTTC CAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGG CCCATCTGGGCAAAGATTCCACACACGGACGGACATTTTCA CCCCTCTCCCCTCATGGGTGGATTCGGACTTAAACACCCTC CTCCACAGATTCTCATCAAGAACACCCCGGTACCTGCGAAT CCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTTCAT CACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGT GGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGAA ATTCAGTACACTTCCAACTACAACAAGTCTGTTAATGTGGA CTTTACTGTGGACACTAATGGCGTGTATTCAGAGCCTCGCC CCATTGGCACCAGATACCTGACTCGTAATCTGTAA
SEQ ID NO:31	Amino acid sequence of AAV2-TT capsid (VP1)	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDD SRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQ LDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKK RILEPLGLVEEPVKTAPGKKRPVEHSPAEPDSSSGTGKSGQ QPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMAS GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRT WALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRF HCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNDG TTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADV FMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFT FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTP SGTTTMSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTA ADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKY FPQSGVLIFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATE QYGSVSTNLQSGNTQAATSDVNTQGVLPGMVWQDRDVYLQG PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPAN

		PSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPE IQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
SEQ ID NO:32	Nucleic acid encoding AAV2-TT-S312N capsid (VP1) (nucleotides that differ from WT AAV2 are underlined)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAC TCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTG GCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGACGAC AGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACC CTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGGCAG ACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCAG CTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGC CGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTT TTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAG AGGATTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAA GACGGCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCTG CGGAGCCAGACTCCTCCTCGGGAACCGGAAAGTCGGGCCAG CAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGA CGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCAC CAGCAGCCCCCTCTGGTCTGGGAACTAATACGATGGCTTCA GGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGA CGGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCA CATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACC TGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAAT TTCCAGCCAATCAGGAGCCTCGAACGACAATCACTACTTTG GCTACAGCACCCCTTGGGGGTATTTTGACTTCAACAGATTC CACTGCCACTTTTCACCACGTGACTGGCAAAGACTCATCAA CAACAACTGGGGATTCCGACCCAAGAGACTCAACTTCAAGC TCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGACGGT ACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGT GTTTACTGACTCGGAGTACCAGCTCCCGTACGTCCTCGGCT CGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGCAGACGTC TTCATGGTGCCACAGTATGGATACCTCACCCTGAACAACGG GAGTCAGGCAGTAGGACGCTCTTCATTTTACTGCCTGGAGT ACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACTTTACC TTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTA CGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCA TCGACCAGTACCTGTATTACTTGAGCAGAACAAACACTCCA AGTGGAACCACCACGATGTCAAGGCTTCAGTTTTCTCAGGC CGGAGCGAGTGACATTCGGGACCAGTCTAGGAACTGGCTTC CTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACAGCT GCGGATAACAACAACAGTGATTACTCGTGGACTGGAGCTAC CAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGG GCCCGGCCATGGCAAGCCACAAGGACGATGAAGAAAAGTAT TTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGACTC AGGAAAAACAAATGTGGACATTGAAAAGGTCATGATTACAG ACGAAGAGGAAATCAGGACAACCAATCCCGTGGCTACGGAG CAGTATGGTTCTGTATCTACCAACCTCCAGAGCGGCAACAC ACAAGCAGCTACCTCAGATGTCAACACACAAGGCGTTCTTC CAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGG CCCATCTGGGCAAAGATTCCACACACGGACGGACATTTTCA CCCCTCTCCCCTCATGGGTGGATTCGGACTTAAACACCCTC CTCCACAGATTCTCATCAAGAACACCCCGGTACCTGCGAAT CCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTTCAT CACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGT GGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGAA ATTCAGTACACTTCCAACTACAACAAGTCTGTTAATGTGGA

		CTTTACTGTGGACACTAATGGCGTGTATTCAGAGCCTCGCC CCATTGGCACCAGATACCTGACTCGTAATCTGTAA
SEQ ID NO:33	Amino acid sequence of AAV2-TT-S312N capsid (VP1)	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDD SRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQ LDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKK RILEPLGLVEEPVKTAPGKKRPVEHSPAEPDSSSGTGKSGQ QPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMAS GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRT WALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRF HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDG TTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADV FMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFT FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTP SGTTTMSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTA ADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKY FPQSGVLIFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATE QYGSVSTNLQSGNTQAATSDVNTQGVLPGMVWQDRDVYLQG PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPAN PSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPE IQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

CLAIMS

What is claimed is:

1. A modified nucleic acid encoding frataxin (FXN) comprising the amino acid sequence set forth in SEQ ID NO:1, wherein said nucleic acid is expressed at a greater level compared with the expression level of the wild type FXN nucleic acid sequence of SEQ ID NO:2, and wherein said modified nucleic acid comprises at least one characteristic selected from the group consisting of: a GC content of at least 55%, a number of CpG dinucleotides not greater than 124, and a codon adaptation index (CAI) of at least 0.76.

2. The modified nucleic acid of claim 1, said nucleic acid comprising at least one characteristic selected from the group consisting of:

(a) a CAI of at least 0.86, at least 0.95, or at least 0.98;

(b) a GC content is at least 57%, at least 61%, or at least 69%;

(c) a number of CpG dinucleotides is less than 124;

(d) a nucleic acid sequence selected from the group consisting of a sequence as set forth in SEQ ID NOs:3-9.

3. A modified nucleic acid encoding FXN, wherein said nucleic acid is expressed at a greater level compared with the level of expression of the wild type FXN nucleic acid sequence of SEQ ID NO:2, and wherein the nucleic acid comprises at least one of:

(a) a nucleic acid sequence selected from the group consisting of SEQ ID NOs:3-9;

(b) a GC content of at least 55%;

(c) a number of CpG dinucleotides not greater than 117; and

(d) a CAI of at least 0.86.

4. The modified nucleic acid of claim 3, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:7.

5. The modified nucleic acid of claim 1 comprising the nucleic acid sequence of SEQ ID NO:7.

6. A vector comprising the modified nucleic acid of claim 1.

7. A vector comprising the modified nucleic acid of claim 3.

8. The vector of claim 7, wherein said vector is a recombinant adeno-associated virus vector (rAAV) and the nucleic acid is self-complementary.

9. The rAAV of claim 8, wherein said rAAV comprises a capsid selected from the group consisting of a capsid of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh10, AAVrh74, RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV Hu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, AAV2i8, AAV2G9, AAV2i8G9, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, and AAV-LK03.

10. The rAAV of claim 9, wherein the modified nucleic acid comprises the sequence of SEQ ID NO:7 and wherein capsid is selected from a capsid of AAV2i8 and a capsid of AAV2-TT-S312N.

11. The rAAV of claim 10, wherein said nucleic acid further comprises at least one element selected from the group consisting of: at least one AAV terminal repeat sequence, an enhancer, a promoter, a stop codon, and a poly-adenylation (polyA) signal sequence.

12. The rAAV of claim 11, said rAAV comprising two AAV terminal repeat sequences, a CBh promoter, a sequence encoding a collagen stabilization sequence (CSS), and a bovine growth hormone poly-adenylation signal sequence (bGHpolyA).

13. A rAAV vector comprising a nucleic acid comprising, from 5' to 3':

(a) an AAV2 terminal repeat,

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:25;

(c) the modified nucleic acid encoding FXN of claim 3;

(d) a CSS having the sequence of SEQ ID NO:24;

(e) a bGHpolyA signal sequence having the sequence of SEQ ID NO:26; and

(f) an AAV2 terminal repeat.

14. The rAAV vector of claim 13, further comprising an AAV2i8 capsid wherein the VP1 comprises the amino acid sequence of SEQ ID NO:29, said vector further comprising a self-complementary nucleic acid comprising, from 5' to 3':

(a) an AAV2 terminal repeat,

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:25;

(c) a modified nucleic acid encoding FXN comprising the sequence of SEQ ID NO:7;

(d) a CSS having the sequence of SEQ ID NO:24;

(e) a bGHpolyA signal sequence having by the sequence of SEQ ID NO:26; and

(f) an AAV2 terminal repeat.

15. The rAAV vector of claim 13, further comprising an AAV2-TT-S312N capsid wherein the VP1 comprises the amino acid sequence of SEQ ID NO:31, said vector further comprising a self-complementary nucleic acid comprising, from 5' to 3':

(a) an AAV2 terminal repeat,

(b) a CBh promoter comprising the nucleic acid sequence of SEQ ID NO:25;

(c) a modified nucleic acid encoding FXN comprising the sequence of SEQ ID NO:7;

(d) a CSS having the sequence of SEQ ID NO:24;

(e) a bGHpolyA signal sequence having the sequence of SEQ ID NO:26; and

(f) an AAV2 terminal repeat.

16. A rAAV vector for treating Friedreich ataxia (FRDA) in a patient in need thereof, wherein said rAAV comprises a modified nucleic acid encoding FXN selected from the group consisting of:

(a) a modified nucleic acid comprising the amino acid sequence set forth in SEQ ID NO:1, wherein said nucleic acid is expressed at a greater level compared with the expression level of the wild type FXN nucleic acid sequence of SEQ ID NO:2, and further comprises a GC content of at least 55%, a number of CpG dinucleotides not greater than 124, and a codon adaptation index (CAI) of at least 0.76;

(b) a modified nucleic comprising a CAI of at least 0.86, a GC content is at least 55%, and a number of CpG dinucleotides not greater than 117;

(c) a modified nucleic comprising the nucleic acid sequence of SEQ ID NO:5; and

(d) a modified nucleic comprising the nucleic acid sequence of SEQ ID NO:7.

17. The modified nucleic acid of claim 16, comprising the nucleic acid sequence of SEQ ID NO:7.

18. A rAAV vector for treating Friedreich ataxia in a patient in need thereof, wherein said vector comprises the modified nucleic acid encoding frataxin of claim 3.

19. A pharmaceutical composition comprising the rAAV vector of claim 18.

20. A method of treating FRDA in a subject, said method comprising administering the rAAV vector of claim 18.

21. The method of 20, wherein said rAAV vector is administered systemically, by direct cardiac administration or by intracranial administration.

22. The method claim 21, wherein the rAAV vector is administered intracranially.

23. The method of claim 21, wherein the rAAV vector is directly administered into the heart.

24. The method of claim 21, wherein said modified nucleic acid encoding FXN comprises the nucleic acid sequence of SEQ ID NO:7.

25. A method of treating a disease, disorder or condition mediated by a decreased level of FXN in a subject, the method comprising administering the rAAV vector claim 18.

26. A host cell comprising the modified nucleic acid encoding FXN of claim 3.

27. The host cell of claim 26, wherein the cell is selected from the group consisting of VERO, WI38, MRC5, A549, HEK293 cells, B-50 or any other HeLa cells, HepG2, Saos-2, HuH7, and HT1080.

28. The host cell of claim 27, wherein the host cell is a HEK293 adapted to growth in suspension culture.

29. The host cell of claim 27, wherein the cell is a HEK293 cell having ATCC No. PTA 13274.

30. The host cell of claim 26, said cell comprising at least one nucleic acid encoding at least one protein selected from the group consisting of a Rep protein, a Cap protein, a E1a protein, a E1b protein, an E2a protein, an E4 protein and a VA RNA.

31. The modified nucleic acid encoding frataxin of claim 1 for use in increasing the level of frataxin in a cell.

32. The modified nucleic acid encoding frataxin of claim 3 for use in increasing the level of frataxin in a cell.

33. The rAAV vector of claim 18 for use in treating Friedreich ataxia in a subject.

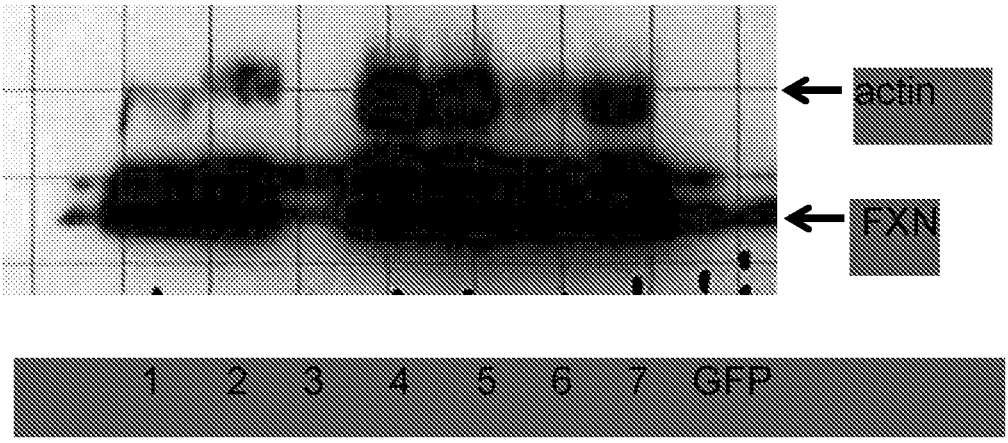


FIGURE 1A

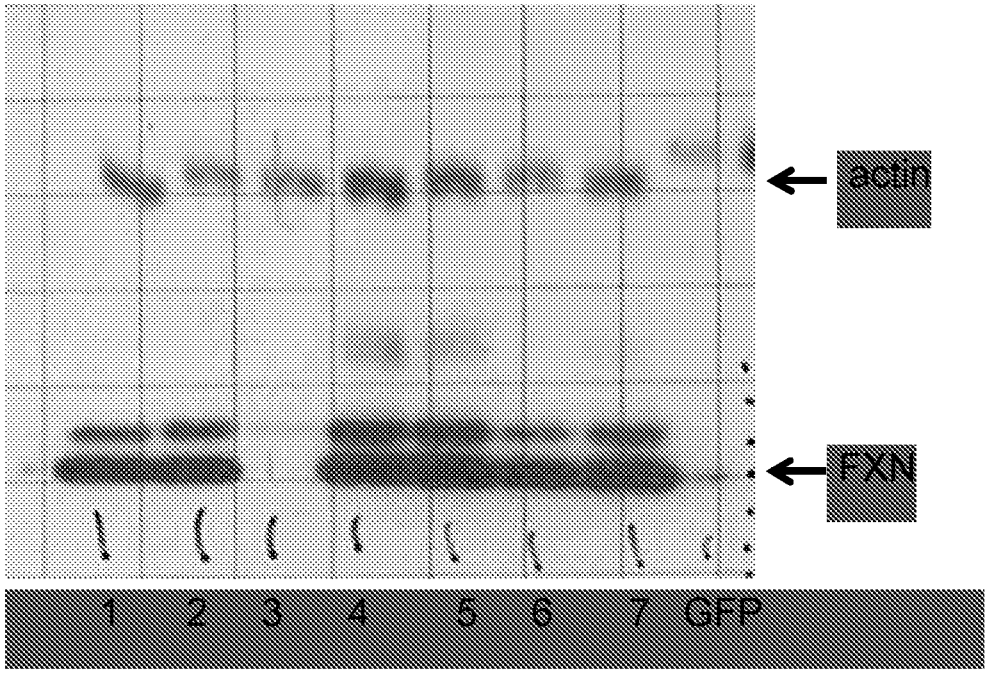


FIGURE 1B

FIGURE 2A

WT FXN (SEQ ID NO:19)

TAGAAG**ACCGGT**CGCCACCatgtggactctcgggcgccgcgcagtagccggcctcctggcgt
cacccagcccagcccaggcccagaccctcacccgggtcccgcggccggcagagttggcccca
ctctgcggccgccgtggcctgcgcaccgacatcgatgcgacctgcacgccccgccgcgcaag
ttcgaaccaacgtggcctcaaccagatttggaatgtcaaaaagcagagtgtctatttgatga
atttgaggaaatctggaactttgggccacccaggctctctagatgagaccacctatgaaaga
ctagcagaggaaacgctggactctttagcagagtttttttgaagaccttgcagacaagccata
cacgtttgaggactatgatgtctcctttgggagtggtgtcttaactgtcaaactgggtggag
atctaggaacctatgtgatcaacaagcagacgccaaacaagcaaatctggctatcttctcca
tccagtggacctaagcgttatgactggactgggaaaaactgggtgtactcccacgacggcgt
gtccctccatgagctgctggccgcagagctcactaaagccttaaaaaccaaactggacttgt
cttccttggcctattccggaaaagatgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

FIGURE 2B

IDT1 codon optimized FXN (SEQ ID NO:20)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggtaggcgagcggtggccggcctgttggcat
ctcctagtcctgcacaagctcaaacgctgactagagtccctcggccagcagaactggcgcca
ctttgcggccggcgcggtcttcgcactgatattgatgccacttgcacaccccggcgcgcctc
cagtaatcagcggggacttaatcaaatttggaatgtgaagaagcagtctgtgtatcttatga
atctgcggaagagcgggaccctgggccaccctggtagccttgatgaaaccacctatgagcgc
ctggccgaagagacactggacagtcttgccgagtttttgaggatctggccgacaaacctta
tacttttgaggactatgacgtgtcctttggatctggtgtattgaccgtaaaactcgggggag
accttgggacgtatgtaataaataagcagaccccaaacaagcagatctggctcagctctcca
agtagtggtcctaagagatatgattggacgggcaagaactgggtctattcccatgatggcgt
ctctttgcatgaactccttgcagcagagctgaccaaggccttgaagaccaaattggatctca
gcagcctcgcctatagtggcaaagatgcatagCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

FIGURE 2C

IDT3 – low expresser (SEQ ID NO:21)

TAGAAG**ACCGGT**CGCCACCatgtggacactgggaaggcgcgccgtggccggtctgttggcat
caccatccccagcccaggctcagacactcacccgagtcccaagacccgcagagctggcccct
ctgtgcgggcgccgaggccttcgcaccgatatcgatgctacatgcacgccacgcagagctag
ctcaaatcagaggggactcaaccagatatggaatgtcaagaagcaaagcgtgtatctcatga
acctccggaaaagcggcaccctgggacatcccgggtctctcgacgagaccacttatgaaaga
ctggcagaggagactcttgacagtctggcggagttcttcgaagacctcgctgacaagccata
taccttcgaagattacgacgtctccttcggctctggggtgctgactgtcaagcttggcggcg
acctggggacctacgtgatcaacaagcagactccaaacaagcaaatctggctcagcagtcca
agctccggacccaagagatacgattggacaggcaagaattgggtttactcccacgacggggt
gtccctccatgagctgctggccgctgagctgacgaaggccctgaagaccaagctggatctct
cctccctggcatacagtggtaaggacgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

FIGURE 2D

IDT4 (SEQ ID NO:22)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggcgggccgtagctggcttgctggcta
gcccaagtcccgcccaggctcagactctcaccagggtacccaggcccgcagagcttgctcca
ctctgcggacgcaggggtctgcgaaccgatatcgacgcaacttgcacgccgcggagggcctc
ttcaaaccagagaggactcaatcaaatttggaatgtaaagaaacagagcgtgtatctcatga
acctccgaaagagtgggactcttgggcaccccggctccctggacgagactacttacgagcgc
ctggccgaagaaaccttggattccctggcggagtttttgaagacttggcagacaagcctta
taccttcgaggattacgacgtgagttttggctctggtgttcttacagtcaagctcggtggcg
accttggcacttatgtaattaacaagcagacacctaacaagcagatctggctttctagtccg
tcttccggtcccaaaaggtacgattggactggaaagaactgggtctacagtcacgacggtgt
ctccctgcacgaattgcttgcggctgagctgactaaggcgctcaaaacaaaactggatctgt
ccagccttgcctatagcgggaaggacgcatgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

FIGURE 2E

GenScript (SEQ ID NO:23)

TAGAAG**ACCGGT**CGCCACCatgtggacactgggccggagagccgtcgctgggctgctggcat
caccatcccccgcacaggcacagaccctgacaagagtccctcggccagcagagctggcccca
ctgtgcgggcggagaggactgcgaaccgacatcgatgctacttgtaccccaaggcgagcaag
ctccaaccagcgagggctgaaccagatttggaatgtgaagaaacagtctgtctacctgatga
atctgagaaagagcggcactctgggacaccctggcagcctggacgagaccacctacgagcgg
ctggccgaggaaaccctggattccctggccgagttctttgaagacctggctgataagccata
caccttcgaagactatgacgtgagcttcggcagcggcgtgctgacagtcaaactgggcgggg
acctgggaacatacgtgatcaacaagcagactcctaacaagcagatttggctgtctagtccc
tcaagcggccctaagaggtacgactggacagggaaaaactgggtgtatagtcacgatggcgt
ctcactgcatgagctgctggccgctgaactgactaaagccctgaaaactaaactggacctgt
cttccctggcatactctggcaaggacgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

FIGURE 2F

GenScript (low CpG) (SEQ ID NO:24)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggagagcagtggcaggactgctggcaa
gtccatcacctgctcaggcacagactctgacaagagtcccaagacctgcagagctggctcca
ctgtgcgggaggcgcggactgagaacagacatcgatgctacatgtactcctcgacgggcaag
ctccaaccagcgagggctgaaccagatttggaatgtgaagaaacagtccgtctacctgatga
atctgaggaagtcaggcaccctggggcacccaggaagtctggacgagaccacatatgaacgg
ctggctgaggaaacactggattctctggccgagttctttgaagacctggctgataagcccta
cacattcgaagactatgatgtgagctttggatccggcgtgctgactgtcaaactgggcgggg
acctgggcacttacgtgatcaacaagcagacccctaacaagcagatttggctgtctagtcct
tcaagcggaccaaagcggtacgactggaccggcaaaaactgggtgtattctcacgatggggt
cagtctgcatgagctgctggccgctgaactgaccaaggccctgaagacaaaactggacctgt
cctctctggcatatagcggaaaagatgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

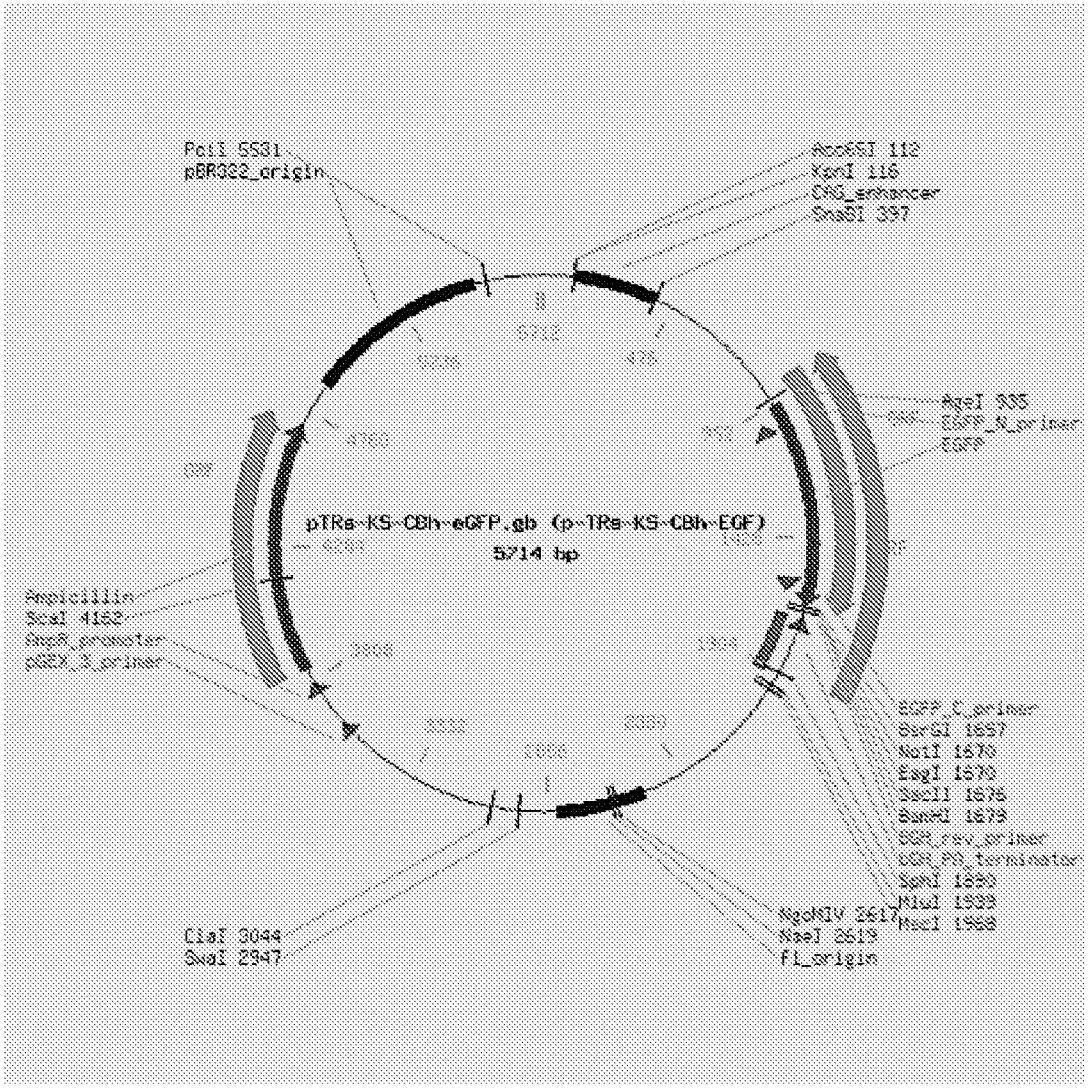
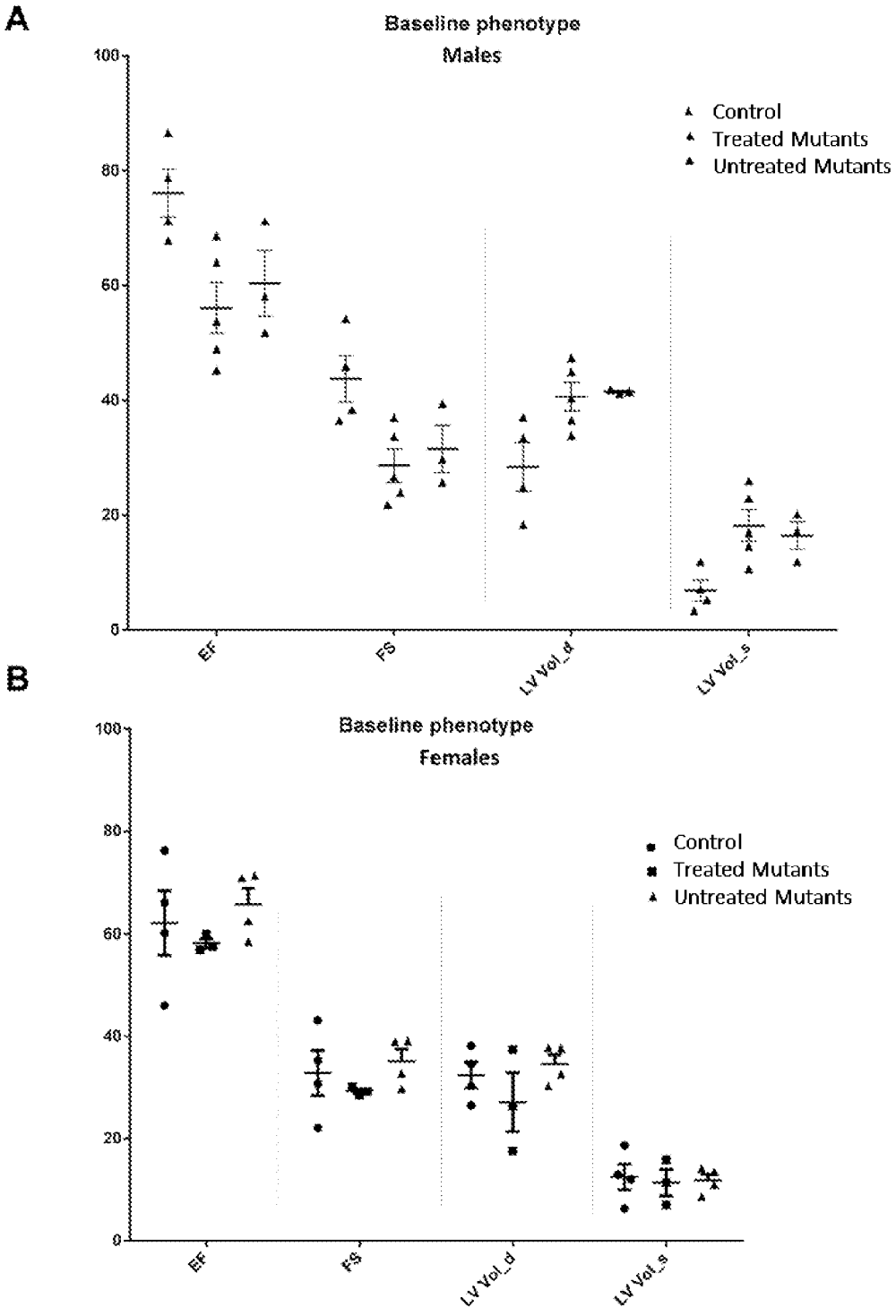
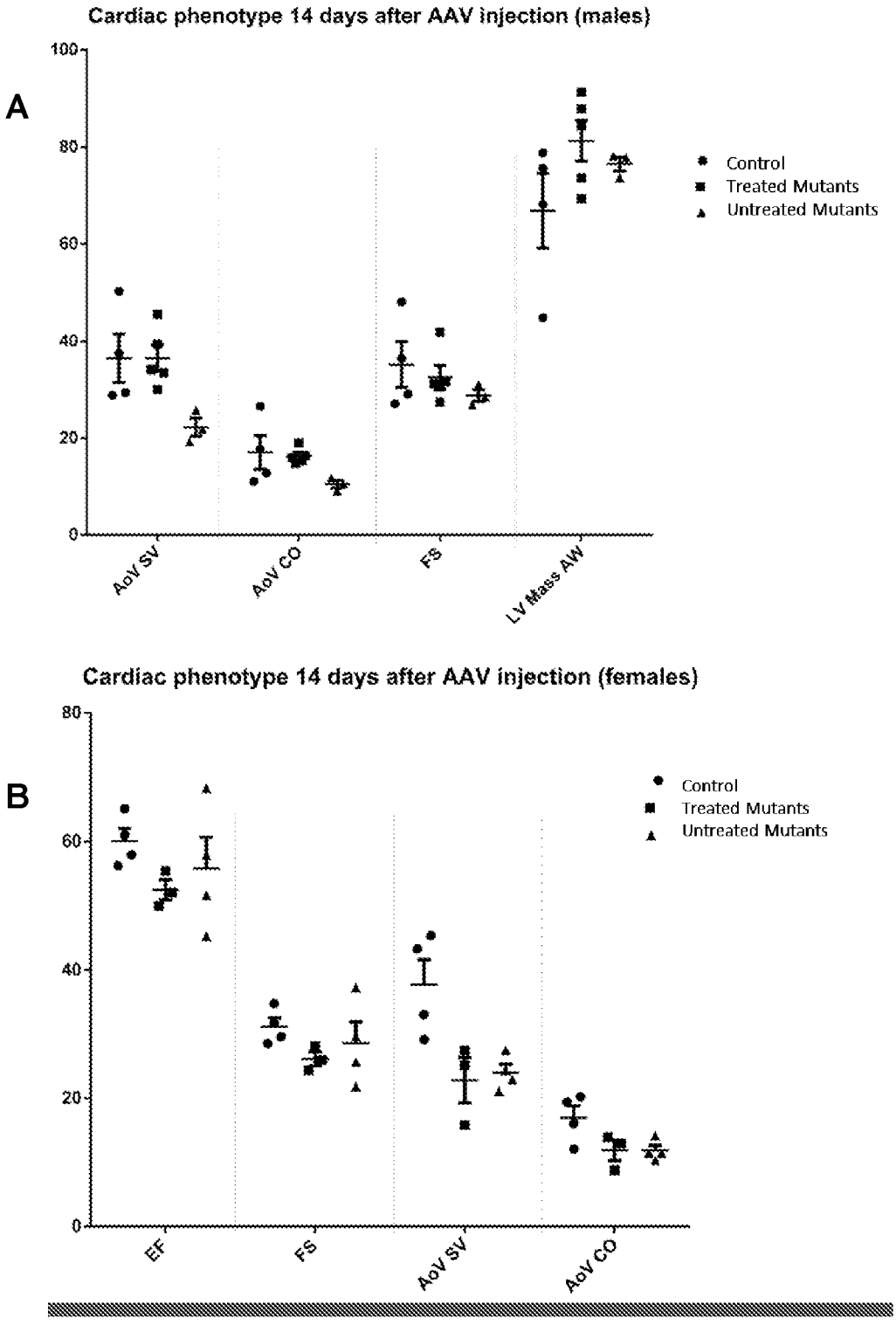

PciI 5531
pBR322_origin
Acc65I 112
KpnI 116
CMV_enhancer
SnaBI 397
AgeI 935
EGFP_N_primer
EGFP
pTRe-KS-CBh-eGFP.gb (p-TRe-KS-CBh-EGF)
5714 bp
Ampicillin
ScaI 4162
AmpR_promoter
pGEX_3_primer
BGFP_C_primer
SrfI 1957
NotI 1970
EagI 1970
SacII 1975
BamHI 1979
CBh_rev_primer
CBh_PA_terminator
SpeI 1990
MluI 1998
NsiI 1998
NgoMIV 2617
NaeI 2619
f1_origin
ClaI 3044
SwaI 2947

FIGURE 3

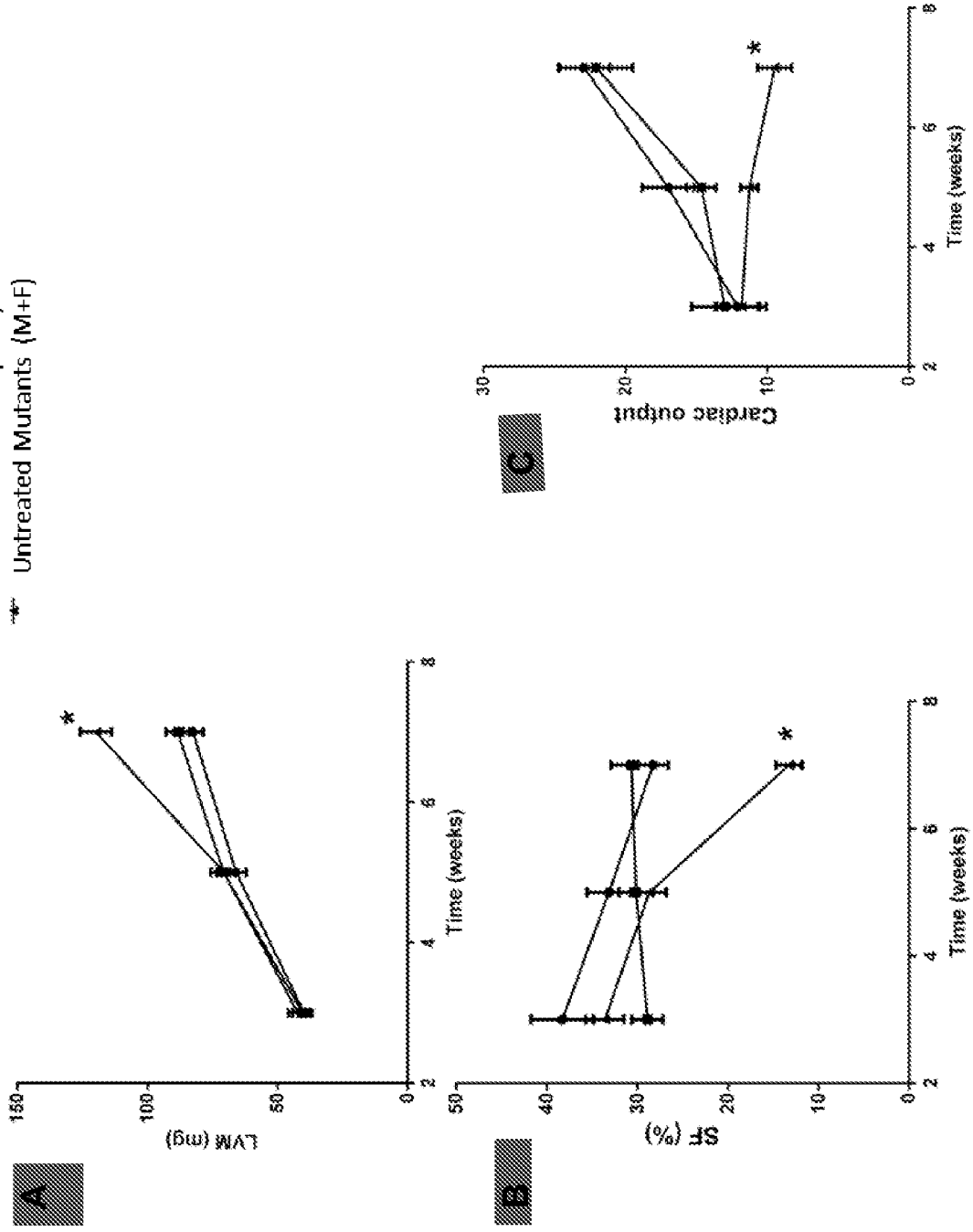


FIGURES 4A-4B



FIGURES 5A-5B

FIGURES 6A-6C



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/056572

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/221462 A1 (PUCCIO HELENE MONIQUE [FR] ET AL) 7 August 2014 (2014-08-07) paragraph [0088]	1-33
A	D. KUMARI ET AL: "Repeat Expansion Affects Both Transcription Initiation and Elongation in Friedreich Ataxia Cells", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 286, no. 6, 11 February 2011 (2011-02-11), pages 4209-4215, XP055329700, US ISSN: 0021-9258, DOI: 10.1074/jbc.M110.194035 the whole document	1-33

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2016	03/01/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schmitz, Till

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/056572

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MORGANE PERDOMINI ET AL: "Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia", NATURE MEDICINE, vol. 20, no. 5, 6 April 2014 (2014-04-06), pages 542-547, XP055210141, ISSN: 1078-8956, DOI: 10.1038/nm.3510 the whole document	1-33
A	MARGUERITE V. EVANS-GALEA ET AL: "FXN methylation predicts expression and clinical outcome in Friedreich ataxia", ANNALS OF NEUROLOGY., vol. 71, no. 4, 1 April 2012 (2012-04-01), pages 487-497, XP055330339, BOSTON, US ISSN: 0364-5134, DOI: 10.1002/ana.22671 the whole document	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/056572

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014221462 A1	07-08-2014	AU 2014211315 A1	13-08-2015
		CA 2900135 A1	07-08-2014
		EA 201500802 A1	29-01-2016
		EP 2950824 A1	09-12-2015
		US 2014221462 A1	07-08-2014
		US 2015313969 A1	05-11-2015
		US 2016024526 A1	28-01-2016
		WO 2014118346 A1	07-08-2014

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 108348621 A
(43)申请公布日 2018.07.31

(21)申请号 201680064440.0

(22)申请日 2016.11.01

(30)优先权数据
62/251,288 2015.11.05 US
62/411,980 2016.10.24 US

(85)PCT国际申请进入国家阶段日
2018.05.04

(86)PCT国际申请的申请数据
PCT/IB2016/056572 2016.11.01

(87)PCT国际申请的公布数据
WO2017/077451 EN 2017.05.11

(71)申请人 竹治疗股份有限公司
地址 美国北卡罗来纳

(72)发明人 R·J·萨穆尔斯基

(74)专利代理机构 中国国际贸易促进委员会专利商标事务所 11038
代理人 唐宏

(51)Int.Cl.
A61K 48/00(2006.01)

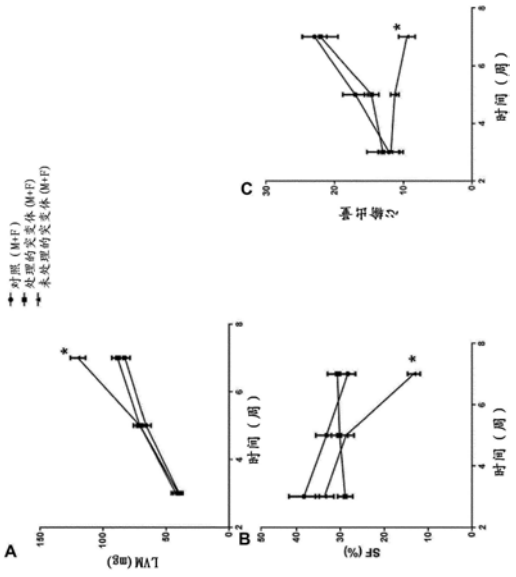
权利要求书3页 说明书66页
序列表29页 附图8页

(54)发明名称

用于基因治疗的经修饰的弗里德赖希共济失调基因及载体

(57)摘要

本发明涉及经修饰的FXN基因，其提供可用于治疗弗里德赖希(Friedreich)共济失调的经编码的共济失调蛋白(frataxin)的增加的表达。



CN 108348621 A

1.一种编码包含在SEQ ID NO:1中所示的氨基酸序列的共济失调蛋白(FXN)的经修饰的核酸,其中所述核酸的表达水平高于SEQ ID NO:2的野生型FXN核酸序列的表达水平,且其中所述经修饰的核酸包含选自由以下各者组成的组的至少一个特性:GC水平为至少55%、CpG二核苷酸数目不超过124,及密码子适应指数(CAI)为至少0.76。

2.权利要求1的经修饰的核酸,所述核酸包含选自由以下各者组成的组的至少一个特性:

(a)CAI为至少0.86、至少0.95或至少0.98;

(b)GC水平为至少57%、至少61%或至少69%;

(c)CpG二核苷酸的数目小于124;

(d)选自由如SEQ ID NO:3至9中所示的序列组成的组的核酸序列。

3.一种编码FXN的经修饰的核酸,其中所述核酸的表达水平高于SEQ ID NO:2的野生型FXN核酸序列的表达水平,且其中所述核酸包含以下各者中的至少一者:

(a)选自由SEQ ID NO:3至9组成的组的核酸序列;

(b)GC水平为至少55%;

(c)CpG二核苷酸数目不超过117;及

(d)CAI为至少0.86。

4.权利要求3的经修饰的核酸,其中所述核酸序列选自由SEQ ID NO:5及SEQ ID NO:7组成的组。

5.权利要求1的经修饰的核酸,其包含SEQ ID NO:7的核酸序列。

6.一种载体,其包含权利要求1的经修饰的核酸。

7.一种载体,其包含权利要求3的经修饰的核酸。

8.权利要求7的载体,其中所述载体为重组腺相关病毒载体(rAAV)且所述核酸为自我互补的。

9.权利要求8的rAAV,其中所述rAAV包含选自由以下各者的衣壳组成的组的衣壳:AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAVrh10、AAVrh74、RHM4-1、RHM15-1、RHM15-2、RHM15-3/RHM15-5、RHM15-4、RHM15-6、AAV Hu.26、AAV1.1、AAV2.5、AAV6.1、AAV6.3.1、AAV9.45、AAV2i8、AAV2G9、AAV2i8G9、AAV2-TT、AAV2-TT-S312N、AAV3B-S312N及AAV-LK03。

10.权利要求9的rAAV,其中所述经修饰的核酸包含SEQ ID NO:7的序列,且其中衣壳选自AAV2i8的衣壳及AAV2-TT-S312N的衣壳。

11.权利要求10的rAAV,其中所述核酸进一步包含选自由以下各者组成的组的至少一个元件:至少一个AAV末端重复序列、增强子、启动子、终止密码子及聚腺苷酸化(polyA)信号序列。

12.权利要求11的rAAV,所述rAAV包含两个AAV末端重复序列、CBh启动子、编码胶原蛋白稳定序列(CSS)的序列、及牛生长激素聚腺苷酸化信号序列(bGHpolyA)。

13.一种rAAV载体,其包含自5'至3'包含以下的核酸:

(a)AAV2末端重复序列;

(b)CBh启动子,其包含SEQ ID NO:25的核酸序列;

(c)权利要求3的编码FXN的经修饰的核酸;

(d)CSS,其具有SEQ ID NO:24的序列;

(e)bGHpolyA信号序列,其具有SEQ ID NO:26的序列;及

(f)AAV2末端重复序列。

14.权利要求13的rAAV载体,其进一步包含AAV2i8衣壳,其中VP1包含SEQ ID NO:29的氨基酸序列,所述载体进一步包含自5'至3'包含以下的自我互补核酸:

(a)AAV2末端重复序列;

(b)CBh启动子,其包含SEQ ID NO:25的核酸序列;

(c)编码FXN的经修饰的核酸,其包含SEQ ID NO:7的序列;

(d)CSS,其具有SEQ ID NO:24的序列;

(e)bGHpolyA信号序列,其具有SEQ ID NO:26的序列;及

(f)AAV2末端重复序列。

15.权利要求13的rAAV载体,其进一步包含AAV2-TT-S312N衣壳,其中所述VP1包含SEQ ID NO:31的氨基酸序列,所述载体进一步包含自5'至3'包含以下的自我互补核酸:

(a)AAV2末端重复序列;

(b)CBh启动子,其包含SEQ ID NO:25的核酸序列;

(c)编码FXN的经修饰的核酸,其包含SEQ ID NO:7的序列;

(d)CSS,其具有SEQ ID NO:24的序列;

(e)bGHpolyA信号序列,其具有SEQ ID NO:26的序列;及

(f)AAV2末端重复序列。

16.一种用于治疗有需要的患者的弗里德赖希(Fr iedreich)共济失调(FRDA)的rAAV载体,其中所述rAAV包含选自由以下各者组成的组的编码FXN的经修饰的核酸:

(a)经修饰的核酸,其包含在SEQ ID NO:1中所示的氨基酸序列,其中所述核酸的表达水平高于SEQ ID NO:2的野生型FXN核酸序列的表达水平,且进一步包含至少55%的GC水平、不超过124的CpG二核苷酸数目、及至少0.76的密码子适应指数(CAI);

(b)经修饰的核酸,其包含至少0.86的CAI、至少55%的GC水平、及不超过117的CpG二核苷酸数目;

(c)经修饰的核酸,其包含SEQ ID NO:5的核酸序列;及

(d)经修饰的核酸,其包含SEQ ID NO:7的核酸序列。

17.权利要求16的经修饰的核酸,其包含SEQ ID NO:7的核酸序列。

18.一种用于治疗有需要的患者的弗里德赖希共济失调的rAAV载体,其中所述载体包含权利要求3的编码共济失调蛋白的经修饰的核酸。

19.一种药物组合物,其包含权利要求18的rAAV载体。

20.一种治疗受试者的FRDA的方法,所述方法包括施用权利要求18的rAAV载体。

21.权利要求20的方法,其中通过直接心脏施用或通过颅内施用来全身性施用所述rAAV载体。

22.权利要求21的方法,其中所述rAAV载体经颅内施用。

23.权利要求21的方法,其中直接向心脏施用所述rAAV载体。

24.权利要求21的方法,其中编码FXN的所述经修饰的核酸包含SEQ ID NO:7的核酸序列。

25.一种治疗受试者中由降低水平的FXN介导的疾病、病症或病状的的方法，所述方法包括施用权利要求18的rAAV载体。

26.一种宿主细胞，其包含权利要求3的编码FXN的经修饰的核酸。

27.权利要求26的宿主细胞，其中所述细胞选自由以下各者组成的组：VERO、WI38、MRC5、A549、HEK293细胞、B-50或任何其他HeLa细胞、HepG2、Saos-2、HuH7及HT1080。

28.权利要求27的宿主细胞，其中所述宿主细胞为适于在悬浮培养物中生长的HEK293。

29.权利要求27的宿主细胞，其中所述细胞为具有ATCC NO.PTA13274的HEK293细胞。

30.权利要求26的宿主细胞，所述细胞包含编码选自由以下各者组成的组的至少一种蛋白质的至少一种核酸：Rep蛋白、Cap蛋白、E1a蛋白、E1b蛋白、E2a蛋白、E4蛋白及VA RNA。

31.权利要求1的编码共济失调蛋白的经修饰的核酸，其用于增加共济失调蛋白在细胞中的水平。

32.权利要求3的编码共济失调蛋白的经修饰的核酸，其用于增加共济失调蛋白在细胞中的水平。

33.权利要求18的rAAV载体，其用于治疗受试者的弗里德赖希共济失调。

用于基因治疗的经修饰的弗里德赖希共济失调基因及载体

发明领域

[0001] 本发明涉及经修饰的共济失调蛋白(frataxin)(FXN)基因、包含经修饰的FXN基因的载体、使用经修饰的FXN基因及含有该等基因的载体通过提供表达水平增加的非突变(野生型)线粒体蛋白共济失调蛋白来治疗弗里德赖希(Friedreich)共济失调的方法,该弗里德赖希共济失调包括心肌病和/或与其相关联的神经退化性疾病。

[0002] 发明背景

[0003] 弗里德赖希共济失调(FRDA)与编码线粒体蛋白共济失调蛋白的FXN基因的表达的降低和/或该FXN基因的突变相关联。FRDA为常染色体隐性疾病,意指个体仅在其自双亲遗传缺陷基因时罹患此疾病。FRDA由导致mRNA及共济失调蛋白的蛋白水平降低的FXN基因的突变引起。缺陷的共济失调蛋白表达引起关键代谢变化,包括氧化还原失衡及ATP缺乏。

[0004] FRDA为影响儿童及青少年且导致进行性残疾及过早死亡的神经退化性疾病。神经体征与感觉神经元的退化相关联,且穿过周边神经及脊髓的感觉信息的流动受到严重影响。还存在来自小脑及脊髓的肌肉控制信号的一些损害。这些问题导致表征FRDA的平衡、协调及肌肉强度的逐渐丧失。此外,患者往往罹患很可能造成过早死亡的肥厚性心肌病。心脏增大、不规则心跳及心脏病的其他症状为明显的。

[0005] 据信,共济失调蛋白调节线粒体内使用氧以产生能量所需的铁的含量。共济失调蛋白似乎充当铁的储存库,仅在需要铁来合成线粒体中的酶时释放铁。因此,共济失调蛋白的缺乏导致这些酶的缺乏且进一步降低线粒体功能,这很可能解释了弗里德赖希共济失调影响神经系统及心脏的细胞的原因。

[0006] 到目前为止,不存在用于停止或减缓FRDA的负面影响的疗法。临床使用的或处于评估的当前治疗方法针对缓解症状且最大化生命质量。物理治疗及言语治疗已用于改良运动。此外,一些药物已用于治疗心脏病。因此,非常需要一种用于治疗与FRDA相关联的症状的新颖治疗方法。

[0007] 发明概述

[0008] 本文公开及例示了编码共济失调蛋白(FXN)的经修饰的核酸及包含该经修饰的核酸的载体及通过向有需要的患者施用经修饰的核酸或包含该核酸的载体来治疗由降低水平的FXN介导的疾病的方法。

[0009] 本领域技术人员使用不超过常规实验的途径即可识别或能够确定本文中所描述的本发明的特定实施方案的许多等效物。此类等效物意欲由以下实施方案(E)涵盖。

[0010] E1.一种用于治疗人类受试者的FRDA的经修饰的FXN基因,其中经修饰的FXN基因已被修饰以改变GC核苷酸的含量和/或具有减少数目的CpG二核苷酸。

[0011] E2.如实施方案1的经修饰的FXN基因,其中由于CpG基序的甲基化,CpG二核苷酸的降低数目呈足以抑制基因表达的沉默的量。

[0012] E3.如实施方案1的经修饰的FXN基因,其中相对于野生型基因,GC核苷酸的含量大于10%、20%、30%、40%、50%、60%或70%。

[0013] E4.如实施方案3的经修饰的FXN基因,其具有>0.75、>0.80、>0.85、>0.90或>0.95

的密码子适应指数。

[0014] E5.如实施方案3的经修饰的FXN基因，其包含选自SEQ ID NO:3至SEQ ID NO:9中的任一者的序列。

[0015] E6.如实施方案1的经修饰的FXN基因，其中相对于野生型基因，GC核苷酸的含量小于10％、20％、30％、40％、50％、60％或70％。

[0016] E7.如实施方案1的经修饰的FXN基因，其包括在病毒载体或质体中。

[0017] E8.如实施方案7的经修饰的FXN基因，其中病毒载体为自我互补的AAV序列。

[0018] E9.如实施方案8的经修饰的FXN基因，其中该病毒载体选自由以下各者组成的组：AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV1.1、AAV2.5、AAV6.1、AAV6.3.1、AAV9.45、AAV Hu.26、AAV2i8、AAV2G9、rhAAV10、rhAAV74、RHM4-1、RHM15-1、RHM15-2、RHM15-3/RHM15-5、RHM15-4、RHM15-6、AAV2-TT、AAV2-TT-S312N、AAV3B-S312N、AAV-LK03，及其组合和变体。

[0019] E10.如实施方案8的经修饰的FXN基因，其中病毒载体为祖AAV载体。

[0020] E11.如实施方案8的经修饰的FXN基因，其中病毒载体为包括来自AAV2、AAV3B、AAV6或AAV8的AAV主链的组合且进一步包含来自AAV9的半乳糖(Gal)结合足迹的嵌合AAV。

[0021] E12.如实施方案1的经修饰的FXN基因，其中共济失调蛋白具有SEQ ID NO.1的氨基酸序列或其功能片段。

[0022] E13.一种用于治疗有需要的受试者的与共济失调蛋白缺乏相关联的疾病的方法，其包括向该受试者施用治疗有效量的经修饰的FXN基因，其中该经修饰的FXN基因已被修饰以增加或减少GC核苷酸的含量和/或减少CpG二核苷酸的数目。

[0023] E14.如实施方案13的方法，其中经修饰的FXN基因编码具有SEQ ID NO.1的氨基酸序列的共济失调蛋白。

[0024] E15.如实施方案13的方法，其中经修饰的FXN基因在靶细胞中表达，其中所述靶细胞为心脏细胞或神经元细胞。

[0025] E16.如实施方案13的方法，其中经修饰的FXN基因在病毒或非病毒载体中递送至靶细胞。

[0026] E17.如实施方案16的方法，其中载体通过全身注射或通过直接心脏或颅内注射递送。

[0027] E18.一种治疗有需要的受试者的弗里德赖希共济失调(FRDA)的方法，该方法包括：提供包含经修饰的FXN基因的至少一个重组病毒载体，其中该经修饰的FXN基因已被修饰以增加或减少GC核苷酸的含量和/或减少CpG二核苷酸的量；及在使经修饰的FXN基因以在受试者的心脏和/或神经元组织中产生治疗有效量的共济失调蛋白的水平表达的条件下向受试者施用重组病毒载体。

[0028] E19.如实施方案18的方法，其中向受试者的神经元或心肌细胞施用重组病毒载体。

[0029] E20.一种用编码共济失调蛋白肽或其功能片段的经修饰的FXN基因转染的宿主细胞，其中经修饰的FXN基因已被修饰以增加或减少GC核苷酸的含量和/或减少CpG二核苷酸的数目。

[0030] E21.一种制备共济失调蛋白肽或其片段的方法，其包括：用编码共济失调蛋白肽

或其功能片段的经修饰的FXN基因转染宿主细胞;及将宿主细胞保持在足以表达共济失调蛋白肽的生物条件下。

[0031] E22.如实施方案21的方法,其中经修饰的FXN基因相较于如SEQ ID NO:2中所示的野生型共济失调蛋白的核酸序列具有增加水平的GC核苷酸和/或降低水平的CpG二核苷酸。

[0032] E23.一种包含经修饰的FXN基因及医药学上可接受的载剂的药物组合物,其中经修饰的FXN基因具有增加或降低含量的GC核苷酸和/或减少数目的CpG二核苷酸。

[0033] E24.一种用于治疗FRDA的方法,其包括向需要治疗的受试者递送包含编码FXN基因的经修饰的多核苷酸序列的载体,其中FXN基因在靶细胞中表达,进而治疗受试者的FRDA。靶细胞优选为心脏或神经元细胞,且载体优选经由直接心脏或颅内注射递送至靶细胞。

[0034] E25.如实施方案1的经修饰的核酸,其中相对于野生型基因,经修饰的核酸具有降低的GC含量,该降低的GC含量比野生型基因小20%、30%、40%、50%或60%,同时仍具有与野生型相同的表达水平。可将沉默突变引入至编码序列中,以便减少基因的GC含量。

[0035] E26.一种具有降低水平的CpG二核苷酸的编码FXN的经修饰的核酸。

[0036] E27.一种用于治疗有需要的人类受试者的FRDA的编码FXN的经修饰的核酸(也被称作"经修饰的FXN基因"),其中经修饰的FXN基因已被修饰以相对于如SEQ ID NO:2所示的编码FXN的野生型核酸序列增加GC含量且减少某些顺式基序。

[0037] E28.一种经修饰的FXN基因,其与存在于如SEQ ID NO:2所示的编码FXN的野生型核酸序列中的CpG二核苷酸的数目相比较,具有由于CpG基序的甲基化而呈抑制基因表达的沉默的量的减少数目的CpG二核苷酸。

[0038] E29.一种治疗受试者的FRDA的方法,该方法包括:提供包含实施方案1至12、23、及25至28中的任一者的经修饰的FXN基因的至少一个重组病毒载体,且在使经修饰的FXN基因以在受试者的心脏和/或神经元组织中产生治疗有效量的共济失调蛋白的水平表达的条件下向受试者施用重组病毒载体。

[0039] E30.一种用于降低有需要的受试者的神经元及心肌细胞的弗里德赖希共济失调的影响或治疗该弗里德赖希共济失调的方法,其包括向该受试者施用治疗有效量的重组病毒载体,该重组病毒载体包含编码蛋白共济失调蛋白的经修饰的FXN核酸。

[0040] E31.一种用于治疗有需要的受试者的弗里德赖希共济失调的方法,其包括基于施用核酸的基因治疗,该核酸包含选自由序列SEQ ID NO:3-9组成的组的核苷酸序列。

[0041] E32.一种包含腺相关病毒(AAV)载体或其功能片段的组合物,该腺相关病毒载体包含经修饰的FXN基因,其中该AAV载体包含单链AAV载体基因组、双链AAV载体基因组或自我互补的(sc)AAV载体基因组。

[0042] E33.一种表达载体,其包含包括经修饰的FXN基因或其片段的多核苷酸。

[0043] E34.如实施方案33的载体,其中AAV包含选自由以下各者组成的组的血清型的衣壳:AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、rhAAV10、rhAAV74、RHM4-1、RHM15-1、RHM15-2、RHM15-3/RHM15-5、RHM15-4、RHM15-6、AAV Hu.26、AAV1.1(SEQ ID NO:15)、AAV2.5(SEQ ID NO.13)、AAV6.1(SEQ ID NO:17)、AAV6.3.1(SEQ ID NO:18)、AAV9.45、AAV2i8(SEQ ID NO:29)、AAV2G9、AAV2-TT(SEQ ID NO:31)、AAV2-TT-S312N(SEQ ID NO:33)、AAV3B-S312N及AAV-LK03。

[0044] E35.如实施方案34的载体，其进一步包含：AAV1.1衣壳，其中氨基酸残基265缺失(SEQ ID NO:15)；AAV 6.1衣壳，其中氨基酸残基265缺失(SEQ ID NO:17)；AAV 6.3.1衣壳，其中氨基酸残基265缺失且氨基酸残基531自Lys变为Glu(SEQ ID NO:18)。野生型AAV1衣壳的核苷酸序列显示于(SEQ ID NO:14)中，且野生型AAV6衣壳的核苷酸序列示于(SEQ ID NO:16)中。

[0045] E36.一种包含实施方案1至12、23、及25至28中的任一者的经修饰的FXN基因的嵌合AAV病毒载体，其进一步包含包括来自AAV2、AAV3、AAV6、AAV8的AAV主链的组合的衣壳以及来自AAV9的半乳糖(Gal)结合足迹。特别地，将来自AAV9的半乳糖(Gal)结合足迹接枝至硫酸肝素结合AAV血清型2以改良转导效率。

[0046] E37.一种包含实施方案1至12、23及25至28中的任一者的经修饰的FXN基因的嵌合AAV病毒载体，其进一步包括其中载体衣壳包含与AAV1和/或AAV6的265缺失突变组合的酪氨酸突变以及将半乳糖结合足迹添加至衣壳蛋白。

[0047] E38.一种包含实施方案1至12、23及25至28中的任一者的经修饰的FXN基因的嵌合AAV病毒载体，其进一步包含嵌入AAV的HI结构环或AAV 2主链中的585aa位置的靶向肽。另外，祖AAV载体可用于治疗性体内基因治疗。值得注意地，与同时代的病毒或其部分相比，自祖病毒序列组装的病毒颗粒的使用在当前人群中呈现对预存免疫降低的易感性。

[0048] E39.一种宿主细胞，其包含实施方案1至12、23及25至28中的任一者的经修饰的FXN基因。

[0049] E40.一种制备共济失调蛋白肽或其片段的方法，其包括：用实施方案1至12、23及25至28中的任一者的经修饰的FXN基因转染宿主细胞，及将宿主细胞保持在足以表达共济失调蛋白肽的生物条件下。

[0050] E41.一种实施方案1至12、23及25至28中的任一者的经修饰的FXN基因在治疗弗里德赖希共济失调中的用途。

[0051] E42.一种包含用于治疗引起人类受试者的心脏组织中的神经元及细胞的退化的弗里德赖希共济失调的经修饰的FXN基因及医药学上可接受的载剂的药物组合物，其中经修饰的FXN基因具有增加量的GC核苷酸、减少量的GC核苷酸和/或具有减少数目的CpG二核苷酸数目。

[0052] E43.一种编码共济失调蛋白的表达优化核酸，其包含选自SEQ ID NO:3-9中的任一者的核酸序列。

[0053] E44.一种编码包含示于SEQ ID NO:1中的氨基酸的共济失调蛋白的经修饰的核酸，其中核酸具有至少55％的GC含量、与SEQ ID NO:2的核酸序列相比减少数目的CpG二核苷酸、至少0.8的密码子适应指数(CAI)，且其中其以与包含SEQ ID NO:2的核酸序列的野生型共济失调蛋白的表达水平相比更高的水平表达。

[0054] E45.如实施方案44的经修饰的核酸，其中CAI为至少0.86。

[0055] E46.如实施方案44的经修饰的核酸，其中CAI为至少0.95。

[0056] E47.如实施方案44的经修饰的核酸，其中CAI为至少0.98。

[0057] E48.如实施方案44至47中的任一者的经修饰的核酸，其中GC含量为至少61％。

[0058] E49.如实施方案44至47中的任一者的经修饰的核酸，其中GC含量为至少69％。

[0059] E50.如实施方案44至49中的任一者的经修饰的核酸，其中CpG二核苷酸的数目为

约114至124。

[0060] E51.一种编码包含在SEQ ID NO:1中所示的氨基酸序列的共济失调蛋白(FXN)的经修饰的核酸,其中该核酸的表达水平高于SEQ ID NO:2的野生型FXN核酸序列的表达水平,且其中该经修饰的核酸包含选自由以下各者组成的组的至少一个特性:至少55%的GC含量、不超过124的CpG二核苷酸数目,及至少0.76的密码子适应指数(CAI)。

[0061] E52.如实施方案51的经修饰的核酸,该核酸包含选自由以下各者组成的组的至少一个特性:至少0.86、至少0.95或至少0.98的CAI;GC含量为至少57%、至少61%,或至少69%;CpG二核苷酸的数目小于124;及选自由如SEQ ID NO:3-9中所示的序列组成的组的核酸序列。

[0062] E53.一种编码FXN的经修饰的核酸,其中该核酸以与SEQ ID NO:2的野生型FXN核酸序列的表达水平相比更高的水平表达,且其中核酸包含以下各者中的至少一者:选自由SEQ ID NO:3-9组成的组的核酸序列;至少55%的GC含量;不超过117的CpG二核苷酸数目;及至少0.86的CAI。

[0063] E54.如实施方案53的经修饰的核酸,其中该核酸序列选自由SEQ ID NO:5及SEQ ID NO:7组成的组。

[0064] E55.如实施方案43至54中的任一者的经修饰的核酸,其包含SEQ ID NO:7的核酸序列。

[0065] E56.如实施方案1至12、23、25至28及43至55中的任一者的经修饰的核酸,其进一步包含编码至少一个AAV末端重复序列(TR)的核酸序列。

[0066] E57.如实施方案55的经修饰的核酸,其中核酸为单链、双链和/或自我互补的。

[0067] E58.如实施方案57的经修饰的核酸,其中该核酸为自我互补的。

[0068] E59.如实施方案1至12、23、25至28及43至58中的任一者的经修饰的核酸,其进一步包含增强子。

[0069] E60.如实施方案59的经修饰的核酸,其中增强子为细胞巨大病毒(CMV)立即早期增强子。

[0070] E61.如实施方案1至12、23、25至28及43至60中的任一者的经修饰的核酸,其进一步包含启动子。

[0071] E62.如实施方案1至12、23、25至28及43至61中的任一者的经修饰的核酸,其中启动子为组成性的或经调节的。

[0072] E63.如实施方案62的经修饰的核酸,其中启动子为经调节的。

[0073] E64.如实施方案63的经修饰的核酸,其中启动子为诱导性的或可抑制的。

[0074] E65.如实施方案1至12、23、25至28及43至64中的任一者的经修饰的核酸,其进一步包含编码胶原蛋白稳定序列(collagen stabilization sequence;CSS)的核酸序列。

[0075] E66.如实施方案1至12、23、25至28及43至65中的任一者的经修饰的核酸,其进一步包含终止密码子。

[0076] E67.如实施方案1至12、23、25至28及43至66中的任一者的经修饰的核酸,其进一步包含聚腺苷酸化(polyA)信号序列。

[0077] E68.如实施方案67的经修饰的核酸,其中启动子选自由以下各者组成的组:鸡β肌动蛋白(CBA)启动子、巨细胞病毒(CMV)启动子、CMV增强子/CBA启动子(CBh),及合成CAG启

动子。

[0078] E69.如实施方案68的经修饰的核酸,其中启动子为CBh启动子。

[0079] E70.如实施方案1至6、12、25至28及44至69中的任一者的经修饰的核酸,其进一步包含编码胶原蛋白稳定序列(CSS)的核酸序列。

[0080] E71.一种重组AAV载体(rAAV),其包含实施方案1至12、23、25至28及43至70中的任一者的编码FXN的经修饰的核酸。

[0081] E72.如实施方案71的rAAV,其中该rAAV包含选自由来自以下各者的衣壳组成的组的衣壳:AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAVrh10、AAVrh74、AAV2.5(SEQ ID NO.13)、AAV hu.26、AAV1.1、AAV2.5、AAV6.1、AAV6.3.1、AAV2i8、AAV2G9、AAV9.45、AAV2i8G9、RHM4-1、RHM15-1、RHM15-2、RHM15-3/RHM15-5、RHM15-4、RHM15-6、AAV2-TT、AAV2-TT-S312N、AAV3B-S312N及AAV-LK03。

[0082] E73.如实施方案72的rAAV,其中该衣壳选自由AAV2-TT、AAV2-TT-S312N及AAV2i8衣壳组成的组。

[0083] E74.如实施方案73的rAAV,其中经修饰的核酸包含SEQ ID NO:7的序列,且其中衣壳选自AAV2i8衣壳及AAV2-TT-S312N衣壳。

[0084] E75.如实施方案74的rAAV,其中核酸进一步包含侧接编码FXN的序列的两个AAV末端重复序列,且进一步包含在编码FXN的序列上游的CBh启动子。

[0085] E76.如实施方案75的rAAV,该核酸进一步包含在编码FXN的序列的3'的胶原蛋白稳定序列(CSS;SEQ ID NO:25)。

[0086] E77.如实施方案71至76中的任一者的rAAV,其中核酸包含牛生长激素polyA(bGHpolyA)信号序列。

[0087] E78.一种rAAV载体,其包含AAV2i8衣壳,其中VP1包含SEQ ID NO:29的氨基酸,且进一步包含自5'至3'包含以下的核酸:(a)AAV2末端重复序列(TR);(b)CBh启动子,其包含SEQ ID NO:26的核酸序列;(c)编码FXN的经修饰的核酸,其包含选自由SEQ ID NO:3-9组成的组的核酸序列;(d)CSS,其具有SEQ ID NO:25的序列;(e)bGHpolyA信号序列,其具有SEQ ID NO:27的序列;及(f)AAV2TR。

[0088] E79.一种包含AAV2-TT衣壳的rAAV载体,其中VP1包含SEQ ID NO:31的氨基酸,且进一步包含自5'至3'包含以下的核酸:(a)AAV2TR;(b)CBh启动子,其包含SEQ ID NO:26的核酸序列;(c)编码FXN的经修饰的核酸,其包含选自由SEQ ID NO:3-9组成的组的核酸序列;(d)CSS,其具有SEQ ID NO:25的序列;(e)bGHpolyA信号序列,其具有SEQ ID NO:27的序列;及(f)AAV2TR。

[0089] E80.一种包含AAV2-TT-S312N衣壳的rAAV载体,其中VP1包含SEQ ID NO:33的氨基酸,且进一步包含自5'至3'包含以下的核酸:(a)AAV2TR;(b)CBh启动子,其包含SEQ ID NO:26的核酸序列;(c)编码FXN的经修饰的核酸,其包含选自由SEQ ID NO:3-9组成的组的核酸序列;(d)CSS,其具有SEQ ID NO:25的序列;(e)bGHpolyA信号序列,其具有SEQ ID NO:27的序列;及(f)AAV2TR。

[0090] E81.如实施方案71至80中的任一者的rAAV载体,其中编码FXN的经修饰的核酸包含SEQ ID NO:7的核酸序列。

[0091] E82.一种用于治疗有需要的受试者的弗里德赖希共济失调的rAAV载体,其中该载

体包含实施方案1至6、12、25至28及71至81中的任一者的编码共济失调蛋白的经修饰的核酸。

[0092] E83.一种药物组合物，其包含实施方案7至11、33至39及71至82中的任一者的rAAV载体及医药学上可接受的载剂。

[0093] E84.一种治疗受试者的FRDA的方法，该方法包括施用以下各者中的至少一者：实施方案1至12、23、25至28及43至70中的任一者的编码共济失调蛋白的经修饰的核酸；实施方案7至11、33至39及71至82中的任一者的rAAV载体；及实施方案83的药物组合物。

[0094] E85.如实施方案84的方法，其中全身性地施用或通过直接心脏或颅内施用来施用实施方案7至11、33至39及71至82中的任一者的rAAV载体。

[0095] E86.如实施方案85的方法，其中颅内施用实施方案71至82中的任一者的rAAV载体。

[0096] E87.如实施方案85的方法，其中直接向心脏施用实施方案71至82中的任一者的rAAV载体。

[0097] E88.如实施方案84的方法，其中编码FXN的经修饰的核酸包含SEQ ID NO:6的核酸序列。

[0098] E89.如实施方案84的方法，其中编码FXN的经修饰的核酸包含SEQ ID NO:7的核酸序列。

[0099] E90.一种治疗由降低水平的FTX介导的疾病、病症或病状的方法，该方法包括施用以下各者中的至少一者：实施方案1至6、12、25至28及43至70中的任一者的编码共济失调蛋白的经修饰的核酸；实施方案7至11、33至39及71至82中的任一者的rAAV载体；及实施方案83的药物组合物。

[0100] E91.一种宿主细胞，其包含实施方案1至6、12、25至28及43至70中的任一者的编码FXN的经修饰的核酸。

[0101] E92.如实施方案91的宿主细胞，其中细胞选自由以下各者组成的组：VERO、WI38、MRC5、A549、HEK293细胞、B-50或任何其他HeLa细胞、HepG2、Saos-2、HuH7及HT1080。

[0102] E93.如实施方案92的宿主细胞，其中宿主细胞为适于在悬浮培养物中生长的HEK293。

[0103] E94.如实施方案91至93中的任一者的宿主细胞，其中细胞为具有ATCC NO.PTA 13274的HEK293细胞。

[0104] E95.一种包含实施方案7至11、33至39及70至82中的任一者的rAAV载体的包装细胞，其中该细胞进一步包含编码AAV Rep蛋白的至少一种核酸、编码AAV Cap蛋白的至少一种核酸，及编码辅助功能的至少一种核酸。

[0105] E96.一种用于产生rAAV载体的方法，该方法包括在产生rAAV的条件下培养实施方案91至95中的任一者的细胞。

[0106] E97.如实施方案96的方法，其进一步包括分离所产生的rAAV。

[0107] E98.以下各者中的至少一者提高细胞中的共济失调蛋白水平的用途：实施方案1至6、12、25至28及43至70中的任一者的编码共济失调蛋白的经修饰的核酸；实施方案7至11、33至39及71至82中的任一者的rAAV载体；及实施方案83的药物组合物。

[0108] E99.如实施方案1至6、12、25至28及43至70中的任一者的编码共济失调蛋白的经

修饰的核酸;实施方案7至11、33至39及71至82中的任一者的rAAV载体;及实施方案83的药物组合物,用于增加受试者的共济失调蛋白的含量。

[0109] E100.如实施方案1至6、12、25至28及43至70中的任一者的编码共济失调蛋白的经修饰的核酸;实施方案7至11、33至39及71至82中的任一者的rAAV载体;及实施方案83的药物组合物,用于治疗受试者的弗里德赖希共济失调。

[0110] 本发明的其他特征及优势根据下面的详细描述、附图、例示性实施方案及权利要求将是明显的。

[0111] 附图简述

[0112] 图1A及图1B。图1A及图1B均显示与野生型核酸相比来自编码FXN的所选经修饰的核酸的共济失调蛋白在HeLa细胞中的表达的结果(泳道1)。来自包含以下经修饰的核酸的HeLa细胞的提取物经检查以检测在细胞中产生的FXN。共济失调蛋白通过使用抗共济失调蛋白抗体的蛋白质印迹检测,该抗体使用与HRP(辣根过氧化酶)缀合以用于通过将蛋白质印迹曝光于光敏薄膜的化学发光检测的二级抗体来检测。泳道装载有来自用编码共济失调蛋白的以下经修饰的核酸转染的HeLa细胞的提取物:泳道1:野生型对照核酸;泳道2:IDT2;泳道3:IDT5;泳道4:JCAT;泳道5:GeneArt;泳道6:Genscr ipt(对照);及泳道7:Genscript(低CpG)。

[0113] 图2A至图2F显示用于克隆至自我互补的rAAV载体pTRs-KS-CBh-EGFP-bGHpolyA的各种经修饰的FXN基因构建体的序列,其中使用野生型FXN基因(SEQ ID NO:2)或其经修饰的形式(例如,SEQ ID NO:3-9)来替换EGFP标记基因。每个图显示WT FXN(图2A)或经修饰的FXN基因(图2B至图2F)。每一构建体包含(自5'至3')AgeI切割位点、FXN/经修饰的FXN基因、AvrII切割位点、胶原蛋白稳定序列(CSS)、SpeI切割位点、bGHpolyA信号序列,及MluI切割位点。图2A显示pTRs-KS-CBh-WTFXN-bGHpolyA构建体(SEQ ID NO:19);图2B显示整合的DNA技术IDT 1(IDT1)修饰的FXN基因构建体pTRs-KS-CBh-IDT1FXN-bGHpolyA(SEQ ID NO:20);图2C显示IDT3修饰的FXN基因构建体pTRs-KS-CBh-IDT3FXN-bGHpolyA(SEQ ID NO:21);图2D显示IDT4修饰的FXN基因构建体pTRs-KS-CBh-IDT4 FXN-bGHpolyA(SEQ ID NO:22);图2E显示GenScript修饰的FXN基因构建体pTRs-KS-CBh-GenScript FXN-bGHpolyA(SEQ ID NO:23);且图2F显示GenScript(低CpG)修饰的FXN基因构建体pTRs-KS-CBh-Genscript(低CpG)FXN-bGHpolyA(SEQ ID NO:24),每一序列包括在图2A至图2F中自5'至3'如下指示的元件(例如,AgeI、AvrII、CSS、SpeI、bGHpolyA及MluI):以粗体指示的AgeI切割位点(ACCGGT);呈小写字母的FXN基因,通过下划线指示的AvrI I切割位点(CCTAGG);通过双下划线指示的编码胶原蛋白稳定序列(CSS)的序列;以粗体带下划线指示的SpeI切割位点(ACTAGT);以斜体指示的牛生长激素聚腺苷酸化信号序列(bGHpolyA);及以粗斜体指示的MluI切割位点(ACGCGT)。构建体中的FXN基因在AgeI切割位点上游的CBh启动子的控制下。CBh启动子的序列并未显示于图2A至图2F中,但示于SEQ ID NO:25中。

[0114] 图3显示描绘包括AgeI切割位点上游的CBh启动子的载体的各种限制(切割)位点及元件的pTRs-KS-CBh-eGFP克隆构建体的载体(质体)图。

[0115] 图4A及图4B显示说明对照组、经治疗的突变体及未治疗的突变体雄性(图4A)及雌性(图4B)小鼠的基线心脏表型的图。图4A在每一分组内自左至右显示对照雄性、经治疗突变小鼠及未治疗突变小鼠的心脏表型,其中所述分组为:EF(射血分数)、FS(缩短分数);LV

Vol_d(左心室容积舒张);及LV Vol_s(左心室容积收缩)。图4B显示雌性小鼠群组的基线心脏表型:对照组(圆形);经治疗突变小鼠(方形);及未治疗突变小鼠(三角形)。

[0116] 图5A及图5B显示说明在5周龄(及对经治疗突变小鼠的治疗后14天)时与未经治疗Mck突变小鼠中的心脏表型相比较的经治疗Mck突变小鼠中的FRDA心脏表型的反转的图。图5A显示在rAAV-FXN注射14天之后的对照组(圆形)、经治疗突变(方形)及未治疗突变(三角形)的雄性小鼠的心脏表型。缩写词如下:AoV SV(主动脉瓣心搏出量);AoV CO(主动脉瓣心输出量);FS(缩短分数);及LV Mass AW(左心室质量前壁)。图5B显示在rAAV-FXN注射14天之后的对照组(圆形)、经治疗突变(方形)及未治疗突变(三角形)的雄性小鼠的心脏表型。缩写词如下:ES(射血分数);FS(缩短分数);AoV SV(主动脉瓣心搏出量);AoV CO(主动脉瓣心输出量)。

[0117] 图6A至图6C显示说明在经治疗Mck突变组的rAAV-FXN治疗后的二十八(28)天的雄性及雌性对照小鼠(圆形)、经治疗突变雄性及雌性小鼠(方形)及未治疗突变雄性及雌性小鼠(三角形)的心脏功能的图。图6A显示对历时连续数周(即,3周龄(rAAV施用时间)、5周龄(rAAV施用后14天)及7周龄(rAAV施用后28天))的所有三个小鼠群组的左心室质量(LVM)超声心动图评估,其中在5周龄施用治疗剂。图6B显示历时连续数周的所有三个小鼠群组的缩短因素(SF)超声心动图评估。图6C显示历时连续数周的所有三个小鼠群组的心输出量超声心动图评估。数据为每组8个小鼠的平均值±S.E.M。使用多重t-测试比较(Sidak-Bonferroni method)将Mck突变小鼠的数据与Mck阳性对照组相比较。*$p<0.05$。

[0118] 发明详述

[0119] 定义

[0120] 除非另外定义,否则本文中所使用的所有技术及科学术语均具有与本发明所属领域中一般技术者通常理解的含义。本文中所使用的术语仅出于描述特定实施方案的目的且并不意欲限制本发明。如本发明的描述及所附权利要求书中所使用的,除非上下文中另有清楚指示,否则单数形式“一个”、“一种”和“该/所述”还意欲包括复数形式。以下术语具有给定的含义:

[0121] 如本文中所使用,当指代可测量值(诸如生物活性的量、多核苷酸或多肽序列的长度、G及C核苷酸的含量、密码子适应指数、CpG二核苷酸的数目、剂量、时间、温度及其类似物)时,术语“约”意指涵盖指定量的20%、10%、5%、1%、0.5%或甚至0.1%的变化。

[0122] 如本文所用,术语“和/或”是指和涵盖相关所列项目中的一个或多个的任何及所有可能组合,以及当以替代性(“或”)解释时不具有组合。

[0123] AAV“rep”及“cap”基因指代编码腺相关病毒的复制及衣壳化蛋白质的多核苷酸序列。AAV rep及cap在本文中被称作AAV“封装基因”。

[0124] 本发明提供重组腺相关病毒(rAAV)载体。“AAV”为腺相关病毒的缩写,且可用于指代病毒自身或其衍生物。除非另有要求,否则该术语覆盖所有亚型和天然存在及重组的形式。缩写词“rAAV”指代重组腺相关病毒,其也被称作重组AAV载体(或“rAAV载体”)或简称为“AAV载体”。术语“AAV”包括例如各种血清型的AAV,例如,AAV 1型(AAV-1)、AAV 2型(AAV-2)、AAV 3型(AAV-3)、AAV 4型(AAV-4)、AAV 5型(AAV-5)、AAV 6型(AAV-6)、AAV 7型(AAV-7)、AAV 8型(AAV-8)、AAV 9型(AAV-9)、AAV 10型(AAV-10,包括AAVrh10)、AAVrh74、AAV 12型(AAV-12)、禽类AAV、牛AAV、犬类AAV、马类AAV、灵长类AAV、非灵长类AAV,及绵羊类AAV。

“灵长类AAV”指代感染灵长类动物的AAV，“非灵长类AAV”指代感染非灵长类哺乳动物的AAV，“牛AAV”指代感染牛哺乳动物的AAV等等。

[0125] 出于若干原因，各种血清型的AAV是有吸引力的，最显著地，AAV被认为是非病原性的且野生型病毒可整合其基因组位点，特异性地整合至人类19号染色体(Linden等人，1996年，Proc Natl Acad Sci USA 93:11288-11294)。人类基因组中的AAV的插入位点被称作AAVS1。与随机整合相反的定点整合被认为很可能产生可预测的长期表达图谱。

[0126] 各种血清型的AAV的基因组序列以及自然末端重复序列(TR)的序列、Rep蛋白质及衣壳亚单元为本领域中已知的。此类序列可发现于文献中或诸如GenBank的公共数据库中。参见，例如，GenBank登录号NC-002077(AAV-1)、AF063497(AAV-1)、NC-001401(AAV-2)、AF043303(AAV-2)、NC-001729(AAV-3)、NC-001829(AAV-4)、U89790(AAV-4)、NC-006152(AAV-5)、AF513851(AAV-7)、AF513852(AAV-8)及NC-006261(AAV-8)；所述GenBank登录号的公开内容以引用的方式并入本文中。还参见，例如，Srivistava等人，1983，J.Virology 45:555;Chiorini等人，1998，J.Virology 71:6823;Chiorini等人，1999，J.Virology 73:1309;Bantel-Schaal等人，1999，J.Virology 73:939;Xiao等人，1999，J.Virology 73:3994;Muramatsu等人，1996，Virology 221:208;Shade等人，1986，J.Virol.58:921;Gao等人，2002，Proc.Nat.Acad.Sci.USA 99:11854;Moris等人，2004，Virology 33:375-383;国际专利公开号WO 00/28061、WO 99/61601、WO 98/11244;WO 2013/063379;WO 2014/194132;WO 2015/121501;及美国专利第6,156,303号及第7,906,111号。

[0127] 如本文所使用的“rAAV载体”是指包含不属于AAV来源的多核苷酸序列(即，与AAV异源的多核苷酸)的AAV载体，通常为细胞的遗传转化所感兴趣的序列。在一些实施方案中，异源多核苷酸可通过至少一个，且有时通过两个AAV末端反向重复序列(ITRs)侧接。术语rAAV载体涵盖rAAV载体颗粒及rAAV载体质粒两者。rAAV载体可为单链(ssAAV)或自我互补的(scAAV)。“AAV病毒”或“AAV病毒颗粒”或“rAAV载体颗粒”是指由至少一个AAV衣壳蛋白(通常通过野生型AAV的所有衣壳蛋白)及衣壳化多核苷酸rAAV载体组成的病毒颗粒。若颗粒包含异源多核苷酸(即，除野生型AAV基因组，诸如待递送至哺乳动物细胞的转基因以外的多核苷酸)，则其通常被称作“rAAV载体颗粒”或简称为“rAAV载体”。因此，rAAV颗粒的产生必定包括rAAV载体的产生，因此载体包含于rAAV颗粒之内。

[0128] 如本文所使用的“重组”意指载体、多核苷酸、多肽或细胞为克隆、限制性或连接步骤(例如与多核苷酸或包含于其中的多肽相关)和/或导致构建体不同于在自然界中发现的产物的其他程序的各种组合的产物。重组病毒或载体为包含重组性多核苷酸的病毒颗粒。所述术语各自包括原始病毒构建体的子代及原始多核苷酸构建体的复制品。

[0129] “AAV Rep”意指AAV复制蛋白及其类似物。

[0130] “AAV Cap”意指AAV衣壳蛋白、VP1、VP2及VP3及其类似物。在野生型AAV病毒中，三种衣壳基因vp1、vp2及vp3彼此重叠。参见，Grieger及Samulski，2005，J.Virol.79(15):9933-9944。单个P40启动子允许所有三种衣壳蛋白vp1、vp2、vp3分别以约1:1:10的比率表达，其与rAAV生产互补。对于重组AAV载体的产生，VP1:VP2:VP3的所需比率在约1:1:1至约1:1:100的范围内，优选在约1:1:2至约1:1:50的范围内，更优选在约1:1:5至约1:1:20的范围内。尽管VP1:VP2的所需比率为1:1，但VP1:VP2的比率范围可在1:50至50:1的范围内变化。

[0131] 已知AAV血清型的衣壳的氨基酸序列的全面列表及比对由Marsic等人,2014,Molecular Therapy 22(11):1900-1909提供,尤其在补充图1中。

[0132] 仅出于说明的目的,野生型AAV2包含通过重叠序列由三种蛋白(VP1、VP2及VP3;总共60个衣壳蛋白组成AAV衣壳)组成的AAV的较小(20nm至25nm)二十面体病毒衣壳。蛋白VP1(735aa;Genbank登录号AAC03780)、VP2(598aa;Genbank登录号AAC03778)及VP3(533aa;Genbank登录号AAC03779)在衣壳中以1:1:10的比率存在。即,对于AAV,VP1为全长蛋白,且VP2及VP3为VP1的进行性更短形式,相对于VP1具有增大的N端截短。

[0133] "AAV TR"意指在AAV基因组的末端或末端附近的回文末端重复序列,包含通常互补、对称地布置的序列,且包括天然AAV TR及其类似物的类似物。

[0134] "顺式基序"包括诸如在基因组序列的末端处或接近末端处发现且经辨识用于复制初始化的保守序列;内部位置处的隐含启动子或序列很可能用于转录初始化、剪接或终止。

[0135] "治疗"意指反转、减缓或阻止此类术语所适用的病症或病状或此类病症或病状的一种或多种症状的进程。

[0136] "治疗有效量"意指必需向受试者提供治疗益处的活性剂的最小量。举例而言,对患者的"治疗有效量"为引发、改良、稳定、减缓进程或以其他方式引起病理症状、与病症相关联的疾病进程或生理条件的改良或屈服于病症的抵抗力的量。

[0137] "基因"意指含有能够在经转录及翻译之后编码特定多肽或蛋白的至少一个开放阅读框架的多核苷酸。

[0138] "编码序列"意指"编码特定蛋白"或"编码核酸"的序列,其表示当置于适当的调节序列的控制下(可操作地连接于适当的调节序列)时,被体外或体内转录(在DNA的情况下)及翻译(在mRNA的情况下)为多肽的核酸序列。编码序列的边界通过5'(氨基)端处的起始密码子及3'(羧基)端处的翻译终止密码子判定。编码序列可包括(但不限于)来自原核或真核mRNA的cDNA、来自原核或真核DNA的基因组DNA序列,和甚至合成DNA序列。

[0139] 关于病毒衣壳或颗粒,"嵌合"意指该衣壳或颗粒包括来自不同细小病毒(优选不同AAV血清型)的序列,如Rabinowitz等人美国专利6,491,907中所描述,该专利的公开内容以全文引用的方式并入于本文中。还参见Rabinowitz等人,2004,J.Virol.78(9):4421-4432。尤其优选的嵌合病毒衣壳为AAV2.5衣壳,其具有含以下突变的AAV2衣壳的序列:263Q至A;265插入T;705N至A;708V至A;及716T至N。其中如WO 2006/066066中所描述将编码此类衣壳的核苷酸序列定义为SEQ ID NO:15。其他优选的嵌合AAV包括(但不限于):描述于WO 2010/093784中的AAV2i8、描述于WO 2014/144229中的AAV2G9及AAV8G9,及AAV9.45(Pulicherla等人,2011,Molecular Therapy 19(6):1070-1078)。

[0140] 关于通过其他元件侧接的序列,"侧接"指相对于序列在上游和/或下游(即,5'和/或3')存在一个或多个侧接元件。术语"侧接"并不意欲指示序列必需为连续的。举例而言,编码转基因的核酸与侧接元件之间可存在插入序列。通过两个其他元件(例如,TR)"侧接"的序列(例如,转基因)指示一个元件位于序列的5'且另一元件位于序列的3';然而,可存在在其之间的插入序列。

[0141] "多核苷酸"意指通过磷酸二酯键连接的核苷酸的序列。在本文中在自5'至3'方向的方向上呈现多核苷酸。本发明的多核苷酸可为脱氧核糖核酸(DNA)分子或核糖核酸(RNA)

分子。其中多核苷酸为DNA分子，该分子可为基因或cDNA分子。在本文中通过单字母码指示核苷酸碱基：腺嘌呤(A)、鸟嘌呤(G)、胸(腺)嘧啶(T)、胞嘧啶(C)、肌核苷(I)及尿嘧啶(U)。可使用本领域技术人员所熟知的标准技术来制备本发明的多核苷酸。

[0142] 通过病毒“转导”细胞意指存在核酸自病毒颗粒至细胞的转移。

[0143] “经修饰的FXN基因”意指与编码FXN的野生型核酸(例如，SEQ ID NO:2)相比较具有至少一个修饰的编码FXN的经修饰的核酸(例如，SEQ ID NO:1的氨基酸序列)，其中该修饰包括(但不限于)增加的GC含量、减少的GC含量或具有减少的CpG含量的FXN基因。优选，经修饰的FXN基因展现经改良的蛋白表达，例如，与由野生型基因提供的蛋白在细胞中的表达水平相比较，经编码蛋白在其他相同细胞中以可检测的较高水平表达。

[0144] 细胞的“转染”意指出于基因修饰细胞的目的将遗传物质引入至细胞中。转染可通过本领域中已知的各种方式实现，诸如磷酸钙、聚乙二亚胺、电穿孔及其类似物。

[0145] 除非另外规定，否则“多肽”涵盖肽及蛋白两者。

[0146] “基因转移”或“基因递送”是指用于可靠地将外来DNA插入至宿主细胞的方法或系统。此类方法可导致非整合式转移DNA的短暂表达、经转移复制子(例如，游离基因体)的染色体外复制及表达，或经转移遗传物质至宿主细胞的基因组DNA中的整合。

[0147] 术语“宿主细胞”、“宿主细胞系”及“宿主细胞培养物”可互换使用且是指已引入外源核酸的细胞，包括此类细胞的子代。宿主细胞包括“转化体”、“经转化细胞”及“经转导细胞”，所述“转化体”、“经转化细胞”及“经转导细胞”包括主要经转化细胞及自其衍生的后代而不考虑继代的数目。

[0148] “转基因”用于意指针对递送至靶细胞(在本文中亦称为“宿主细胞”)且包括在靶细胞中的表达的掺入载体(包括病毒载体)中的任何异源核苷酸序列及相关联表达控制序列，诸如启动子。本领域技术人员应理解，将基于促进转基因在靶细胞中的表达的能力选择表达控制序列。转基因的实例为编码治疗性多肽的核酸。

[0149] “载体”意指包含待体外或体内递送至宿主细胞中的多核苷酸的重组质粒或病毒。

[0150] 当指代核酸或其片段时，“实质同源性”或“实质相似性”意指指示：当以适当的核苷酸插入或缺失与另一核酸(或其互补链)最佳地对准时，在该序列的至少约95％至99％中存在核苷酸序列一致性。

[0151] “重组病毒载体”意指包含一个或多个异源序列(即，不属于病毒来源的多核苷酸序列)的重组性多核苷酸载体。在重组细小病毒载体的情况下，重组性多核苷酸通过至少一个、优选两个末端反向重复序列(ITR)侧接。

[0152] “同源”在关于肽使用时是指两种肽之间的氨基酸序列相似性。当两种肽中的氨基酸位置由相同氨基酸占据时，其在该位置同源。因此“实质上同源”意指很大程度上但不完全同源的氨基酸序列，且当该序列同源时其保留大部分或所有活性。如本文中所使用，如本文所使用的“实质上同源”意指序列与参考肽至少50％相同，且同源性为优选至少75％且更优选95％。当肽具有与未经修饰的肽实质上相同的活性或功能的时候，其它肽序列修饰包括在内，诸如本文公开的序列的氨基酸序列的微小变化、缺失、替代物或衍生物。氨基酸的衍生物可包括(但不限于)：三氟亮氨酸、六氟亮氨酸、5,5,5-三氟异亮氨酸、4,4,4-三氟缬氨酸、p-氟苯丙氨酸、o-氟酪氨酸、m-氟酪氨酸、2,3-二氟酪氨酸、4-氟组氨酸、2-氟组氨酸、2,4-二氟组氨酸、氟脯氨酸、二氟脯氨酸、4-羟脯氨酸、硒甲硫氨酸、碲甲硫氨酸、硒半胱氨

酸、硒色氨酸、4-氨基色氨酸、5-氨基色氨酸、5-羟色氨酸、7-氮杂色氨酸、4-氟色氨酸、5-氟色氨酸、6-氟色氨酸、高烯丙基甘氨酸、高炔丙基甘氨酸、2-丁炔基甘氨酸、顺式-巴豆基甘氨酸、烯丙基甘氨酸、脱氢亮氨酸、脱氢脯氨酸、2-氨基-3-甲基-4-戊烯酸、叠氮基高丙氨酸、叠氮基丙氨酸(as idoalanine)、叠氮基正亮氨酸、p-乙炔基苯丙氨酸、p-叠氮基苯丙氨酸、p-溴苯基丙氨酸、p-乙酰基苯丙氨酸及苯并呋喃基丙氨酸。值得注意地,经修饰的肽将保留与未经修饰的肽相关联的活性或功能,经修饰的肽将大体上具有与未经修饰的序列的氨基酸序列"实质上同源"的氨基酸序列。

[0153] 多核苷酸或多肽与另一多核苷酸或多肽具有一定百分比"序列一致性",意指,当比对时,在比较两个序列时相同的碱基或氨基酸的百分比。可以多种不同方式判定序列相似性。为判定序列一致性,可使用方法及计算机程序(包括BLAST,在全球信息网ncbi.nlm.nih.gov/BLAST/上可用)比对序列。另一比对算法为FASTA,其在遗传学计算群组(Genetics Computing Group;GCG)包(来自Wis.,Madison,美国)中可用。用于比对的其他技术描述于Methods in Enzymology第266卷:Computer Methods for Macromolecular Sequence Analysis(1996),Doolittle编,Academic Press公司中。尤其受关注的是允许序列中的空隙的比对程序。Smith-Waterman为一种允许序列比对中的空隙的算法。参见Meth.Mol.Biol.70:173-187(1997)。使用Needleman及Wunsch比对方法的GAP程序可用于比对序列。参见J.Mol.Biol.48:443-453(1970)。

[0154] 所关注的是BestFit程序,其使用Smith and Waterman的局部同源算法(1981,Advances in Applied Mathematics 2:482-489)以判定序列一致性。空隙产生罚分将大体在1至5、通常2至4的范围内,且在许多实施方案中将为3。空隙扩展罚分将大体在约0.01至0.20的范围内,且在许多情况下将为0.10。程序具有由经输入以相比较的序列判定的预设参数。优选,使用由程序判定的默认参数来判定序列一致性。还可从遗传学计算群组(GCG)包(来自Madison,WI,美国)商购此程序。

[0155] 所关注的另一程序为FastDB算法。FastDB描述于Current Methods in Sequence Comparison and Analysis,Macromolecule Sequencing and Synthesis,Selected Methods and Applications,第127页至149页,1988,Alan R.Liss公司中。

[0156] 基于以下参数通过FastDB计算百分比序列一致性:不匹配罚分:1.00;空隙罚分:1.00;空隙大小罚分:0.33;及连接罚分:30.0。

[0157] 本发明提供经修饰的FXN基因。本发明还提供核酸构建体,诸如载体,其包括作为其序列的部分,经修饰的FXN基因(例如,GC含量优化的FXN基因序列)与野生型FXN基因序列相比较包含更大或更小量的GC核苷酸,和/或与野生型FXN基因中存在的CpG二核苷酸的含量相比较,FXN基因序列具有降低水平的CpG二核苷酸。举例而言,本发明包括质粒和/或其他载体,其包括经修饰的FXN序列以及其他元件(诸如调节元件)。此外,本发明提供包括经修饰的FXN序列的包装的基因运载工具,诸如病毒衣壳。本发明还包括递送方法,且优选,通过将经修饰的序列递送至细胞以及促进细胞中的表达所需的元件来表达经修饰的FXN基因。本发明还提供基因治疗方法,其中向受试者施用经修饰的FXN基因序列,例如,作为载体的组分和/或经包装为病毒基因运载工具的组分。治疗可例如有效提高受试者的共济失调蛋白的含量且治疗受试者的共济失调蛋白缺乏。在随后部分中进一步论述本发明的这些方面中的每一者。

[0158] 用于共济失调蛋白的表达的经修饰的核酸

[0159] 本发明提供编码共济失调蛋白的经修饰的核苷酸序列。经修饰的核苷酸序列包括含有一个或多个修饰的野生型或天然FXN基因序列。

[0160] 在一个方面中，与来自SEQ ID NO:2的野生型核酸序列的共济失调蛋白在细胞中的表达相比较，经修饰的核酸序列提供共济失调蛋白在其他相同细胞中的可检测的更高表达水平。此可被称作“表达优化”或“增强表达”核酸，或简称为“经修饰的核酸”。

[0161] 如在本文中可互换地指代，“优化”或“经密码子优化”是指编码序列已相对于野生型编码序列(例如，共济失调蛋白的编码序列)经优化以提高编码序列的表达，例如，通过最小化罕见密码子的使用、降低CpG二核苷酸的数目、移除隐含剪接供体或受体位点、移除Kozak序列、移除核糖体进入位点，及类似者。

[0162] 修饰的实例包括消除一个或多个顺式作用基序及引入一个或多个Kozak序列。在一个实施方案中，消除一个或多个顺式作用基序且引入一个或多个Kozak序列。

[0163] 可被消除的顺式作用基序的实例包括：内部TATA盒；chi位点；核糖体进入位点；ARE、INS、和/或CRS序列元件；重复序列和/或RNA二级结构；(隐含)剪接供体和/或受体位点、分支点；及Sall。

[0164] 在一个实施方案中，相对于SEQ ID NO:2的野生型FXN基因序列，GC含量(例如核酸序列中存在的G及C核苷酸的数目)增加。GC含量比野生型基因(SEQ ID NO:2)多优选至少5％、更优选至少6％、更优选至少7％、甚至更优选至少8％、更优选至少9％、甚至更优选至少10％、更优选至少12％、甚至更优选至少14％、更优选至少15％、更优选至少17％、甚至更优选至少20％、甚至更优选至少30％、更优选至少40％、更优选至少50％、甚至更优选至少60％且最优选至少70％。

[0165] 在另一实施方案中，GC含量以G(鸟嘌呤)及C(胞嘧啶)核苷酸在序列中的百分比表示。即，编码共济失调蛋白的野生型核酸(SEQ ID NO:1)的GC含量为约55％，而本发明的代表性经修饰的FXN基因的GC含量的范围为IDT-3(SEQ ID NO:8)约57％、Genescript(SEQ ID NO:6)57％；GeneArt(SEQ ID NO:5)61％及JCAT(SEQ ID NO:4)69％。因此，与如SEQ ID NO:2中所示的编码共济失调蛋白的野生型核酸序列约55％的GC含量相比较，本发明的经修饰的核酸包含至少57％、更优选至少61％的GC含量，甚至更优选至少69％的GC含量。

[0166] 在一个实施方案中，本发明的经修饰的核酸的GC含量大于包含SEQ ID NO:2的核酸序列的编码共济失调蛋白的野生型核酸的GC含量。本领域技术人员应理解，在知晓核酸代码的简并性的情况下，与编码蛋白的核酸序列无关，自其表达的共济失调蛋白的氨基酸序列优选为SEQ ID NO:1的氨基酸序列。

[0167] 在一个实施方案中，本发明的编码FXN的经修饰的核酸的GC含量与野生型FNX基因(SEQ ID NO:2)的GC含量相同(即，为约55％)。

[0168] 另外，编码共济失调蛋白的经修饰的核酸(即，经修饰的FXN基因)的密码子适应指数为优选至少0.74、优选至少0.76、甚至更优选至少0.77、更优选至少0.80、优选至少0.85、更优选至少0.86、更优选至少0.87、甚至更优选至少0.90、更优选至少0.95，且最优选至少0.98。

[0169] 在另一实施方案中，与编码FXN的野生型核酸序列(例如，SEQ ID NO:2)相比较，经修饰的FXN序列具有降低水平的CpG二核苷酸，该含量减少约10％、20％、30％、50％或更多。

[0170] 已知,CpG二核苷酸的甲基化在真核细胞的基因表达的调节中起重要作用。特别地,真核细胞中的CpG二核苷酸的甲基化基本上用于经由干扰转录机制来沉默基因表达。因此,由于通过CpG基序的甲基化诱发的基因沉默,具有减少数目的CpG二核苷酸的本发明的核酸及载体将提供较高且长效的转基因表达水平。

[0171] 在一个实施方案中,经修饰的FXN基因包含比野生型FXN基因少的潜在CpG岛区域,即,128个。优选,经修饰的FXN基因包含约124个潜在CpG岛区域,更优选约123个,甚至更优选约117个,且更优选约114个潜在CpG岛区域。

[0172] 经修饰的FXN基因序列还可包括促进亚克隆至表达载体中的侧接限制位点。许多此类限制位点为本领域中熟知的且包括(但不限于)图2A至图2F、及图3(scAAV质粒载体pTRs-KS-CBh-EGFP-BGH的质粒图)及表8(SEQ ID NO:19-23)中所显示的那些位点,诸如,AgeI、AvrII、SpeI及MluI。

[0173] 本发明还包括编码功能活性片段共济失调蛋白的序列SEQ ID NO:3至9中的任一者的片段。“功能活性”或“功能性共济失调蛋白”指示片段提供与全长共济失调蛋白相同或类似的生物活性。即,片段提供相同活性包括(但不限于):校正主要Fe-S集群缺乏;降低线粒体铁积累(Puccio等人,2001,Nature Genetics 27:181-186;Seznec等人,2004,Human Mol.Genet.13:1017-1024);及如Perdomini等人,2014,Nature Med.20(5):542-547中所论述的其他缺乏。FXN或其功能片段的生物活性还涵盖逆转或防止Mck小鼠中与如本文中其他处表明的FRDA相关联的心脏表型。

[0174] 本发明包括含有经修饰的FXN基因序列及各种调节或对照元件的核酸载体。适用于基因表达的调节元件的精确性质将在自生物体至生物体及自细胞类型至细胞类型的范围内变化。大体而言,其包括引导所关注的细胞中的RNA转录的初始化的启动子。启动子可为组成性或经调节的。组成性启动子为使得可操作地连接的基因基本上始终表达的那些。经调节的启动子为可经活化或去活化的那些。经调节的启动子包括诱导性启动子,其通常为“关闭”但可经诱发以变为“打开”,及“可抑制”启动子,其通常为“打开”但可变为“关闭”。许多不同调节子为已知的,包括温度、激素、细胞激素、重金属及调节蛋白。区别不是绝对的;组成性启动子可常常被调节至某一含量。在一些情况下,内源性路径可被利用以提供转基因表达的调节,例如,使用在病理条件改良时天然地下调的启动子。

[0175] 合适的启动子的实例包括:腺病毒启动子,诸如腺病毒主要晚期启动子;异源启动子,诸如巨细胞病毒(CMV)启动子;呼吸道融合性病毒启动子;劳斯肉瘤病毒(RSV)启动子;白蛋白启动子;诱导性启动子,诸如小鼠乳腺肿瘤病毒(MMTV)启动子;金属硫蛋白启动子;热休克启动子;α-1-抗胰蛋白酶启动子;B型肝炎表面抗原启动子;运铁蛋白启动子;脂蛋白元A-1启动子;鸡β肌动蛋白CBA)启动子,CBh启动子(SEQ ID NO:25)及CAG启动子(巨细胞病毒早期增强子元件及启动子、第一外显子、及鸡β肌动蛋白基因的第一内含子,及家兔β血球蛋白基因的剪接受体)(Alexopoulou等人,2008,BioMed.Central Cell Biol.9:2),及人类FXN启动子。启动子可为组织特异性启动子,诸如小鼠白蛋白启动子,其如同甲状腺素运载蛋白启动子(TTR)一样在肝细胞中具活性。

[0176] 在另一方面中,编码FXN的经修饰的核酸进一步包含增加FXN蛋白的表达的增强子。许多增强子在本领域中已知,包括(但不限于)巨细胞病毒主要立即早期增强子。更特定而言,CMV MIE启动子包含三个区域:调节子、独特区域及增强子(Isomura及Stinski,2003,

J.Virol.77(6):3602-3614)。CMV增强子区域可与其他启动子或其部分组合,从而形成混合式启动子以进一步增加可操作地连接其的核酸的表达。举例而言,鸡β-肌动蛋白(CBA)启动子或其部分可与CMV启动子/增强子或其部分组合,从而使CBA的形式称为"CBh"启动子,其表示鸡β肌动蛋白混合式启动子,如Gray等人(2011,Human Gene Therapy 22:1143-1153)中所描述。

[0177] 此外,控制元件可包括胶原蛋白稳定序列(CSS)、终止密码子、终止序列,及聚腺苷酸化信号序列,诸如(但不限于)牛生长激素聚A信号序列(bGHpolyA),从而在真核mRNA的3'端处驱使聚腺苷"尾部"的有效添加(参见,例如,Goodwin及Rottman,1992,J.Biol.Chem.267(23):16330-16334)。

[0178] 非病毒载体

[0179] 在一特定实施方案中,根据本发明使用的载体为非病毒载体。通常,非病毒载体可为包括描述经修饰的FXN基因或其变体的核酸序列的质粒。

[0180] 经包装的经修饰的FXN序列

[0181] 经修饰的FXN基因序列还可提供为经包装病毒载体的组分。大体而言,经包装病毒载体包括包装于衣壳中的病毒载体。病毒载体及病毒衣壳论述于随后部分中。包装于rAAV载体中的核酸可为单链(ss)、自我互补的(sc)或双链(ds)。

[0182] 病毒载体

[0183] 通常,携载转基因的病毒载体由编码转基因的多核苷酸、合适的调节元件及产生介导细胞转导的病毒蛋白所需的元件组装。病毒载体的实例包括但不限于腺病毒、逆转录病毒、慢病毒、疱疹病毒及腺相关联病毒(AAV)载体。

[0184] 根据本发明的方法产生的经包装病毒载体的病毒载体组分包括至少一个转基因,例如,经修饰的FXN基因序列及用于控制经修饰的FXN基因序列的表达的相关联表达控制序列。

[0185] 在优选的实施方案中,病毒载体包括细小病毒基因组(诸如rep及cap缺失和/或由经修饰的FXN基因序列及其相关联表达控制序列替代的AAV基因组)的一部分。经修饰的FXN基因序列通常嵌入至与适合病毒复制的一个或两个AAV TR或TR元件相邻(即,由其侧接)(Xiao等人,1997,J.Virol.71(2):941-948),替代编码病毒rep及cap蛋白的核酸。还可包括适用于促进经修饰的FXN基因序列在靶细胞中的组织特异性表达的其他调节序列。

[0186] 本领域技术人员应理解,包含转基因且不具有病毒复制所需的病毒蛋白(例如,cap及rep)的AAV载体不能复制,这是由于此类蛋白质为病毒复制及包装所必要。此外,AAV为一种依赖病毒(Dependovirus),原因在于其不能在无通过辅助病毒的细胞协同感染的情况下在细胞中复制。辅助病毒通常包括腺病毒或单纯疱疹病毒。替代地,如下文所论述,可提供给包装细胞的辅助功能(E1a、E1b、E2a、E4及VA RNA)包括通过用编码各种辅助元件的一个或多个核酸转染细胞,和/或该细胞可包含编码辅助蛋白的核酸。举例而言,HEK 293由用腺病毒5DNA转化人类细胞而产生,且目前表达许多种腺病毒基因,包括(但不限于)E1及E3(参见,例如,Graham等人,1977,J.Gen.Virol.36:59-72)。因此,那些辅助功能可由HEK 293包装细胞提供,而无需通过例如编码其的质粒将其供应至细胞。

[0187] 病毒载体可为任何合适的核酸构建体,诸如DNA或RNA构建体,且可为单链、双链、或双螺旋(即,如WO 2001/92551中所描述的自我互补的)。

[0188] 本领域技术人员应理解,rAAV载体可进一步包括“填充片段”或“填充”序列(填充/填充片段),其中包含转基因的核酸小于大约4.1kb至4.9kb大小时,最适合包装核酸进入AAV衣壳中。参见,Grieger及Samulski,2005,J.Virol.79(15):9933-9944。即,AAV载体通常接受具有指定大小范围(通常约4kb至约5.2kb,或略多)的DNA的插入序列。因此,对于较短序列,于插入序列中包含填充/填充片段,以便将长度调整至接近或处于正常大小的病毒基因组序列,以让AAV载体接受用于包装进入病毒颗粒中。在各种实施方案中,填充/填充片段核酸序列为核酸的未翻译(非蛋白质编码)片段。在rAAV载体的特定实施方案中,异源多核苷酸序列具有小于4.7Kb的长度,且填充/填充片段多核苷酸序列具有的长度使其在与异源多核苷酸序列组合(例如,插入至载体)时,具有在约3.0kb至5.5Kb之间、或约4.0kb至5.0Kb之间,或约4.3kb至4.8Kb之间的总长度。

[0189] 内含子还可充当填充/填充片段多核苷酸序列,以便获得包装至病毒颗粒中的AAV载体的长度。充当填充/填充片段多核苷酸序列的内含子及内含子片段也可增强表达。举例而言,与在不存在内含子元件的情况下的表达相比较,包括内含子元件可增强表达(Kurachi等人,1995,J.Biol.Chem.270(10):5276-5281)。此外,填充/填充片段多核苷酸序列为本领域中熟知且包括(但不限于)WO 2014/144486中所描述的那些。

[0190] 病毒衣壳

[0191] 经包装病毒载体的病毒衣壳组分可为细小病毒衣壳。AAV Cap及嵌合衣壳为优选的。合适的细小病毒衣壳组分的实例为来自细小病毒科的衣壳组分,诸如自发性细小病毒或依赖病毒。举例而言,病毒衣壳可为AAV衣壳(例如,AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAV1.1、AAV2.5、AAV6.1、AAV6.3.1、AAV9.45、AAVrh10、AAVrh74、RHM4-1(WO 2015/013313的SEQ ID NO:5)、AAV2-TT、AAV2-TT-S312N、AAV3B-S312N、AAV-LK03、蛇AAV、禽类AAV、牛AAV、大类AAV、马类AAV、绵羊类AAV、山羊AAV、虾AAV,及目前已知或后续发现的任何其他AAV。参见,例如,Fields等人,VIROLOGY,第2卷,第69章(第4版,Lippincott-Raven Publishers)。衣壳可衍生自以下各者中所公开的多个AAV血清型:美国专利第7,906,111号;Gao等人,2004,J.Virol.78:6381;Moris等人,2004,Virol.33:375;WO 2013/063379;WO 2014/194132;且包括WO 2015/121501中所公开的理想类型AAV(AAV-TT)变体,及WO 2015/013313中所公开的RHM4-1、RHM15-1至RHM15-6,及其变体,且本领域技术人员应知晓,很可能存在尚未识别的执行相同或类似功能的其他变体,或可包括来自两个或多于两个AAV衣壳的组分。AAV Cap蛋白的全集包括VP1、VP2及VP3。包含编码AAV VP衣壳蛋白的核苷酸序列的ORF可包含小于全集AAV Cap蛋白或可提供AAV Cap蛋白的全集。

[0192] AAV Cap蛋白中的一个或多个可为嵌合蛋白,其包括来自两个或多于两个病毒(优选两个或多于两个AAV)的AAV Cap的氨基酸序列,如Rabinowi tz等人,美国专利6,491,907中所描述,该专利的全部公开内容以引用的方式并入本文中。举例而言,嵌合病毒衣壳可包括AAV1Cap蛋白或亚单元及至少一个AAV2Cap或亚单元。嵌合衣壳可例如包括具有一个或多个B19Cap亚单元的AAV衣壳,例如,AAV Cap蛋白或亚单元可由B19Cap蛋白或亚单元替换。举例而言,在优选的实施方案中,AAV衣壳的Vp3亚单元可由B19的Vp2亚单元替换。

[0193] 另一实施方案包括嵌合病毒株,其经合成包括来自AAV2、AAV3、AAV6、AAV8等的AAV主链的组合以及来自AAV9的半乳糖(Gal)结合足迹。腺相关病毒(AAV)为将硫酸乙酰肝素

(HS)、半乳糖(Gal)或唾液酸(Sia)用作细胞表面结合的主要受体的辅助依赖型细小病毒。举例而言,AAV血清型2及3b利用HS.AAV1、4及5结合具有不同键联特异性的Sia,AAV血清型6辨识Sia及HS两者,而AAV9将Gal用于宿主细胞连接。特别地,来自AAV9的半乳糖(Gal)结合足迹接枝至硫酸肝素结合AAV血清型2上,且仅正交聚糖结合足迹的接枝改良转导效率。通过使用结构性比对及定点诱变将来自AAV9的Gal结合足迹掺入AAV2VP3主链或嵌合AAV2i8衣壳模板中来产生新双聚糖结合病毒株(AAV2G9)及嵌合肌肉向性病毒株(AAV2i8G9)。体外结合及转导测定通过用于细胞进入的AAV2G9确认HS及Gal受体两者的采用。转基因表达动力学的后续体内特征化及载体基因组生物分布特征指示通过此经合理改造的嵌合AAV病毒株的较快、持久、及经提高的转基因表达。类似经改良的转导特征用肝脏去靶向肌肉特异性AAV2i8G9嵌合体观察到(Shen等人,2013,J.Biol.Chem.288(4):28814-28823)。此类新接枝组合完全地描述于WO2014/144229中,WO2014/144229的内容以引用的方式并入本文中。另外的肝去靶向AAV(诸如AAV9.45)描述于Pulicherla等人,2011,Molecular Therapy 19(6):1070-1078中,其内容以全文引用的方式并入本文中,以供参考。

[0194] 在又一实施方案中,本发明提供用于治疗性体内基因治疗的祖AAV载体的使用。特别地,计算机衍生序列经重新合成且针对生物活性进行表征。此项工作引起九个功能性推定的祖AAV的产生及Anc80(AAV血清型1、2、8及9的经预测祖先)的识别(Zinn等人,2015,Cell Reports 12:1056-1068)。除了组装至病毒颗粒中之外,此类祖序列的预测及合成可通过使用描述于WO 2015/054653中的方法实现,WO 2015/054653的内容以引用的方式并入本文中。值得注意地,与当代病毒或其部分相比,自祖病毒序列组装的病毒颗粒的使用在当前人群中呈现对预存免疫性降低的易感性。

[0195] 经包装病毒载体的产生

[0196] 本发明包括由“宿主细胞”涵盖的包装细胞,所述包装细胞可经培养以产生本发明的经包装病毒载体。本发明的包装细胞大体上包括具有异源(1)病毒载体功能、(2)包装功能及(3)辅助功能的细胞。这些组分功能中的每一者论述于随后部分中。

[0197] 最初,载体可通过本领域技术人员已知的若干方法制得(参见,例如,WO 2013/063379)。优选的方法描述于Grieger等人,2015,Molecular Therapy 24(2):287-297中,其内容出于所有目的以引用的方式并入本文中。简言之,将HEK293细胞的有效转染用作起点,其中将来自合格临床主细胞库的黏附HEK293细胞用于在允许快速及可调式rAAV产生的摇瓶及WAVE生物反应器中的无动物组分悬浮条件下生长。使用三重转染方法(例如,WO 96/40240),在转染之后48小时收获时,悬浮HEK293细胞系产生大于1×10^5含有颗粒(vg)/细胞的载体基因组或大于1×10^{14}vg/L的细胞培养。更特定而言,三重转染是指用三个质粒转染包装细胞的事实:一个质粒编码AAV rep及cap基因,另一质粒编码各种辅助功能(例如,腺病毒或HSV蛋白,诸如E1a、E1b、E2a、E4及VA RNA,且另一质粒编码转基因及其各种控制元件(例如,经修饰的FXN基因及CBh启动子)。

[0198] 为实现所需产量,优化多个变量:诸如选择支持生长及转染两者的兼容的无血清悬浮培养基,选择转染试剂、转染条件及细胞密度。基于离子交换层析方法,还开发了通用纯化策略,其导致AAV血清型1至6、8、9的高纯度载体预备及各种嵌合衣壳。此使用者友好制程可在一周内完成,导致较高的满至空颗粒比率(>90%全部颗粒),提供纯化后产量(>1×10^{13}vg/L)及适用于临床应用且关于所有血清型及嵌合颗粒通用的纯度。此可调式产生技术

已用于针对视网膜新血管生成(AAV2)、B型血友病(scAAV8)、巨轴突神经病(scAAV9)及色素性视网膜炎(AAV2)产生GMP I期临床AAV载体,已向患者施用所述AAV载体。另外,整个载体产生中通过实施灌注方法的5倍增加的最小值需要在转染后的大量时间点自培养基收获rAAV。

[0199] 病毒载体功能

[0200] 本发明的包装细胞包括病毒载体功能以及包装及载体功能。病毒载体功能通常包括细小病毒基因组(诸如rep及cap缺失及由经修饰的FXN序列及其相关联表达控制序列替代的AAV基因组)的一部分。病毒载体功能包括导致用于包装的病毒载体的复制的充足表达控制序列。通常,病毒载体包括细小病毒基因组(诸如rep及cap缺失及由转基因及其相关联表达控制序列替代的AAV基因组)的一部分。转基因通常由两个AAV TR侧接,替代缺失的病毒rep及cap ORF。适当的表达控制序列(诸如适用于促进转基因在靶细胞中的组织特异性表达的组织特异性启动子及其他调节序列)包括在内。转基因通常为可经表达以产生治疗性多肽或标志物多肽的核酸序列。

[0201] “复式载体”在本文中可互换地被称作“二聚”或“自我互补的”载体。复式细小病毒颗粒可例如包含含有病毒颗粒DNA(vDNA)的细小病毒衣壳。vDNA为自我互补的以使得在自病毒衣壳释放之后其可形成发夹结构。复式vDNA似乎将可通过宿主细胞表达(即,转录及视情况翻译)的双链DNA提供至宿主细胞而无需第二链合成,常规细小病毒载体有此需要。复式/自我互补的rAAV载体为本领域中熟知的且描述于例如WO 2001/92551、WO 2015/006743及许多其他文献中。

[0202] 病毒载体功能可合适地提供为复式载体模板,如Samulski等人的美国专利第7,465,583号(针对关于复式载体的其教导,其全部公开内容以引用的方式并入本文中)中所描述。复式载体为二聚自我互补的(sc)多核苷酸(通常,DNA)。复式载体基因组针对所选细小病毒衣壳(例如,AAV衣壳)内的衣壳化优选含有足够的包装序列。本领域技术人员将了解,复式vDNA在所有条件下可能不会以双链形式存在,但在促进互补核苷酸碱基的退火的条件下有此能力。“复式细小病毒颗粒”涵盖混合式、嵌合及靶向病毒颗粒。优选,复式细小病毒颗粒具有AAV衣壳,该衣壳可进一步为嵌合或靶向衣壳,如上文所描述。

[0203] 病毒载体功能可合适地提供为复式载体模板,如Samulski等人的美国专利第7,465,583号(针对关于复式载体的其教导其全部公开内容以引用的方式并入本文中)中所描述。复式载体为二聚自我互补的(sc)多核苷酸(通常,DNA)。举例而言,由于链内碱基配对,复式载体的DNA可经所选以便形成双链发夹结构。复式DNA载体的两条链可包装于病毒衣壳内。复式载体提供与双链DNA病毒载体相当的功能且可缓解靶细胞将互补DNA合成至通常通过病毒囊封的单链基因组的需要。

[0204] 经所选用于病毒载体的TR(可解析及非可解析)优选为AAV序列,其中血清型1、2、3、4、5及6为优选的。可解析AAV TR无需具有野生型TR序列(例如,野生型序列可通过插入、缺失、截短或错义突变改变),只要TR介导所需功能(例如,病毒包装、整合和/或原病毒救援及类似者)即可。TR可为充当AAV末端反向重复的合成序列,诸如如Samulski等人的美国专利第5,478,745号中所描述的“双D序列”,该美国专利的全部公开内容以全文引用的方式掺入本文中。通常但未必,TR来自相同细小病毒,例如,两个TR序列均来自AAV2。

[0205] 包装功能包括衣壳组分。衣壳组分优选来自细小病毒衣壳,诸如AAV衣壳或嵌合

AAV衣壳功能。合适的细小病毒衣壳组分的实例为来自细小病毒科的衣壳组分，诸如自发性细小病毒或依赖病毒。举例而言，衣壳组分可选自AAV衣壳，例如，AAV1、AAV2、AAV3、AAV4、AAV5、AAV6、AAV7、AAV8、AAV9、AAV10、AAV11、AAV12、AAVrh10、AAVrh74、RHM4-1、RHM15-1、RHM15-2、RHM15-3/RHM15-5、RHM15-4、RHM15-6、AAV Hu.26、AAV1.1(SEQ ID NO:15)、AAV2.5(SEQ ID NO:13)、AAV6.1(SEQ ID NO:17)、AAV6.3.1(SEQ ID NO:18)、AAV9.45、AAV2i8(SEQ ID NO:29)、AAV2G9、AAV2i8G9、AAV2-TT(SEQ ID NO:31)、AAV2-TT-S312N(SEQ ID NO:33)、AAV3B-S312N及AAV-LK03，及如尚未识别的或来自非人类灵长类源的其他新颖衣壳。衣壳组分可包括来自两个或多于两个AAV衣壳的组分。

[0206] 在一个更优选的实施方案中，VP衣壳蛋白中的一个或多个为嵌合蛋白，包含来自两个或多于两个病毒、优选两个或多于两个AAV的氨基酸序列，如Rabinowitz等人，美国专利6,491,907中所描述。嵌合衣壳在本文中描述为具有来自与另一血清型组合的一个血清型的足以修饰a)病毒产量、b)免疫反应、c)靶向、d)去靶向等的至少一个氨基酸残基。

[0207] 其他嵌合蛋白可通过Li等人，2008，Mol.Ther.16(7):1252-1260中所示的指令制得，其内容以引用的方式并入本文中。特别地，将基于DNA改组的方法用于经由定向进化开发细胞型特异性载体。腺相关病毒(AAV)血清型1至9的衣壳基因组使用PCR随机地分段及重组以产生嵌合衣壳库。含有来自AAV1、2、8及9的基因组片段的单个感染性克隆(嵌合-1829)与先前经显示具有至AAV的低复制容许度的整合素负型仓鼠黑素瘤细胞系隔离。分子模型化研究表明AAV2促成对称的二十面体三重轴线处的表面环，而AAV1及9分别促成两倍及五倍对称相互作用。将C端结构域(AAV9)识别为黑素瘤向性至合理诱变的关键结构性决定子。嵌合-1829将硫酸乙酰肝素用作主要受体且比所有血清型更有效地转导黑素瘤细胞。很可能使用AAV或其他病毒衣壳序列将此技术应用于替代细胞/组织类型以产生作为人类基因转移的载体的一类新生物纳米颗粒。

[0208] 经包装病毒载体大体上包括由TR元件侧接的经修饰的FXN基因序列及表达控制序列，在本文中被称作“转基因”或“转基因表达盒”，其足以导致载体DNA的包装及经修饰的FXN基因序列在经转导细胞中的后续表达。病毒载体功能可例如作为质粒或扩增子的组分供应至细胞。病毒载体功能可染色体外地存在于细胞系内和/或可整合至细胞的染色体DNA中。

[0209] 可采用将携载病毒载体功能核苷酸序列引入至细胞宿主以供复制及包装的任何方法，包括但不限于：电穿孔、磷酸钙沈淀、显微注射、阳离子或阴离子脂质体，及与核定位信号组合的脂质体。在其中通过使用病毒载体的转染提供病毒载体功能的实施方案中，可使用用于产生病毒感染的标准方法。

[0210] 包装功能

[0211] 包装功能包括用于病毒载体复制及包装的基因。因此，例如，包装功能视需要可包括：病毒基因表达、病毒载体复制、自整合式状态救援病毒载体、病毒基因表达及将病毒载体包装至病毒颗粒中所需的功能。可使用诸如质粒或扩增子、杆状病毒或HSV辅助构建体的基因构建体将包装功能共同或单独地供应至包装细胞。包装功能可染色体外地存在于包装细胞内，但优选整合于细胞的染色体DNA中。实例包括编码AAV Rep及Cap蛋白的基因。

[0212] 辅助功能

[0213] 辅助功能包括建立包装细胞的活动性感染所需的辅助病毒元件，此为起始病毒载

体的包装所需要的。实例包括衍生自腺病毒、杆状病毒和/或疱疹病毒的足以引起病毒载体的包装的功能。举例而言，腺病毒辅助功能通常将包括腺病毒组分E1a、E1b、E2a、E4及VA RNA。包装功能可通过用所需病毒感染包装细胞而提供。可使用诸如质粒或扩增子的基因构建体将包装功能共同或单独地供应至包装细胞。参见，例如，如Rabinowitz等人，2002，J.Virol.76:791中所描述的pXR辅助质粒，及Grimm等人，1998，Human Gene Therapy 9:2745-2760中所描述的pDG质粒。包装功能可染色体外地存在于包装细胞内，但优选整合于细胞的染色体DNA(例如，HEK 293细胞中的E1或E3)中。

[0214] 可采用任何合适的辅助病毒功能。举例而言，在包装细胞为昆虫细胞的情况下，杆状病毒可充当辅助病毒。在AAV包装方法中还可将疱疹病毒用作辅助病毒。编码AAV Rep蛋白的混合式疱疹病毒可有利地促进更可调式AAV载体产生方案。

[0215] 可采用将携载辅助功能的核苷酸序列引入至细胞宿主以供复制及包装的任何方法，包括但不限于：电穿孔、磷酸钙沈淀、显微注射、阳离子或阴离子脂质体，及与核定位信号组合的脂质体。在其中通过使用病毒载体的转染或使用辅助病毒的感染提供辅助功能的实施方案中，可使用用于产生病毒感染的标准方法。

[0216] 包装细胞

[0217] 在经包装病毒载体的产生中可采用本领域中已知的任何合适的容许或包装细胞。哺乳动物细胞或昆虫细胞为优选的。在本发明的实践中适用于包装细胞的产生的细胞的实例包括例如：诸如VERO的人细胞系、WI38、MRC5、A549、HEK 293细胞(其表达在组成性启动子的控制下的功能性腺病毒E1)、B-50或任何其他HeLa细胞、HepG2、Saos-2、HuH7及HT1080细胞系。在一个方面中，包装细胞能够在悬浮培养物中生长，更优选，细胞能够在无血清培养中生长。在一个实施方案中，包装细胞为在不含血清的培养基中悬浮生长的HEK293。在另一实施方案中，包装细胞为US专利第9,441,206号中所描述的HEK293细胞，且经保藏为ATCC NO.PTA 13274。大量rAAV包装细胞系在本领域中已知，包括(但不限于)WO 2002/46359中所公开的那些。

[0218] 用作包装细胞的细胞系包括昆虫细胞系。可根据本发明使用允许AAV的复制且可维持培养的任何昆虫细胞。实例包括：草地黏虫，诸如Sf9或Sf21细胞系；果蝇属细胞系；或蚊子细胞系，例如，白蚊伊蚊衍生细胞系。优选的细胞系为草地黏虫Sf9细胞系。将针对关于异源多肽的表达的昆虫细胞的使用的其教导、将核酸引入至此类细胞中的方法、及维持培养此类细胞的方法的以下参考文件并入本文中：Methods in Molecular Biology，Richard编，Humana Press，NJ(1995)；O'Reilly等人，Baculovirus Expression Vectors:A Laboratory Manual，Oxford Univ.Press(1994)；Samulski等人，1989，J.Virol.63:3822-3828；Kajigaya等人，1991，Proc.Nat'l.Acad.Sci.USA 88:4646-4650；Ruffing等人，1992，J.Virol.66:6922-6930；Kimbauer等人，1996，Virol.219:37-44；Zhao等人，2000，Virol.272:382-393；及Samulski等人，美国专利第6,204,059号。

[0219] 可使用本领域中已知的任何方法产生根据本发明的病毒衣壳，例如，通过来自杆状病毒的表达(Brown等人，(1994)Virology 198:477-488)。作为另一替代，本发明的病毒载体可使用杆状病毒载体在昆虫细胞中产生以如例如由Urabe等人，2002，Human Gene Therapy 13:1935-1943所描述的递送rep/cap基因及rAAV模板。

[0220] 在另一方面中，本发明提供在昆虫细胞中产生rAAV的方法，其中杆状病毒包装系

统或载体可经构建以通过将这些基因改造至杆状病毒载体的多角体蛋白编码区域中且通过至宿主细胞中的转染产生病毒重组体来携载AAV Rep及Cap编码区域。在针对AAV使用Baculavirus产生时值得注意地是，优选，AAV DNA载体产物为类似分子的自我互补的AAV而无需使用至AAV ITR的突变。此似乎为昆虫细胞中的低效AAV rep基因巧合的副产物，其藉助于功能性Rep酵素活性的缺少产生自我互补的DNA分子。宿主细胞为感染杆状病毒的细胞或其中已引入编码杆状病毒辅助功能的额外核酸或其中包括这些杆状病毒辅助功能。这些杆状病毒病毒可表达AAV组分且随后促进衣壳的产生。

[0221] 在产生期间，包装细胞大体上包括一个或多个病毒载体功能以及足以引起病毒载体的复制及包装的辅助功能及包装功能。可使用基因构建体(诸如质粒或扩增子)将这些不同功能共同或单独地供应至包装细胞，且所述功能可染色体外地存在于细胞系内或整合于细胞的染色体中。

[0222] 可向细胞供应已并入的所陈述功能中的任何一个或多个，例如，具有染色体外地并入或整合于细胞的染色体DNA中的一个或多个载体功能的细胞系；具有染色体外地并入或整合于细胞的染色体DNA中的一个或多个包装功能的细胞系；或具有染色体外地并入或整合于细胞的染色体DNA中的辅助功能的细胞系。

[0223] rAAV纯化

[0224] rAAV载体可通过本领域的标准方法(诸如通过管柱层析法或氯化铯梯度)纯化。用于纯化rAAV载体的方法为本领域中已知的且包括以下各者中描述的方法：Clark等人，1999，Human Gene Therapy 10(6)：1031-1039；Schenpp及Clark，2002，Methods Mol.Med.69：427-443；美国专利第6,566,118号及WO 98/09657。

[0225] 治疗方法

[0226] 经修饰的FXN基因可用于弗里德赖希共济失调相关病症(诸如，退化性神经肌肉病症和/或与弗里德赖希共济失调相关联的心肌病)的基因治疗。个体可能需要基因治疗，此由于作为FXN基因的编码序列中的一个或多个突变的结果，FXN经不适当地表达，例如，具有不正确氨基酸序列，或在错误组织中或在错误时间处表达或表达不足。本发明的经修饰的FXN基因可用作基因治疗以提高蛋白共济失调蛋白的产量且藉此提高线粒体中的能量生产。参见，例如，美国专利第9,066,966号。

[0227] 本发明的载体的靶细胞为能够表达共济失调蛋白的细胞，诸如哺乳动物的心脏系统的那些，神经元细胞、肌肉细胞及其他细胞具有恰当细胞机制以处理前驱体从而产生具有共济失调蛋白活性的蛋白。

[0228] 药物组合物

[0229] 在特定实施方案中，本发明提供用于预防或治疗由共济失调蛋白的减少表达介导或与共济失调蛋白的减少表达相关联的疾病或病状(例如，弗里德赖希共济失调)的药物组合物。组合物包含治疗有效量的载体，该载体包含可增加FXN在细胞中的表达水平的经修饰的FXN基因。组合物包含载体，该载体包含编码FXN的经修饰的(例如，经优化)核酸，其中该组合物进一步包含医药学上可接受的载剂和/或其他药剂、医药剂、载剂、佐剂、稀释剂等。对于注射，载剂将通常为液体。作为注射介质，使用含有对于注射溶液而言常见的添加剂(诸如稳定剂、盐或盐水和/或缓冲剂)的水为优选的。

[0230] 例示性医药学上可接受的载剂包括无菌、无热原质水及无菌、无热原质磷酸盐缓

冲盐水。生理学上可接受的载剂包括医药学上可接受的载剂。医药学上可接受的载剂为在生物学上或其他方面不会不适宜的那些载剂，即，可向受试者施用该物质而不引起超过该物质的有利生物作用的不适宜生物作用。

[0231] 药物组合物可例如用于离体细胞的转染或直接向受试者施用病毒载体或细胞。

[0232] 优选以生物学上有效量向细胞施用包含经修饰的FXN基因的重组病毒载体。若体内向细胞施用病毒载体(例如，如下文所描述向受试者施用病毒)，则病毒载体的生物学上有效量为足以引起转基因在靶细胞中的转导及表达的量。

[0233] 在一个实施方案中，本发明包括通过单独地或在包含编码共济失调蛋白的经修饰的核酸的载体中(包括质粒、病毒、纳米颗粒、脂质体，或用于将核酸提供至细胞的任何已知方法)向细胞施用核酸来提高共济失调蛋白在细胞中的含量的方法。该方法包括一种方法，其中编码共济失调蛋白的mRNA的含量和/或所表达共济失调蛋白的含量可检测地大于共济失调蛋白(mRNA和/或蛋白)在并未施用核酸的其他相同细胞中的含量。本领域技术人员应理解，细胞可在体外培养或生长或可存在于生物体中(即，体内)。此外，细胞可表达内源性共济失调蛋白，使得共济失调蛋白在细胞中的含量可增加，和/或细胞可表达为野生型共济失调蛋白的突变体或变体的内源性共济失调蛋白，例如，具有SEQ ID NO:2的序列的共济失调蛋白，尤其当人类共济失调蛋白可存在多于一个野生型等位基因时。因此，与表达于其他相同但未经治疗的细胞中的共济失调蛋白的含量相比较共济失调蛋白的含量增加。

[0234] 本发明的另一方面为用含有经修饰的基因的载体体内治疗受试者的方法。可通过本领域中已知的用于施用病毒载体的任何方式向有需要的人类受试者或动物施用载体。

[0235] 可另外施用载体且将其作为照护标准的佐剂。即，载体可与另一治疗剂或化合物同步、同时或以本领域技术人员使用常规方法确定的确定给药间隔共施用。

[0236] 在一个方面中，本发明的rAAV可与包含与rAAV-FXN载体相同或不同的衣壳蛋白的空衣壳(即，不含有核酸分子的病毒衣壳)共施用。此由于本领域技术人员应理解，空衣壳的共施用可降低本发明的rAAV的免疫反应，例如，中和反应。即，空衣壳可充当允许rAAV-FXN载体的诱饵以避免如例如WO 2015/013313中所论述的中和抗体(Nab)免疫反应。

[0237] 全身施用的例示性施用模式包括(但不限于)：静脉内、皮下、皮内、肌内及关节内施用，及类似者，以及直接组织或器官注射。

[0238] 在一个实施方案中，载体经全身性地施用。本领域技术人员应理解，全身施用可将编码FXN的治疗性基因递送至受其中降低水平的FXN影响的所有组织，包括所有肌肉。

[0239] 尽管如此，本领域技术人员应理解，载体可直接递送至受FXN缺乏影响的区域，即，大脑及心脏。

[0240] 因此，在其他优选的实施方案中，通过直接注射将包含经修饰的FXN基因的发明性载体施用至心脏或中枢神经系统(CNS)组织中。

[0241] 在一个实施方案中，编码FNX的经修饰的核酸、载体或包含该载体的组合物经颅内递送，包括鞘内、神经内、脑内、心室内施用。

[0242] 在一个实施方案中，编码FNX的经修饰的核酸、载体或包含该载体的组合物通过利用心外注射随后小切口、利用冠状动脉内注射、利用心内膜心肌注射或利用可用于心脏的另一注射类型直接施用至心肌中递送至心脏。

[0243] 额外施用途径还可包含在直接目测下的载体的局部应用，例如，浅皮层应用或其

他非定向性应用。载体还可例如鞘内或通过静脉注射递送至心室中。

[0244] 本发明的载体的靶细胞为罹患与弗里德赖希共济失调相关联的心肌病的受试者的心肌细胞。优选，受试者为人类、成人或儿童。然而，还预期兽医应用。

[0245] 本发明的载体的靶细胞还包括CNS(优选神经元)的细胞。递送至大脑以治疗弗里德赖希共济失调的神经退化性方面可通过鞘内施用。

[0246] 在一个方面中，全身性地(例如，静脉内)递送编码FNX的经修饰的核酸、载体或包含该载体的组合物以治疗FA相关联的心肌病和/或该疾病的神经退化性方面。

[0247] 在另一实施方案中，通过至少两个途径施用载体。即，可全身性地且亦直接地将载体施用至大脑和/或心脏或其任何组合中。

[0248] 若经由至少两个途径执行，则载体的施用可以但无需同步或同时。实际上，经由不同途径的施用可以每一施用之间的时间间隔单独地执行。通过本领域技术人员例行地判定适当的给药方案以实现每一个别患者的最大治疗益处。

[0249] 在一个方面中，本发明包括用于提高受试者的共济失调蛋白水平的编码本发明的共济失调蛋白的至少一个经修饰的核酸，包括(但不限于)载体或药物组合物中的核酸。

[0250] 在一个方面中，本发明包括用于治疗受试者的弗里德赖希共济失调的至少一个经修饰的核酸、包含该核酸的rAAV载体、及包含该核酸或该载体的药物组合物。

[0251] 除了如本领域中已知的FRDA的照护标准之外和/或与该照护标准并行，用途涵盖施用经修饰的核酸或包含经修饰的核酸的载体。

[0252] 可注射剂可呈熟知的形式，以液体溶液或悬浮液形式，以适用于在注射之前溶解或悬浮的固体形式或以乳液形式制备。

[0253] 具有经修饰的FXN基因的病毒载体的剂量将取决于施用模式、待治疗的疾病或病状、个别受试者的病状、特定病毒载体，及待递送的基因，且可以例行方式判定。用于实现疗效的例示性剂量为至少约10^5、10^6、10^7、10^8、10^9、10^{10}、10^{11}、10^{12}、10^{13}、10^{14}、10^{15}个转导单位或更多的病毒滴定量，优选约10^8至10^{13}个转导单位，更优选10^{12}个转导单位/kg体重。

[0254] 经修饰的FXN基因可作为具有适合于在靶细胞中表达的调节元件的DNA分子的组分经施用。经修饰的FXN基因可作为病毒质粒的组分(诸如rAAV载体)经施用。病毒颗粒可仅作为病毒颗粒经施用，无论在体内直接递送至门静脉脉管时抑或在离体治疗时，该离体治疗包含在体外将载体病毒颗粒施用至来自接受治疗的动物的细胞，随后引入返回至供体的经转导细胞。

[0255] 等效物

[0256] 前述书面说明书被视为足以使本领域技术人员实践本发明。前述描述及实例详述了本发明的某些例示性实施方案。然而，将了解，无论以文字呈现之前述内容如何详细，本发明可以许多方式实践，且本发明应根据所附权利要求书及其任何等效物解释。

[0257] 本文中所引用的全部参考文献(包括专利、专利申请、论文、教科书及类似者)及其中引用的参考文献就其尚未引用的程度而言以全文引用的方式特此并入本文中。

[0258] 例示性实施方案

[0259] 参考以下实验性实施例来进一步详细描述本发明。除非另外规定，否则提供这些实施例仅出于说明的目的，且不意欲为限制性的。因此，本发明决不应解释为限于以下实施例，而是应解释为涵盖由于本文所提供的教导而变得明显的任何及所有变化形式。

实施例

[0260] 实施例1:自我互补的rAAV-FXN构建体的产生

[0261] 材料及方法

[0262] 载体构建

[0263] 将编码自我互补的AAV基因组的pTRs-KS-CBh-EGFP-bGHpolyA构建体(在图3中图解显示)用作转基因表达构建体的主链(Gray等人,2011,Human Gene Therapy 22:1143-1153)。两个密码子优化FXN基因插入序列自pUC57中的GenScript订购,即,Genscript及Genscript(低CpG),且用于替换主链载体中的EGFP。Genscript(SEQ ID NO:6)及Genscript(低CpG)(SEQ ID NO:7)经修饰的FXN基因如图3中所说明的各自可操作地连接至CBh启动子。GenScript FXN(SEQ ID NO:6及7)构建体包括如图2E(Genscript)及图2F(Genscript(低CpG))中所显示的N端AgeI位点、FXN终止密码子下游的胶原蛋白稳定序列(CSS)(5-CCCAGCCCACTTTTCCCCAA-3')、CSS下游的牛生长激素(BGH)polyA序列、BGH polyA下游的MluI位点。插入至pTRs-KS-CBh-FXN-bGHpolyA构建体中的例示性插入序列显示于图2A至图2F中且示于表8中。更特定而言,野生型共济失调蛋白基因(WT FXN;SEQ ID NO:2)经克隆至pTRs-KS-CBh-WT FXN-bGHpolyA中(图2A);IDT1经修饰的FXN基因(SEQ ID NO:11)经克隆至pTRs-KS-CBh-IDT1-bGHpolyA中(图2B);编码IDT3低表达经修饰的FXN基因(SEQ ID NO:8)的核酸经克隆至pTRs-KS-CBh-IDT3-bGHpolyA中(图2C);IDT4经修饰的FXN基因(SEQ ID NO:12)经克隆至pTRs-KS-CBh-IDT4-bGHpolyA中(图2D);Genscript(对照)经修饰的FXN基因(SEQ ID NO:6)经克隆至pTRs-KS-CBh-Genescript-bGHpolyA中(图2E);且Genscript(低CpG)经修饰的FXN基因(SEQ ID NO:7)经克隆至pTRs-KS-CBh-Genescript(低CpG)-bGHpolyA中(图2F)。编码FXN基因的每一插入序列经克隆至载体中,且该基因由5'侧上的AgeI位点及由3'侧上的AvrII切割位点侧接,随后是AvrII位点之后的CSS序列、CSS之后的SpeI切割位点、SpeI切割位点之后的bGHpolyA信号序列,及polyA信号序列之后的MluI切割位点。

[0264] 用AgeI及MluI(New England Biolabs,各别地R0552S及R0198S)消化、凝胶提取及使用ExTaq聚合酶(Clontech,RR001A)连接主链pTRs-KS-CBh-EGFP-bGHpolyA及FXN基因构建体。连接反应转化至SURE细胞(Agilent,200227),在37℃下放置于SOC回收培养基(目录号15544-034,Invitrogen)中历时一小时,随后用(10mg/ml)涂覆于LB板上。针对病毒产生的扩增测序及选择菌落。具有AAV血清型2衣壳的重组AAV(rAAV)载体如所描述通过三重转染方法在人胚肾293(HEK293)细胞中产生UNC载体核心(Grieger等人,2006,Nature Protocols 1:1412-1428)。替代地,类似地产生具有血清型2i8衣壳(SEQ ID NO:28的氨基酸序列)的rAAV载体。含有自我互补的基因组的高纯度重组病毒通过非离子碘克沙醇梯度接着离子交换层析回收。峰值级分由qPCR判定,随后以含有5%d-山梨醇的磷酸盐缓冲盐水(PBS)渗析。病毒滴定量由qPCR判定(Gray等人,2010,J.Amer.Soc.Gene Therapy 18:570-578)。遵循体外初步测试(下文),将GenScript(低CpG)用于产生具有在pTRs-KS-CBh-Genescript(低CpG)-bGHpolyA中的FXN终止密码子之前嵌入的HA标签TACCCATACGATGTTCCAGATTACGCT的构建体。

[0265] 北卡罗莱纳大学(UNC)载体核心用rAAV TK血清型产生具有FXN-HA构建体的病毒。

[0266] sc rAAV-FXN的体外测试

[0267] HEK293(ATCC:CRL-1573)及HeLa(ATCC:CCL-2)细胞维持于Dulbecco的经修饰的Eagle培养基中(DMEM,Gibco)。细胞生长培养基补充有9%的胎牛血清(FBS,Gibco)、3.4mM l-麸酰氨酸、100U/ml青霉素及100μg/ml链霉素(Gibco)。细胞在37℃下保持于5%CO2氛围中。量杆测定:细胞接种于24孔板中以使得其在24小时(h)内达至大约60%汇合,随后在MOI 10,000(VG/细胞)下用scAAV-FXN(在本文中可互换地被称作"rAAV-FXN"或"rAAV-FXN-HA"一式三份地模拟处理或感染。在转导后60h(h p.t.),细胞系根据针对Frataxin Protein Quantity Dipstick Assay的制造商协议(Abcam,ab109881)。使用ImageJ处理数据。

[0268] 蛋白质印迹:

[0269] 细胞接种于6孔板中以使得其在24h内达至大约60%汇合,随后在MOI 10,000(VG/细胞)下用scAAV-FXN模拟处理或感染。在转导后60h,用细胞溶解缓冲液(0.0625M三-HCl pH 6.8、10%甘油、2%SDS、5%2-巯基乙醇、0.02%(w/v)溴酚蓝)来溶解细胞。通过使用15-4%TGX凝胶来凝胶电泳分离十五(15)μl HeLa蛋白溶解物,且蛋白经电转染至硝化纤维素膜(NCM)。使用PBS-T中的5%脱脂奶粉阻断NCM。抗共济失调蛋白抗体(Abcam,18A5DB1)用于具有5%牛奶的PBS-T中。将具有5%乳抗体的PBS-T中的缀合辣根过氧化酶(HRP)二级抗体用于检测抗共济失调蛋白的存在。将WesternBright ECL蛋白质印迹检测试剂盒(Advansta,K-12045-D50)用于根据每一制造商的说明书进行检测。

[0270] 图1A至图1B显示与编码FXN(SEQ ID NO:1)的未优化(即,野生型)序列在HeLa细胞中的表达相比较的不同优化序列的表达的结果。更特定而言,图1A及图1B两者显示蛋白质印迹的像片,该像片显示共济失调蛋白(FXN)在用包含编码共济失调蛋白的插入序列的表达载体转染的HeLa细胞中的表达。图1A在暴露了1秒的WesternBright印迹薄膜的像片中显示FXN在HeLa细胞中的表达。图1B显示图1A中所显示的表明如暴露了1秒的WesternBright印迹薄膜的像片中所显示的FXN在HeLa细胞中的表达的实验的重复。图1A及图1B中的每一凝胶泳道显示与来自编码FXN的野生型核酸序列的表达相比较的来自本发明的经修饰的FXN基因的FXN的表达。即,泳道1显示由编码FXN的野生型非经修饰的核酸(SEQ ID NO:2)驱动的表达;泳道2显示由IDT2经修饰的FXN基因(SEQ ID NO:3)驱动的表达;泳道3显示由IDT5经修饰的FXN基因(SEQ ID NO:9)驱动的表达;泳道4显示由JCAT经修饰的FXN基因(SEQ ID NO:4)驱动的表达;泳道5显示由GeneArt经修饰的FXN基因(SEQ ID NO:5)驱动的表达;泳道6显示由GenScript(对照)经修饰的FXN基因(SEQ ID NO:6)驱动的表达;泳道7显示由Genscript(低CpG)经修饰的FXN基因(SEQ ID NO:7)驱动的表达;且泳道GFP显示编码绿色荧光蛋白的转基因的表达,由插入序列而非编码FXN的核酸编码的可检测标记。

[0271] 所显示数据表明若干经修饰的FXN核酸序列--尤其泳道4(JCAT)、5(GeneArt)、6(Genscript)及7(Genscript低CpG)--相对于野生型核酸序列(泳道1)提供共济失调蛋白在HeLa细胞中的更高表达。每一泳道中的肌动蛋白内参考物作为蛋白内参考物。

[0272] 核酸序列中的GC核苷酸含量(通常表达为序列中的核苷酸总数目的百分比)可具有多个影响,包括(但不限于):mRNA的稳定性增加,二级结构及转基因通常受增加的GC含量负面地影响。因此,本领域技术人员应理解,相对于负面影响,经修饰的核酸的GC含量反映核酸的增加的稳定性与自其转录的mRNA之间的平衡,例如,在由增加的GC含量介导的二级结构上。

[0273] CAI(密码子适应指数)为同义密码子使用偏向的测量值。该指数使用来自物种的高表达基因的参考集以分析每一密码子的相对值,且基因的分值自该基因中的所有密码子的使用频率计算。指数评估在选择密码子使用的模式中选择已生效的程度。其可用于预测基因的表达水平且用于比较不同生物/物种中的密码子使用。对共济失调蛋白基因执行人类密码子优化以实现以下因素的平衡:转录效率--GC含量、CpG二核苷酸含量、隐含剪接位点等;翻译效率--密码子使用偏向、GC含量、mRNA二级结构、过早polyA位点、RNA不稳定基序、内部核糖体结合位点;及蛋白再折叠--密码子使用偏向、密码子及抗密码子的相互作用,RNA二级结构。

[0274] 基本上,密码子优化平衡这些变量以(优选)实现更高表达共济失调蛋白基因序列、增加信息(GC含量、DNA及RNA两者中的二级结构)的稳定性及类似者,如本领域中已知的。

[0275] CpG岛可由经转导细胞中的类Tol受体九(TLR9)辨识且可将免疫反应引发至外来(外源)DNA。因此,在一个实施方案中,本发明涵盖编码共济失调蛋白的经修饰的核酸,其中CpG岛的数目与编码共济失调蛋白的野生型核酸序列(例如,SEQ ID NO:2)中的CpG岛基序的数目相比已减少。

[0276] CAI、GC百分比含量及本文中例示的每一经修饰的FXN基因的潜在CpG岛区域数目显示于下文表1中。

[0277] 表1

[0278]

图 1A 和 1B 凝胶泳道编号	FXN 基因名称	密码子适应指数(CAI)	%GC 含量	潜在 CpG 岛区域的数量	SEQ ID NO:
1	WT-FXN	0.71	55	128	2
	核苷酸序列 22	0.71	55	--	10
	IDT-1	0.73	52	114	11
2	IDT-2	0.76	56	124	3
	IDT-3	0.80	57	123	8
	IDT-4	0.74	54	123	12
3	IDT-5	0.77	55	124	9
4	JCAT	0.98	69	144	4
5	GeneART	0.95	61	117	5
6	Genescript(对照)	0.87	57	257	6
7	Genescript(低 CpG)	0.86	55	117	7

[0279] 即,对于编码FXN的野生型核酸(WT-FXN;SEQ ID NO:2),核酸序列表明CAI为0.71且%GC含量为55%。相反地,JCAT经修饰的FXN基因表明CAI为0.98及GC含量为69%,其两者实质上高于WT-FXN的值。

[0280] 使用在http://www.bioinformatics.org/sms2/cpg_islands.html发现的公开可用软件识别潜在CpG岛。CpG岛软件使用由Gardiner-Garden及Frommer,1987,J.Mol.Biol.196(2):261-282描述的方法报告潜在CpG岛区域。在1个碱基对(bp)时间间隔处使用跨越序列的200个bp窗口执行计算。将CpG岛定义为序列范围,其中Obs/Exp值大于0.6且GC含量大于50%。如下计算窗口中的CpG二聚体的期望数目:窗口中的"C"的数目乘以窗口中的"G"的数目,除以窗口长度。因此,存在于核酸序列中的潜在CpG岛可易于通过将讨论的序列输入至由软件提供的窗口中来判定(通过"将原始序列或一个或多个FASTA序列粘贴至以下文字区域中。输入限制为100000个字符"的指令指示)。CpG岛往往发现于脊椎动物基因的5'区域中,因此,此程序可用于突出显示基因组序列中的潜在基因。

[0281] 由于较高表达水平及较高GC含量(55%)、较高CAI(0.86)及较低数目个CpG二核苷酸(117),Genscript(低CpG)经修饰的FXN基因经选择用于产生以下所示的动物实验中所使用的scAAV-2i8载体。

[0282] 实施例2:弗里德赖希共济失调的小鼠模型中的体内治疗

[0283] 将本领域中公认的FRDA的小鼠模型(Perdomini等人,2014,Nature Med.20(5):542)用于分析rAAV介导的FXN基因治疗的潜在功效。即,检查三组小鼠:未经治疗Mck阳性对照小鼠(Mck-Cre x FXN L3/WT)、未经治疗Mck突变小鼠(Mck-Cre x FXN L3/L-),及接收包含FXN基因的rAAV的一剂量的经治疗Mck突变小鼠,其中经修饰的FXN基因包含SEQ ID NO:7的核酸序列(GenScript(低CpG))且FXN基因经克隆至如上文所描述的pTRs-KS-CBh-EGFP-BGH构建体中以提供pTRs-KS-CBh-Genscript(低CpG)-bGHpolyA。

[0284] 小鼠研究中使用的rAAV-FXN载体进一步包含AAV2i8衣壳。此外,pTRs-KS-CBh-Genscript(低CpG)-bGHpolyA构建体进一步包含编码可检测血球凝集素标签(rAAV-FXN-HA)的核酸序列,其中编码HA标签的序列位于经修饰的FXN基因的3',使得共济失调蛋白的表达可易于通过检测HA的存在而检测及定位,例如,使用抗HA抗体,诸如抗HA小鼠mAb(HA.11克隆16B12,Covance Research Products公司,Princeton,NJ)。载体为指定的rAAV-FXN-HA。

[0285] 研究的三个动物组列于且描述于表2中。

[0286] 表2

[0287]

组标记	组编号	剂量水平 vg/kg	混合性别动物的数量	终止周龄

[0288]

Mck 阳性对照	*Mck*-Cre x FXN L3/WT	0	8	8 周
未治疗的 Mck 突变小鼠	未治疗的 *Mck*-Cre x FXN L3/L-	0	8	8 周
经治疗的 Mck 突变小鼠	rAAV-FXN-HA 治疗的 *Mck*-Cre x FXN L3/L-	$1x10^{13}$	8	8 周

[0289] A.生物标记物研究

[0290] 方法

[0291] 血浆中的半乳糖凝集素-3及H-FABP的测量

[0292] 在5周龄(治疗后2周)及8周龄(治疗后5周)时在异氟醚麻醉之后通过眼眶后穿刺采集血液。

[0293] 使用来自根据制造商的说明书的RayBiotech的小鼠半乳糖凝集素-3Elisa试剂盒在血浆中测量半乳糖凝集素-3。

[0294] 使用来自HycultBiotech的小鼠H-FABP Elisa试剂盒根据制造商的说明书在血浆中测量H-FABP。

[0295] 心脏均质物中的琥珀酸去氢酶活性的测量

[0296] 在处死之后,心脏经采集且每组4个小鼠的心脏的一半经快速冷冻以用于测量SDH活性。

[0297] 心脏均质物的SDH活性测量遵循来自Biovision的琥珀酸去氢酶活性比色测定试剂盒(目录#K660-100)的指令执行。

[0298] 组织中的人类共济失调蛋白的测量

[0299] 在处死之后,心脏(一半)、骨骼肌(腓肠肌)及肝组织经采集及快速冷冻以用于共济失调蛋白的测量。

[0300] 组织均质物的测量遵循人类共济失调蛋白Elisa试剂盒(Abcam;ab176112)的指令执行。

[0301] 组织学

[0302] 小脑(包括齿状核)、性腺、心脏、肾脏、肝脏、肺脏、胰腺、骨骼肌(腓肠肌及比目鱼肌)、脾脏及颈椎、胸及腰椎为甲醛液固定的。随后使用EDTA溶液脱钙脊椎。所有器官经石蜡包埋以获得5μm厚部分;脊椎(包括脊髓及背根节)及心脏的横向部分。用苏木精及伊红染色

所有器官;使用梅森氏(Masson)三色染色法评估心脏纤维化。

[0303] 超声心动图:

[0304] 通过Vevo-2100Visual Sonics回声仪上的30MHz线性探针(MS400)在经麻醉小鼠(异氟醚1-2%)中捕获的经胸超声心动影像。

[0305] 以下参数经测量以分析:a)心脏形态及心室收缩功能(短轴,SAX):左心室端部舒张(LVEDD)及端部收缩直径(LVESD)、中隔(SW)及后壁厚度(PW)、左心室质量(LVM=1.055×[(EDD+SW+PW)3-EDD3)])、射出及缩短分数及心输出量;b)血液动力学特征:肺及主动脉速度及检测心内压力变化的压力(AoV及RV功能)。

[0306] 小鼠

[0307] 小鼠保持在控制温度及湿度的动物设施中,该动物设施具有12h光-暗循环,可以自由饮水及摄取标准啮齿动物食物(D03,SAFE,Villemoisson-sur-Orge,法国)。所有动物程序及实验由针对动物照护及使用的地方道德委员会(Comité d'Ethique en Expérimentation Animale IGBMC-ICS)核准(Com'Eth 2011-007)。

[0308] 每天两次执行小鼠临床观察,每周一次记录体重及每2天记录摄食量,直至过程结束为止。

[0309] 对于生物分布及基因治疗研究,用异氟醚(1-2%)麻醉3周龄小鼠,且针对治疗组,将剂量为1×10^{13}vg/kg的rAAV-FXN-HA载体经静脉注射至眼眶后静脉,且针对未经治疗MCK突变小鼠及对照组,则使用相等体积的盐水。

[0310] 在开始治疗之前2天(基线表型)、在5周龄(治疗后14天)及7周龄(治疗后28天)时,通过超声心动图,在异氟醚麻醉(1-2%)下,评估小鼠心脏功能。在5周龄及8周龄时,采集血液,如本文中其他处所详述,测量心脏型脂肪酸结合蛋白(H-FABP)、半乳糖凝集素-3及琥珀酸去氢酶(SDH)的浓度。

[0311] 在处死之后,记录所有动物的体重、身长、心脏、脾脏、肾脏、肾上腺及肝脏重量。自每组4只动物采集肾上腺、小脑、颈椎、胸及腰椎、性腺(睾丸及卵巢)、心脏、肾脏、肝脏、肺、胰腺、雄性之前列腺、骨骼肌(腓肠肌及比目鱼肌)、脾脏及胸腺,用于病理评估及ELISA测定。

[0312] 采集每组其他4只动物的小脑(包括齿状核)、颈椎、胸及腰背根节、心脏、肾脏、肝脏、肺、性腺、胰腺、骨骼肌(腓肠肌及比目鱼肌)及脾脏,且迅速冷冻,用于分子生物学。

[0313] 结果

[0314] 潜在生物标记物的识别

[0315] 在三组小鼠中判定各种生物标记物的含量:未经治疗Mck阳性对照组、未经治疗Mck突变小鼠,及接受包含FXN基因且进一步包含编码HA标签肽的核酸的rAAV2i8(AAV-FXN-HA)的剂量的经治疗Mck突变小鼠。

[0316] 血浆中的半乳糖凝集素-3及H-FABP的测量

[0317] 在5周龄(对经治疗Mck突变小鼠的AAV治疗的2周)及8周龄(对经治疗Mck突变小鼠群组的rAAV治疗的5周)时在异氟醚麻醉之后通过眼眶后穿刺采集血液,且使用标准方法测量半乳糖凝集素-3及H-FABP的含量。

[0318] 半乳糖凝集素-3:

[0319] 在5周龄时,3个群组之间的半乳糖凝集素-3水平为相当的,即使与未经治疗Mck突

变小鼠群组相比较未经治疗Mck阳性对照组及经治疗Mck突变小鼠群组中的半乳糖凝集素-3水平趋于更高。

[0320] 如表3中所显示，在8周龄时，未经治疗Mck突变组中的半乳糖凝集素-3水平明显地低于阴性对照组。实验性群组中的半乳糖凝集素-3水平趋于比阴性对照组更低，而实验群组与阳性对照组之间的半乳糖凝集素-3水平相当。

[0321] 表3

[0322]

	血浆半乳糖凝集素-3 (ng/ml)	
	第5周	第8周
	平均值+/- sem	平均值+/- sem
未治疗的Mck阳性对照小鼠(n=8)	41.2 +/- 3.5	68 +/- 7.8
未治疗的Mck突变小鼠(n=8)	49.7 +/- 3.6	42 +/- 2.1
经治疗的Mck突变小鼠(n=8)	49.7 +/- 4.4	48.9 +/- 4.5

[0323] 出人意料地，未治疗的Mck阳性对照组的小鼠在8周龄时比在5周龄时显示更高半乳糖凝集素-3水平，而针对未治疗的Mck突变小鼠群组及经治疗的Mck突变小鼠群组，在8周龄时的半乳糖凝集素-3水平与在5周龄时的水平相当。

[0324] 总的，其呈现出半乳糖凝集素-3对关于Mck小鼠的此研究而言并非适当的心脏生物标记物。未治疗的Mck突变小鼠群组中的小鼠在半乳糖凝集素-3为适当的生物标记物时并未显示此参数的预期增加。

[0325] H-FABP：

[0326] 使用标准检测方法在同一样本群组内的小鼠之间观测到H-FABP水平的极大可变性。

[0327] 如表4中所显示，可对在5周龄及8周龄两者时的3组小鼠之间的H-FABP血液水平进行比较。

[0328] 表4

[0329]

[0330]

	血浆 H-FABP (ng/ml)	
	week 5	week 8
	mean +/- sem	mean +/- sem
未治疗的 Mck 阳性对照(n=8)	177.8 +/- 33.9	98.8 +/- 25.0
未治疗的 Mck 突变小鼠(n=8)	187.1 +/- 38.8	139.8 +/- 41.5
经治疗的 Mck 突变小鼠(n=8)	232.2 +/- 53.3	129.6 +/- 25.8

[0331] 在每组中的5周龄及8周龄之间没有观测到H-FABP水平的显著改变。

[0332] 总之,由此看来,H-FABP对关于Mck小鼠的此研究而言并非适当的心脏生物标记物;在未经治疗Mck突变组中未观测到此参数的预期增加,且在同一群组中的小鼠之间观测到重要可变性。

[0333] 心脏均质物中的SDH活性

[0334] 使用标准方法测量来自在研究结束(8周龄、经治疗突变小鼠群组的AAV治疗的5周)时所采集的心脏的心脏均质物中的SDH活性。每组由四个(4)小鼠组成。

[0335] 表5中所显示的结果显示可在3组小鼠之间比较SDH活性。与未经治疗Mck突变组相比较,在未经治疗Mck阳性对照组中未观测到SDH活性降低。

[0336] 表5

[0337]

	心脏均质物中的 SDH 活性 (U/g 蛋白质)
未治疗的 Mck 阳性对照(n=4)	4.14 +/- 0.52
未治疗的 Mck 突变小鼠(n=4)	3.64 +/- 0.77
经治疗的 Mck 突变小鼠(n=4)	4.24 +/- 0.62

[0338] 在同一群组中的小鼠之间观测到SDH活性的重要可变性。此外,在未经治疗Mck阳性对照组中未观测到SDH活性的预期降低。

[0339] 心脏、骨骼肌及肝组织均质物中的共济失调蛋白水平

[0340] 如表6中所显示使用标准方法自在处死时采集的心脏、骨骼肌及肝脏测量人类共济失调蛋白水平。

[0341] 在未经治疗Mck阳性对照组及未经治疗Mck突变组的经检查组织(心脏、骨骼肌及

肝脏)中的任一者中没有检测到人类共济失调蛋白(即,水平低于测定的检测下限[LLD])。

[0342] 在接受rAAV-FXN的经治疗Mck突变小鼠中,在水平为38.35+/-1.99ng/mg的心脏均质物中及在4.57+/-0.39ng/mg的较低浓度下的骨骼肌中检测到人类共济失调蛋白。此外,在肝脏中检测到人类共济失调蛋白的迹象(0.07+/-0.01ng/mg蛋白)。

[0343] 表6

[0344]

	组织中的共济失调蛋白(ng/mg 蛋白质)		
	心脏	骨骼肌	肝脏
	平均值 +/- sem	平均值 +/- sem	平均值 +/- sem
阴性对照(n=4)	<LLD	<LLD	<LLD
阳性对照(n=4)	<LLD	<LLD	<LLD
实验组(n=4)	38.35 +/- 1.99	4.57 +/- 0.39	0.07 +/- 0.01

[0345] 这些数据表明用包含FXN基因的rAAV载体进行的治疗可增加弗里德赖希共济失调(FRDA)的小鼠模型的共济失调蛋白水平。另外,这些数据显示FXN水平可通过rAAV-FXN全身施用体内增加,使得心脏中的FXN水平增加,且在骨骼肌增加较小程度,其中在肝脏中水平低得多。因此,这些数据表明体内FXN水平可在受影响组织中选择性地增加,例如,心脏及骨骼肌,同时在FXN为非所需和/或所需(例如,关于肝脏)的情况下最小化FXN的递送。

[0346] 肉眼病理学

[0347] 未经治疗Mck阳性对照雄性小鼠与未经治疗及经AAV治疗的Mck突变动物相比较明显更长(9.39cm与8.89cm[+5.62%]。P=0.011[t-测试])。未观测到其他显著肉眼可见损伤,尤其在雄性及雌性两者的心脏重量中未观测到肉眼可见损伤或显著变化。

[0348] 组织学

[0349] 心脏

[0350] 在一个未经治疗Mck阳性对照动物(#58)中观测到最小间质纤维化。所有其他3个未经治疗Mck阳性对照组心脏为正常的。

[0351] 然而,在经分析的所有4个未经治疗Mck突变动物中观测到最小(小鼠#38)及中度(小鼠#41、#49及81)间质纤维化。此损伤与小鼠#38(最小)及#81(轻微)中肿胀的心肌细胞的心内膜病灶相关联。在小鼠#41及#49中,纤维化与心肌细胞的中度巨噬细胞发炎、最小播散性肿胀及轻微液胞化相关联。在小鼠#41及#81中观测到阿尼契柯氏(Anitschkow)(鹰眼形)核。

[0352] 与未经治疗Mck突变群形成鲜明对比,在整个评估中,除在小鼠#47及#13中观测到极少阿尼契柯氏核以外,经rAAV-FXN治疗的Mck突变小鼠的心脏呈现正常。

[0353] 肾脏

[0354] 在所有群组中,常常在多个髓质小管的管腔中观测到显著矿化。频率针对未经治

疗Mck阳性对照动物为4/4(尽管其表达Cre转基因),针对未经治疗Mck突变动物为3/4,且针对接受一剂量的rAAV-FXN的经治疗Mck突变动物为2/4。与未经治疗Mck阳性对照组及未经治疗Mck突变组相比较,经rAAV治疗的Mck突变组中呈现矿化强度的降低。管状嗜碱性(再生)在2/4的未经治疗Mck阳性对照组中的动物中、在3/4的未经治疗Mck突变组中的动物中及在经AAV治疗的Mck突变组中的1个动物中观测到。

[0355] 肝脏:在一个未经治疗Mck阳性对照动物(#38)中观测到最小门静脉周发炎。

[0356] 肺脏:在一个未经治疗Mck突变动物(#41)中观测到轻微支气管周发炎。

[0357] 未观测到其他显著显微损伤。特别地,脊髓、背根节及小脑均正常。

[0358] 超声心动图

[0359] 治疗之前的基本表型

[0360] 超声心动图测量结果显示与未经治疗Mck阳性对照组相比较,未经治疗Mck突变雄性小鼠的左心室功能降低。此心机能不全通过左心室(LV)收缩性(缩短分数及射血分数)的减少及LV容积(收缩及舒张两者)的增加表征。在彼阶段中,在未经治疗Mck突变雌性小鼠中未观测到心脏表型。

[0361] 图4A显示通过3周龄(A)雄性及(B)雌性的超声心动图评估收缩功能及LV容积的图。资料为每组8个小鼠的平均值±S.E.M。使用多个t-测试比较(Sidak-Bonferroni法)将Mck突变(经治疗及未经治疗两者)小鼠的数据与未经治疗Mck阳性对照组进行比较。*p<0.05

[0362] rAAV治疗之后14天(5周龄)的结果:

[0363] 为调查用于治疗FRDA心肌病的基因治疗方法的潜能,以单独静脉内注射向3周龄突变小鼠(经治疗Mck突变组)施用剂量为1×10^{13}vg/kg的AAV.FXN-HA。在注射之后14天(5周龄),超声心动图测量结果显示经治疗Mck突变雄性的心脏血流动力学(心输出量)的改良及接近正常形态发展(收缩性、LV质量)。相反地,仍在未经治疗Mck突变小鼠中观测到心机能不全。出人意料地,在雌性中,在所有Mck突变小鼠(无论经治疗或未经治疗)中观测到心肌收缩性缺陷。

[0364] 图5A至图5B显示通过5周龄雄性(图5A)及雌性(图5B)的超声心动图评估收缩功能及LV容积。资料为每组8个小鼠的平均值±S.E.M。使用多个t-测试比较(Sidak-Bonferroni法)将突变小鼠的数据与对照组进行比较。*p<0.05.

[0365] rAAV治疗之后28天(7周龄)的结果:

[0366] 在rAAV治疗二十八(28)天(7周龄),经治疗Mck突变雄性及雌性完全归一化且变得不能在彼此之间及与未经治疗Mck阳性对照小鼠区分开来。这样,在经治疗Mck突变小鼠中表明心脏疾病的完全校正(雄性)及预防(雌性)。相反地,未经治疗Mck突变小鼠出现急进性心机能不全,伴随左心室缩短分数及心输出量明显减少以及左心室肥大。

[0367] 图6A至图6C显示描绘使用对历时连续数周的未经治疗Mck阳性对照组、及经治疗及未经治疗Mck突变小鼠的左心室质量(LVm)、缩短分数(SF)及心输出量的超声心动图评估获得的数据的图。资料为每组8个小鼠的平均值±S.E.M。使用多个t-测试比较(Sidak-Bonferroni法)将Mck突变小鼠的数据与未经治疗Mck阳性对照群组进行比较。*p<0.05.

[0368] 结论

[0369] 超声心动图的结果

[0370] 在此研究中，分析剂量为1×10^{13}vg/kg的视情况进一步包含可检测血球凝集素标签(HA)载体的rAAV-FXN(在本文中被称作rAAV-FXN-HA)的疗效。此剂量比先前在同一Mck小鼠心脏特异性弗里德赖希共济失调小鼠模型中所描述的剂量低大约5倍，该弗里德赖希共济失调小鼠模型使用编码野生型FXN的rrhAAV10载体(Perdomini等人，2014，Nature Medicine 20(5):542)。

[0371] 如图5A中所显示，在3周龄时，未经治疗Mck突变雄性小鼠开始出现左心室(LV)功能不全，在相同周龄的同一群组的雌性中未观测到该左心室功能不全(图5B)。在AAV.FXN-HA注射之后十四(14)天(在5周龄时)，在Mck突变雄性中观测到心脏表型的进行性校正，但该进行性校正在Mck突变雌性中较少。在不希望受任何特定理论束缚的情况下，此差异可由于与雄性相比较雌性中的心脏表型的开始更晚或由于到目前为止此协议中所使用的小鼠数目的减少。

[0372] 这些结果表明经rAAV修饰的FXN的全身施用逆转FRDA的Mck突变小鼠模型中的心脏疾病表型。这些结果进一步表明经rAAV修饰的FXN施用可预防和/或逆转有需要的受试者的FRDA。

[0373] rAAV-FXN注射后二十八(28)天，在经治疗Mck突变雄性及雌性中观测到心脏功能的完全恢复，其表明利用所注射FXN转基因的病变的稳健校正。即，图6A至图6C中所显示的数据表明rAAV-FXN施用之后二十八(28)天的FRDA心脏表型的校正。更特定而言，图6A显示当未经治疗(三角形)Mck突变小鼠呈现明显地更高左心室质量(LVm)时，经治疗Mck突变小鼠及对照组(WT野生型Mck-Cre小鼠)两者的LVm为不可区分的(*$p<0.05$)。图6B显示数据，该数据表明在rAAV-FXN治疗之后28天，阳性对照组(WT L3Mck-Cre小鼠;圆形)及经治疗Mck突变(L-)小鼠(方形)两者表明实质上相同缩短分数(SF)测量结果。相反地，图6B表明未经治疗Mck突变小鼠(三角形)显示极大地减少的SF(*$p<0.05$)。另外，图6C显示资料:该数据表明在用rAAV进行治疗之后28天，与显示明显减少的心输出量(三角形;*$p<0.05$)的未经治疗Mck突变小鼠(三角形)相比较，经治疗Mck突变小鼠(方形)显现不可与对照小鼠(圆形)区分的心输出量。所有(经治疗及未经治疗)Mck突变小鼠使用多个t-测试比较(Sidak-Bonferroni法)与对照未经治疗小鼠进行比较。对于图6A至图6C中所显示的每一个图，指示*<0.05。

[0374] 这些数据充分地表明包含编码共济失调蛋白的经修饰的核酸的rAAV的施用可逆转和/或预防FRDA的本领域中公认的小鼠模型中的Mck表型。因此，这些数据证明经rAAV修饰的FXN介导的治疗可为用于治疗或预防有需要的受试者的降低水平的FRDA或由野生型(例如，功能性)共济失调蛋白介导的疾病、病症或病状的潜在有用疗法。尽管这些数据表明全身性地施用rAAV经修饰的FXN可治疗或预防FRDA，但这些结果进一步证明治疗还包括rAAV-FXN施用的其他(例如，更直接)途径，诸如(但不限于)，颅内或直接心脏施用。即，由于全身施用表明为疗法，故本领域技术人员基于本文中所提供的本发明应理解，更直接施用途径还可提供治疗益处。

[0375] 这些结果强有力地证明使用经修饰的FXN基因的rAAV载体递送的基因治疗为用于患有FRDA的患者及用于治疗或预防显示野生型/功能性共济失调蛋白水平减少的受试者的肾结石的潜在疗法。

[0376] 实施例3:在弗里德赖希共济失调的小鼠模型中体内施用rAAV-FXN的组织学研究

[0377] 研究设计

[0378] 二十四(24)个8周龄C57BL6/N雄性及雌性小鼠经分析用于组织病理学分析。

[0379] 八(8)个小鼠持有与功能性经改造人类共济失调蛋白等位基因相关联的Mck-Cre转基因(MCK:Muscular Creatine Kinase)(Mck-Cre x FXN L3/WT;下文中被称作“Mck阳性对照小鼠”)。十六(16)个小鼠持有目前与无活性经改造共济失调蛋白等位基因相关联的相同转基因(Mck-Cre x FXN L3/L-;下文中被称作“Mck突变小鼠”)。在Mck突变小鼠当中,八(8)个注射编码共济失调蛋白的rAAV2i8(rAAV-FXN;10^{13}vg/kg)(下文中为“经治疗Mck突变小鼠”)。剩余八(8)个Mck突变小鼠接受相等体积的盐水(下文中为“未经治疗Mck突变小鼠”)。阳性对照小鼠群组(Mck-Cre x FXN L3/WT)经施用盐水。参见下表7。

[0380] 表7

[0381]

组标记	组编号	剂量水平 vg/kg	(混合性别)动物的数量	终止周龄
Mck 阳性对照小鼠	*Mck*-Cre x FXN L3/WT	0	8	8 周
未治疗的 Mck 突变小鼠	未治疗的 *Mck*-Cre x FXN L3/L-	0	8	8 周
经治疗的 Mck 突变小鼠	rAAV-FXN-HA 治疗的 *Mck*-Cre x FXN L3/L-	$1x10^{13}$	8	8 周

[0382] 方法

[0383] 在处死之后,自所有动物记录体重、身长及心脏、脾脏、肾脏、肾上腺及肝脏重量。自每组四(4)个动物采集肾上腺、小脑、颈椎、胸及腰椎、性腺(睾丸及卵巢)、心脏、肾脏、肝脏、肺、胰腺、雄性前列腺、骨骼肌(腓肠肌及比目鱼肌)、脾脏及胸腺以用于病理评估及ELISA测定。

[0384] 每组4个其他动物的小脑(包括齿状核)、颈椎、胸及腰背根节、心脏、肾脏、肝脏、肺、性腺、胰腺、骨骼肌(腓肠肌及比目鱼肌)及脾脏经采集且迅速冷冻以用于分子生物学。

[0385] 组织学

[0386] 小脑(包括齿状核)、性腺、心脏、肾脏、肝脏、肺脏、胰腺、骨骼肌(腓肠肌及比目鱼

肌)、脾脏及颈椎、胸及腰椎为甲醛液固定的。随后使用EDTA溶液脱钙脊椎。所有器官经石蜡包埋以获得5μm厚部分,脊椎(包括脊髓及背根节)及心脏的横向部分。用苏木精及伊红染色所有器官。使用梅森氏三色染色法评估心脏纤维化。

[0387] ELISA测定

[0388] 在采集之后立即快速冷冻心脏的一半、肝脏的右叶、及比目鱼肌及腓肠肌。

[0389] 结果

[0390] 肉眼病理学

[0391] Mck阳性对照雄性小鼠与未经治疗Mck突变小鼠及经治疗Mck突变小鼠相比较明显更长(9.39cm与8.89cm[+5.62%]。P=0.011[t-测试])。未观测到其他显著肉眼可见损伤,尤其在雄性及雌性两者的心脏重量中未观测到肉眼可见损伤或显著变化。

[0392] 组织学

[0393] 心脏

[0394] Mck-Cre x FXN L3/WT:在一个Mck阳性对照动物(#58)中观测到最小间质纤维化。所有其他3个阳性对照小鼠心脏为正常的。

[0395] 未经治疗Mck-Cre x FXN L3/L-:相反地,在经分析的所有4个未经治疗Mck突变小鼠中观测到最小(小鼠#38)及中度(小鼠#41、#49及81)间质纤维化。此损伤与小鼠#38(最小)及#81(轻微)中肿胀的心肌细胞的心内膜病灶相关联。在小鼠#41及#49中,纤维化与心肌细胞的中度巨噬细胞发炎、最小播散性肿胀及轻微液胞化相关联。在小鼠#41及#81中观测到阿尼契柯氏(鹰眼形)核。

[0396] 经治疗Mck-Cre x FXN L3/L-:与未经治疗Mck突变小鼠相反,除在小鼠#47及#13中观测到极少阿尼契柯氏核以外,经rAAV-FXN治疗的Mck突变小鼠的心脏呈现正常。

[0397] 肾脏

[0398] 在所有三组中,常常在多个髓质小管的管腔中观测到显著矿化。矿化频率针对Mck阳性对照动物为4/4,针对未经治疗Mck突变动物为3/4,且针对经rAAV-FXN治疗的Mck突变动物为2/4。与Mck阳性对照组及未经治疗Mck突变组相比较,经rAAV-FXN治疗的Mck突变组中呈现矿化强度的降低。管状嗜碱性(再生)在2/4的Mck阳性对照小鼠中的动物中、在3/4的未经治疗Mck突变组中的动物中及在经rAAV-FXN治疗的Mck突变组中的1个动物中观测到。

[0399] 肝脏:在一个Mck阳性对照动物(#38)中观测到最小门静脉周发炎。

[0400] 肺脏:在一个未经治疗Mck突变动物(#41)中观测到轻微支气管周发炎。

[0401] 未观测到其他显著显微损伤。特别地,所有群组的脊髓、背根节及小脑均为正常的。

[0402] 组织学结果

[0403] 关于组织学,在未经治疗Mck突变小鼠的心肌细胞中观测到的鹰眼核、肿胀及液胞化均为心脏退化的标志。与巨噬细胞发炎相关联之间质纤维化可对应于心肌细胞死亡。因此,未经治疗Mck突变小鼠的心肌细胞退化,意指细胞经受下降的功能及演变至细胞死亡及后续心脏衰竭的病变。

[0404] 引人注目地,rAAV-FXN全身递送逆转此表型且经rAAV治疗的Mck突变小鼠(雄性及雌性两者)呈现正常且未显示显著的心肌细胞退化迹象。这些数据表明rAAV-huFXN转导足以逆转内源性小鼠Fxn基因不活化影响。因此,这些数据进一步使证明经rAAV修饰的FXN介

导的全身施用可逆转和/或预防由有需要的受试者的降低水平的野生型(功能性)共济失调蛋白降低水平的减少介导的疾病、病症或病状，诸如(但不限于)，弗里德赖希共济失调。如先前所指出，拥有本发明的教导的本领域技术人员应理解，可将其他施用途径(例如，包括颅内及至心脏的更直接途径)用于向有需要的受试者提供治疗益处。

[0405] 对肾脏的分析识别为不常见损伤的未经治疗Mck阳性对照组及未经治疗Mck突变动物两者的髓质中的肾结石(其为不常见损伤)的存在。由于未向这些动物提议特定膳食且考虑到其年龄，病变的频率及强度强有力地表明此并非偶发损伤，而系与基因型相关的损伤。引人关注地，此损伤强度通过rAAV-FXN治疗部分地降低，其表明肾结石发展涉及Fxn功能和/或蛋白水平的改变。因此，所谓的L3等位基因(其中小鼠共济失调蛋白基因由loxP序列侧接(尽管在内含子区域中))可为减效等位基因。此应与维持经上皮电解质活性传输的这些细胞中的线粒体的关键作用一致。就申请人的知识及信念而言，到目前为止，这些病变尚未报告于弗里德赖希共济失调临床观测中，或报告于FRDA小鼠模型中。

[0406] 因此，本发明涵盖治疗或预防肾脏疾病、病症或病状的方法，包括(但不限于)：有需要的受试者罹患或生长肾结石，其中肾脏疾病、病症或病状由受试者的降低水平的共济失调蛋白(例如，功能性和/或野生型共济失调蛋白)介导。

[0407] 在一个实施方案中，本发明包括评定受试者的共济失调蛋白水平；将受试者的共济失调蛋白水平与已知未罹患肾结石的受试者的共济失调蛋白水平相比较和/或将共济失调蛋白水平与针对其他健康个体判定的或本领域中已知的“标准共济失调蛋白水平”相比较，及在受试者的共济失调蛋白水平低于其他健康个体的共济失调蛋白水平和/或低于标准共济失调蛋白水平时向该受试者施用经rAAV修饰的共济失调蛋白，藉此治疗和/或预防受试者的肾结石。

[0408] 最终，HA染色可用于检测细胞内的rAAV-FXN-HA。首先，定量应表达FXN以恢复或维持心脏功能的细胞的数目将为重要的。其次，并未预期在肾脏中表达Mck基因(且因此由Mck启动子驱动的Cre重组酶)。是否检测肾脏中的rAAV-FXN-HA可有助于阐明肾结石形成是否为对髓质内稳定的HA-FXN的未预期直接影响。

[0409] 结论

[0410] 总之，本文中呈现的数据表明施用(甚至全身性地)编码经修饰的FXN基因的rAAV可治疗(预防和/或逆转)与共济失调蛋白的减少或缺失相关联的影响。因此，数据证明介导共济失调蛋白在细胞及有需要的受试者中的表达的rAAV可为用以治疗与共济失调蛋白的缺少或缺乏相关联或由共济失调蛋白的缺少或缺乏介导的疾病或病症(诸如(但不限于)弗里德赖希共济失调)的有用疗法。

[0411] 尽管已参考不同申请、方法、试剂盒及组合物描述所公开的教导，但应了解，可在不脱离本文中的教导及下文所主张的本发明的情况下进行各种变化及修改。提供前述实施例以更好地说明本发明的教导，且不意欲限制本文中所呈现的教导的范畴。虽然已在这些例示性实施方案方面描述本发明教导，但本领域技术人员将容易理解在不过度实验情况下这些例示性实施方案的大量变化及修改为可能的。所有所述变化及修改均在本教导的范畴内。

[0412] 本文中所引用的全部参考文献(包括专利、专利申请、论文、课本及类似者)及其中引用的参考文献至其尚未引用的程度，在此以全文引用的方式并入本文中。在所并入文献

及类似材料中的一个或多个(包括(但不限于)定义术语、术语用法、所述技术等)与本申请案不同或抵触的情况下,以本申请案为准。

[0413] 前述描述及实施例详述本发明的某些特定实施方案,且描述本发明人预期的最佳模式。然而,将了解,无论以文字呈现之前述内容如何详细,本发明可以许多方式实践,且本发明应根据所附申请专利范围及其任何等效物解释。

[0414] 表8序列

[0415]

SEQ ID NO: 1	人类野生型共济失调蛋白的氨基酸序列	MWTLGRRAVA TGLLASPSPA QAQTLTRVPR PAELAPLCGR RGLRTDIDAT CPRRASSNQR GLNQIWNVKK QSVYLMNLRK SGTLGHPGSL DETTYERLAE ETLDSLAEFF EDLADKPYTF EDYDVSFGSG VLTVKLGGDL GTYVINKQTP NKQIWLSSPS SGPKRYDWTG KNWVYSHDGV SLHELLAAEL TKALKTKLDL SSLAYSGKDA
SEQ ID NO: 2	编码野生型共济失调蛋白的核苷酸序列（图 1A-1B；泳道 1）	ATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGCGTCACCCA GCCCAGCCCAGGCCCAGACCCTCACCCGGGTCCCGCGGCCGGCAGAGTT GGCCCCACTCTGCGGCCGCCGTGGCCTGCGCACCGACATCGATGCGACC TGCACGCCCCGCCGCGCAAGTTCGAACCAACGTGGCCTCAACCAGATTT GGAATGTCAAAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAATCTGG AACTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAAAGACTA GCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTTTTGAAGACCTTGCAG ACAAGCCATACACGTTTGAGGACTATGATGTCTCCTTTGGGAGTGGTGT CTTAACTGTCAAACTGGGTGGAGATCTAGGAACCTATGTGATCAACAAG CAGACGCCAAACAAGCAAATCTGGCTATCTTCTCCATCCAGTGGACCTA AGCGTTATGACTGGACTGGGAAAAACTGGGTGTACTCCCACGACGGCGT GTCCCTCCATGAGCTGCTGGCCGCAGAGCTCACTAAAGCCTTAAAAACC AAACTGGACTTGTCTTCCTTGGCCTATTCCGGAAAAGATGCT
SEQ ID NO: 3	编码共济失调蛋白的 IDT2 优化核苷酸序列（图 1A-1B；泳道 2）	ATGTGGACACTGGGCAGAAGGGCGGTGGCCGGACTGTTGGCGAGTCCCA GTCCCGCGCAGGCGCAGACCCTTACTAGGGTGCCGCGGCCCGCGGAGCT GGCGCCACTCTGCGGTCGCCGCGGTCTGAGAACGGACATTGATGCCACT TGTACACCTCGGAGGGCCAGCTCCAACCAAAGGGGCCTTAATCAAATTT GGAACGTGAAGAAGCAGTCCGTCTACCTGATGAACCTTCGGAAGTCAGG GACCCTGGGCCACCCGGGAAGCTTGGATGAAACAACTTACGAAAGGTTG GCGGAGGAGACCTTGGATTCTCTTGCAGAGTTCTTCGAAGACCTGGCTG ATAAGCCTTACACCTTTGAGGACTACGATGTGTCTTTTGGATCTGGAGT

[0416]

		GCTGACCGTTAAACTGGGCGGGGATCTGGGCACCTACGTGATTAACAAG CAAACTCCAAACAAGCAGATCTGGCTTTCAAGCCCCAGTAGCGGGCCAA AACGCTACGATTGGACCGGAAAGAATTGGGTTTACAGCCACGATGGCGT TTCACTGCACGAGCTTCTGGCAGCAGAACTGACAAAAGCACTCAAGACG AAGCTCGACTTGTCATCCTTGGCATACTCCGGAAAGGATGCC
SEQ ID NO: 4	编码共济失调蛋白的 JCAT 优化核苷酸序列(图 1A-1B; 泳道 4)	ATGTGGACCCTGGGCCGCCGCGCCGTGGCCGGCCTGCTGGCCAGCCCCA GCCCCGCCCAGGCCCAGACCCTGACCCGCGTGCCCCGCCCCGCCGAGCT GGCCCCCCTGTGCGGCCGCCGCGGCCTGCGCACCGACATCGACGCCACC TGCACCCCCCGCCGCGCCAGCAGCAACCAGCGCGGCCTGAACCAGATCT GGAACGTGAAGAAGCAGAGCGTGTACCTGATGAACCTGCGCAAGAGCGG CACCCTGGGCCACCCCGGCAGCCTGGACGAGACCACCTACGAGCGCCTG GCCGAGGAGACCCTGGACAGCCTGGCCGAGTTCTTCGAGGACCTGGCCG ACAAGCCCTACACCTTCGAGGACTACGACGTGAGCTTCGGCAGCGGCGT GCTGACCGTGAAGCTGGGCGGCGACCTGGGCACCTACGTGATCAACAAG CAGACCCCCAACAAGCAGATCTGGCTATCTAGCCCCAGCAGCGGCCCCA AGCGCTACGACTGGACCGGCAAGAACTGGGTGTACAGCCACGACGGCGT GAGCCTGCACGAGCTGCTGGCCGCCGAGCTGACCAAGGCCCTGAAGACC AAGCTGGACCTGAGCAGCCTGGCCTACAGCGGCAAGGACGCC
SEQ ID NO: 5	编码共济失调蛋白的 GeneArt 优化核苷酸序列(图 1A-1B; 泳道 5)	ATGTGGACACTGGGGAGAAGGGCTGTGGCCGGACTGCTGGCTTCTCCAT CTCCAGCCCAGGCCCAGACCCTGACCAGAGTGCCTAGACCTGCCGAACT GGCCCCTCTGTGTGGCAGAAGAGGCCTGAGAACCGACATCGACGCCACC TGTACCCCCAGAAGGGCCAGCAGCAATCAGCGGGGCCTGAATCAGATCT GGAACGTGAAGAAACAGAGCGTGTACCTGATGAACCTGAGAAAGAGCGG CACCCTGGGCCACCCTGGAAGCCTGGATGAGACAACCTACGAGCGGCTG GCCGAGGAAACCCTGGATTCCCTGGCCGAGTTCTTCGAGGACCTGGCCG ACAAGCCCTACACCTTCGAGGATTACGACGTGTCCTTCGGCAGCGGCGT GCTGACAGTGAAGCTGGGCGGAGATCTGGGCACCTACGTGATCAACAAG CAGACCCCCAACAAACAGATCTGGCTATCTAGCCCCAGCAGCGGCCCCA

[0417]

		AGAGATACGATTGGACCGGCAAGAACTGGGTGTACAGCCACGACGGCGT GTCCCTGCATGAGCTGCTGGCTGCCGAGCTGACCAAGGCCCTGAAAACA AAGCTGGACCTGTCCAGCCTGGCCTACAGCGGCAAGGATGCC
SEQ ID NO: 6	编码共济失调蛋白的 Genscript(对照)优化核苷酸序列 图 1A-1B; 泳道 6)	ATGTGGACACTGGGCCGGAGAGCCGTCGCTGGGCTGCTGGCATCACCAT CCCCCGCACAGGCACAGACCCTGACAAGAGTCCCTCGGCCAGCAGAGCT GGCCCCACTGTGCGGGCGGAGAGGACTGCGAACCGACATCGATGCTACT TGTACCCCAAGGCGAGCAAGCTCCAACCAGCGAGGGCTGAACCAGATTT GGAATGTGAAGAAACAGTCTGTCTACCTGATGAATCTGAGAAAGAGCGG CACTCTGGGACACCCTGGCAGCCTGGACGAGACCACCTACGAGCGGCTG GCCGAGGAAACCCTGGATTCCCTGGCCGAGTTCTTTGAAGACCTGGCTG ATAAGCCATACACCTTCGAAGACTATGACGTGAGCTTCGGCAGCGGCGT GCTGACAGTCAAACTGGGCGGGGACCTGGGAACATACGTGATCAACAAG CAGACTCCTAACAAGCAGATTTGGCTGTCTAGTCCCTCAAGCGGCCCTA AGAGGTACGACTGGACAGGGAAAAACTGGGTGTATAGTCACGATGGCGT CTCACTGCATGAGCTGCTGGCCGCTGAACTGACTAAAGCCCTGAAAACT AAACTGGACCTGTCTTCCCTGGCATACTCTGGCAAGGACGCC
SEQ ID NO: 7	编码共济失调蛋白的 Genscript(低 CpG)核苷酸序列(图 1A-1B; 泳道 7)	ATGTGGACTCTGGGCCGGAGAGCAGTGGCAGGACTGCTGGCAAGTCCAT CACCTGCTCAGGCACAGACTCTGACAAGAGTCCCAAGACCTGCAGAGCT GGCTCCACTGTGCGGGAGGCGCGGACTGAGAACAGACATCGATGCTACA TGTACTCCTCGACGGGCAAGCTCCAACCAGCGAGGGCTGAACCAGATTT GGAATGTGAAGAAACAGTCCGTCTACCTGATGAATCTGAGGAAGTCAGG CACCCTGGGGCACCCAGGAAGTCTGGACGAGACCACATATGAACGGCTG GCTGAGGAAACACTGGATTCTCTGGCCGAGTTCTTTGAAGACCTGGCTG ATAAGCCCTACACATTCGAAGACTATGATGTGAGCTTTGGATCCGGCGT GCTGACTGTCAAACTGGGCGGGGACCTGGGCACTTACGTGATCAACAAG CAGACCCCTAACAAGCAGATTTGGCTGTCTAGTCCTTCAAGCGGACCAA AGCGGTACGACTGGACCGGCAAAAACTGGGTGTATTCTCACGATGGGGT CAGTCTGCATGAGCTGCTGGCCGCTGAACTGACCAAGGCCCTGAAGACA AAACTGGACCTGTCCTCTCTGGCATATAGCGGAAAAGATGCC

[0418]

SEQ ID NO: 8	编码共济失调蛋白的 IDT3 优化核苷酸序列	ATGTGGACACTGGGAAGGCGCGCCGTGGCCGGTCTGTTGGCATCACCAT CCCCAGCCCAGGCTCAGACACTCACCCGAGTCCCAAGACCCGCAGAGCT GGCCCCTCTGTGCGGGCGCCGAGGCCTTCGCACCGATATCGATGCTACA TGCACGCCACGCAGAGCTAGCTCAAATCAGAGGGGACTCAACCAGATAT GGAATGTCAAGAAGCAAAGCGTGTATCTCATGAACCTCCGGAAAAGCGG CACCCTGGGACATCCCGGGTCTCTCGACGAGACCACTTATGAAAGACTG GCAGAGGAGACTCTTGACAGTCTGGCGGAGTTCTTCGAAGACCTCGCTG ACAAGCCATATACCTTCGAAGATTACGACGTCTCCTTCGGCTCTGGGGT GCTGACTGTCAAGCTTGGCGGCGACCTGGGGACCTACGTGATCAACAAG CAGACTCCAAACAAGCAAATCTGGCTATCTAGTCCAAGCTCCGGACCCA AGAGATACGATTGGACAGGCAAGAATTGGGTTTACTCCCACGACGGGGT GTCCCTCCATGAGCTGCTGGCCGCAGAGCTGACGAAGGCCCTGAAGACC AAGCTGGATCTCTCCTCCCTGGCATACAGTGGTAAGGACGCT
SEQ ID NO: 9	编码共济失调蛋白的 IDT5 优化核苷酸序列 (图 1A-1B; 泳道 3)	ATGTGGACACTGGGCCGGCGCGCCGTCGCTGGGCTGCTCGCAAGCCCCA GCCCAGCCCAAGCGCAGACTCTGACTAGGGTGCCGCGGCCTGCCGAGTT GGCCCCCCTGTGCGGTAGGAGAGGCCTGCGCACAGACATCGATGCCACT TGCACACCCCGGCGGGCCAGCTCTAACCAAAGGGGCCTGAATCAAATTT GGAACGTCAAAAAACAGTCTGTATATCTGATGAATCTCCGGAAATCTGG AACGCTCGGGCATCCCGGATCTCTTGACGAGACCACCTACGAGCGACTG GCCGAGGAAACCCTTGACAGCCTGGCAGAATTCTTTGAGGATCTGGCTG ATAAACCCTATACCTTTGAAGATTACGATGTGAGTTTTGGTAGCGGAGT ACTGACTGTTAAGCTGGGCGGTGATCTCGGTACGTATGTTATCAATAAA CAAACCCCCAATAAACAGATTTGGCTCTCCTCCCCATCCTCTGGGCCTA AGCGCTATGACTGGACAGGAAAGAATTGGGTCTATTCACACGACGGAGT CAGTTTGCACGAGCTCCTCGCCGGCAGAGTTACCAAGGCCCTTAAGACT AAGCTCGACCTGTCAAGCCTCGCTTACTCTGGTAAGGACGCT
SEQ ID NO: 10	编码共济失调蛋白的核苷酸序列(核酸 22)	ATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGCGTCACCCA GCCCGGCCCAGGCCCAGACCCTCACCCGGGTCCCGCGGCCGGCAGAGTT GGCCCCACTCTGCGGCCGCCGTGGCCTGCGCACCGACATCGATGCGACC

[0419]

		TGCACGCCCCGCCGCGCAAGTTCGAACCAACGTGGCCTCAACCAGATTT GGAATGTCAAAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAATCTGG AACTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAAAGACTA GCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTTTTGAAGACCTTGCAG ACAAGCCATACACGTTTGAGGACTATGATGTCTCCTTTGGGAGTGGTGT CTTAACTGTCAAACTGGGTGGAGATCTAGGAACCTATGTGATCAACAAG CAGACGCCAAACAAGCAAATCTGGCTATCTTCTCCATCCAGTGGACCTA AGCGTTATGACTGGACTGGGAAAAACTGGGTGTACTCCCACGACGGCGT GTCCCTCCATGAGCTGCTGGCCGCAGAGCTCACTAAAGCCTTAAAAACC AAACTGGACTTGTCTTCCTTGGCCTATTCCGGAAAAGATGCT
SEQ ID NO:11	编码共济失调蛋白的 IDT-1 优化核苷酸序列(核酸 23)	ATGTGGACTCTGGGTAGGCGAGCGGTGGCCGGCCTGTTGGCATCTCCTA GTCCTGCACAAGCTCAAACGCTGACTAGAGTCCCTCGGCCAGCAGAACT GGCGCCACTTTGCGGCCGGCGCGGTCTTCGCACTGATATTGATGCCACT TGCACACCCCGGCGCGCCTCCAGTAATCAGCGGGGACTTAATCAAATTT GGAATGTGAAGAAGCAGTCTGTGTATCTTATGAATCTGCGGAAGAGCGG GACCCTGGGCCACCCTGGTAGCCTTGATGAAACCACCTATGAGCGCCTG GCCGAAGAGACACTGGACAGTCTTGCCGAGTTTTTTGAGGATCTGGCCG ACAAACCTTATACTTTTGAGGACTATGACGTGTCCTTTGGATCTGGTGT ATTGACCGTAAAACTCGGGGGAGACCTTGGGACGTATGTAATAAATAAG CAGACCCCAAACAAGCAGATCTGGCTATCTTCTCCAAGTAGTGGTCCTA AGAGATATGATTGGACGGGCAAGAACTGGGTCTATTCCCATGATGGCGT CTCTTTGCATGAACTCCTTGCAGCAGAGCTGACCAAGGCCTTGAAGACC AAATTGGATCTCAGCAGCCTCGCCTATAGTGGCAAAGATGCA
SEQ ID NO:12	编码共济失调蛋白的 IDT-4 优化核苷酸序列(核酸 26)	ATGTGGACTCTGGGCCGGCGGGCCGTAGCTGGCTTGCTGGCTAGCCCAA GTCCCGCCCAGGCTCAGACTCTCACCAGGGTACCCAGGCCCGCAGAGCT TGCTCCACTCTGCGGACGCAGGGGTCTGCGAACCGATATCGACGCAACT TGCACGCCGCGGAGGGCCTCTTCAAACCAGAGAGGACTCAATCAAATTT GGAATGTAAAGAAACAGAGCGTGTATCTCATGAACCTCCGAAAGAGTGG GACTCTTGGGCACCCCGGCTCCCTGGACGAGACTACTTACGAGCGCCTG

[0420]

		GCCGAAGAAACCTTGGATTCCCTGGCGGAGTTTTTTGAAGACTTGGCAG ACAAGCCTTATACCTTCGAGGATTACGACGTGAGTTTTGGCTCTGGTGT TCTTACAGTCAAGCTCGGTGGCGACCTTGGCACTTATGTAATTAACAAG CAGACACCTAACAAGCAGATCTGGCTTTCTAGTCCGTCTTCCGGTCCCA AAAGGTACGATTGGACTGGAAAGAACTGGGTCTACAGTCACGACGGTGT CTCCCTGCACGAATTGCTTGCGGCAGAGCTGACTAAGGCGCTCAAAACA AAACTGGATCTGTCCAGCCTTGCCTATAGCGGGAAGGACGCA
SEQ ID NO:13	编码嵌合 AAV2.5 载体衣壳 VP1 的核苷酸序列	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTG AAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCACCAAA GCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGG TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCA ACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCA GCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCG GAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTTTTGGGGGCAACCTCG GACGAGCAGTCTTCCAGGCGAAAAAGAGGGTTCTTGAACCTCTGGGCCT GGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGTAGAG CACTCTCCTGTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCC AGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAGA CTCAGTACCTGACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCT GGTCTGGGAACTAATACGATGGCTACAGGCAGTGGCGCACCAATGGCAG ACAATAACGAGGGCGCCGACGGAGTGGGTAATTCCTCGGGAAATTGGCA TTGCGATTCCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGA ACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAATTTCCA GCGCTTCAACGGGAGCCTCGAACGACAATCACTACTTTGGCTACAGCAC CCCTTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCA CGTGACTGGCAAAGACTCATCAACAACAACTGGGGATTCCGACCCAAGA GACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAA TGACGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTG TTTACTGACTCGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATC

[0421]

		AAGGATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCACAGTA TGGATACCTCACCCTGAACAACGGGAGTCAGGCAGTAGGACGCTCTTCA TTTTACTGCCTGGAGTACTTTCCTTCTCAGATGCTGCGTACCGGAAACA ACTTTACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTA CGCTCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCATCGACCAG TACCTGTATTACTTGAGCAGAACAAACACTCCAAGTGGAACCACCACGC AGTCAAGGCTTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGACCA GTCTAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCA AAGACATCTGCGGATAACAACAACAGTGAATACTCGTGGACTGGAGCTA CCAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCGGC CATGGCAAGCCACAAGGACGATGAAGAAAAGTTTTTTCCTCAGAGCGGG GTTCTCATCTTTGGGAAGCAAGGCTCAGAGAAAACAAATGTGGACATTG AAAAGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGT GGCTACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGGCAAC AGACAAGCAGCTACCGCAGATGTCAACACACAAGGCGTTCTTCCAGGCA TGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCATCTGGGCAAA GATTCCACACACGGACGGACATTTTCACCCCTCTCCCCTCATGGGTGGA TTCGGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAACACCCCGG TACCTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTT CATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGTGGGAG CTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTACACTT CCAACTACGCCAAGTCTGTCAATGTGGACTTTACTGTGGACAATAATGG CGTGTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGACTCGTAAT CTGTAA
SEQ ID NO: 14	编码野生型 AAV1 衣壳(VP1)的核苷酸序列	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTG AGGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAGCCCAA AGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGC TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCA ACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCA

[0422]

		GCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCC
		GAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCG
		GGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCT
		GGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAG
		CAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATCGGCAAGACAGGCC
		AGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGA
		GTCAGTCCCCGATCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCT
		GCTGTGGGACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAG
		ACAATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCA
		TTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGC
		ACCTGGGCCTTGCCCACCTACAATAACCACCTCTACAAGCAAATCTCCA
		GTGCTTCAACGGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCAC
		CCCCTGGGGGTATTTTGATTTCAACAGATTCCACTGCCACTTTTCACCA
		CGTGACTGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGA
		GACTCAACTTCAAACTCTTCAACATCCAAGTCAAGGAGGTCACGACGAA
		TGATGGCGTCACAACCATCGCTAATAACCTTACCAGCACGGTTCAAGTC
		TTCTCGGACTCGGAGTACCAGCTTCCGTACGTCCTCGGCTCTGCGCACC
		AGGGCTGCCTCCCTCCGTTCCCGGCGGACGTGTTCATGATTCCGCAATA
		CGGCTACCTGACGCTCAACAATGGCAGCCAAGCCGTGGGACGTTCATCC
		TTTTACTGCCTGGAATATTTCCCTTCTCAGATGCTGAGAACGGGCAACA
		ACTTTACCTTCAGCTACACCTTTGAGGAAGTGCCTTTCCACAGCAGCTA
		CGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAA
		TACCTGTATTACCTGAACAGAACTCAAAATCAGTCCGGAAGTGCCCAAA
		ACAAGGACTTGCTGTTTAGCCGTGGGTCTCCAGCTGGCATGTCTGTTCA
		GCCCAAAAACTGGCTACCTGGACCCTGTTATCGGCAGCAGCGCGTTTCT
		AAAACAAAAACAGACAACAACAACAGCAATTTTACCTGGACTGGTGCTT
		CAAAATATAACCTCAATGGGCGTGAATCCATCATCAACCCTGGCACTGC
		TATGGCCTCACACAAAGACGACGAAGACAAGTTCTTTCCCATGAGCGGT
		GTCATGATTTTTGGAAAAGAGAGCGCCGGAGCTTCAAACACTGCATTGG

[0423]

		ACAATGTCATGATTACAGACGAAGAGGAAATTAAAGCCACTAACCCTGT GGCCACCGAAAGATTTGGGACCGTGGCAGTCAATTTCCAGAGCAGCAGC ACAGACCCTGCGACCGGAGATGTGCATGCTATGGGAGCATTACCTGGCA TGGTGTGGCAAGATAGAGACGTGTACCTGCAGGGTCCCATTTGGGCCAA AATTCCTCACACAGATGGACACTTTCACCCGTCTCCTCTTATGGGCGGC TTTGGACTCAAGAACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTG TTCCTGCGAATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATT CATCACCCAATACTCCACAGGACAAGTGAGTGTGGAAATTGAATGGGAG CTGCAGAAAGAAAACAGCAAGCGCTGGAATCCCGAAGTGCAGTACACAT CCAATTATGCAAAATCTGCCAACGTTGATTTTACTGTGGACAACAATGG ACTTTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTTACCCGTCCC CTGTAA
SEQ ID NO:15	编码经修饰的 AAV1.1 衣壳 VP1 的核苷酸序列 (氨基酸残基编号 265 缺失)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTG AGGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAGCCCAA AGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGC TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCA ACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCA GCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCC GAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCG GGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCT GGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAG CAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATCGGCAAGACAGGCC AGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGA GTCAGTCCCCGATCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCT GCTGTGGGACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAG ACAATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCA TTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGC ACCTGGGCCTTGCCCACCTACAATAACCACCTCTACAAGCAAATCTCCA GTGCTTCAGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCACCCC

[0424]

		CTGGGGGTATTTTGATTTCAACAGATTCCACTGCCACTTTTCACCACGT GACTGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGAGAC TCAACTTCAAACTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGA TGGCGTCACAACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTTC TCGGACTCGGAGTACCAGCTTCCGTACGTCCTCGGCTCTGCGCACCAGG GCTGCCTCCCTCCGTTCCCGGCGGACGTGTTCATGATTCCGCAATACGG CTACCTGACGCTCAACAATGGCAGCCAAGCCGTGGGACGTTCATCCTTT TACTGCCTGGAATATTTCCCTTCTCAGATGCTGAGAACGGGCAACAACT TTACCTTCAGCTACACCTTTGAGGAAGTGCCTTTCCACAGCAGCTACGC GCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAATAC CTGTATTACCTGAACAGAACTCAAAATCAGTCCGGAAGTGCCCAAAACA AGGACTTGCTGTTTAGCCGTGGGTCTCCAGCTGGCATGTCTGTTCAGCC CAAAAACTGGCTACCTGGACCCTGTTATCGGCAGCAGCGCGTTTCTAAA ACAAAAACAGACAACAACAACAGCAATTTTACCTGGACTGGTGCTTCAA AATATAACCTCAATGGGCGTGAATCCATCATCAACCCTGGCACTGCTAT GGCCTCACACAAAGACGACGAAGACAAGTTCTTTCCCATGAGCGGTGTC ATGATTTTTGGAAAAGAGAGCGCCGGAGCTTCAAACACTGCATTGGACA ATGTCATGATTACAGACGAAGAGGAAATTAAAGCCACTAACCCTGTGGC CACCGAAAGATTTGGGACCGTGGCAGTCAATTTCCAGAGCAGCAGCACA GACCCTGCGACCGGAGATGTGCATGCTATGGGAGCATTACCTGGCATGG TGTGGCAAGATAGAGACGTGTACCTGCAGGGTCCCATTTGGGCCAAAAT TCCTCACACAGATGGACACTTTCACCCGTCTCCTCTTATGGGCGGCTTT GGACTCAAGAACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTC CTGCGAATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATTCAT CACCCAATACTCCACAGGACAAGTGAGTGTGGAAATTGAATGGGAGCTG CAGAAAGAAAACAGCAAGCGCTGGAATCCCGAAGTGCAGTACACATCCA ATTATGCAAAATCTGCCAACGTTGATTTTACTGTGGACAACAATGGACT TTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTTACCCGTCCCCTG TAA

[0425]

SEQ ID NO: 16	编码野生型 AAV6 衣壳 (VP1)的核苷酸序列	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTG AGGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAA AGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGC TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCA ACGCGGCGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCA GCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCC GAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCG GGCGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCGAACCTTTTGGTCT GGTTGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAG CAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCC AGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGA GTCAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCT GCTGTGGGACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAG ACAATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCA TTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGA ACATGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAATCTCCA GTGCTTCAACGGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCAC CCCCTGGGGGTATTTTGATTTCAACAGATTCCACTGCCATTTCTCACCA CGTGACTGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGA GACTCAACTTCAAGCTCTTCAACATCCAAGTCAAGGAGGTCACGACGAA TGATGGCGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGTC TTCTCGGACTCGGAGTACCAGTTGCCGTACGTCCTCGGCTCTGCGCACC AGGGCTGCCTCCCTCCGTTCCCGGCGGACGTGTTCATGATTCCGCAGTA CGGCTACCTAACGCTCAACAATGGCAGCCAGGCAGTGGGACGGTCATCC TTTTACTGCCTGGAATATTTCCCATCGCAGATGCTGAGAACGGGCAATA ACTTTACCTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTA CGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAG TACCTGTATTACCTGAACAGAACTCAGAATCAGTCCGGAAGTGCCCAAA ACAAGGACTTGCTGTTTAGCCGGGGGTCTCCAGCTGGCATGTCTGTTCA

[0426]

		GCCCAAAAACTGGCTACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCT AAAACAAAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGCTT CAAAATATAACCTTAATGGGCGTGAATCTATAATCAACCCTGGCACTGC TATGGCCTCACACAAAGACGACAAAGACAAGTTCTTTCCCATGAGCGGT GTCATGATTTTTGGAAAGGAGAGCGCCGGAGCTTCAAACACTGCATTGG ACAATGTCATGATCACAGACGAAGAGGAAATCAAAGCCACTAACCCCGT GGCCACCGAAAGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAGC ACAGACCCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTACCTGGAA TGGTGTGGCAAGACAGAGACGTATACCTGCAGGGTCCTATTTGGGCCAA AATTCCTCACACGGATGGACACTTTCACCCGTCTCCTCTCATGGGCGGC TTTGGACTTAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTG TTCCTGCGAATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATT CATCACCCAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAATGGGAG CTGCAGAAAGAAAACAGCAAACGCTGGAATCCCGAAGTGCAGTATACAT CTAACTATGCAAAATCTGCCAACGTTGATTTCACTGTGGACAACAATGG ACTTTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTCCC CTGTAA
SEQ ID NO:17	编码经修饰的 AAV6.1 衣壳 VP1 的核苷酸序列(氨基酸残基编号 265 缺失)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTDGA GGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAA GCCAACCAGCAAAAGCAGGACGACGGCCGGGGTDGGTGCTTCCTGGCTA CAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAAC GCGGCGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGC TCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGA GTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGG CGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCGAACCTTTTGGTDGGT TGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAG TCGCCACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCCAGC AGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGAGTC AGTCCCCGACCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCTGCT

[0427]

		GTGGGACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAGACA
		ATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCATTG
		CGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGAACA
		TGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAATCTCCAGTG
		CTTCAGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCACCCCCTG
		GGGGTATTTTGATTTCAACAGATTCCACTGCCATTTCTCACCACGTGAC
		TGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGAGACTCA
		ACTTCAAGCTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGATGG
		CGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTTCTCG
		GACTCGGAGTACCAGTTGCCGTACGTCCTCGGCTCTGCGCACCAGGGCT
		GCCTCCCTCCGTTCCCGGCGGACGTGTTCATGATTCCGCAGTACGGCTA
		CCTAACGCTCAACAATGGCAGCCAGGCAGTGGGACGGTCATCCTTTTAC
		TGCCTGGAATATTTCCCATCGCAGATGCTGAGAACGGGCAATAACTTTA
		CCTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTACGCGCA
		CAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAGTACCTG
		TATTACCTGAACAGAACTCAGAATCAGTCCGGAAGTGCCCAAAACAAGG
		ACTTGCTGTTTAGCCGGGGGTCTCCAGCTGGCATGTCTGTTCAGCCCAA
		AAACTGGCTACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCTAAAACA
		AAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGCTTCAAAAT
		ATAACCTTAATGGGCGTGAATCTATAATCAACCCTGGCACTGCTATGGC
		CTCACACAAAGACGACAAAGACAAGTTCTTTCCCATGAGCGGTGTCATG
		ATTTTTGGAAAGGAGAGCGCCGGAGCTTCAAACACTGCATTGGACAATG
		TCATGATCACAGACGAAGAGGAAATCAAAGCCACTAACCCCGTGGCCAC
		CGAAAGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAGCACAGAC
		CCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTACCTGGAATGGTGT
		GGCAAGACAGAGACGTATACCTGCAGGGTCCTATTTGGGCCAAAATTCC
		TCACACGGATGGACACTTTCACCCGTCTCCTCTCATGGGCGGCTTTGGA
		CTTAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTG
		CGAATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATTCATCAC

[0428]

		CCAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAATGGGAGCTGCAG AAAGAAAACAGCAAACGCTGGAATCCCGAAGTGCAGTATACATCTAACT ATGCAAAATCTGCCAACGTTGATTTCACTGTGGACAACAATGGACTTTA TACTGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTCCCCTGTAA
SEQ ID NO:18	编码经修饰的AAV6.3.1衣壳VP1的核苷酸序列(氨基酸残基265缺失，Lys 531改变为Glu)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTG AGGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAA AGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGC TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCA ACGCGGCGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCA GCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCC GAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCG GGCGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCGAACCTTTTGGTCT GGTTGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAG CAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCC AGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGA GTCAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCT GCTGTGGGACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAG ACAATAACGAAGGCGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCA TTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGA ACATGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAATCTCCA GTGCTTCAGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCACCCC CTGGGGGTATTTTGATTTCAACAGATTCCACTGCCATTTCTCACCACGT GACTGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGAGAC TCAACTTCAAGCTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGA TGGCGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTTC TCGGACTCGGAGTACCAGTTGCCGTACGTCCTCGGCTCTGCGCACCAGG GCTGCCTCCCTCCGTTCCCGGCGGACGTGTTCATGATTCCGCAGTACGG CTACCTAACGCTCAACAATGGCAGCCAGGCAGTGGGACGGTCATCCTTT TACTGCCTGGAATATTTCCCATCGCAGATGCTGAGAACGGGCAATAACT

[0429]

		TTACCTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAGCTACGC GCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAGTAC CTGTATTACCTGAACAGAACTCAGAATCAGTCCGGAAGTGCCCAAAACA AGGACTTGCTGTTTAGCCGGGGGTCTCCAGCTGGCATGTCTGTTCAGCC CAAAAACTGGCTACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCTAAA ACAAAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGCTTCAA AATATAACCTTAATGGGCGTGAATCTATAATCAACCCTGGCACTGCTAT GGCCTCACACAAAGACGACGAAGACAAGTTCTTTCCCATGAGCGGTGTC ATGATTTTTGGAAAGGAGAGCGCCGGAGCTTCAAACACTGCATTGGACA ATGTCATGATCACAGACGAAGAGGAAATCAAAGCCACTAACCCCGTGGC CACCGAAAGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAGCAGCACA GACCCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTACCTGGAATGG TGTGGCAAGACAGAGACGTATACCTGCAGGGTCCTATTTGGGCCAAAAT TCCTCACACGGATGGACACTTTCACCCGTCTCCTCTCATGGGCGGCTTT GGACTTAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTC CTGCGAATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATTCAT CACCCAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAATGGGAGCTG CAGAAAGAAAACAGCAAACGCTGGAATCCCGAAGTGCAGTATACATCTA ACTATGCAAAATCTGCCAACGTTGATTTCACTGTGGACAACAATGGACT TTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTCCCCTG TAA
SEQ ID NO:19	用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编码人类野生型共济失调蛋白(WT FXN)的核苷酸序列 AgeI 位点为粗体; AvrII 加下划线;	TAGAAGACCGGTCGCCACCatgtggactctcgggcgccgcgcagtagcc ggcctcctggcgtcacccagcccagcccaggcccagaccctcacccggg tcccgcggccggcagagttggccccactctgcggccgccgtggcctgcg caccgacatcgatgcgacctgcacgccccgccgcgcaagttcgaaccaa cgtggcctcaaccagatttggaatgtcaaaaagcagagtgtctatttga tgaatttgaggaaatctggaactttgggccacccaggctctctagatga gaccacctatgaaagactagcagaggaaacgctggactctttagcagag ttttttgaagaccttgcagacaagccatacacgtttgaggactatgatg

[0430]

	CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体且为斜体 (参见图 2A)	tctcctttgggagtggtgtcttaactgtcaaactgggtggagatctagg aacctatgtgatcaacaagcagacgccaaacaagcaaatctggctatct tctccatccagtggacctaagcgttatgactggactgggaaaaactggg tgtactcccacgacggcgtgtccctccatgagctgctggccgcagagct cactaaagccttaaaaaccaaactggacttgtcttccttggcctattcc ggaaaagatgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG TGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO:20	用于克隆至 pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编码 FXN 的 IDT1 密码子优化核苷酸序列 AgeI 位点为粗体; AvrII 加下划线; CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体且为斜体 (参见图 2B)	TAGAAGACCGGTCGCCACCatgtggactctgggtaggcgagcggtggcc ggcctgttggcatctcctagtcctgcacaagctcaaacgctgactagag tccctcggccagcagaactggcgccactttgcggccggcgcggtcttcg cactgatattgatgccacttgcacaccccggcgcgcctccagtaatcag cggggacttaatcaaatttggaatgtgaagaagcagtctgtgtatctta tgaatctgcggaagagcgggaccctgggccaccctggtagccttgatga aaccacctatgagcgcctggccgaagagacactggacagtcttgccgag ttttttgaggatctggccgacaaaccttatacttttgaggactatgacg tgtcctttggatctggtgtattgaccgtaaaactcgggggagaccttgg gacgtatgtaataaataagcagaccccaaacaagcagatctggctcagc tctccaagtagtggtcctaagagatatgattggacgggcaagaactggg tctattcccatgatggcgtctctttgcatgaactccttgcagcagagct gaccaaggccttgaagaccaaattggatctcagcagcctcgcctatagt ggcaaagatgcatagCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG*

[0431]

		*GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG TGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO:21	用于克隆至 pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编码 FXN IDT3(低表达)的密码子优化核苷酸序列 AgeI 位点为粗体; AvrII 加下划线; CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体且为斜体 (参见图 2C)	TAGAAGACCGGTCGCCACCatgtggacactgggaaggcgcgccgtggcc ggtctgttggcatcaccatccccagcccaggctcagacactcacccgag tcccaagacccgcagagctggcccctctgtgcgggcgccgaggccttcg caccgatatcgatgctacatgcacgccacgcagagctagctcaaatcag aggggactcaaccagatatggaatgtcaagaagcaaagcgtgtatctca tgaacctccggaaaagcggcaccctgggacatcccgggtctctcgacga gaccacttatgaaagactggcagaggagactcttgacagtctggcggag ttcttcgaagacctcgctgacaagccatataccttcgaagattacgacg tctccttcggctctggggtgctgactgtcaagcttggcggcgacctggg gacctacgtgatcaacaagcagactccaaacaagcaaatctggctcagc agtccaagctccggacccaagagatacgattggacaggcaagaattggg tttactcccacgacggggtgtccctccatgagctgctggccgctgagct gacgaaggccctgaagaccaagctggatctctcctccctggcatacagt ggtaaggacgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG TGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO:22	用于克隆至 pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编	TAGAAGACCGGTCGCCACCatgtggactctgggccggcgggccgtagct ggcttgctggctagcccaagtcccgcccaggctcagactctcaccaggg tacccaggcccgcagagcttgctccactctgcggacgcaggggtctgcg

[0432]

	码 FXN IDT4 的密码子优化核苷酸序列 AgeI 位点为粗体; AvrII 加下划线; CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体且为斜体 (参见图 2D)	aaccgatatcgacgcaacttgcacgccgcggagggcctcttcaaaccag agaggactcaatcaaatttggaatgtaaagaaacagagcgtgtatctca tgaacctccgaaagagtgggactcttgggcaccccggctccctggacga gactacttacgagcgcctggccgaagaaaccttggattccctggcggag ttttttgaagacttggcagacaagccttataccttcgaggattacgacg tgagttttggctctggtgttcttacagtcaagctcggtggcgaccttgg cacttatgtaattaacaagcagacacctaacaagcagatctggctttct agtccgtcttccggtcccaaaaggtacgattggactggaaagaactggg tctacagtcacgacggtgtctccctgcacgaattgcttgcggctgagct gactaaggcgctcaaaacaaaactggatctgtccagccttgcctatagc gggaaggacgcatgaCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG TGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO: 23	用于克隆至 pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编码 FXN GenScript 的密码子优化核苷酸序列 AgeI 位点为粗体; AvrII 加下划线; CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体	TAGAAGACCGGTCGCCACCatgtggacactgggccggagagccgtcgct gggctgctggcatcaccatcccccgcacaggcacagaccctgacaagag tccctcggccagcagagctggccccactgtgcgggcggagaggactgcg aaccgacatcgatgctacttgtaccccaaggcgagcaagctccaaccag cgagggctgaaccagatttggaatgtgaagaaacagtctgtctacctga tgaatctgagaaagagcggcactctgggacaccctggcagcctggacga gaccacctacgagcggctggccgaggaaaccctggattccctggccgag ttctttgaagacctggctgataagccatacaccttcgaagactatgacg tgagcttcggcagcggcgtgctgacagtcaaactgggcggggacctggg aacatacgtgatcaacaagcagactcctaacaagcagatttggctgtct agtccctcaagcggccctaagaggtacgactggacagggaaaaactggg

[0433]

	且为斜体 (参见图 2E)	tgtatagtcacgatggcgtctcactgcatgagctgctggccgctgaact gactaaagccctgaaaactaaactggacctgtcttccctggcatactct ggcaaggacgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC* *CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG* *GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA* *TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC* *AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG* *TGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO:24	用于克隆至 pTRs-KS-CBh-EGFP-BGH scAAV 载体中的编码 FXN GenScript 的密码子优化核苷酸序列 AgeI 位点为粗体; AvrII 加下划线; CSS 加双下划线; SpeI 为粗体且加下划线的; bGHpolyA 为斜体; MluI 位点为粗体且为斜体 (参见图 2F)	TAGAAGACCGGTCGCCACCatgtggactctgggccggagagcagtggca ggactgctggcaagtccatcacctgctcaggcacagactctgacaagag tcccaagacctgcagagctggctccactgtgcgggaggcgcggactgag aacagacatcgatgctacatgtactcctcgacgggcaagctccaaccag cgagggctgaaccagatttggaatgtgaagaaacagtccgtctacctga tgaatctgaggaagtcaggcaccctggggcacccaggaagtctggacga gaccacatatgaacggctggctgaggaaacactggattctctggccgag ttctttgaagacctggctgataagccctacacattcgaagactatgatg tgagctttggatccggcgtgctgactgtcaaactgggcggggacctggg cacttacgtgatcaacaagcagacccctaacaagcagatttggctgtct agtccttcaagcggaccaaagcggtacgactggaccggcaaaaactggg tgtattctcacgatggggtcagtctgcatgagctgctggccgctgaact gaccaaggccctgaagacaaaactggacctgtcctctctggcatatagc ggaaaagatgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGATCCCA GCCCACTTTTCCCCAATACGACTAGTA*CTCGACTGTGCCTTCTAGTTGC* *CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAG* *GTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA* *TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC* *AGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCGG*

[0434]

		*TGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG*ACGCGT*CTT AAG
SEQ ID NO: 25	编码胶原蛋白稳定序列(CSS)的核酸序列	CCCAGCCCACTTTTCCCCAA
SEQ ID NO: 26	CBh 启动子的核酸序列	tacataacttacggtaaatggcccgcctggctgaccgcccaacgacccc cgcccattgacgtcaatagtaacgccaatagggactttccattgacgtc aatgggtggagtatttacggtaaactgcccacttggcagtacatcaagt gtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatgg cccgcctggcattgtgcccagtacatgaccttatgggactttcctactt ggcagtacatctacgtattagtcatcgctattaccatggtcgaggtgag ccccacgttctgcttcactctccccatctccccccccctccccacccca attttgtatttatttatttttaattattttgtgcagcgatgggggcgg ggggggggggggcgcgcgccaggcggggcggggcggggcgaggggcg gggcggggcgaggcggagaggtgcggcggcagccaatcagagcggcgcg ctccgaaagtttccttttatggcgaggcggcggcggcggccctata aaaagcgaagcgcgcggcgggcgggagtcgctgcgacgctgccttcgcc ccgtgccccgctccgccgccgcctcgcgccgcccgccccggctctgact gaccgcgttactcccacaggtgagcgggcgggacggcccttctcctccg ggctgtaattagctgagcaagaggtaagggtttaagggatggttggttg gtggggtattaatgtttaattacctggagcacctgcctgaaatcactt ttttcaggttgga
SEQ ID NO: 27	bGHpoly A信号序列的核酸序列	CTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCC GTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAAT AAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCT GGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAC AGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAA GAACCAGCT
SEQ ID NO: 28	编码 AAV2i8 衣壳(VP1)的核苷酸序列	atggctgccgatggttatcttccagattggctcgaggacactctctctg aaggaataagacagtggtggaagctcaaacctggcccaccaccaccaaa

[0435]

		gcccgcagagcggcataaggacgacagcaggggtcttgtgcttcctggg
		tacaagtacctcggacccttcaacggactcgacaagggagagccggtca
		acgaggcagacgccgcggccctcgagcacgacaaagcctacgaccggca
		gctcgacagcggagacaacccgtacctcaagtacaaccacgccgacgcg
		gagtttcaggagcgccttaaagaagatacgtcttttgggggcaacctcg
		gacgagcagtcttccaggcgaaaaagagggttcttgaacctctgggcct
		ggttgaggaacctgttaagacggctccgggaaaaaagaggccggtagag
		cactctcctgtggagccagactcctcctcgggaaccggaaaggcgggcc
		agcagcctgcaagaaaaagattgaattttggtcagactggagacgcaga
		ctcagtacctgacccccagcctctcggacagccaccagcagcccccctct
		ggtctgggaactaatacgatggctacaggcagtggcgcaccaatggcag
		acaataacgagggcgccgacggagtgggtaattcctcgggaaattggca
		ttgcgattccacatggatgggcgacagagtcatcaccaccagcacccga
		acctgggccctgcccacctacaacaaccacctctacaaacaaatttcca
		gccaatcaggagcctcgaacgacaatcactactttggctacagcacccc
		ttgggggtattttgacttcaacagattccactgccacttttcaccacgt
		gactggcaaagactcatcaacaacaactggggattccgacccaagagac
		tcaacttcaagctctttaacattcaagtcaaagaggtcacgcagaatga
		cggtacgacgacgattgccaataaccttaccagcacggttcaggtgttt
		actgactcggagtaccagctcccgtacgtcctcggctcggcgcatcaag
		gatgcctcccgccgttcccagcagacgtcttcatggtgccacagtatgg
		atacctcaccctgaacaacgggagtcaggcagtaggacgctcttcattt
		tactgcctggagtactttccttctcagatgctgcgtaccggaaacaact
		ttaccttcagctacacttttgaggacgttcctttccacagcagcta
		cgctcacagccagagtctggaccgtctcatgaatcctctcatcgaccag
		tacctgtattacttgagcagaacaaacactccaagtggaaccaccacgc
		agtcaaggcttcagttttctgtggccggacccagtaacatggctgtcca
		gggaaggaactggcttcctggaccctgttaccgccagcagcgagtatca
		aagacatctgcggataacaacaacagtgaatttgcttggactggagcta

[0436]

		ccaagtaccacctcaatggcagagactctctggtgaatccgggcccggc catggcaagccacaaggacgatgaagaaaagtttttttcctcagagcggg gttctcatctttgggaagcaaggctcagagaaaacaaatgtggacattg aaaaggtcatgattacagacgaagaggaaatcaggacaaccaatcccgt ggctacggagcagtatggttctgtatctaccaacctccagcaacagaac acagcaccagctaccgcagatgtcaacacacaaggcgttcttccaggca tggtctggcaggacagagatgtgtaccttcaggggcccatctgggcaaa gattccacacacggacggacattttcacccctctcccctcatgggtgga ttcggacttaaacaccctcctccacagattctcatcaagaacaccccgg tacctgcgaatccttcgaccaccttcagtgcggcaaagtttgcttcctt catcacacagtactccacgggacaggtcagcgtggagatcgagtgggag ctgcagaaggaaaacagcaaacgctggaatcccgaaattcagtacactt ccaactacaacaagtctgttaatgtggactttactgtggacactaatgg cgtgtattcagagcctcgccccattggcaccagatacctgactcgtaat ctgtaa
SEQ ID NO: 29	AAV2i8 衣壳(VP1)的氨基酸序列	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPG YKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVE HSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPS GLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTR TWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPR DWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVF TDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSF YCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQY LYYLSRTNTPSGTTTQSRLQFSVAGPSNMAVQGRNWLPGPCYRQQRVSK TSADNNNSEFAWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGV LIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQQQNT APATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGF GLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWEL

[0437]

		QKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
SEQ ID NO: 30	编码 AAV2-TT 衣壳(VP1)的核酸(不同于 WT AAV2 的核苷酸加下划线)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTG AAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCACCAAA GCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGG TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCA ACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCA GCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCG GAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTTTTGGGGGCAACCTCG GACGAGCAGTCTTCCAGGCGAAAAAGAGGATTCTTGAACCTCTGGGCCT GGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGTAGAG CACTCTCCTGCGGAGCCAGACTCCTCCTCGGGAACCGGAAAGTCGGGCC AGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAGA CTCAGTACCTGACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCT GGTCTGGGAACTAATACGATGGCTTCAGGCAGTGGCGCACCAATGGCAG ACAATAACGAGGGCGCCGACGGAGTGGGTAATTCCTCGGGAAATTGGCA TTGCGATTCCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGA ACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAATTTCCA GCCAATCAGGAGCCTCGAACGACAATCACTACTTTGGCTACAGCACCCC TTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGT GACTGGCAAAGACTCATCAACAACAACTGGGGATTCCGACCCAAGAGAC TCAGCTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGA CGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTT ACTGACTCGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATCAAG GATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCACAGTATGG ATACCTCACCCTGAACAACGGGAGTCAGGCAGTAGGACGCTCTTCATTT TACTGCCTGGAGTACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACT TTACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTACGC TCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCATCGACCAGTAC CTGTATTACTTGAGCAGAACAAACACTCCAAGTGGAACCACCACGATGT

[0438]

		CAAGGCTTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGACCAGTC TAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAG ACAGCTGCGGATAACAACAACAGTGATTACTCGTGGACTGGAGCTACCA AGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCGGCCAT GGCAAGCCACAAGGACGATGAAGAAAAGTATTTTCCTCAGAGCGGGGTT CTCATCTTTGGGAAGCAAGACTCAGGAAAAACAAATGTGGACATTGAAA AGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGC TACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGCGGCAACACA CAAGCAGCTACCTCAGATGTCAACACACAAGGCGTTCTTCCAGGCATGG TCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCATCTGGGCAAAGAT TCCACACACGGACGGACATTTTCACCCCTCTCCCCTCATGGGTGGATTC GGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAACACCCCGGTAC CTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTTCAT CACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGTGGGAGCTG CAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTACACTTCCA ACTACAACAAGTCTGTTAATGTGGACTTTACTGTGGACACTAATGGCGT GTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGACTCGTAATCTG TAA
SEQ ID NO: 31	AAV2-TT 衣壳(VP1)的氨基酸序列	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPG YKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EFQERLKEDTSFGGNLGRAVFQAKKRILEPLGLVEEPVKTAPGKKRPVE HSPAEPDSSSGTGKSGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPS GLGTNTMASGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTR TWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPR DWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVF TDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSF YCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQY LYYLSRTNTPSGTTTMSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSK TAADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKYFPQSGV

[0439]

		LIFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQSGNT QAATSDVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGF GLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWEL QKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
SEQ ID NO: 32	编码 AAV2-TT-S312N 衣壳(VP1)的核酸(不同于 WT AAV2 的核苷酸加下划线)	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTG AAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCACCAAA GCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGG TACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCA ACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGGCA GCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCG GAGTTTCAGGAGCGCCTTAAAGAAGATACGTCTTTTGGGGGCAACCTCG GACGAGCAGTCTTCCAGGCGAAAAAGAGGATTCTTGAACCTCTGGGCCT GGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGTAGAG CACTCTCCTGCGGAGCCAGACTCCTCCTCGGGAACCGGAAAGTCGGGCC AGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAGA CTCAGTACCTGACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCT GGTCTGGGAACTAATACGATGGCTTCAGGCAGTGGCGCACCAATGGCAG ACAATAACGAGGGCGCCGACGGAGTGGGTAATTCCTCGGGAAATTGGCA TTGCGATTCCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGA ACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAACAAATTTCCA GCCAATCAGGAGCCTCGAACGACAATCACTACTTTGGCTACAGCACCCC TTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGT GACTGGCAAAGACTCATCAACAACAACTGGGGATTCCGACCCAAGAGAC TCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGA CGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTT ACTGACTCGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATCAAG GATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCACAGTATGG ATACCTCACCCTGAACAACGGGAGTCAGGCAGTAGGACGCTCTTCATTT TACTGCCTGGAGTACTTTCCTTCTCAGATGCTGCGTACCGGAAACAACT

[0440]

		TTACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTACGC TCACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCATCGACCAGTAC CTGTATTACTTGAGCAGAACAAACACTCCAAGTGGAACCACCACGATGT CAAGGCTTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGACCAGTC TAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAG ACAGCTGCGGATAACAACAACAGTGATTACTCGTGGACTGGAGCTACCA AGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCGGCCAT GGCAAGCCACAAGGACGATGAAGAAAAGTATTTTCCTCAGAGCGGGGTT CTCATCTTTGGGAAGCAAGACTCAGGAAAAACAAATGTGGACATTGAAA AGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGC TACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGCGGCAACACA CAAGCAGCTACCTCAGATGTCAACACACAAGGCGTTCTTCCAGGCATGG TCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCATCTGGGCAAAGAT TCCACACACGGACGGACATTTTCACCCCTCTCCCCTCATGGGTGGATTC GGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAACACCCCGGTAC CTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCTTCAT CACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGTGGGAGCTG CAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTACACTTCCA ACTACAACAAGTCTGTTAATGTGGACTTTACTGTGGACACTAATGGCGT GTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGACTCGTAATCTG TAA
SEQ ID NO: 33	AAV2-TT-S312N 衣壳(VP1)的氨基酸序列	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPG YKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EFQERLKEDTSFGGNLGRAVFQAKKRILEPLGLVEEPVKTAPGKKRPVE HSPAEPDSSSGTGKSGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPS GLGTNTMASGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTR TWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPR DWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVF TDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSF

[0441]

		YCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQY LYYLSRTNTPSGTTTMSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSK TAADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKYFPQSGV LIFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQSGNT QAATSDVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGF GLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWEL QKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

序列表

<110> Bamboo Therapeutics, Inc.

Samulski, Richard J.

<120> 用于基因治疗的经修饰的弗里德赖希共济失调基因及载体

<130> PC45291A

<150> 62/251,288

<151> 2015-11-05

<150> 62/411,980

<151> 2016-10-24

<160> 33

<170> PatentIn version 3.5

<210> 1

<211> 210

<212> PRT

<213> 人工序列

<220>

<223> 人类野生型共济失调蛋白的氨基酸序列

<400> 1

Met Trp Thr Leu Gly Arg Arg Ala Val Ala Thr Gly Leu Leu Ala Ser
1 5 10 15
Pro Ser Pro Ala Gln Ala Gln Thr Leu Thr Arg Val Pro Arg Pro Ala
20 25 30
Glu Leu Ala Pro Leu Cys Gly Arg Arg Gly Leu Arg Thr Asp Ile Asp
35 40 45
Ala Thr Cys Pro Arg Arg Ala Ser Ser Asn Gln Arg Gly Leu Asn Gln
50 55 60
Ile Trp Asn Val Lys Lys Gln Ser Val Tyr Leu Met Asn Leu Arg Lys
65 70 75 80
Ser Gly Thr Leu Gly His Pro Gly Ser Leu Asp Glu Thr Thr Tyr Glu
85 90 95
Arg Leu Ala Glu Glu Thr Leu Asp Ser Leu Ala Glu Phe Phe Glu Asp
100 105 110
Leu Ala Asp Lys Pro Tyr Thr Phe Glu Asp Tyr Asp Val Ser Phe Gly
115 120 125
Ser Gly Val Leu Thr Val Lys Leu Gly Gly Asp Leu Gly Thr Tyr Val
130 135 140
Ile Asn Lys Gln Thr Pro Asn Lys Gln Ile Trp Leu Ser Ser Pro Ser
145 150 155 160

Ser Gly Pro Lys Arg Tyr Asp Trp Thr Gly Lys Asn Trp Val Tyr Ser
165 170 175
His Asp Gly Val Ser Leu His Glu Leu Leu Ala Ala Glu Leu Thr Lys
180 185 190
Ala Leu Lys Thr Lys Leu Asp Leu Ser Ser Leu Ala Tyr Ser Gly Lys
195 200 205
Asp Ala
210

<210> 2
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码野生型共济失调蛋白的核苷酸序列
<400> 2
atgtggactc tcgggcgccg cgcagtagcc ggcctcctgg cgtcacccag cccagcccag 60
gcccagaccc tcacccgggt cccgcggccg gcagagttgg ccccactctg cggccgccgt 120
ggcctgcgca ccgacatcga tgcgacctgc acgccccgcc gcgcaagttc gaaccaacgt 180
ggcctcaacc agatttggaa tgtcaaaaag cagagtgtct atttgatgaa tttgaggaaa 240
tctggaactt tgggccaccc aggctctcta gatgagacca cctatgaaag actagcagag 300
gaaacgctgg actctttagc agagtttttt gaagaccttg cagacaagcc atacacgttt 360
gaggactatg atgtctcctt tgggagtggt gtcttaactg tcaaactggg tggagatcta 420
ggaacctatg tgatcaacaa gcagacgcca aacaagcaaa tctggctatc ttctccatcc 480
agtggaccta agcgttatga ctggactggg aaaaactggg tgtactccca cgacggcgtg 540
tccctccatg agctgctggc cgcagagctc actaaagcct taaaaaccaa actggacttg 600
tcttccttgg cctattccgg aaaagatgct 630

<210> 3
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的IDT2优化核苷酸序列
<400> 3
atgtggacac tgggcagaag ggcggtggcc ggactgttgg cgagtcccag tcccgcgcag 60
gcgcagaccc ttactagggt gccgcggccc gcggagctgg cgccactctg cggtcgccgc 120
ggtctgagaa cggacattga tgccacttgt acacctcgga gggccagctc caaccaaagg 180
ggccttaatc aaatttggaa cgtgaagaag cagtccgtct acctgatgaa ccttcggaag 240
tcagggaccc tgggccaccc gggaagcttg gatgaaacaa cttacgaaag gttggcggag 300
gagaccttgg attctcttgc agagttcttc gaagacctgg ctgataagcc ttacaccttt 360

gaggactacg atgtgtcttt tggatctgga gtgctgaccg ttaaactggg cggggatctg 420
ggcacctacg tgattaacaa gcaaactcca aacaagcaga tctggctttc aagccccagt 480
agcgggccaa aacgctacga ttggaccgga aagaattggg tttacagcca cgatggcgtt 540
tcactgcacg agcttctggc agcagaactg acaaaagcac tcaagacgaa gctcgacttg 600
tcatccttgg catactccgg aaaggatgcc 630

<210> 4
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的JCAT优化核苷酸序列
<400> 4
atgtggaccc tgggccgccg cgccgtggcc ggcctgctgg ccagccccag ccccgcccag 60
gcccagaccc tgacccgcgt gccccgcccc gccgagctgg ccccccctgtg cggccgccgc 120
ggcctgcgca ccgacatcga cgccacctgc acccccccgcc gcgccagcag caaccagcgc 180
ggcctgaacc agatctggaa cgtgaagaag cagagcgtgt acctgatgaa cctgcgcaag 240
agcggcaccc tgggccaccc cggcagcctg gacgagacca cctacgagcg cctggccgag 300
gagaccctgg acagcctggc cgagttcttc gaggacctgg ccgacaagcc ctacaccttc 360
gaggactacg acgtgagctt cggcagcggc gtgctgaccg tgaagctggg cggcgacctg 420
ggcacctacg tgatcaacaa gcagaccccc aacaagcaga tctggctatc tagccccagc 480
agcggcccca agcgctacga ctggaccggc aagaactggg tgtacagcca cgacggcgtg 540
agcctgcacg agctgctggc cgccgagctg accaaggccc tgaagaccaa gctggacctg 600
agcagcctgg cctacagcgg caaggacgcc 630

<210> 5
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的GeneArt优化核苷酸序列
<400> 5
atgtggacac tggggagaag ggctgtggcc ggactgctgg cttctccatc tccagcccag 60
gcccagaccc tgaccagagt gcctagacct gccgaactgg cccctctgtg tggcagaaga 120
ggcctgagaa ccgacatcga cgccacctgt acccccagaa gggccagcag caatcagcgg 180
ggcctgaatc agatctggaa cgtgaagaaa cagagcgtgt acctgatgaa cctgagaaag 240
agcggcaccc tgggccaccc tggaagcctg gatgagacaa cctacgagcg gctggccgag 300
gaaaccctgg attccctggc cgagttcttc gaggacctgg ccgacaagcc ctacaccttc 360
gaggattacg acgtgtcctt cggcagcggc gtgctgacag tgaagctggg cggagatctg 420
ggcacctacg tgatcaacaa gcagaccccc aacaaacaga tctggctatc tagccccagc 480
agcggcccca agagatacga ttggaccggc aagaactggg tgtacagcca cgacggcgtg 540

tccctgcatg agctgctggc tgccgagctg accaaggccc tgaaaacaaa gctggacctg 600
tccagcctgg cctacagcgg caaggatgcc 630
<210> 6
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的Genscript(对照)优化核苷酸序列
<400> 6
atgtggacac tgggccggag agccgtcgct gggctgctgg catcaccatc ccccgcacag 60
gcacagaccc tgacaagagt ccctcggcca gcagagctgg ccccactgtg cgggcggaga 120
ggactgcgaa ccgacatcga tgctacttgt accccaaggc gagcaagctc caaccagcga 180
gggctgaacc agatttggaa tgtgaagaaa cagtctgtct acctgatgaa tctgagaaag 240
agcggcactc tgggacaccc tggcagcctg gacgagacca cctacgagcg gctggccgag 300
gaaaccctgg attccctggc cgagttcttt gaagacctgg ctgataagcc atacaccttc 360
gaagactatg acgtgagctt cggcagcggc gtgctgacag tcaaactggg cggggacctg 420
ggaacatacg tgatcaacaa gcagactcct aacaagcaga tttggctgtc tagtccctca 480
agcggcccta agaggtacga ctggacaggg aaaaactggg tgtatagtca cgatggcgtc 540
tcactgcatg agctgctggc cgctgaactg actaaagccc tgaaaactaa actggacctg 600
tcttccctgg catactctgg caaggacgcc 630
<210> 7
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的Genscript(低CpG)核苷酸序列
<400> 7
atgtggactc tgggccggag agcagtggca ggactgctgg caagtccatc acctgctcag 60
gcacagactc tgacaagagt cccaagacct gcagagctgg ctccactgtg cgggaggcgc 120
ggactgagaa cagacatcga tgctacatgt actcctcgac gggcaagctc caaccagcga 180
gggctgaacc agatttggaa tgtgaagaaa cagtccgtct acctgatgaa tctgaggaag 240
tcaggcaccc tggggcaccc aggaagtctg gacgagacca catatgaacg gctggctgag 300
gaaacactgg attctctggc cgagttcttt gaagacctgg ctgataagcc ctacacattc 360
gaagactatg atgtgagctt tggatccggc gtgctgactg tcaaactggg cggggacctg 420
ggcacttacg tgatcaacaa gcagacccct aacaagcaga tttggctgtc tagtccttca 480
agcggaccaa agcggtacga ctggaccggc aaaaactggg tgtattctca cgatggggtc 540
agtctgcatg agctgctggc cgctgaactg accaaggccc tgaagacaaa actggacctg 600
tcctctctgg catatagcgg aaaagatgcc 630
<210> 8

<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的IDT3优化核苷酸序列
<400> 8
atgtggacac tgggaaggcg cgccgtggcc ggtctgttgg catcaccatc cccagcccag 60
gctcagacac tcacccgagt cccaagaccc gcagagctgg cccctctgtg cgggcgccga 120
ggccttcgca ccgatatcga tgctacatgc acgccacgca gagctagctc aaatcagagg 180
ggactcaacc agatatggaa tgtcaagaag caaagcgtgt atctcatgaa cctccggaaa 240
agcggcaccc tgggacatcc cgggtctctc gacgagacca cttatgaaag actggcagag 300
gagactcttg acagtctggc ggagttcttc gaagacctcg ctgacaagcc atataccttc 360
gaagattacg acgtctcctt cggctctggg gtgctgactg tcaagcttgg cggcgacctg 420
gggacctacg tgatcaacaa gcagactcca aacaagcaaa tctggctatc tagtccaagc 480
tccggaccca agagatacga ttggacaggc aagaattggg tttactccca cgacggggtg 540
tccctccatg agctgctggc cgcagagctg acgaaggccc tgaagaccaa gctggatctc 600
tcctccctgg catacagtgg taaggacgct 630
<210> 9
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的IDT5优化核苷酸序列
<400> 9
atgtggacac tgggccggcg cgccgtcgct gggctgctcg caagccccag cccagcccaa 60
gcgcagactc tgactagggt gccgcggcct gccgagttgg ccccccctgtg cggtaggaga 120
ggcctgcgca cagacatcga tgccacttgc acaccccggc gggccagctc taaccaaagg 180
ggcctgaatc aaatttggaa cgtcaaaaaa cagtctgtat atctgatgaa tctccggaaa 240
tctggaacgc tcgggcatcc cggatctctt gacgagacca cctacgagcg actggccgag 300
gaaaccctg acagcctggc agaattcttt gaggatctgg ctgataaacc ctatacctt 360
gaagattacg atgtgagttt tggtagcgga gtactgactg ttaagctggg cggtgatctc 420
ggtacgtatg ttatcaataa acaaaccccc aataaacaga tttggctctc ctccccatcc 480
tctgggccta agcgctatga ctggacagga aagaattggg tctattcaca cgacggagtc 540
agtttgcacg agctcctcgc cggcagagtt accaaggccc ttaagactaa gctcgacctg 600
tcaagcctcg cttactctgg taaggacgct 630
<210> 10
<211> 630
<212> DNA
<213> 人工序列

<220>
<223> 编码共济失调蛋白的核苷酸序列
<400> 10
atgtggactc tcgggcgccg cgcagtagcc ggcctcctgg cgtcacccag cccggcccag 60
gcccagaccc tcacccgggt cccgcggccg gcagagttgg ccccactctg cggccgccgt 120
ggcctgcgca ccgacatcga tgcgacctgc acgccccgcc gcgcaagttc gaaccaacgt 180
ggcctcaacc agatttggaa tgtcaaaaag cagagtgtct atttgatgaa tttgaggaaa 240
tctggaactt tgggccaccc aggctctcta gatgagacca cctatgaaag actagcagag 300
gaaacgctgg actctttagc agagtttttt gaagaccttg cagacaagcc atacacgttt 360
gaggactatg atgtctcctt tgggagtggt gtcttaactg tcaaactggg tggagatcta 420
ggaacctatg tgatcaacaa gcagacgcca aacaagcaaa tctggctatc ttctccatcc 480
agtggaccta agcgttatga ctggactggg aaaaactggg tgtactccca cgacggcgtg 540
tccctccatg agctgctggc cgcagagctc actaaagcct taaaaaccaa actggacttg 600
tcttccttgg cctattccgg aaaagatgct 630
<210> 11
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的IDT-1优化核苷酸序列
<400> 11
atgtggactc tgggtaggcg agcggtggcc ggcctgttgg catctcctag tcctgcacaa 60
gctcaaacgc tgactagagt ccctcggcca gcagaactgg cgccactttg cggccggcgc 120
ggtcttcgca ctgatattga tgccacttgc acaccccggc gcgcctccag taatcagcgg 180
ggacttaatc aaatttggaa tgtgaagaag cagtctgtgt atcttatgaa tctgcggaag 240
agcgggaccc tgggccaccc tggtagcctt gatgaaacca cctatgagcg cctggccgaa 300
gagacactgg acagtcttgc cgagtttttt gaggatctgg ccgacaaacc ttatactttt 360
gaggactatg acgtgtcctt tggatctggt gtattgaccg taaaactcgg gggagacctt 420
gggacgtatg taataaataa gcagacccca aacaagcaga tctggctatc ttctccaagt 480
agtggtccta agagatatga ttggacgggc aagaactggg tctattccca tgatggcgtc 540
tctttgcatg aactccttgc agcagagctg accaaggcct tgaagaccaa attggatctc 600
agcagcctcg cctatagtgg caaagatgca 630
<210> 12
<211> 630
<212> DNA
<213> 人工序列
<220>
<223> 编码共济失调蛋白的IDT-4优化核苷酸序列
<400> 12

atgtggactc tgggccggcg ggccgtagct ggcttgctgg ctagcccaag tcccgcccag 60
gctcagactc tcaccagggt acccaggccc gcagagcttg ctccactctg cggacgcagg 120
ggtctgcgaa ccgatatcga cgcaacttgc acgccgcgga gggcctcttc aaaccagaga 180
ggactcaatc aaatttggaa tgtaaagaaa cagagcgtgt atctcatgaa cctccgaaag 240
agtgggactc ttgggcaccc cggctccctg gacgagacta cttacgagcg cctggccgaa 300
gaaaccttgg attccctggc ggagtttttt gaagacttgg cagacaagcc ttataccttc 360
gaggattacg acgtgagttt tggctctggt gttcttacag tcaagctcgg tggcgacctt 420
ggcacttatg taattaacaa gcagacacct aacaagcaga tctggctttc tagtccgtct 480
tccggtccca aaaggtacga ttggactgga aagaactggg tctacagtca cgacggtgtc 540
tccctgcacg aattgcttgc ggcagagctg actaaggcgc tcaaaacaaa actggatctg 600
tccagccttg cctatagcgg gaaggacgca 630

<210> 13
<211> 2211
<212> DNA
<213> 人工序列
<220>
<223> 编码嵌合AAV2.5载体衣壳VP1的核苷酸序列
<400> 13

atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga 60
cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac 120
gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccttcaa cggactcgac 180
aagggagagc cggtcaacga ggcagacgcc gcggccctcg agcacgacaa agcctacgac 240
cggcagctcg acagcggaga caacccgtac ctcaagtaca accacgccga cgcggagttt 300
caggagcgcc ttaaagaaga tacgtctttt gggggcaacc tcggacgagc agtcttccag 360
gcgaaaaaga gggttcttga acctctgggc ctggttgagg aacctgttaa gacggctccg 420
ggaaaaaaga ggccggtaga gcactctcct gtggagccag actcctcctc gggaaccgga 480
aaggcgggcc agcagcctgc aagaaaaaga ttgaattttg gtcagactgg agacgcagac 540
tcagtacctg acccccagcc tctcggacag ccaccagcag ccccctctgg tctgggaact 600
aatacgatgg ctacaggcag tggcgcacca atggcagaca ataacgaggg cgccgacgga 660
gtgggtaatt cctcgggaaa ttggcattgc gattccacat ggatgggcga cagagtcatc 720
accaccagca cccgaacctg ggccctgccc acctacaaca accacctcta caaacaaatt 780
tccagcgctt caacgggagc ctcgaacgac aatcactact ttggctacag cacccctgg 840
gggtatttg acttcaacag attccactgc cacttttcac cacgtgactg gcaaagactc 900
atcaacaaca actggggatt ccgacccaag agactcaact tcaagctctt taacattcaa 960
gtcaaagagg tcacgcagaa tgacggtacg acgacgattg ccaataacct taccagcacg 1020
gttcaggtgt ttactgactc ggagtaccag ctcccgtacg tcctcggctc ggcgcatcaa 1080
ggatgcctcc cgccgttccc agcagacgtc ttcatggtgc cacagtatgg atacctcacc 1140
ctgaacaacg ggagtcaggc agtaggacgc tcttcatttt actgcctgga gtactttcct 1200
tctcagatgc tgcgtaccgg aaacaacttt accttcagct acacttttga ggacgttcct 1260

ttccacagca gctacgctca cagccagagt ctggaccgtc tcatgaatcc tctcatcgac 1320
cagtacctgt attacttgag cagaacaaac actccaagtg gaaccaccac gcagtcaagg 1380
cttcagtttt ctcaggccgg agcgagtgac attcgggacc agtctaggaa ctggcttcct 1440
ggaccctgtt accgccagca gcgagtatca aagacatctg cggataacaa caacagtgaa 1500
tactcgtgga ctggagctac caagtaccac ctcaatggca gagactctct ggtgaatccg 1560
ggcccggcca tggcaagcca caaggacgat gaagaaaagt tttttcctca gagcggggtt 1620
ctcatctttg ggaagcaagg ctcagagaaa acaaatgtgg acattgaaaa ggtcatgatt 1680
acagacgaag aggaaatcag gacaaccaat cccgtggcta cggagcagta tggttctgta 1740
tctaccaacc tccagagagg caacagacaa gcagctaccg cagatgtcaa cacacaaggc 1800
gttcttccag gcatggtctg gcaggacaga gatgtgtacc ttcaggggcc catctgggca 1860
aagattccac acacggacgg acattttcac ccctctcccc tcatgggtgg attcggactt 1920
aaacaccctc ctccacagat tctcatcaag aacaccccgg tacctgcgaa tccttcgacc 1980
accttcagtg cggcaaagtt tgcttccttc atcacacagt actccacggg acaggtcagc 2040
gtggagatcg agtgggagct gcagaaggaa aacagcaaac gctggaatcc cgaaattcag 2100
tacacttcca actacgccaa gtctgtcaat gtggacttta ctgtggacaa taatggcgtg 2160
tattcagagc ctcgccccat tggcaccaga tacctgactc gtaatctgta a 2211

<210> 14
<211> 2211
<212> DNA
<213> 人工序列
<220>
<223> 编码野生型AAV1衣壳(VP1)的核苷酸序列
<400> 14

atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc 60
gagtggtggg acttgaaacc tggagccccg aagcccaaag ccaaccagca aaagcaggac 120
gacggccggg gtctggtgct tcctggctac aagtacctcg gacccttcaa cggactcgac 180
aaggggggagc ccgtcaacgc ggcggacgca gcggccctcg agcacgacaa ggcctacgac 240
cagcagctca aagcgggtga caatccgtac ctgcggtata accacgccga cgccgagttt 300
caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360
gccaagaagc gggttctcga acctctcggt ctggttgagg aaggcgctaa gacggctcct 420
ggaaagaaac gtccggtaga gcagtcgcca caagagccag actcctcctc gggcatcggc 480
aagacaggcc agcagcccgc taaaaagaga ctcaattttg gtcagactgg cgactcagag 540
tcagtccccg atccacaacc tctcggagaa cctccagcaa ccccccgctgc tgtgggacct 600
actacaatgg cttcaggcgg tggcgcacca atggcagaca ataacgaagg cgccgacgga 660
gtgggtaatg cctcaggaaa ttggcattgc gattccacat ggctgggcga cagagtcatc 720
accaccagca cccgcacctg ggccttgccc acctacaata accacctcta caagcaaatc 780
tccagtgctt caacgggggc cagcaacgac aaccactact tcggctacag caccccctgg 840
gggtatttg atttcaacag attccactgc cacttttcac cacgtgactg gcagcgactc 900
atcaacaaca attggggatt ccggcccaag agactcaact tcaaactctt caacatccaa 960

gtcaaggagg tcacgacgaa tgatggcgtc acaaccatcg ctaataacct taccagcacg 1020
gttcaagtct tctcggactc ggagtaccag cttccgtacg tcctcggctc tgcgcaccag 1080
ggctgcctcc ctccgttccc ggcggacgtg ttcatgattc cgcaatacgg ctacctgacg 1140
ctcaacaatg gcagccaagc cgtgggacgt tcatcctttt actgcctgga atatttccct 1200
tctcagatgc tgagaacggg caacaacttt accttcagct acacctttga ggaagtgcct 1260
ttccacagca gctacgcgca cagccagagc ctggaccggc tgatgaatcc tctcatcgac 1320
caatacctgt attacctgaa cagaactcaa aatcagtccg gaagtgccca aaacaaggac 1380
ttgctgttta gccgtgggtc tccagctggc atgtctgttc agcccaaaaa ctggctacct 1440
ggaccctgtt atcggcagca gcgcgtttct aaaacaaaaa cagacaacaa caacagcaat 1500
tttacctgga ctggtgcttc aaaatataac ctcaatgggc gtgaatccat catcaaccct 1560
ggcactgcta tggcctcaca caaagacgac gaagacaagt tctttcccat gagcggtgtc 1620
atgatttttg gaaaagagag cgccggagct tcaaacactg cattggacaa tgtcatgatt 1680
acagacgaag aggaaattaa agccactaac cctgtggcca ccgaaagatt tgggaccgtg 1740
gcagtcaatt tccagagcag cagcacagac cctgcgaccg gagatgtgca tgctatggga 1800
gcattacctg gcatggtgtg gcaagataga gacgtgtacc tgcagggtcc catttgggcc 1860
aaaattcctc acacagatgg acactttcac ccgtctcctc ttatgggcgg ctttggactc 1920
aagaacccgc ctcctcagat cctcatcaaa aacacgcctg ttcctgcgaa tcctccggcg 1980
gagttttcag ctacaaagtt tgcttcattc atcacccaat actccacagg acaagtgagt 2040
gtggaaattg aatgggagct gcagaaagaa aacagcaagc gctggaatcc cgaagtgcag 2100
tacacatcca attatgcaaa atctgccaac gttgatttta ctgtggacaa caatggactt 2160
tatactgagc ctcgccccat tggcacccgt taccttaccc gtcccctgta a 2211

<210> 15
<211> 2208
<212> DNA
<213> 人工序列
<220>
<223> 编码经修饰的AAV1.1衣壳VP1的核苷酸序列
<400> 15

atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc 60
gagtggtggg acttgaaacc tggagccccg aagcccaaag ccaaccagca aaagcaggac 120
gacggccggg gtctggtgct tcctggctac aagtacctcg gaccccttcaa cggactcgac 180
aaggggggagc ccgtcaacgc ggcggacgca gcggccctcg agcacgacaa ggcctacgac 240
cagcagctca aagcgggtga caatccgtac ctgcggtata accacgccga cgccgagttt 300
caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360
gccaagaagc gggttctcga acctctcggt ctggttgagg aaggcgctaa gacggctcct 420
ggaaagaaac gtccggtaga gcagtcgcca caagagccag actcctcctc gggcatcggc 480
aagacaggcc agcagcccgc taaaaagaga ctcaattttg gtcagactgg cgactcagag 540
tcagtccccg atccacaacc tctcggagaa cctccagcaa cccccgctgc tgtgggacct 600
actacaatgg cttcaggcgg tggcgcacca atggcagaca ataacgaagg cgccgacgga 660

gtgggtaatg cctcaggaaa ttggcattgc gattccacat ggctgggcga cagagtcatc 720
accaccagca cccgcacctg ggccttgccc acctacaata accacctcta caagcaaatc 780
tccagtgctt caggggccag caacgacaac cactacttcg gctacagcac cccctggggg 840
tattttgatt tcaacagatt ccactgccac ttttcaccac gtgactggca gcgactcatc 900
aacaacaatt ggggattccg gcccaagaga ctcaacttca aactcttcaa catccaagtc 960
aaggaggtca cgacgaatga tggcgtcaca accatcgcta ataaccttac cagcacggtt 1020
caagtcttct cggactcgga gtaccagctt ccgtacgtcc tcggctctgc gcaccagggc 1080
tgcctccctc cgttcccggc ggacgtgttc atgattccgc aatacggcta cctgacgctc 1140
aacaatggca gccaagccgt gggacgttca tccttttact gcctggaata tttcccttct 1200
cagatgctga gaacgggcaa caactttacc ttcagctaca cctttgagga agtgcctttc 1260
cacagcagct acgcgcacag ccagagcctg gaccggctga tgaatcctct catcgaccaa 1320
tacctgtatt acctgaacag aactcaaaat cagtccggaa gtgcccaaaa caaggacttg 1380
ctgtttagcc gtgggtctcc agctggcatg tctgttcagc ccaaaaactg gctacctgga 1440
ccctgttatc ggcagcagcg cgtttctaaa acaaaaacag acaacaacaa cagcaatttt 1500
acctggactg gtgcttcaaa atataacctc aatgggcgtg aatccatcat caaccctggc 1560
actgctatgg cctcacacaa agacgacgaa gacaagttct ttcccatgag cggtgtcatg 1620
atttttggaa aagagagcgc cggagcttca aacactgcat tggacaatgt catgattaca 1680
gacgaagagg aaattaaagc cactaaccct gtggccaccg aaagatttgg gaccgtggca 1740
gtcaatttcc agagcagcag cacagaccct gcgaccggag atgtgcatgc tatgggagca 1800
ttacctggca tggtgtggca agatagagac gtgtacctgc agggtcccat ttgggccaaa 1860
attcctcaca cagatggaca ctttcacccg tctcctctta tgggcggctt tggactcaag 1920
aacccgcctc ctcagatcct catcaaaaac acgcctgttc ctgcgaatcc tccggcggag 1980
ttttcagcta caaagtttgc ttcattcatc acccaatact ccacaggaca agtgagtgtg 2040
gaaattgaat gggagctgca gaaagaaaac agcaagcgct ggaatcccga agtgcagtac 2100
acatccaatt atgcaaaatc tgccaacgtt gattttactg tggacaacaa tggactttat 2160
actgagcctc gccccattgg cacccgttac cttacccgtc ccctgtaa 2208

<210> 16
<211> 2211
<212> DNA
<213> 人工序列
<220>
<223> 编码野生型AAV6衣壳(VP1)的核苷酸序列
<400> 16

atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc 60
gagtggtggg acttgaaacc tggagccccg aaacccaaag ccaaccagca aaagcaggac 120
gacggccggg gtctggtgct tcctggctac aagtacctcg gacccttcaa cggactcgac 180
aagggggagc ccgtcaacgc ggcggatgca gcggccctcg agcacgacaa ggcctacgac 240
cagcagctca aagcgggtga caatccgtac ctgcggtata accacgccga cgccgagttt 300
caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360

gccaagaaga gggttctcga acctttggt ctggttgagg aaggtgctaa gacggctcct 420
ggaaagaaac gtccggtaga gcagtcgcca caagagccag actcctcctc gggcattggc 480
aagacaggcc agcagcccgc taaaaagaga ctcaattttg gtcagactgg cgactcagag 540
tcagtccccg acccacaacc tctcggagaa cctccagcaa ccccccgctgc tgtgggacct 600
actacaatgg cttcaggcgg tggcgcacca atggcagaca ataacgaagg cgccgacgga 660
gtgggtaatg cctcaggaaa ttggcattgc gattccacat ggctgggcga cagagtcatc 720
accaccagca cccgaacatg ggccttgccc acctataaca accacctcta caagcaaatc 780
tccagtgctt caacgggggc cagcaacgac aaccactact tcggctacag caccccctgg 840
gggtattttg atttcaacag attccactgc catttctcac cacgtgactg gcagcgactc 900
atcaacaaca attggggatt ccggcccaag agactcaact tcaagctctt caacatccaa 960
gtcaaggagg tcacgacgaa tgatggcgtc acgaccatcg ctaataacct taccagcacg 1020
gttcaagtct tctcggactc ggagtaccag ttgccgtacg tcctcggctc tgcgcaccag 1080
ggctgcctcc ctccgttccc ggcggacgtg ttcatgattc cgcagtacgg ctacctaacg 1140
ctcaacaatg gcagccaggc agtgggacgg tcatcctttt actgcctgga atatttccca 1200
tcgcagatgc tgagaacggg caataacttt accttcagct acaccttcga ggacgtgcct 1260
ttccacagca gctacgcgca cagccagagc ctggaccggc tgatgaatcc tctcatcgac 1320
cagtacctgt attacctgaa cagaactcag aatcagtccg gaagtgccca aaacaaggac 1380
ttgctgttta gccgggggtc tccagctggc atgtctgttc agcccaaaaa ctggctacct 1440
ggaccctgtt accggcagca gcgcgtttct aaaacaaaaa cagacaacaa caacagcaac 1500
tttacctgga ctggtgcttc aaaatataac cttaatgggc gtgaatctat aatcaaccct 1560
ggcactgcta tggcctcaca caaagacgac aaagacaagt tctttcccat gagcggtgtc 1620
atgatttttg gaaaggagag cgccggagct tcaaacactg cattggacaa tgtcatgatc 1680
acagacgaag aggaaatcaa agccactaac cccgtggcca ccgaaagatt tgggactgtg 1740
gcagtcaatc tccagagcag cagcacagac cctgcgaccg gagatgtgca tgttatggga 1800
gccttacctg gaatggtgtg gcaagacaga gacgtatacc tgcagggtcc tatttgggcc 1860
aaaattcctc acacggatgg acactttcac ccgtctcctc tcatgggcgg ctttggactt 1920
aagcacccgc ctcctcagat cctcatcaaa aacacgcctg ttcctgcgaa tcctccggca 1980
gagttttcgg ctacaaagtt tgcttcattc atcacccagt attccacagg acaagtgagc 2040
gtggagattg aatgggagct gcagaaagaa aacagcaaac gctggaatcc cgaagtgcag 2100
tatacatcta actatgcaaa atctgccaac gttgatttca ctgtggacaa caatggactt 2160
tatactgagc ctcgccccat tggcacccgt tacctcaccc gtcccctgta a 2211

<210> 17
<211> 2205
<212> DNA
<213> 人工序列
<220>
<223> 编码经修饰的AAV6.1衣壳VP1的核苷酸序列
<400> 17
atggctgccg atggttatct tccagattgg ctcgaggaca acctctdgag ggcattcgcg 60

agtggtggga cttgaaacct ggagccccga aacccaaagc caaccagcaa aagcaggacg 120
acggccgggg tdggtgcttc ctggctacaa gtacctcgga cccttcaacg gactcgacaa 180
gggggagccc gtcaacgcgg cggatgcagc ggccctcgag cacgacaagg cctacgacca 240
gcagctcaaa gcgggtgaca atccgtacct gcggtataac cacgccgacg ccgagtttca 300
ggagcgtctg caagaagata cgtcttttgg gggcaacctc gggcgagcag tcttccaggc 360
caagaagagg gttctcgaac cttttggtdg gttgaggaag gtgctaagac ggctcctgga 420
aagaaacgtc cggtagagca gtcgccacaa gagccagact cctcctcggg cattggcaag 480
acaggccagc agcccgctaa aaagagactc aattttggtc agactggcga ctcagagtca 540
gtccccgacc cacaacctct cggagaacct ccagcaaccc ccgctgctgt gggacctact 600
acaatggctt caggcggtgg cgcaccaatg gcagacaata acgaaggcgc cgacggagtg 660
ggtaatgcct caggaaattg gcattgcgat tccacatggc tgggcgacag agtcatcacc 720
accagcaccc gaacatgggc cttgcccacc tataacaacc acctctacaa gcaaatctcc 780
agtgcttcag gggccagcaa cgacaaccac tacttcggct acagcacccc ctgggggtat 840
tttgatttca acagattcca ctgccatttc tcaccacgtg actggcagcg actcatcaac 900
aacaattggg gattccggcc caagagactc aacttcaagc tcttcaacat ccaagtcaag 960
gaggtcacga cgaatgatgg cgtcacgacc atcgctaata accttaccag cacggttcaa 1020
gtcttctcgg actcggagta ccagttgccg tacgtcctcg gctctgcgca ccagggctgc 1080
ctccctccgt tcccggcgga cgtgttcatg attccgcagt acggctacct aacgctcaac 1140
aatggcagcc aggcagtggg acggtcatcc ttttactgcc tggaatattt cccatcgcag 1200
atgctgagaa cgggcaataa ctttaccttc agctacacct tcgaggacgt gcctttccac 1260
agcagctacg cgcacagcca gagcctggac cggctgatga atcctctcat cgaccagtac 1320
ctgtattacc tgaacagaac tcagaatcag tccggaagtg cccaaaacaa ggacttgctg 1380
tttagccggg ggtctccagc tggcatgtct gttcagccca aaaactggct acctggaccc 1440
tgttaccggc agcagcgcgt ttctaaaaca aaaacagaca acaacaacag caactttacc 1500
tggactggtg cttcaaaata taaccttaat gggcgtgaat ctataatcaa ccctggcact 1560
gctatggcct cacacaaaga cgacaaagac aagttctttc ccatgagcgg tgtcatgatt 1620
tttggaaagg agagcgccgg agcttcaaac actgcattgg acaatgtcat gatcacagac 1680
gaagaggaaa tcaaagccac taaccccgtg gccaccgaaa gatttgggac tgtggcagtc 1740
aatctccaga gcagcagcac agaccctgcg accggagatg tgcatgttat gggagcctta 1800
cctggaatgg tgtggcaaga cagagacgta tacctgcagg gtcctatttg ggccaaaatt 1860
cctcacacgg atggacactt tcacccgtct cctctcatgg gcggctttgg acttaagcac 1920
ccgcctcctc agatcctcat caaaaacacg cctgttcctg cgaatcctcc ggcagagttt 1980
tcggctacaa agtttgcttc attcatcacc cagtattcca caggacaagt gagcgtggag 2040
attgaatggg agctgcagaa agaaaacagc aaacgctgga atcccgaagt gcagtataca 2100
tctaactatg caaaatctgc caacgttgat ttcactgtgg acaacaatgg actttatact 2160
gagcctcgcc ccattggcac ccgttacctc acccgtcccc tgtaa 2205

<210> 18

<211> 2208

<212> DNA

<213> 人工序列

<220>

<223> 编码经修饰的AAV6.3.1衣壳VP1的核苷酸序列

<400> 18

atggctgccg atggttatct tccagattgg ctcgaggaca acctctctga gggcattcgc 60
gagtggtggg acttgaaacc tggagccccg aaacccaaag ccaaccagca aaagcaggac 120
gacggccggg gtctggtgct tcctggctac aagtacctcg gacccttcaa cggactcgac 180
aagggggagc ccgtcaacgc ggcggatgca gcggccctcg agcacgacaa ggcctacgac 240
cagcagctca aagcgggtga caatccgtac ctgcggtata accacgccga cgccgagttt 300
caggagcgtc tgcaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360
gccaagaaga gggttctcga acctttggt ctggttgagg aaggtgctaa gacggctcct 420
ggaaagaaac gtccggtaga gcagtcgcca caagagccag actcctcctc gggcattggc 480
aagacaggcc agcagcccgc taaaaagaga ctcaattttg gtcagactgg cgactcagag 540
tcagtccccg acccacaacc tctcggagaa cctccagcaa cccccgctgc tgtgggacct 600
actacaatgg cttcaggcgg tggcgcacca atggcagaca ataacgaagg cgccgacgga 660
gtgggtaatg cctcaggaaa ttggcattgc gattccacat ggctgggcga cagagtcatc 720
accaccagca cccgaacatg ggccttgccc acctataaca accacctcta caagcaaatc 780
tccagtgctt caggggccag caacgacaac cactacttcg gctacagcac cccctggggg 840
tattttgatt tcaacagatt ccactgccat ttctcaccac gtgactggca gcgactcatc 900
aacaacaatt ggggattccg gcccaagaga ctcaacttca agctcttcaa catccaagtc 960
aaggaggtca cgacgaatga tggcgtcacg accatcgcta ataaccttac cagcacggtt 1020
caagtcttct cggactcgga gtaccagttg ccgtacgtcc tcggctctgc gcaccagggc 1080
tgcctccctc cgttcccggc ggacgtgttc atgattccgc agtacggcta cctaacgctc 1140
aacaatggca gccaggcagt gggacggtca tccttttact gcctggaata tttcccatcg 1200
cagatgctga gaacgggcaa taactttacc ttcagctaca ccttcgagga cgtgcctttc 1260
cacagcagct acgcgcacag ccagagcctg gaccggctga tgaatcctct catcgaccag 1320
tacctgtatt acctgaacag aactcagaat cagtccggaa gtgcccaaaa caaggacttg 1380
ctgtttagcc gggggtctcc agctggcatg tctgttcagc ccaaaaactg gctacctgga 1440
ccctgttacc ggcagcagcg cgtttctaaa acaaaaacag acaacaacaa cagcaacttt 1500
acctggactg gtgcttcaaa atataacctt aatgggcgtg aatctataat caaccctggc 1560
actgctatgg cctcacacaa agacgacgaa gacaagttct ttcccatgag cggtgtcatg 1620
atttttggaa aggagagcgc cggagcttca aacactgcat tggacaatgt catgatcaca 1680
gacgaagagg aaatcaaagc cactaacccc gtggccaccg aaagatttgg gactgtggca 1740
gtcaatctcc agagcagcag cacagaccct gcgaccggag atgtgcatgt tatgggagcc 1800
ttacctggaa tggtgtggca agacagagac gtatacctgc agggtcctat ttgggccaaa 1860
attcctcaca cggatggaca ctttcacccg tctcctctca tgggcggctt tggacttaag 1920
cacccgcctc ctcagatcct catcaaaaac acgcctgttc ctgcgaatcc tccggcagag 1980
ttttcggcta caaagtttgc ttcattcatc acccagtatt ccacaggaca agtgagcgtg 2040
gagattgaat gggagctgca gaaagaaaac agcaaacgct ggaatcccga agtgcagtat 2100

acatctaact atgcaaaatc tgccaacgtt gatttcactg tggacaacaa tggactttat 2160
actgagcctc gccccattgg cacccgttac ctcacccgtc ccctgtaa 2208

<210> 19
<211> 983
<212> DNA
<213> 人工序列
<220>
<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码人类野生型共济失调蛋白(WT FXN)的核苷酸序列

<400> 19
tagaagaccg gtcgccacca tgtggactct cgggcgccgc gcagtagccg gcctcctggc 60
gtcacccagc ccagcccagg cccagaccct cacccgggtc ccgcggccgg cagagttggc 120
cccactctgc ggccgccgtg gcctgcgcac cgacatcgat gcgacctgca cgccccgccg 180
cgcaagttcg aaccaacgtg gcctcaacca gatttggaat gtcaaaaagc agagtgtcta 240
tttgatgaat ttgaggaaat ctggaacttt gggccaccca ggctctctag atgagaccac 300
ctatgaaaga ctagcagagg aaacgctgga ctctttagca gagtttttg aagaccttgc 360
agacaagcca tacacgtttg aggactatga tgtctccttt gggagtggtg tcttaactgt 420
caaactgggt ggagatctag gaacctatgt gatcaacaag cagacgccaa acaagcaaat 480
ctggctatct tctccatcca gtggacctaa gcgttatgac tggactggga aaaactgggt 540
gtactcccac gacggcgtgt ccctccatga gctgctggcc gcagagctca ctaaagcctt 600
aaaaaccaaa ctggacttgt cttccttggc ctattccgga aaagatgctt gacgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 20
<211> 983
<212> DNA
<213> 人工序列
<220>
<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码FXN的IDT1密码子优化核苷酸序列

<400> 20
tagaagaccg gtcgccacca tgtggactct gggtaggcga gcggtggccg gcctgttggc 60
atctcctagt cctgcacaag ctcaaacgct gactagagtc cctcggccag cagaactggc 120
gccactttgc ggccggcgcg gtcttcgcac tgatattgat gccacttgca caccccggcg 180
cgcctccagt aatcagcggg gacttaatca aatttggaat gtgaagaagc agtctgtgta 240

tcttatgaat ctgcggaaga gcgggaccct gggccaccct ggtagccttg atgaaaccac 300
ctatgagcgc ctggccgaag agacactgga cagtcttgcc gagtttttg aggatctggc 360
cgacaaacct tatacttttg aggactatga cgtgtccttt ggatctggtg tattgaccgt 420
aaaactcggg ggagaccttg ggacgtatgt aataaataag cagaccccaa acaagcagat 480
ctggctcagc tctccaagta gtggtcctaa gagatatgat tggacgggca agaactgggt 540
ctattcccat gatggcgtct ctttgcatga actccttgca gcagagctga ccaaggcctt 600
gaagaccaaa ttggatctca gcagcctcgc ctatagtggc aaagatgcat agcgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 21

<211> 983

<212> DNA

<213> 人工序列

<220>

<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码FXN IDT3(低表达)的密码子优化核苷酸序列

<400> 21

tagaagaccg gtcgccacca tgtggacact gggaaggcgc gccgtggccg gtctgttggc 60
atcaccatcc ccagcccagg ctcagacact cacccgagtc ccaagacccg cagagctggc 120
ccctctgtgc gggcgccgag gccttcgcac cgatatcgat gctacatgca cgccacgcag 180
agctagctca aatcagaggg gactcaacca gatatggaat gtcaagaagc aaagcgtgta 240
tctcatgaac ctccggaaaa gcggcaccct gggacatccc gggtctctcg acgagaccac 300
ttatgaaaga ctggcagagg agactcttga cagtctggcg gagttcttcg aagacctcgc 360
tgacaagcca tataccttcg aagattacga cgtctccttc ggctctgggg tgctgactgt 420
caagcttggc ggcgacctgg ggacctacgt gatcaacaag cagactccaa acaagcaaat 480
ctggctcagc agtccaagct ccggacccaa gagatacgat tggacaggca agaattgggt 540
ttactcccac gacggggtgt ccctccatga gctgctggcc gctgagctga cgaaggccct 600
gaagaccaag ctggatctct cctccctggc atacagtggt aaggacgctt gacgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 22

<211> 983

<212> DNA

<213> 人工序列

<220>

<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码FXN IDT4的密码子优化核苷酸序列

<400> 22

tagaagaccg gtcgccacca tgtggactct gggccggcgg gccgtagctg gcttgctggc 60
tagcccaagt cccgcccagg ctcagactct caccagggta cccaggcccg cagagcttgc 120
tccactctgc ggacgcaggg gtctgcgaac cgatatcgac gcaacttgca cgccgcggag 180
ggcctcttca aaccagagag gactcaatca aatttggaat gtaaagaaac agagcgtgta 240
tctcatgaac ctccgaaaga gtgggactct tgggcacccc ggctccctgg acgagactac 300
ttacgagcgc ctggccgaag aaaccttgga ttccctggcg gagtttttg aagacttggc 360
agacaagcct tataccttcg aggattacga cgtgagtttt ggctctggtg ttcttacagt 420
caagctcggt ggcgaccttg gcacttatgt aattaacaag cagacaccta acaagcagat 480
ctggctttct agtccgtctt ccggtcccaa aaggtacgat tggactggaa agaactgggt 540
ctacagtcac gacggtgtct ccctgcacga attgcttgcg gctgagctga ctaaggcgct 600
caaaacaaaa ctggatctgt ccagccttgc ctatagcggg aaggacgcat gacgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 23

<211> 983

<212> DNA

<213> 人工序列

<220>

<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码FXN GenScript的密码子优化核苷酸序列

<400> 23

tagaagaccg gtcgccacca tgtggacact gggccggaga gccgtcgctg ggctgctggc 60
atcaccatcc cccgcacagg cacagaccct gacaagagtc cctcggccag cagagctggc 120
cccactgtgc gggcggagag gactgcgaac cgacatcgat gctacttgta ccccaaggcg 180
agcaagctcc aaccagcgag ggctgaacca gatttggaat gtgaagaaac agtctgtcta 240
cctgatgaat ctgagaaaga gcggcactct gggacaccct ggcagcctgg acgagaccac 300
ctacgagcgg ctggccgagg aaaccctgga ttccctggcc gagttctttg aagacctggc 360
tgataagcca tacaccttcg aagactatga cgtgagcttc ggcagcggcg tgctgacagt 420

caaactgggc ggggacctgg gaacatacgt gatcaacaag cagactccta acaagcagat 480
ttggctgtct agtccctcaa gcggccctaa gaggtacgac tggacaggga aaaactgggt 540
gtatagtcac gatggcgtct cactgcatga gctgctggcc gctgaactga ctaaagccct 600
gaaaactaaa ctggacctgt cttccctggc atactctggc aaggacgcct gacgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 24

<211> 983

<212> DNA

<213> 人工序列

<220>

<223> 用于克隆至pTRs-KS-CBh-EGFP-BGH scAAV载体中的编码FXN GenScript的密码子优化核苷酸序列

<400> 24

tagaagaccg gtcgccacca tgtggactct gggccggaga gcagtggcag gactgctggc 60
aagtccatca cctgctcagg cacagactct gacaagagtc ccaagacctg cagagctggc 120
tccactgtgc gggaggcgcg gactgagaac agacatcgat gctacatgta ctcctcgacg 180
ggcaagctcc aaccagcgag ggctgaacca gatttggaat gtgaagaaac agtccgtcta 240
cctgatgaat ctgaggaagt caggcaccct ggggcaccca ggaagtctgg acgagaccac 300
atatgaacgg ctggctgagg aaacactgga ttctctggcc gagttctttg aagacctggc 360
tgataagccc tacacattcg aagactatga tgtgagcttt ggatccggcg tgctgactgt 420
caaactgggc ggggacctgg gcacttacgt gatcaacaag cagaccccta acaagcagat 480
ttggctgtct agtccttcaa gcggaccaaa gcggtacgac tggaccggca aaaactgggt 540
gtattctcac gatggggtca gtctgcatga gctgctggcc gctgaactga ccaaggccct 600
gaagacaaaa ctggacctgt cctctctggc atatagcgga aaagatgcct gacgagcggc 660
cgctcctagg agcagtatcg atcccagccc acttttcccc aatacgacta gtactcgact 720
gtgccttcta gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg 780
gaaggtgcca ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg 840
agtaggtgtc attctattct gggggggtggg gtggggcagg acagcaaggg ggaggattgg 900
gaagacaaca gcaggcatgc tggggatgcg gtgggctcta tggcttctga ggcggaaaga 960
accagctttg gacgcgtctt aag 983

<210> 25

<211> 20

<212> DNA

<213> 人工序列

<220>
<223> 编码胶原蛋白稳定序列(CSS)的核酸序列
<400> 25
cccagcccac ttttccccaa 20
<210> 26
<211> 797
<212> DNA
<213> 人工序列
<220>
<223> CBh启动子的核酸序列
<400> 26
tacataactt acggtaaatg gcccgcctgg ctgaccgccc aacgaccccc gcccattgac 60
gtcaatagta acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta 120
aactgcccac ttggcagtac atcaagtgta tcatatgcca agtacgcccc ctattgacgt 180
caatgacggt aaatggcccg cctggcattg tgcccagtac atgaccttat gggactttcc 240
tacttggcag tacatctacg tattagtcat cgctattacc atggtcgagg tgagccccac 300
gttctgcttc actctcccca tctccccccc ctccccaccc ccaattttgt atttatttat 360
tttttaatta ttttgtgcag cgatgggggc ggggggggag gggggcgcgg cgccaggcgg 420
ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 480
gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 540
aaagcgaagc gcgcggcggg cgggagtcgc tgcgacgctg ccttcgcccc gtgccccgct 600
ccgccgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc ccacaggtga 660
gcgggcggga cggcccttct cctccgggct gtaattagct gagcaagagg taagggttta 720
agggatggtt ggttggtggg gtattaatgt ttaattacct ggagcacctg cctgaaatca 780
ctttttttca ggttgga 797
<210> 27
<211> 254
<212> DNA
<213> 人工序列
<220>
<223> bGHpoly A信号序列的核酸序列
<400> 27
ctcgactgtg ccttctagtt gccagccatc tgttgtttgc ccctcccccg tgccttcctt 60
gaccctggaa ggtgccactc ccactgtcct ttcctaataa aatgaggaaa ttgcatcgca 120
ttgtctgagt aggtgtcatt ctattctggg gggtggggtg gggcaggaca gcaaggggga 180
ggattgggaa gacaacagca ggcatgctgg ggatgcggtg ggctctatgg cttctgaggc 240
ggaaagaacc agct 254
<210> 28
<211> 2208

<212> DNA

<213> 人工序列

<220>

<223> 编码AAV2i8衣壳(VP1)的核苷酸序列

<400> 28

atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga 60
cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac 120
gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccttcaa cggactcgac 180
aagggagagc cggtcaacga ggcagacgcc gcggccctcg agcacgacaa agcctacgac 240
cggcagctcg acagcggaga caacccgtac ctcaagtaca accacgccga cgcggagttt 300
caggagcgcc ttaaagaaga tacgtctttt gggggcaacc tcggacgagc agtcttccag 360
gcgaaaaaga gggttcttga acctctgggc ctggttgagg aacctgttaa gacggctccg 420
ggaaaaaaga ggccggtaga gcactctcct gtggagccag actcctcctc gggaaccgga 480
aaggcgggcc agcagcctgc aagaaaaaga ttgaattttg gtcagactgg agacgcagac 540
tcagtacctg acccccagcc tctcggacag ccaccagcag ccccctctgg tctgggaact 600
aatacgatgg ctacaggcag tggcgcacca atggcagaca ataacgaggg cgccgacgga 660
gtgggtaatt cctcgggaaa ttggcattgc gattccacat ggatgggcga cagagtcatc 720
accaccagca cccgaacctg ggccctgccc acctacaaca accacctcta caaacaaatt 780
tccagccaat caggagcctc gaacgacaat cactactttg gctacagcac cccttggggg 840
tattttgact tcaacagatt ccactgccac ttttcaccac gtgactggca aagactcatc 900
aacaacaact ggggattccg acccaagaga ctcaacttca agctctttaa cattcaagtc 960
aaagaggtca cgcagaatga cggtacgacg acgattgcca ataaccttac cagcacggtt 1020
caggtgttta ctgactcgga gtaccagctc ccgtacgtcc tcggctcggc gcatcaagga 1080
tgcctcccgc cgttcccagc agacgtcttc atggtgccac agtatggata cctcaccctg 1140
aacaacggga gtcaggcagt aggacgctct tcattttact gcctggagta ctttccttct 1200
cagatgctgc gtaccggaaa caactttacc ttcagctaca cttttgagga cgttcctttc 1260
cacagcagct acgctcacag ccagagtctg gaccgtctca tgaatcctct catcgaccag 1320
tacctgtatt acttgagcag aacaaacact ccaagtggaa ccaccacgca gtcaaggctt 1380
cagttttctg tggccggacc cagtaacatg gctgtccagg gaaggaactg gcttcctgga 1440
ccctgttacc gccagcagcg agtatcaaag acatctgcgg ataacaacaa cagtgaattt 1500
gcttggactg gagctaccaa gtaccacctc aatggcagag actctctggt gaatccgggc 1560
ccggccatgg caagccacaa ggacgatgaa gaaaagtttt ttcctcagag cggggttctc 1620
atctttggga agcaaggctc agagaaaaca aatgtggaca ttgaaaaggt catgattaca 1680
gacgaagagg aaatcaggac aaccaatccc gtggctacgg agcagtatgg ttctgtatct 1740
accaacctcc agcaacagaa cacagcacca gctaccgcag atgtcaacac acaaggcgtt 1800
cttccaggca tggtctggca ggacagagat gtgtaccttc aggggcccat ctgggcaaag 1860
attccacaca cggacggaca ttttcacccc tctcccctca tgggtggatt cggacttaaa 1920
caccctcctc cacagattct catcaagaac accccggtac ctgcgaatcc ttcgaccacc 1980
ttcagtgcgg caaagtttgc ttccttcatc acacagtact ccacgggaca ggtcagcgtg 2040

gagatcgagt gggagctgca gaaggaaaac agcaaacgct ggaatcccga aattcagtac 2100
acttccaact acaacaagtc tgttaatgtg gactttactg tggacactaa tggcgtgtat 2160
tcagagcctc gccccattgg caccagatac ctgactcgta atctgtaa 2208

<210> 29
<211> 735
<212> PRT
<213> 人工序列
<220>
<223> AAV2i8衣壳(VP1)的氨基酸序列
<400> 29

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1 5 10 15
Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
20 25 30
Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80
Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125
Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140
Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160
Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175
Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190
Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly
195 200 205
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile

225 230 235 240
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255
Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270
Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285
Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300
Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320
Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335
Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350
Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365
Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380
Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400
Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
405 410 415
Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430
Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr
435 440 445
Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser Arg Leu Gln Phe Ser Val
450 455 460
Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Trp Leu Pro Gly
465 470 475 480
Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp Asn Asn
485 490 495
Asn Ser Glu Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly
500 505 510
Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
515 520 525
Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys
530 535 540

Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
545 550 555 560
Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr
565 570 575
Gly Ser Val Ser Thr Asn Leu Gln Gln Gln Asn Thr Ala Pro Ala Thr
580 585 590
Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
595 600 605
Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
610 615 620
Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
625 630 635 640
His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn
645 650 655
Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln
660 665 670
Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys
675 680 685
Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr
690 695 700
Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr
705 710 715 720
Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<210> 30
<211> 2208
<212> DNA
<213> 人工序列
<220>
<223> 编码AAV2-TT衣壳(VP1)的核酸
<400> 30

atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga 60
cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac 120
gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccttcaa cggactcgac 180
aagggagagc cggtcaacga ggcagacgcc gcggccctcg agcacgacaa agcctacgac 240
cggcagctcg acagcggaga caacccgtac ctcaagtaca accacgccga cgcggagttt 300
caggagcgcc ttaaagaaga tacgtctttt gggggcaacc tcggacgagc agtcttccag 360
gcgaaaaaga ggattcttga acctctgggc ctggttgagg aacctgttaa gacggctccg 420
ggaaaaaaga ggccggtaga gcactctcct gcggagccag actcctcctc gggaaccgga 480

aagtcgggcc agcagcctgc aagaaaaaga ttgaattttg gtcagactgg agacgcagac 540
tcagtacctg acccccagcc tctcggacag ccaccagcag ccccctctgg tctgggaact 600
aatacgatgg cttcaggcag tggcgcacca atggcagaca ataacgaggg cgccgacgga 660
gtgggtaatt cctcgggaaa ttggcattgc gattccacat ggatgggcga cagagtcatc 720
accaccagca cccgaacctg ggccctgccc acctacaaca accacctcta caaacaaatt 780
tccagccaat caggagcctc gaacgacaat cactactttg gctacagcac cccttggggg 840
tattttgact tcaacagatt ccactgccac ttttcaccac gtgactggca aagactcatc 900
aacaacaact ggggattccg acccaagaga ctcagcttca agctctttaa cattcaagtc 960
aaagaggtca cgcagaatga cggtacgacg acgattgcca ataaccttac cagcacggtt 1020
caggtgttta ctgactcgga gtaccagctc ccgtacgtcc tcggctcggc gcatcaagga 1080
tgcctcccgc cgttcccagc agacgtcttc atggtgccac agtatggata cctcaccctg 1140
aacaacggga gtcaggcagt aggacgctct tcattttact gcctggagta ctttccttct 1200
cagatgctgc gtaccggaaa caactttacc ttcagctaca cttttgagga cgttcctttc 1260
cacagcagct acgctcacag ccagagtctg gaccgtctca tgaatcctct catcgaccag 1320
tacctgtatt acttgagcag aacaaacact ccaagtggaa ccaccacgat gtcaaggctt 1380
cagttttctc aggccggagc gagtgacatt cgggaccagt ctaggaactg gcttcctgga 1440
ccctgttacc gccagcagcg agtatcaaag acagctgcgg ataacaacaa cagtgattac 1500
tcgtggactg gagctaccaa gtaccacctc aatggcagag actctctggt gaatccgggc 1560
ccggccatgg caagccacaa ggacgatgaa gaaaagtatt ttcctcagag cggggttctc 1620
atctttggga agcaagactc aggaaaaaca aatgtggaca ttgaaaaggt catgattaca 1680
gacgaagagg aaatcaggac aaccaatccc gtggctacgg agcagtatgg ttctgtatct 1740
accaacctcc agagcggcaa cacacaagca gctacctcag atgtcaacac acaaggcgtt 1800
cttccaggca tggtctggca ggacagagat gtgtaccttc aggggcccat ctgggcaaag 1860
attccacaca cggacggaca ttttcacccc tctcccctca tgggtggatt cggacttaaa 1920
caccctcctc cacagattct catcaagaac accccggtac ctgcgaatcc ttcgaccacc 1980
ttcagtgcgg caaagtttgc ttccttcatc acacagtact ccacgggaca ggtcagcgtg 2040
gagatcgagt gggagctgca gaaggaaaac agcaaacgct ggaatcccga aattcagtac 2100
acttccaact acaacaagtc tgttaatgtg gactttactg tggacactaa tggcgtgtat 2160
tcagagcctc gccccattgg caccagatac ctgactcgta atctgtaa 2208

<210> 31
<211> 735
<212> PRT
<213> 人工序列
<220>
<223> AAV2-TT衣壳(VP1)的氨基酸序列
<400> 31

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1 5 10 15
Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro

20 25 30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80
Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Ile Leu Glu Pro
115 120 125
Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140
Pro Val Glu His Ser Pro Ala Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160
Lys Ser Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175
Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190
Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Ser Gly Ser Gly
195 200 205
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225 230 235 240
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255
Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270
Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285
Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300
Gly Phe Arg Pro Lys Arg Leu Ser Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320
Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350
Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365
Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380
Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400
Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
405 410 415
Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430
Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr
435 440 445
Asn Thr Pro Ser Gly Thr Thr Thr Met Ser Arg Leu Gln Phe Ser Gln
450 455 460
Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly
465 470 475 480
Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ala Ala Asp Asn Asn
485 490 495
Asn Ser Asp Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly
500 505 510
Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
515 520 525
Asp Glu Glu Lys Tyr Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys
530 535 540
Gln Asp Ser Gly Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
545 550 555 560
Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr
565 570 575
Gly Ser Val Ser Thr Asn Leu Gln Ser Gly Asn Thr Gln Ala Ala Thr
580 585 590
Ser Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
595 600 605
Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
610 615 620
Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
625 630 635 640
His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn

645 650 655
Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln
660 665 670
Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys
675 680 685
Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr
690 695 700
Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr
705 710 715 720
Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<210> 32
<211> 2208
<212> DNA
<213> 人工序列
<220>
<223> 编码AAV2-TT-S312N衣壳(VP1)的核酸
<400> 32
atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga aggaataaga 60
cagtggtgga agctcaaacc tggcccacca ccaccaaagc ccgcagagcg gcataaggac 120
gacagcaggg gtcttgtgct tcctgggtac aagtacctcg gacccttcaa cggactcgac 180
aagggagagc cggtcaacga ggcagacgcc gcggccctcg agcacgacaa agcctacgac 240
cggcagctcg acagcggaga caacccgtac ctcaagtaca accacgccga cgcggagttt 300
caggagcgcc ttaaagaaga tacgtctttt gggggcaacc tcggacgagc agtcttccag 360
gcgaaaaaga ggattcttga acctctgggc ctggttgagg aacctgttaa gacggctccg 420
ggaaaaaaga ggccggtaga gcactctcct gcggagccag actcctcctc gggaaccgga 480
aagtcgggcc agcagcctgc aagaaaaaga ttgaattttg gtcagactgg agacgcagac 540
tcagtacctg acccccagcc tctcggacag ccaccagcag ccccctctgg tctgggaact 600
aatacgatgg cttcaggcag tggcgcacca atggcagaca ataacgaggg cgccgacgga 660
gtgggtaatt cctcgggaaa ttggcattgc gattccacat ggatgggcga cagagtcatc 720
accaccagca cccgaacctg ggccctgccc acctacaaca accacctcta caaacaaatt 780
tccagccaat caggagcctc gaacgacaat cactactttg gctacagcac cccttggggg 840
tattttgact tcaacagatt ccactgccac ttttcaccac gtgactggca aagactcatc 900
aacaacaact ggggattccg acccaagaga ctcaacttca agctctttaa cattcaagtc 960
aaagaggtca cgcagaatga cggtacgacg acgattgcca ataaccttac cagcacggtt 1020
caggtgttta ctgactcgga gtaccagctc ccgtacgtcc tcggctcggc gcatcaagga 1080
tgcctcccgc cgttcccagc agacgtcttc atggtgccac agtatggata cctcaccctg 1140
aacaacggga gtcaggcagt aggacgctct tcattttact gcctggagta ctttccttct 1200
cagatgctgc gtaccggaaa caactttacc ttcagctaca cttttgagga cgttcctttc 1260

cacagcagct acgctcacag ccagagtctg gaccgtctca tgaatcctct catcgaccag 1320
tacctgtatt acttgagcag aacaaacact ccaagtggaa ccaccacgat gtcaaggctt 1380
cagttttctc aggccggagc gagtgacatt cgggaccagt ctaggaactg gcttcctgga 1440
ccctgttacc gccagcagcg agtatcaaag acagctgcgg ataacaacaa cagtgattac 1500
tcgtggactg gagctaccaa gtaccacctc aatggcagag actctctggt gaatccgggc 1560
ccggccatgg caagccacaa ggacgatgaa gaaaagtatt ttcctcagag cggggttctc 1620
atctttggga agcaagactc aggaaaaaca aatgtggaca ttgaaaaggt catgattaca 1680
gacgaagagg aaatcaggac aaccaatccc gtggctacgg agcagtatgg ttctgtatct 1740
accaacctcc agagcggcaa cacacaagca gctacctcag atgtcaacac acaaggcgtt 1800
cttccaggca tggtctggca ggacagagat gtgtaccttc aggggcccat ctgggcaaag 1860
attccacaca cggacggaca ttttcacccc tctcccctca tgggtggatt cggacttaaa 1920
caccctcctc cacagattct catcaagaac accccggtac ctgcgaatcc ttcgaccacc 1980
ttcagtgcgg caaagtttgc ttccttcatc acacagtact ccacgggaca ggtcagcgtg 2040
gagatcgagt gggagctgca gaaggaaaac agcaaacgct ggaatcccga aattcagtac 2100
acttccaact acaacaagtc tgttaatgtg gactttactg tggacactaa tggcgtgtat 2160
tcagagcctc gccccattgg caccagatac ctgactcgta atctgtaa 2208

<210> 33
<211> 735
<212> PRT
<213> 人工序列
<220>
<223> AAV2-TT-S312N衣壳(VP1)的氨基酸序列
<400> 33

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1 5 10 15
Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
20 25 30
Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80
Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Ile Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140
Pro Val Glu His Ser Pro Ala Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160
Lys Ser Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175
Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190
Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Ser Gly Ser Gly
195 200 205
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225 230 235 240
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255
Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270
Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285
Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300
Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320
Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335
Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350
Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365
Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380
Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400
Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
405 410 415
Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430
Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr

435 440 445

Asn Thr Pro Ser Gly Thr Thr Thr Met Ser Arg Leu Gln Phe Ser Gln

450 455 460

Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly

465 470 475 480

Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ala Ala Asp Asn Asn

485 490 495

Asn Ser Asp Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly

500 505 510

Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp

515 520 525

Asp Glu Glu Lys Tyr Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys

530 535 540

Gln Asp Ser Gly Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr

545 550 555 560

Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr

565 570 575

Gly Ser Val Ser Thr Asn Leu Gln Ser Gly Asn Thr Gln Ala Ala Thr

580 585 590

Ser Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp

595 600 605

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr

610 615 620

Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys

625 630 635 640

His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn

645 650 655

Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln

660 665 670

Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys

675 680 685

Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr

690 695 700

Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr

705 710 715 720

Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu

725 730 735

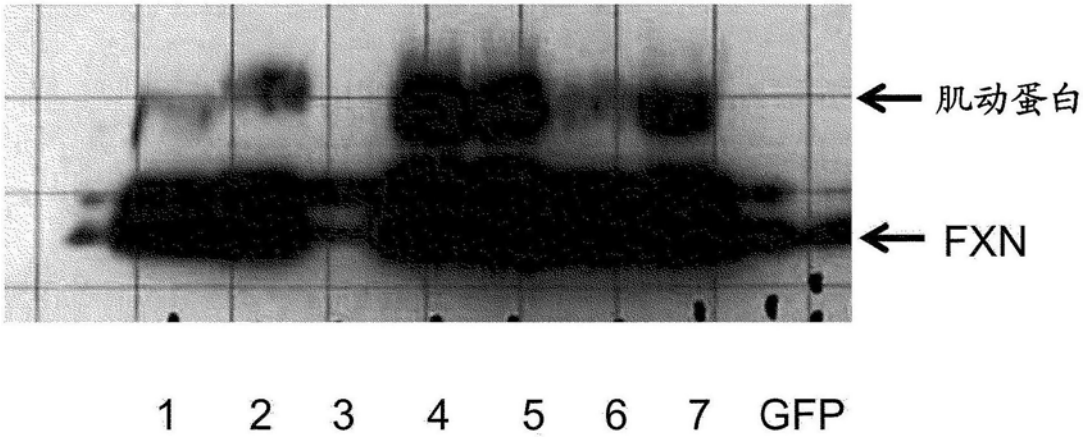


图1A

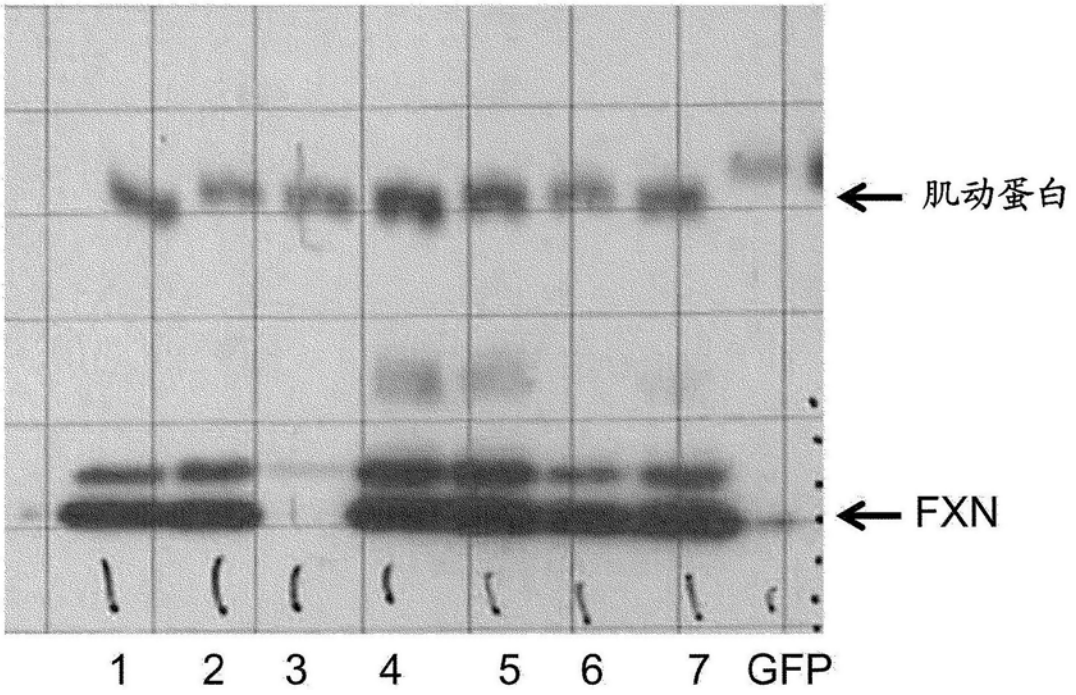


图1B

WT FXN (SEQ ID NO:19)

TAGAAG**ACCGGT**CGCCACCatgtggactctcgggcgccgcgcagtagccggcctcctggcgt
cacccagcccagcccaggcccagaccctcacccgggtcccgcggccggcagagttggcccca
ctctgcggccgccgtggcctgcgcaccgacatcgatgcgacctgcacgccccgccgcgcaag
ttcgaaccaacgtggcctcaaccagatttggaatgtcaaaaagcagagtgtctatttgatga
atttgaggaaatctggaactttgggccacccaggctctctagatgagaccacctatgaaaga
ctagcagaggaaacgctggactctttagcagagtttttgaagaccttgcagacaagccata
cacgtttgaggactatgatgtctcctttgggagtggtgtcttaactgtcaaactgggtggag
atctaggaacctatgtgatcaacaagcagacgccaaacaagcaaatctggctatcttctcca
tccagtggacctaagcgttatgactggactgggaaaaactgggtgtactcccacgacggcgt
gtccctccatgagctgctggccgcagagctcactaaagccttaaaaaccaaactggacttgt
cttccttggcctattccggaaaagatgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2A

IDT1 密码子优化的 FXN (SEQ ID NO:20)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggtaggcgagcggtggccggcctgttggcat
ctcctagtcctgcacaagctcaaacgctgactagagtccctcggccagcagaactggcgcca
ctttgcggccggcgcggtcttcgcactgatattgatgccacttgcacaccccggcgcgcctc
cagtaatcagcggggacttaatcaaatttggaatgtgaagaagcagtctgtgtatcttatga
atctgcggaagagcgggaccctgggccaccctggtagccttgatgaaaccacctatgagcgc
ctggccgaagagacactggacagtcttgccgagtttttgaggatctggccgacaaaccttа
tacttttgaggactatgacgtgtcctttggatctggtgtattgaccgtaaaactcgggggag
accttgggacgtatgtaataaataagcagaccccaaacaagcagatctggctcagctctcca
agtagtggtcctaagagatatgattggacgggcaagaactgggtctattcccatgatggcgt
ctctttgcatgaactccttgcagcagagctgaccaaggccttgaagaccaaattggatctca
gcagcctcgcctatagtggcaaagatgcatagCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2B

IDT3 - 低表达(SEQ ID NO:21)

TAGAAG**ACCGGT**CGCCACCatgtggacactgggaaggcgcgccgtggccggtctgttggcat
caccatccccagcccaggctcagacactcacccgagtcccaagacccgcagagctggcccct
ctgtgcgggcgccgaggccttcgcaccgatatcgatgctacatgcacgccacgcagagctag
ctcaaatcagaggggactcaaccagatatggaatgtcaagaagcaaagcgtgtatctcatga
acctccggaaaagcggcaccctgggacatcccgggtctctcgacgagaccacttatgaaaga
ctggcagaggagactcttgacagtctggcggagttcttcgaagacctcgctgacaagccata
taccttcgaagattacgacgtctccttcggctctggggtgctgactgtcaagcttggcggcg
acctggggacctacgtgatcaacaagcagactccaaacaagcaaatctggctcagcagtcca
agctccggacccaagagatacgattggacaggcaagaattgggtttactcccacgacggggt
gtccctccatgagctgctggccgctgagctgacgaaggccctgaagaccaagctggatctct
cctccctggcatacagtggtaaggacgcttgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2C

IDT4 (SEQ ID NO:22)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggcgggccgtagctggcttgctggcta
gcccaagtcccgcccaggctcagactctcaccagggtacccaggcccgcagagcttgctcca
ctctgcggacgcaggggtctgcgaaccgatatcgacgcaacttgcacgccgcggagggcctc
ttcaaaccagagaggactcaatcaaatttggaatgtaaagaaacagagcgtgtatctcatga
acctccgaaagagtgggactcttgggcaccccggctccctggacgagactacttacgagcgc
ctggccgaagaaaccttggattccctggcggagtttttgaagacttggcagacaagcctta
taccttcgaggattacgacgtgagttttggctctggtgttcttacagtcaagctcggtggcg
accttggcacttatgtaattaacaagcagacacctaacaagcagatctggctttctagtccg
tcttccggtcccaaaaggtacgattggactggaaagaactgggtctacagtcacgacggtgt
ctccctgcacgaattgcttgcggctgagctgactaaggcgctcaaaacaaaactggatctgt
ccagccttgcctatagcgggaaggacgcatgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2D

GenScript (SEQ ID NO:23)

TAGAAG**ACCGGT**CGCCACCatgtggacactgggccggagagccgtcgctgggctgctggcat
caccatcccccgcacaggcacagaccctgacaagagtccctcggccagcagagctggcccca
ctgtgcgggcggagaggactgcgaaccgacatcgatgctacttgtaccccaaggcgagcaag
ctccaaccagcgagggctgaaccagatttggaatgtgaagaaacagtctgtctacctgatga
atctgagaaagagcggcactctgggacaccctggcagcctggacgagaccacctacgagcgg
ctggccgaggaaaccctggattccctggccgagttctttgaagacctggctgataagccata
caccttcgaagactatgacgtgagcttcggcagcggcgtgctgacagtcaaactgggcgggg
acctgggaacatacgtgatcaacaagcagactcctaacaagcagatttggctgtctagtccc
tcaagcggccctaagaggtacgactggacagggaaaaactgggtgtatagtcacgatggcgt
ctcactgcatgagctgctggccgctgaactgactaaagccctgaaaactaaactggacctgt
cttccctggcatactctggcaaggacgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2E

GenScript (低 CpG) (SEQ ID NO:24)

TAGAAG**ACCGGT**CGCCACCatgtggactctgggccggagagcagtggcaggactgctggcaa
gtccatcacctgctcaggcacagactctgacaagagtcccaagacctgcagagctggctcca
ctgtgcgggaggcgcggactgagaacagacatcgatgctacatgtactcctcgacgggcaag
ctccaaccagcgagggctgaaccagatttggaatgtgaagaaacagtccgtctacctgatga
atctgaggaagtcaggcaccctggggcacccaggaagtctggacgagaccacatatgaacgg
ctggctgaggaaacactggattctctggccgagttctttgaagacctggctgataagcccta
cacattcgaagactatgatgtgagctttggatccggcgtgctgactgtcaaactgggcgggg
acctgggcacttacgtgatcaacaagcagacccctaacaagcagatttggctgtctagtcct
tcaagcggaccaaagcggtacgactggaccggcaaaaactgggtgtattctcacgatggggt
cagtctgcatgagctgctggccgctgaactgaccaaggccctgaagacaaaactggacctgt
cctctctggcatatagcggaaaagatgcctgaCGAGCGGCCGCTCCTAGGAGCAGTATCGAT
CCCAGCCCACTTTTCCCCAATACG**ACTAGT***ACTCGACTGTGCCTTCTAGTTGCCAGCCATCT*
GTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAACAGCAGGCATGCTGGGGATGCG
*GTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCT*TTGG***ACGCGT***CTTAAG

图2F

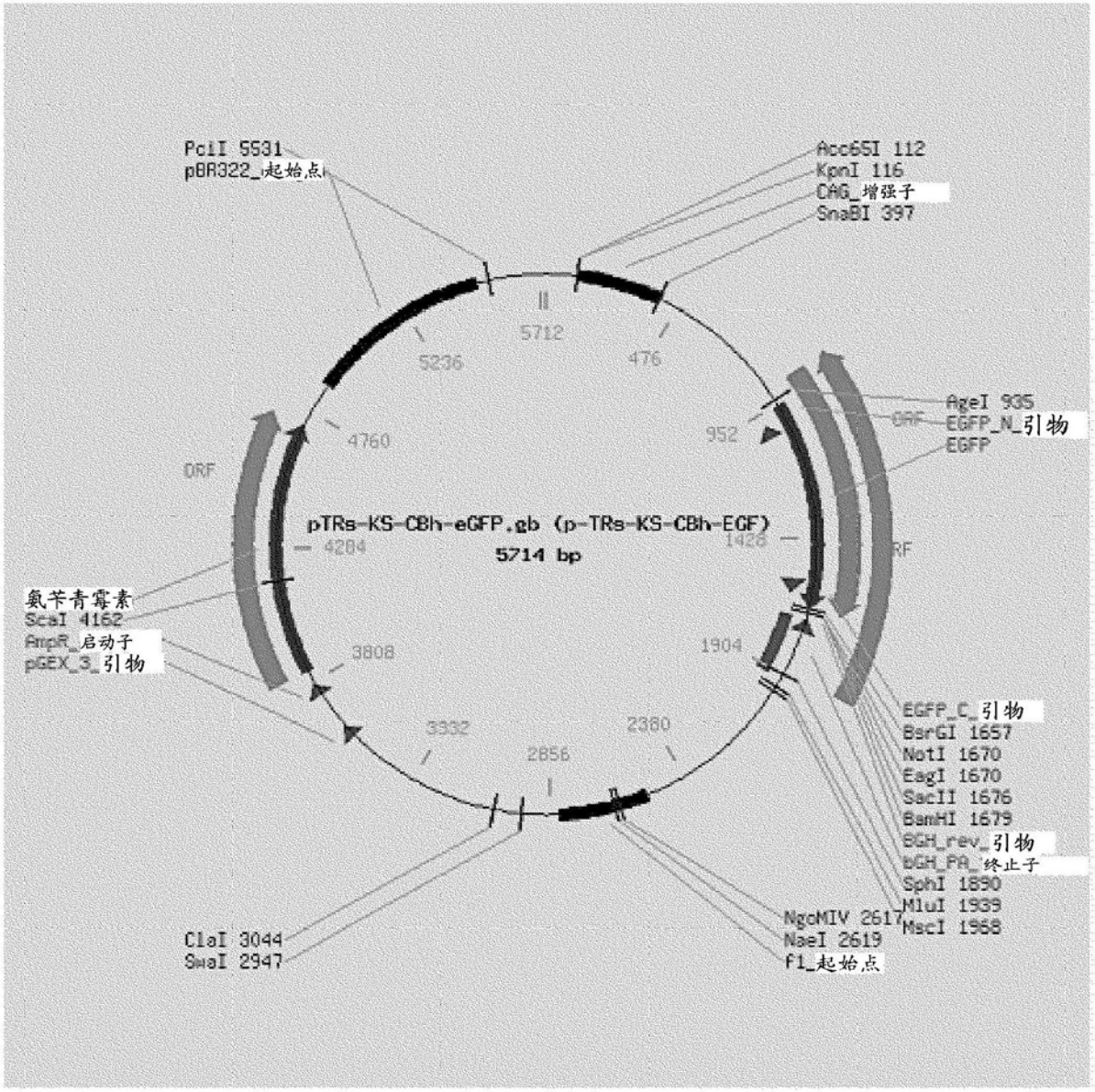

PciI 5531
pBR322_起始点
Acc65I 112
KpnI 116
CAG_增强子
SnaBI 397
5712
5236
476
4760
952
AgeI 935
ORF
EGFP_N_引物
EGFP
ORF
pTRs-KS-CBh-eGFP.gb (p-TRs-KS-CBh-EGF)
5714 bp
4204
1428
ORF
氨苄青霉素
ScaI 4162
AmpR_启动子
pGEX_3_引物
3808
1904
3332
2380
2856
EGFP_C_引物
BsrGI 1657
NotI 1670
EagI 1670
SacII 1676
BamHI 1679
BGH_rev_引物
bGH_PA_终止子
SphI 1890
MluI 1939
MscI 1968
NgoMIV 2617
NaeI 2619
f1_起始点
ClaI 3044
SwaI 2947

图3

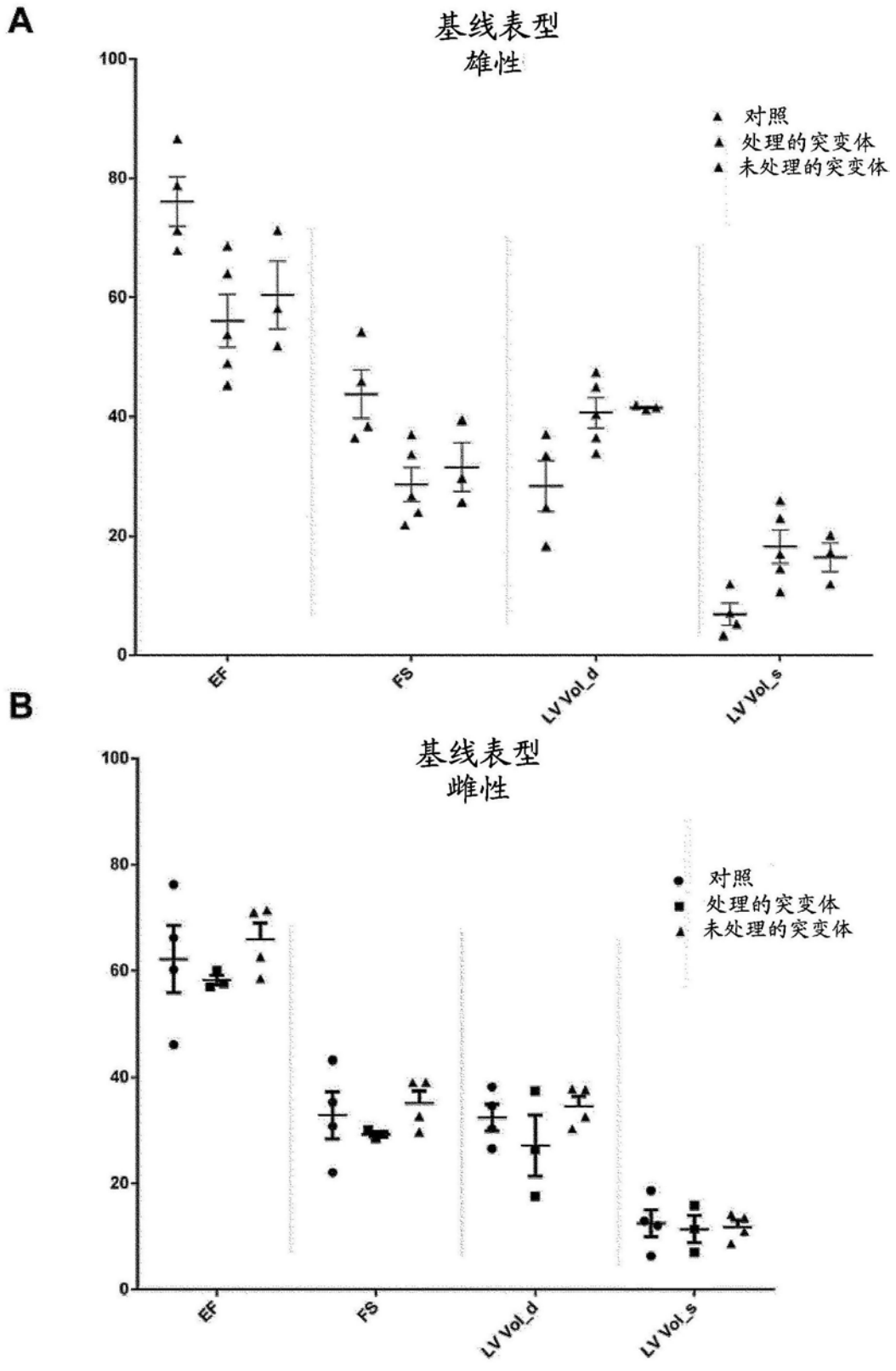

A
基线表型
雄性
对照
处理的突变体
未处理的突变体
100
80
60
40
20
0
EF
FS
LV Vol_d
LV Vol_s
B
基线表型
雌性
对照
处理的突变体
未处理的突变体
100
80
60
40
20
0
EF
FS
LV Vol_d
LV Vol_s

图4A-4B

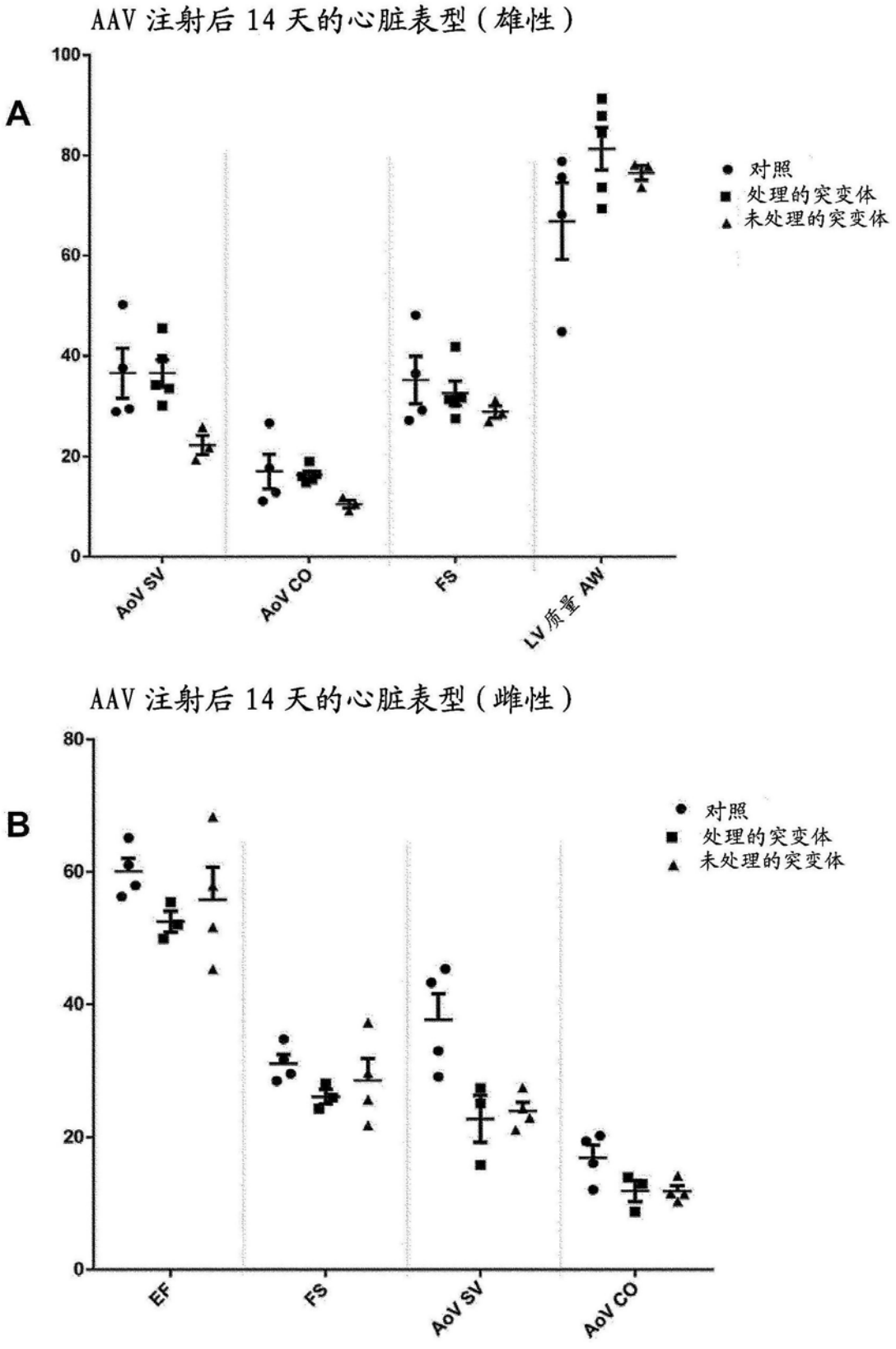

AAV 注射后 14 天的心脏表型（雄性）
A
100
80
60
40
20
0
AoV SV
AoV CO
FS
LV 质量 AW
对照
处理的突变体
未处理的突变体
AAV 注射后 14 天的心脏表型（雌性）
B
80
60
40
20
0
EF
FS
AoV SV
AoV CO
对照
处理的突变体
未处理的突变体

图5A-5B

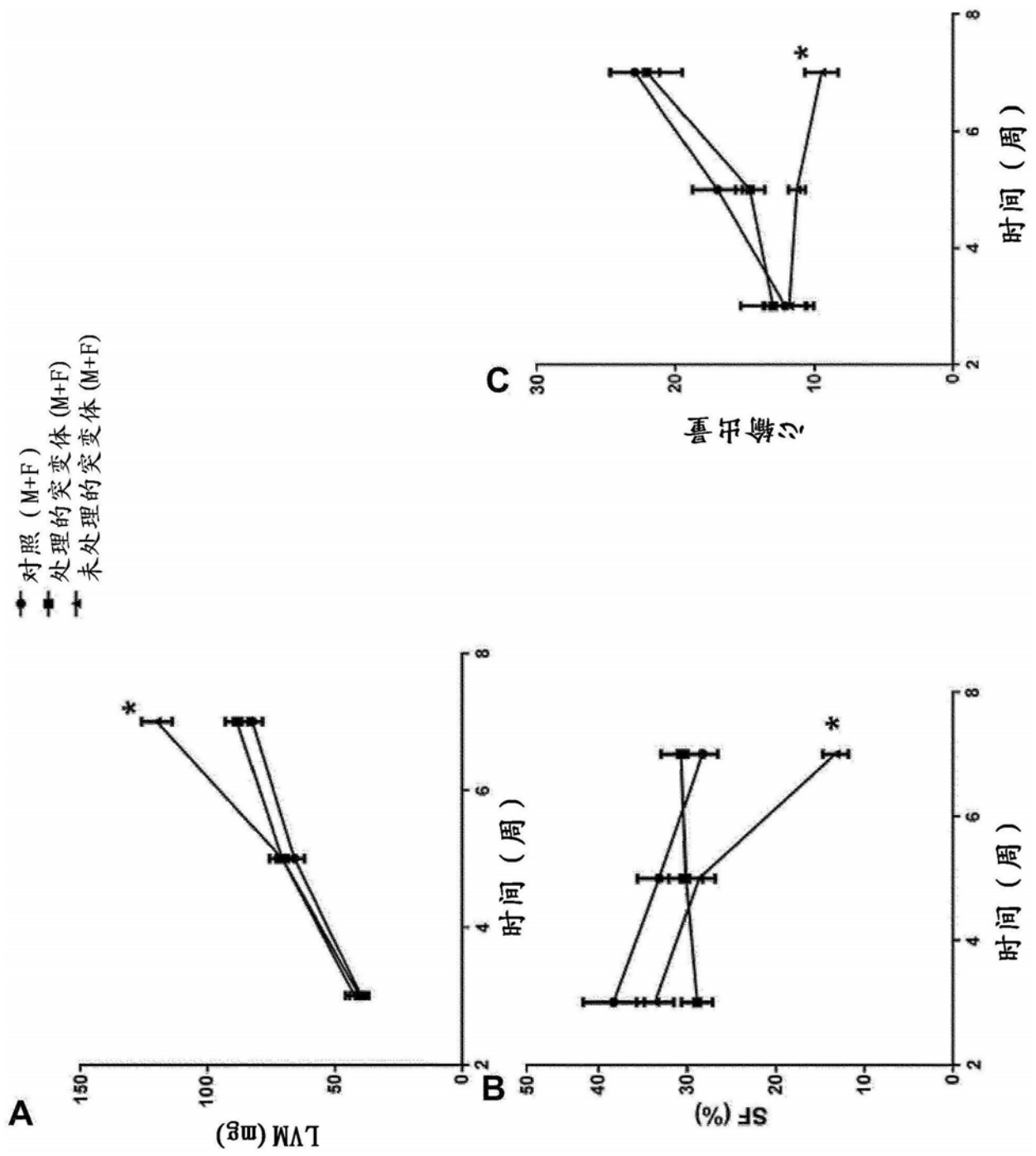

对照（M+F）
处理的突变体 (M+F)
未处理的突变体 (M+F)
A
LVM (mg)
150
100
50
0
2
4
6
8
时间（周）
B
SF (%)
50
40
30
20
10
0
2
4
6
8
时间（周）
C
心输出量
30
20
10
0
2
4
6
8
时间（周）
*

图6A-6C