Claims

1. A DNA polynucleotide encoding human frataxin (h*FXN*) or an RNA equivalent thereof, wherein the polynucleotide has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 1.

2. The polynucleotide of claim 1, wherein the polynucleotide has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 1.

3. The polynucleotide of claim 2, wherein the polynucleotide has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 1.

4. The polynucleotide of claim 3, wherein the polynucleotide has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 1.

5. The polynucleotide of claim 4, wherein the polynucleotide has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 1.

6. The polynucleotide of claim 5, wherein the polynucleotide has the nucleic acid sequence of SEQ ID NO: 1.

7. A vector comprising the polynucleotide of any one of claims 1-6, optionally wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector.

8. The vector of claim 7, wherein:
(i) the vector is a viral vector;
(ii) the polynucleotide is operably linked to a muscle specific promoter, optionally wherein the promoter is positioned 5' to the polynucleotide; or
(iii) the vector further comprises a polyadenylation site (pA), optionally wherein the pA is positioned 3' to the polynucleotide.

9. The vector of claim 8, wherein:
(i) the viral vector is selected from the group consisting of an adeno-associated virus (AAV), an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus;
(ii) the muscle specific promotor is a phosphoglycerate kinase (PGK) promoter, a desmin promoter, a muscle creatine kinase promoter, a myosin light chain promoter, a myosin heavy chain promoter, a cardiac troponin C promoter, a troponin I promoter, a myoD gene family promoter, an actin alpha

promoter, an actin beta promoter, an actin gamma promoter, or a promoter within intron 1 of ocular paired like homeodomain 3;
(iii) the pA site comprises the simian virus 40 (SV40) late polyadenylation site, the SV40 early polyadenylation site, the human β-globin polyadenylation site, or the bovine growth hormone polyadenylation site; or
(iv) the vector further comprises an intron, optionally wherein the intron is positioned 3' to the promoter and 5' to the polynucleotide.

10. The vector of claim 9, wherein:
(i) the viral vector is an AAV;
(ii) the muscle specific promoter is a PGK promoter;
(iii) the pA site comprises the SV40 late polyadenylation site; or
(iv) the intron is an SV40 intron.

11. The vector of claim 10, wherein:
(i) the AAV comprises capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, and AAVrh74;
(ii) the viral vector is a pseudotyped AAV;
(iii) the AAV comprises a recombinant capsid protein;
(iv) the AAV further comprises two inverted terminal repeats (ITRs), wherein the two ITRs comprise a first ITR (ITR1) and a second ITR (ITR2), wherein ITR1 is positioned 5' to the polynucleotide and ITR2 is positioned 3' to the polynucleotide to form a cassette comprising the structure ITR1-h*FXN*-ITR2; or
(v) the PGK promoter has a nucleic acid sequence that is at least 85% identical to the nucleic acid of sequence of SEQ ID NO: 2.

12. The vector of claim 11, wherein:
(i) the pseudotyped AAV is AAV2/8 or AAV2/9, optionally wherein the pseudotyped AAV is AAV2/8; or
(ii) the PGK promoter has a nucleic acid sequence that is at least 90% identical to the nucleic acid of sequence of SEQ ID NO: 2.

13. The vector of claim 12, wherein the PGK promoter has a nucleic acid sequence that is at least 95% identical to the nucleic acid of sequence of SEQ ID NO: 2, optionally wherein the PGK promoter has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 2.

14. The vector of claim 13, wherein the PGK promoter has the nucleic acid of SEQ ID NO: 2.

15. The vector of claim 11, wherein:
(i) the length of the nucleic acid between ITR1 and ITR2 is from about 3.7 Kb to about 4.3 Kb;
(ii) the length of the nucleic acid between and including ITR1 and ITR2 is from about 3.9 Kb to about 4.7 Kb; or
(iii) the two ITRs are AAV serotype 2 ITRs.

16. The vector of claim 15, wherein:
(i) the length of the nucleic acid between ITR1 and ITR2 is from about 3.8 Kb to about 4.2 Kb; or
(ii) the length of the nucleic acid between and including ITR1 and ITR2 is from about 4.1 Kb to about 4.5 Kb.

17. The vector of claim 16, wherein:
(i) the length of the nucleic acid between ITR1 and ITR2 is from about 3.9 Kb to about 4.1 Kb; or
(ii) the length of the nucleic acid between and including ITR1 and ITR2 is about 4.3 Kb.

18. The vector of claim 17, wherein the length of the nucleic acid between ITR1 and ITR2 is about 4.0 Kb.

19. A plasmid encoding the viral vector of any one of claims 8-18.

20. The plasmid of claim 19, wherein the plasmid further comprises one or more spacer elements (SS).

21. The plasmid of claim 20, wherein:
(i) the one or more SS do not comprise an open reading frame that is greater than 100 amino acids in length;
(ii) the one or more SS do not comprise prokaryotic transcription factor binding sites;
(iii) the vector comprises two spacer elements: a first spacer element (SS1) and a second spacer element (SS2); or
(iv) the SS is positioned 5' to ITR1 and/or 3' to ITR2.

22. The plasmid of claim 21, wherein:
(i) the SS1 is from about 1.0 Kb to about 5.0 Kb in length;
(ii) the SS2 is from about 1.0 Kb to about 5.0 Kb in length; or
(iii) the SS1 is positioned 5' to ITR1 and the SS2 is positioned 3' to ITR2.

23. The plasmid of claim 22, wherein:
(i) the SS1 is from about 2.0 Kb to about 5.0 Kb in length; or

(ii) the SS2 is from about 2.0 Kb to about 5.0 Kb in length.

24. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-6, the vector of any one of claims 7-18, or the plasmid of any one of claims 19-23 and a pharmaceutically acceptable carrier, diluent, or excipient.

25. A nucleic acid molecule comprising:

(i) an ITR1;

(ii) a h*FXN* or an RNA equivalent thereof; and

(iii) an ITR2;

wherein the components are operably linked to each other in a 5'-to-3' direction as:

ITR1-h*FXN*-ITR2; and wherein the length of the nucleic acid between ITR1 and ITR2 is from about 3.7 Kb to about 4.3 Kb.

26. The nucleic acid molecule of claim 25, wherein:

(a) the length of the nucleic acid between ITR1 and ITR2 is from about 3.8 Kb to about 4.2 Kb;

(b) the length of the nucleic acid between and including ITR1 and ITR2 is from about 3.9 Kb to about 4.7 Kb;

(c) the nucleic acid molecule further comprises:

(iv) a eukaryotic promoter (P_{Euk}),

wherein the components are operably linked to each other in a 5'-to-3' direction as:

ITR1-P_{Euk}-h*FXN*-ITR2;

(d) the h*FXN,* or an RNA equivalent thereof, encodes a protein having an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 3; or

(e) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 85% identical to the nucleic acid of sequence of SEQ ID NO: 1.

27. The nucleic acid molecule of claim 26, wherein:

(a) the length of the nucleic acid between ITR1 and ITR2 is from about 3.9 Kb to about 4.1 Kb;

(b) the length of the nucleic acid between and including ITR1 and ITR2 is from about 4.1 Kb to about 4.5 Kb;

(c) the P_{Euk} is a muscle specific promoter;

(d) the h*FXN,* or an RNA equivalent thereof, encodes a protein having an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 3;

(e) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 90% identical to the nucleic acid of sequence of SEQ ID NO: 1; or

(f) the nucleic acid molecule further comprises:

(v) a pA,

wherein the components are operably linked to each other in a 5'-to-3' direction as:

ITR1-P_{Euk}-h*FXN*-pA-ITR2.

28. The nucleic acid molecule of claim 27, wherein:

(a) the length of the nucleic acid between ITR1 and ITR2 is about 4.0 Kb;

(b) the length of the nucleic acid between and including ITR1 and ITR2 is about 4.3 Kb;

(c) the muscle specific promoter is a PGK promoter, a desmin promoter, a muscle creatine kinase promoter, a myosin light chain promoter, a myosin heavy chain promoter, a cardiac troponin C promoter, a troponin I promoter, a myoD gene family promoter, an actin alpha promoter, an actin beta promoter, an actin gamma promoter, or a promoter within intron 1 of ocular paired like homeodomain 3, a cytomegalovirus promoter, or a chicken-β-actin promoter;

(d) the h*FXN,* or an RNA equivalent thereof, encodes a protein having the amino acid sequence of SEQ ID NO: 3;

(e) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 95% identical to the nucleic acid of sequence of SEQ ID NO: 1;

(f) the pA site comprises the SV40 late polyadenylation site, the SV40 early polyadenylation site, the human β-globin polyadenylation site, or the bovine growth hormone polyadenylation site; or

(g) the nucleic acid molecule further comprises:

(vi) an intron,

wherein the components are operably linked to each other in a 5'-to-3' direction as: ITR1-P_{Euk}-intron-h*FXN*-pA-ITR2.

29. The nucleic acid molecule of claim 28, wherein:

(a) the muscle specific promoter is a PGK promoter;

(b) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 97% identical to the nucleic acid of sequence of SEQ ID NO: 1;

(c) the pA site comprises the SV40 late polyadenylation site; or

(d) the intron is an SV40 intron.

30. The nucleic acid molecule of claim 29, wherein:

(a) the PGK promoter has a nucleic acid sequence that is at least 85% identical to the nucleic acid of sequence of SEQ ID NO: 2; or

(b) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 98% identical to the nucleic acid of sequence of SEQ ID NO: 1.

31. The nucleic acid molecule of claim 30, wherein:

(a) the PGK promoter has a nucleic acid sequence that is at least 90% identical to the nucleic acid of sequence of SEQ ID NO: 2; or

(b) the h*FXN,* or an RNA equivalent thereof, has a nucleic acid sequence that is at least 99% identical to the nucleic acid of sequence of SEQ ID NO: 1.

32. The nucleic acid molecule of claim 31, wherein:
(a) the PGK promoter has a nucleic acid sequence that is at least 95% identical to the nucleic acid of sequence of SEQ ID NO: 2, optionally wherein the PGK promoter has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 2; or
(b) the h*FXN,* or an RNA equivalent thereof, has the nucleic acid sequence of SEQ ID NO: 1.

33. The nucleic acid molecule of claim 32, wherein the PGK promoter has the nucleic acid of SEQ ID NO: 2.

34. A vector comprising the nucleic acid molecule of any one of claims 25-33, optionally wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector.

35. The vector of claim 34, wherein:
(i) the vector is a viral vector; or
(ii) ITR1 and/or ITR2 is a parvoviral ITR.

36. The vector of claim 35, wherein:
(i) the viral vector is selected from the group consisting of an AAV, an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus; or
(ii) the parvoviral ITR is an AAV ITR.

37. The vector of claim 36, wherein:
(i) the viral vector is an AAV; or
(ii) the AAV ITR is an AAV serotype 2 ITR.

38. The vector of claim 37, wherein:
(i) the AAV is comprises capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, and AAVrh74;
(ii) the viral vector is a pseudotyped AAV; or
(iii) the AAV comprises a recombinant capsid protein.

39. The vector of claim 38, wherein the pseudotyped AAV is AAV2/8 or AAV2/9, optionally wherein the pseudotyped AAV is AAV2/8.

40. The vector of any one of claims 7-18 and 34-39, wherein the vector has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO: 4.

41. The vector of claim 40, wherein the vector has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 4.

42. The vector of claim 41, wherein the vector has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 4, optionally wherein the vector has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 4.

43. The vector of claim 42, wherein the vector has the nucleic acid of SEQ ID NO: 4.

44. A plasmid encoding the viral vector of any one of claims 35-43.

45. The plasmid of claim 44, wherein the plasmid further comprises one or more SS, wherein the one or more SS is positioned 5' to ITR1 and/or 3' to ITR2.

46. The plasmid of claim 45, wherein:

(i) the plasmid comprises two SS, wherein the two SS comprise a SS1 and a SS2, wherein SS1 is positioned 5' to ITR1 and SS2 is positioned 3' to ITR2;

(ii) the one or more SS does not comprise an open reading frame that is greater than 100 amino acids in length; or

(iii) the one or more SS does not comprise prokaryotic transcription factor binding sites.

47. The plasmid of claim 46, wherein:

(i) SS1 is about 1.0 Kb to about 5.0 Kb in length;

(ii) SS2 is about 1.0 Kb to about 5.0 Kb in length; or

(iii) the plasmid further comprises a prokaryotic promoter operably linked to a selectable marker gene positioned 5' to the one or more SS positioned 5' to ITR1 or positioned 3' to the one or more SS positioned 3' to ITR2.

48. The plasmid of claim 47, wherein:

(i) SS1 is about 2.0 Kb to about 5.0 Kb in length;

(ii) SS2 is about 2.0 Kb to about 5.0 Kb in length;

(iii) the selectable marker gene is an antibiotic resistance gene; or

(iv) the plasmid further comprises a prokaryotic origin of replication positioned 5' to the one or more SS positioned 5' to ITR1 and/or positioned 3' to the one or more SS positioned 3' to ITR2.

49. A pharmaceutical composition comprising the nucleic acid molecule of any one of claims 25-33, the vector of any one of claims 34-43, or the plasmid of any one of claims 44-48 and a pharmaceutically acceptable carrier, diluent, or excipient.

50. Use of the polynucleotide of any one of claims 1-6, the nucleic acid molecule of any one of claims 25-33, the vector of any one of claims 7-18 and 34-43, the plasmid of any one of claims 19-23 and 44-48, or the pharmaceutical composition of claim 24 or 49, in the manufacture of a medicament for:

(i) treating Friedreich Ataxia in a human patient in need thereof; or
(ii) increasing frataxin expression in a human patient diagnosed as having Friedreich Ataxia.

51. The use of claim 50, wherein:
(i) the patient is from 3 years of age to 17 years of age;
(ii) upon administration of the polynucleotide, nucleic acid molecule, vector, plasmid, or pharmaceutical composition to the patient, the patient exhibits an increase in whole blood frataxin level, optionally wherein the patient exhibits the increase in whole blood frataxin level by about 12 weeks after administration; or
(iii) upon administration of the polynucleotide, nucleic acid molecule, vector, plasmid, or pharmaceutical composition to the patient, the patient exhibits a reduction in Total Friedreich Ataxia Rating Scale Score, optionally wherein the patient exhibits the reduction in Total FARS Score by about 12 weeks after administration.

52. A kit comprising (i) the polynucleotide of any one of claims 1-6, the nucleic acid molecule of any one of claims 25-33, the vector of any one of claims 7-18 and 34-43, the plasmid of any one of claims 19-23 and 44-48, or the pharmaceutical composition of claim 24 or 49, and (ii) a package insert, wherein the package insert instructs a user of the kit to administer the polynucleotide, nucleic acid molecule, vector, plasmid, or pharmaceutical composition to a human patient diagnosed as having Friedreich Ataxia.