FRATAXIN GENE THERAPY

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on September 9, 2022, is named 51037-060WO4_Sequence_Listing_9_8_22_ST26.XML and is 23,124 bytes in size.

Field of the Invention

The invention relates to the field of nucleic acid biotechnology and provides, for instance, compositions and methods for production of codon-optimized nucleic acids for enhancing gene expression in a target cell or tissue

Background of the Invention

Friedreich ataxia is the most common autosomal recessive inherited movement disorder, with 6,000 Americans diagnosed with this disease, and a prevalence of approximately 15,000 to 20,000 patients diagnosed worldwide. The clinical manifestations of Friedreich ataxia are the result of deficiency of the frataxin protein, with 95% of cases resulting from mutations that cause a GAA repeat expansion. Frataxin is a mitochondrial iron-binding protein that is ubiquitously expressed and is critical to the function of the heart, cerebellum, and spinal cord, including dorsal root ganglia neurons. The phenotype of Friedreich ataxia includes degeneration and demyelination of the spinocerebellar dorsal root ganglion neurons, leading to progressive weakness, spasticity, and sensory loss; and most Friedreich patients are wheelchair-bound by the age of 20 (Dun and Brice, *Curr Opin Neurol* (2000) 13:407-413). Furthermore, most patients with Friedreich ataxia develop cardiac abnormalities (e.g., left ventricular hypertrophy), resulting in death from heart failure by the age of about 30 to 40 in 60% of patients. The development of gene therapies for the treatment of Friedreich ataxia have been hindered by the difficulty associated with achieving expression of therapeutically effective amounts of frataxin in affected tissues, and currently no approved disease-modifying treatments exist. There remains a need for a set of compositions and methods that address these hindrances.

Summary

The present disclosure provides compositions and methods that can be used for treating Frederich ataxia. Using the compositions and methods of the disclosure, a patient (e.g., a mammalian patient, such as a human patient) having Frederich ataxia may be administered a plasmid (e.g., a viral vector) that contains a human frataxin gene (h*FXN*) or an RNA equivalent thereof.

In one example, the disclosure provides a DNA polynucleotide encoding h*FXN*, or an RNA equivalent thereof, wherein the polynucleotide has a nucleic acid sequence that is at least 95% (e.g., 95%, 96%, 97%, 98%, or 99%) identical to the nucleic acid sequence of SEQ ID NO: 1. In one aspect, the disclosure provides 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.

In another example, the disclosure provides a vector including the composition of the foregoing example, wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector. In one aspect, the disclosure provides a vector comprising the polynucleotide disclosed herein, optionally wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector. For

example, in some examples, the vector is a viral vector. In some examples, the viral vector is an adeno-associated virus (AAV).

In some examples of the foregoing aspect, the polynucleotide is operably linked to a muscle specific promoter, optionally wherein the promoter is positioned 5' to the polynucleotide.

In some examples, the vector further includes a polyadenylation site (pA), optionally wherein the pA is positioned 3' to the polynucleotide.

In some examples, the vector further includes an intron, optionally wherein the intron is positioned 3' to the promoter and 5' to the polynucleotide.

In some examples, 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.

In some examples, the length of the nucleic acid between ITR1 and ITR2 is from about 3.7 Kb to about 4.3 Kb (e.g., about 3.8 Kb to about 4.2 Kb or about 3.9 Kb to about 4.1 Kb). For example, in some examples, the length of the nucleic acid between ITR1 and ITR2 is from about 3.8 Kb to about 4.2 Kb. In some examples, the length of the nucleic acid between ITR1 and ITR2 is from about 3.9 Kb to about 4.1 Kb. In some examples, the length of the nucleic acid between ITR1 and ITR2 is about 4.0 Kb.

In another example, the disclosure provides a plasmid encoding the viral vector of the foregoing example. In one aspect, the disclosure provides a plasmid encoding the viral vector disclosed herein.

In another example, the disclosure provides a nucleic acid molecule including: an ITR1; a h*FXN* or an RNA equivalent thereof; and 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 (e.g., about 3.8 Kb to about 4.2 Kb or about 3.9 Kb to about 4.1 Kb). In one aspect, the disclosure provides 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. For example, in some examples, the length of the nucleic acid between ITR1 and ITR2 is from about 3.8 Kb to about 4.2 Kb. In some examples, the length of the nucleic acid between ITR1 and ITR2 is from about 3.9 Kb to about 4.1 Kb. In some examples, the length of the nucleic acid between ITR1 and ITR2 is about 4.0 Kb.

In some examples of any of the foregoing aspects, the length of the nucleic acids between and including ITR1 and ITR2 is about 3.9 Kb to about 4.7 Kb (e.g., about 4.0 Kb to about 4.6 Kb, about 4.1 Kb to about 4.5 Kb, about 4.2 Kb to about 4.4 Kb, or about 4.3 Kb). For example, in some examples, the length of the nucleic acids between and including ITR1 and ITR2 is about 4.0 Kb to about 4.6 Kb. In some examples, the length of the nucleic acids between and including ITR1 and ITR2 is about 4.1 Kb to about 4.5 Kb. In some examples, the length of the nucleic acids between and including ITR1 and ITR2 is about 4.2 Kb to about 4.4 Kb. In some examples, the length of the nucleic acids between and including ITR1 and ITR2 is about 4.3 Kb.

In some examples, the nucleic acid molecule further includes: 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.

In some examples, the P_{Euk} is a muscle specific promoter.

In some examples of any of the foregoing aspects, the muscle specific promoter 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, a cytomegalovirus promoter, or a chicken-β-actin promoter. For example, in some examples, the muscle specific promoter is a PGK promoter.

In some examples of any of the foregoing aspects, the PGK promoter has a nucleic acid sequence that is at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the nucleic acid of sequence of SEQ ID NO: 2. For example, in some examples, 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. In some examples, 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. In some examples, the PGK promoter has a nucleic acid of SEQ ID NO: 2.

In some examples, the nucleic acid molecule further includes: 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.

In some examples of any of the foregoing aspects, the pA site includes 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. For example, in some examples, the pA site includes the SV40 late polyadenylation site.

In some examples, the nucleic acid molecule further includes: 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.

In some examples of any of the foregoing aspects, the intron is an SV40 intron.

In some examples, the h*FXN*, or an RNA equivalent thereof, encodes a protein that is at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequence of SEQ ID NO: 3. For example, in some examples, the h*FXN*, or an RNA equivalent thereof, encodes a protein that is at least 90% identical to the amino acid sequence of SEQ ID NO: 3, optionally wherein the h*FXN*, or an RNA equivalent thereof, encodes a protein that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 3. In some examples, the h*FXN*, or an RNA equivalent thereof, encodes a protein having the amino acid sequence of SEQ ID NO: 3.

In some examples of any of the foregoing aspects, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the nucleic acid sequence of SEQ ID NO: 1. For example, in some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 86% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 87% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof,

has a nucleic acid sequence that is at least 88% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 89% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 91% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 92% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 93% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 94% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the h*FXN*, or an RNA equivalent thereof, has the nucleic acid sequence of SEQ ID NO: 1.

In another example, the disclosure provides a vector including the composition or the nucleic acid molecule of any of the foregoing examples, wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector. For example, in some examples, the vector is a viral vector. In one aspect, the disclosure provides a vector comprising the nucleic acid molecule disclosed herein, optionally wherein the vector is a plasmid, a DNA vector, an RNA vector, a virion, or a viral vector.

In some examples of any of the foregoing aspects, 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. For example, in some examples, the viral vector is an AAV. In some examples, the AAV is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAVrh74 serotype. In some examples, the viral vector is a pseudotyped AAV. In some examples, the pseudotyped AAV is AAV2/8 or AAV2/9, optionally wherein the pseudotyped AAV is AAV2/8.

In some examples of any of the foregoing aspects, ITR1 and/or ITR2 is a parvoviral ITR. For example, in some examples, the parvoviral ITR is an AAV ITR. In some examples, the AAV ITR is an AAV serotype 2 ITR.

In some examples of any of the foregoing aspects, the AAV includes a recombinant capsid protein.

In some examples of any of the foregoing aspects, the vector has a nucleic acid sequence that is at least 85% e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the nucleic acid sequence of SEQ ID NO: 4. For example, in some examples, the vector has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID

NO: 4. In some examples, 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. In some examples, the vector has a nucleic acid of SEQ ID NO: 4.

In another example, the disclosure provides a plasmid encoding the viral vector of any one of the foregoing examples.

In some examples of the foregoing aspect, the plasmid further includes one or more spacers (SS), wherein the one or more SS is positioned 5' to ITR1 and/or 3' to ITR2. For example, in some examples, the plasmid includes two SS, wherein the two SS include a first spacer (SS1) and a second spacer (SS2), wherein SS1 is positioned 5' to ITR1 and the SS2 is positioned 3' to ITR2.

In some examples, the one or more SS does not include an open reading frame that is greater than 100 amino acids in length.

In some examples, the one or more SS does not include prokaryotic transcription factor binding sites.

In some examples of any of the foregoing aspects, SS1 is about 1.0 Kb to about 5.0 Kb (e.g., 1.5 Kb to about 4.5 Kb, about 2.0 Kb to about 4.0 Kb, or about 3.0 Kb) in length. For example, in some examples, SS1 is about 2.0 Kb to about 5.0 Kb in length.

In some examples of any of the foregoing aspects, SS2 is about 1.0 Kb to about 5.0 Kb (e.g., 1.5 Kb to about 4.5 Kb, about 2.0 Kb to about 4.0 Kb, or about 3.0 Kb) in length. For example, in some examples, SS2 is about 2.0 Kb to about 5.0 Kb in length.

In some examples, the plasmid further includes 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. For example, in some examples, the selectable marker gene is an antibiotic resistance gene.

In some examples, the plasmid further includes 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.

In another example, the disclosure provides a pharmaceutical composition including the composition, the nucleic acid molecule, the vector, or the plasmid of any of the foregoing examples and a pharmaceutically acceptable carrier, diluent, or excipient. In one aspect, the disclosure provides a pharmaceutical composition comprising the polynucleotide disclosed herein, the vector disclosed herein, or the plasmid disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient. In one aspect, the disclosure provides a pharmaceutical composition comprising the nucleic acid molecule disclosed herein, the vector disclosed herein, or the plasmid disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient.

In one aspect, the present disclosure provides use of the polynucleotide disclosed herein, the nucleic acid molecule disclosed herein, the vector disclosed herein, the plasmid disclosed herein, or the pharmaceutical composition disclosed herein, 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.

In another example, the disclosure provides a method of treating Friedreich Ataxia in a human patient in need thereof, the method including administering to the patient a therapeutically effective amount of the composition, the nucleic acid molecule, the vector, the plasmid, or the pharmaceutical composition of any of the foregoing examples.

In another example, the disclosure provides a method of increasing frataxin expression in a human patient diagnosed as having Friedreich Ataxia, the method including administering to the patient a therapeutically effective amount of the composition, the nucleic acid molecule, the vector, the plasmid, or the pharmaceutical composition of any of the foregoing examples.

In some examples, the patient is from 3 years of age to 17 years (e.g., 4 years of age to 16 years, 5 years of age to 15 years, 6 years of age to 14 years, 7 years of age to 13 years, 8 years of age to 12 years, 9 years of age to 11 years, or 10 years) of age.

In some examples, upon administering the composition, the nucleic acid molecule, the vector, the plasmid, or the pharmaceutical composition of any of the foregoing examples to the patient, the patient displays a change in whole blood frataxin levels, optionally wherein the patient displays the change in whole blood frataxin levels by about 12 weeks after administration.

In some examples, upon administering the composition, the nucleic acid molecule, the vector, the plasmid, or the pharmaceutical composition of any of the foregoing examples to the patient, the patient displays a reduction in Total Friedreich Ataxia Rating Scale (FARS) Score, optionally wherein the patient displays the reduction in Total FARS Score by about 12 weeks after administration.

In another example, the disclosure provides a kit including the composition, the nucleic acid molecule, the vector, the plasmid, or the pharmaceutical composition of any of the foregoing examples; and a package insert, wherein the package insert instructs a user of the kit to administer the composition or vector to a human patient diagnosed as having Friedreich Ataxia. In one aspect, the disclosure provides a kit comprising i) the polynucleotide disclosed herein, the nucleic acid molecule disclosed herein, the vector disclosed herein, the plasmid disclosed herein, or the pharmaceutical composition disclosed herein, 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.

Brief Description of the Drawings

FIG. 1 is a schematic depicting the probability of nucleic acid variations in residue positions 1-270, respectively, of the gene encoding human frataxin (*FXN*) variant 1 (H.*FXN*.WT). Using the Integrated DNA Technologies (IDT) and GENEWIZ (GeneWiz) codon optimization tools, residue optimizations were compared across databases and residue positions. Whilst residues 1-270 are depicted herein, codon optimization was performed across all residues of the human *FXN* variant 1 gene, including residues 1-633. The sequence at the top indicates the most frequent nucleic acid at the respective residue position across the datasets. Arrows indicate a residue chosen for modification in a codon-optimized human *FXN* variant 1 construct (abbreviated interchangeably as H.*FXN*.ATX.Co or h*FXN*co). For example, residue 12 denotes that wild-type cytosine (C) was changed to guanine (G).

FIG. 2 is a map of an exemplary pseudotyped adeno-associated virus (AAV) 2/8 (AAV2/8) viral vector for the expression of a codon-optimized human *FXN* variant 1 gene (h*FXN*co). From left to right, the shaded arrows represent a plasmid containing a nucleic acid molecule including from 5'-to-3' a first spacer, a first inverted terminal repeat (ITR1), a human phosphoglycerate kinase (hPGK) promoter, an h*FXN*co, a simian virus 40 (SV40) late polyadenylation site (SV4 LpA), a second ITR (ITR2), a prokaryotic origin of replication (ori), and a kanamycin (kan) selection gene, wherein the length between ITR1 and ITR2, including the payload (e.g., hPGK and h*FXN*co), is about 4.3 Kb in length.

FIGS. 3A and 3B are photographs and a graph, respectively, showing an anti-frataxin immunoflourescence expression-based potency assay. **FIG. 3A** is a set of photographs of cells derived from murine skeletal muscle (C2C12) stained for frataxin following their transduction with 1 x 10^7 (1e7) viral genome (vg)/cell of an AAV2/8 viral vector for expression of h*FXN*co. **FIG. 3B** is a graph depicting the frataxin immunofluorescence described in FIG. 3A as a function of the multiplicity of infection (MOI), as normalized to the intensity of a Hoechst counter-stain.