Claims

What is claimed is:

1. An *in vitro* method for editing the titin gene in a human cell by genome editing comprising introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within the titin gene that results in permanent correction of one or more mutations within the titin gene and restoration of titin protein activity.

2. Use of one or more deoxyribonucleic acid (DNA) endonucleases in the manufacture of a medicament for treating a patient with a titin-based cardiomyopathy or other titinopathy by editing the titin gene in endogenous cardiac stem cells (eCSC) or primary cardiomyocytes isolated from a heart biopsy of a patient with a titin-based cardiomyopathy or other titinopathy comprising:
i) using the medicament comprising one or more DNA endonucleases to edit the titin gene of the eCSC or primary cardiomyocytes; and wherein
ii) the genome edited eCSC or primary cardiomyocytes are to be implanted into the patient.

3. The use of claim 2, wherein the editing step comprises introducing into the eCSC or primary cardiomyocyte one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within the titin gene that results in permanent correction of one or more mutations within the titin gene and restoration of titin protein activity.

4. Use of one or more deoxyribonucleic acid (DNA) endonucleases in the manufacture of a medicament for treating a patient with a titin-based cardiomyopathy or other titinopathy comprising using the medicament comprising one or more DNA endonucleases to edit the titin gene in a cell of the patient.

5. The use of claim 4, wherein the editing step comprises introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within the titin gene that results in permanent correction of one or more mutations within the titin gene and restoration of titin protein activity.

6. The method of claim 1 or the use of any one of claims 3, 4 and 5, wherein the one or more DNA endonucleases is a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a homolog, codon-optimized, or modified version thereof.

7. The method or the use of claim 6, comprising introducing into the cell one or more polynucleotides encoding the DNA endonuclease.

8. The method or the use of claim 6, comprising introducing into the cell one or more ribonucleic acids (RNAs) encoding the DNA endonuclease.

9. The method or the use of claim 7 or 8, wherein the one or more polynucleotides or one or more RNAs is a modified polynucleotide or RNA.

10. The method or the use of any one of the preceding claims, further comprising introducing into the cell one or more guide ribonucleic acids (gRNAs).

11. The method or the use of claim 10, wherein the one or more DNA endonucleases is pre-complexed with one or more gRNAs or single-molecule guide RNA (sgRNAs).

12. The method or the use of any one of the preceding claims, further comprising introducing into the cell a polynucleotide donor template comprising a part of the wild-type titin gene or cDNA.

13. The method or the use of claim 12, wherein the part of the wild-type titin gene or cDNA is the entire titin gene or cDNA.

14. The method of claim 1 or the use of any one of the preceding claims, further comprising introducing into the cell one guide ribonucleic acid (gRNA) and a polynucleotide donor template comprising a part of the wild-type titin gene, and wherein the one or more DNA endonucleases is one or more Cas9 endonucleases that effect one double-strand break (DSB) at a DSB locus within the titin gene that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA at the DSB locus that results in permanent correction of a part of the chromosomal DNA of the titin gene proximal to the DSB locus and restoration of titin protein activity, and wherein the gRNA comprises a spacer sequence that is complementary to a segment of the DSB locus.

15. The method or the use of claim 14, wherein proximal means nucleotides both upstream and downstream of the DSB locus.

16. The method of claim 1 or the use of any one of the preceding claims, further comprising introducing into the cell two guide ribonucleic acid (gRNAs) and a polynucleotide donor template comprising a part of the wild-type titin gene, and wherein the one or more DNA endonucleases is two or more Cas9 endonucleases that effect a pair of double-strand breaks (DSBs), the first at a 5' DSB locus and the second at a 3' DSB locus, within the titin gene that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA between the 5' DSB locus and the 3' DSB locus that results in permanent correction of the chromosomal DNA between the 5' DSB locus and the 3' DSB locus within the titin gene and restoration of titin protein activity, and wherein the first guide RNA comprises a spacer sequence that is complementary to a segment of the 5' DSB locus and the second guide RNA comprises a spacer sequence that is complementary to a segment of the 3' DSB locus.

17. The method or the use of any one of claims 14-16, wherein the one or more DNA endonucleases is pre-complexed with one or two gRNAs or sgRNAs.

18. The method or the use of any one of claims 14-17, wherein the part of the wild-type titin gene or cDNA is the entire titin gene or cDNA.

19. The method or the use of claim 18, wherein the DSB, or 5' DSB and 3' DSB are in exon 219, intron 242, or both exon 219 and intron 242 of the titin gene.

20. The method of claim 1 or the use of any one of claims 2-4, wherein the gRNA or sgRNA is directed to one or more of the following pathological variants: c.32854G>C, c.37112G>A, c.43628insAT, p.EE3359 W33362delinsVKEK, c.62890delG, mutations located in exons 112-225 which are located in the PEVK region of titin, mutations in exon 48 (Novek 3 isoform), and mutations in the cardiac N2B isoform.

21. The method of claim 1 or the use of any one of claims 2-4, wherein the correction is by homology directed repair (HDR).

22. The method of claim 1 or the use of any one of claims 2-4, wherein the Cas9 mRNA, gRNA, and donor template are either each formulated separately into lipid nanoparticles or all co-formulated into a lipid nanoparticle.

23. The method of claim 1 or the use of any one of claims 2-4, wherein the Cas9 mRNA is formulated into a lipid nanoparticle, and both the gRNA and donor template are delivered by an adeno-associated virus (AAV).

24. The method or the use of any one of the preceding claims, wherein the titin gene is located on Chromosome 2q31 (Genome Reference Consortium – GRCh38/hg38).

25. One or more guide ribonucleic acids (gRNAs) comprising a spacer sequence selected from the group consisting of the sequences in the Figures for editing the titin gene in a cell from a patient with a cardiac myopathy or other titinopathy.

26. The one or more gRNAs of claim 25, wherein the one or more gRNAs are single-molecule guide RNAs (sgRNAs).