Claims

1. A guide RNA (gRNA) molecule, wherein the gRNA molecule comprises a targeting domain complementary to a target sequence, and the target sequence is located within an HBG1 promoter region located between chr11: 5,248,269 and 5,249,857 of human genome hg19, or within an HBG2 promoter region located between chr11: 5,253,188 and 5,254,781 of human genome hg19, wherein the target sequence is located between about 210 bp upstream and about 100 bp upstream of transcription initiation sites of the HBG1 and HBG2.
2. The gRNA molecule according to claim 1, wherein the gRNA molecule is a single guide RNA molecule (sgRNA).
3. The gRNA molecule according to claim 2, wherein (1) a CRISPR/Cas system comprising the gRNA molecule is contacted with the target sequence, or (2) a CRISPR/Cas system comprising the gRNA molecule is introduced into a cell in which a gene comprising the target sequence is located, enabling a base insertion or deletion (indel) formed at or near the target sequence; wherein the CRISPR/Cas system comprises a Cas12b nuclease and/or a functionally active fragment thereof.
4. The gRNA molecule according to claim 3, comprising a nucleotide sequence selected from any one of SEQ ID NOs: 12-59 and 90.
5. A nucleic acid molecule encoding the gRNA molecule according to claim 1.
6. A composition comprising:

(1) one or more gRNA molecules according to claim 1, and a nuclease of a CRISPR/Cas system, wherein the one or more gRNA molecules and the nuclease of a CRISPR/Cas system are present in a ribonucleoprotein (RNP) complex;

(2) a nucleic acid encoding one or more gRNA molecules according to claim 1, and a nuclease of a CRISPR/Cas system;

(3) one or more gRNA molecules according to claim 1, and a nucleic acid encoding a nuclease of a CRISPR/Cas system; or

(4) a nucleic acid encoding one or more gRNA molecules according to claim 1, and a nucleic acid encoding a nuclease of a CRISPR/Cas system;

wherein the composition further comprises a pharmaceutically acceptable carrier.

7. The composition according to claim 6, wherein the nuclease is a class II type V Cas enzyme.

8. The composition according to claim 7, wherein the nuclease is a Cas12b nuclease or a variant thereof, an ortholog thereof and/or a functionally active fragment thereof.

9. The composition according to claim 8, wherein the Cas12b nuclease is a Cas12b nuclease from *Alicyclobacillus acidiphilus* (AaCas12b).

10. The composition according to claim 9, wherein the AaCas12b comprises one or more of the following mutations relative to wild-type AaCas12b nuclease:

(1) a substitution of one or more amino acid residues in the wild-type AaCas12b nuclease that interact with a protospacer adjacent motif (PAM) with a positively charged amino acid residue(s);

(2) a substitution of one or more amino acid residues involved in opening a DNA double strand in the wild-type AaCas12b nuclease with an amino acid residue(s) having an aromatic ring; and/or

(3) a substitution of one or more amino acid residues in RuvC domain of the wild-type AaCas12b nuclease that interact with a single-stranded DNA substrate with a positively charged amino acid residue(s) or a hydrophobic amino acid residue(s),

wherein the amino acid sequence of the wild-type AaCas12b nuclease is as set forth in SEQ ID NO: 91.

11. The composition according to claim 10, wherein the AaCas12b comprises amino acid residue substitutions relative to the wild-type AaCas12b nuclease at one or more of the following positions:

(1) positions located on the one or more amino acid residues that interact with PAM: 116, 123, 130, 132, 144, 145, 153, 173, 222, 395, 400 and 475;

(2) positions located on the one or more amino acid residues involved in opening the DNA double strand: 118 and 119; and/or

(3) positions located on the one or more amino acid residues in RuvC domain that interact with a single-stranded DNA substrate: 300, 301, 304, 329, 636, 639, 647, 682, 757, 758, 761, 764, 768, 852, 854, 856, 857, 858, 860, 862, 863, 865, 866, 867, 869, 938, 956, 957, 958, 994, 1093 and 1097;

and wherein the amino acid residues are numbered according to SEQ ID NO: 91.

12. The composition according to claim 10, wherein the AaCas12b comprises one or more of the following amino acid residue substitutions relative to the wild-type AaCas12b nuclease:

(1) one or more of substitutions of amino acid residues in the wild-type AaCas12b nuclease that interact with PAM with a positively charged amino acid residue(s): D116R and E475R;

(2) one or more substitutions of amino acid residues involved in opening the DNA double strand in the wild-type AaCas12b nuclease with an amino acid residue(s) having an aromatic ring: Q119Y, Q119F or Q119W; and/or

(3) one or more substitutions of amino acid residues in RuvC domain of the wild-type AaCas12b nuclease that interact with a single-stranded DNA substrate: E636R, Q639R, T647R, Q682R, I757R, E758R, E761R, Q854R, N857K, D858R, I994R, Q1093R and W1097R;

and wherein the amino acid residues are numbered according to SEQ ID NO: 91.

13. The composition according to claim 8, wherein the gRNA molecule comprises a nucleotide sequence set forth in any one of SEQ ID NOs: 12-59 and 90.

14. The composition according to claim 6, wherein the N-terminus and/or C-terminus of the nuclease is linked to one or more nuclear localization signals.

15. The composition according to claim 6, wherein the nuclease comprises an amino acid sequence selected from any one of SEQ ID NOs: 1-8.

16. A cell, comprising the composition according to claim 6.

17. A cell population or progeny thereof, comprising a gene product produced by administration of the composition according to claim 6, wherein the gene product comprises an HBG gene having one or more modifications, wherein the modification is a base insertion or deletion (indel) at or near a target sequence; wherein the target sequence is located within an HBG1 promoter region between chr11: 5,248,269 and 5,249,857 of human genome hg19, or within an HBG2 promoter region between chr11: 5,253,188 and 5,254,781 of human genome hg19, wherein the target sequence is located between about 210 bp upstream and about 100 bp upstream of transcription initiation sites of the HBG1 and HBG2.

18. A kit, comprising the composition according to claim 6.

19. A method for regulating fetal hemoglobin (HbF) expression in a cell, cell population or progeny thereof, comprising administering the composition according to claim 6, wherein the cell or cell population is a hematopoietic stem/progenitor cell (HSPC).

20. A method for treating, preventing or alleviating a hemoglobinopathy and/or related condition thereof, comprising administering to a subject in need thereof an effective amount of the composition according to claim 6, wherein the hemoglobinopathy and/or related condition thereof is selected from: sickle cell disease, sickle cell anemia, hemoglobin C disease, hemoglobin C trait, hemoglobin S/C disease, hemoglobin D disease, hemoglobin E disease, thalassemia, a hemoglobinopathy with decreased oxygen affinity, and an unstable hemoglobinopathy.