

double strand break in the absence of an externally supplied repair template (*e.g.* “donor” or “transgene”) is commonly used for the inactivation of the targeted gene via mutations (insertions and/or deletions known as “indels”) introduced by the cellular NHEJ pathway. For instance, U.S. Patent Publication 20110082093 discloses specific zinc finger proteins targeted to Htt and U.S. Patent Publication No. 20130253040 relates to DNA-binding proteins that modulate expression of an HD allele such as Htt. U.S. Publication No. 20150335708 relates to methods of modifying medium spiny neurons.

[0011] However, there remains a need for methods for the diagnosis, study, treatment and/or prevention of Huntington’s Disease, including for modalities that exhibit widespread delivery to the brain.

SUMMARY

[0012] In one aspect, the present disclosure refers to a non-naturally occurring zinc finger protein that binds to an Htt gene, the zinc finger protein comprising 5 zinc finger domains ordered F1 to F5, wherein the zinc finger domains comprise the recognition helix regions sequences ordered as shown in a single row of Table 1. In another aspect, the present disclosure refers to a fusion protein comprising a zinc finger protein of claim 1 and a functional domain, wherein the functional domain is a transcriptional repression domain or a nuclease domain. In another aspect, the present disclosure refers to a polynucleotide encoding one or more zinc finger proteins as described herein. In another aspect, the present disclosure refers to a polynucleotide encoding one or more fusion proteins as described herein. In another aspect, there is provided an AAV vector comprising the polynucleotide as described herein. In another aspect, there is provided an AAV vector comprising the polynucleotide also described herein. In another aspect, the present disclosure refers to a host cell comprising one or more zinc finger proteins, one or more fusion proteins, a polynucleotide and/or an AAV vector as described herein. In another aspect, the present disclosure refers to a pharmaceutical composition comprising one or more polynucleotides as described herein and/or one or more AAV vectors as described herein. In another aspect, the present disclosure refers to a pharmaceutical composition comprising one or more polynucleotides also described herein and/or one or more AAV vectors also described herein. In another aspect, the present disclosure refers to an *in vitro* method of modifying expression of an Htt gene in a cell, the

method comprising administering to the cell one or more polynucleotides, one or more AAV vectors and/or one or more pharmaceutical compositions as described herein. In yet another aspect, the present disclosure refers the use of a composition in the manufacture of a medicament for treating and/or preventing Huntington's Disease

5 (HD) in a subject in need thereof, the composition comprising one or more polynucleotides, one or more AAV vectors and/or one or more pharmaceutical compositions as described herein. In one example, the present disclosure describes a non-naturally occurring zinc finger protein that binds to an Htt gene, the zinc finger protein comprising 5 zinc finger domains ordered F1 to F5, wherein the zinc finger

10 domains comprise the recognition helix regions sequences shown in a single row of Table 1. In another example, the present disclosure describes a fusion protein comprising a zinc finger protein of the present invention and a functional domain. In yet another example, the present disclosure describes a polynucleotide encoding one or more zinc finger proteins of any of the present disclosure. In yet another example,

15 the present disclosure describes an AAV vector comprising the polynucleotide of the present disclosure. In yet another example, the present disclosure describes a host cell comprising one or more zinc finger proteins of the present disclosure, a polynucleotide of the present disclosure and/or an AAV vector of the present disclosure. In yet another example, the present disclosure describes a pharmaceutical

20 composition comprising one or more polynucleotides according to the present disclosure and/or one or more AAV vectors according to the present disclosure. In yet another example, the present disclosure describes a method of modifying expression of an Htt gene in a cell, the method comprising administering to the cell one or more polynucleotides according to the present disclosure, one or more AAV vectors

25 according to the present disclosure and/or one or more pharmaceutical compositions according the present disclosure. In yet another example, the present disclosure describes the use of a composition in the manufacture of a medicament for treating and/or preventing Huntington's Disease in a subject in need thereof, the composition comprising one or more polynucleotides according to the present disclosure, one or

30 more AAV vectors according to the present disclosure and/or one or more pharmaceutical compositions according to the present disclosure.

Disclosed herein are methods and compositions for diagnosing, preventing and/or treating Huntington's Disease. In particular, provided herein are methods and compositions for modifying (*e.g.*, modulating expression of) an HD Htt allele so as to treat Huntington Disease, including Htt repressors (that repress Htt expression). The compositions (Htt repressors) described herein provide a therapeutic benefit in subjects, for example by reducing cell death, decreasing apoptosis, increasing cellular function (metabolism) and/or reducing motor deficiency in the subjects. Also provided are methods and compositions that allow for bi-directional axonal transport in a primate brain. Surprisingly and unexpectedly, the present inventors have found that unlike other AAV serotypes that have been used, AAV9 exhibits widespread delivery throughout the brain, including anterograde and retrograde axonal transport to brain regions distant from the site of AAV9 administration. Thus, described herein is a non-naturally occurring zinc finger protein that binds to an Htt gene, the zinc finger protein comprising 5 zinc finger domains ordered F1 to F5, wherein the zinc finger domains comprise the recognition helix regions sequences shown in a single row of Table 1.

[0013] Thus, in one example, engineered (non-naturally occurring) Htt repressors are provided. The repressors may comprise systems (*e.g.*, zinc finger proteins, TAL effector (TALE) proteins or CRISPR/dCas-TF) that modulate expression of a HD allele (*e.g.*, Htt). Engineered zinc finger proteins or TALEs are

CLAIMS

What is claimed is:

- 5 1. A non-naturally occurring zinc finger protein that binds to an Htt gene, the zinc finger protein comprising 5 zinc finger domains ordered F1 to F5, wherein the zinc finger domains comprise the recognition helix regions sequences ordered as shown in a single row of Table 1.
- 10 2. A fusion protein comprising a zinc finger protein of claim 1 and a functional domain, wherein the functional domain is a transcriptional repression domain or a nuclease domain.
3. A polynucleotide encoding one or more zinc finger proteins of claim 1.
- 15 4. A polynucleotide encoding one or more fusion proteins of claim 2.
5. An AAV vector comprising the polynucleotide of claim 3.
- 20 6. An AAV vector comprising the polynucleotide of claim 4.
7. A host cell comprising one or more zinc finger proteins of claim 1, one or more fusion proteins of claim 2, a polynucleotide of claim 3 or claim 4 and/or an AAV vector of claim 5 or claim 6.
- 25 8. A pharmaceutical composition comprising one or more polynucleotides according to claim 3 and/or one or more AAV vectors according to claim 5.
9. A pharmaceutical composition comprising one or more polynucleotides according to claim 4 and/or one or more AAV vectors according to claim 6.
- 30 10. An *in vitro* method of modifying expression of an Htt gene in a cell, the method comprising administering to the cell one or more polynucleotides according to

claim 4, one or more AAV vectors according to claim 6 and/or one or more pharmaceutical compositions according to claim 9.

5 11. The *in vitro* method of claim 10, wherein the Htt gene comprises at least wild-type and/or one mutant allele.

12. The *in vitro* method of any claim 10 or claim 11, wherein the fusion protein comprises a repression domain and expression of the Htt gene is repressed.

10 13. The *in vitro* method of any of claims 10 to 112, wherein the cell is a neuronal cell.

14. The polynucleotide according to claim 4, the AAV vector according to claim 6 and/or the pharmaceutical composition according to claim 9 for use in
15 therapy.

15. Use of a composition in the manufacture of a medicament for treating and/or preventing Huntington's Disease (HD) in a subject in need thereof, the composition comprising one or more polynucleotides according to claim 4, one or
20 more AAV vectors according to claim 6 and/or one or more pharmaceutical compositions according to claim 9.