

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 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 the present invention and a functional domain. In yet another aspect, the present invention refers to a polynucleotide encoding one or more zinc finger proteins of any of the present invention. In yet another aspect, the present invention refers to an AAV vector comprising the polynucleotide of the present invention. In yet another aspect, the present invention refers to a host cell comprising one or more zinc finger proteins of the present invention, a polynucleotide of the present invention and/or an AAV vector of the present invention. In yet another aspect, the present invention refers to a pharmaceutical composition comprising one or more polynucleotides according to the present invention and/or one or more AAV vectors according to the present invention. In yet another aspect, the present invention refers to 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 invention, one or more AAV vectors according to the present invention and/or one or more pharmaceutical compositions according to the present invention. In yet another aspect, the present invention refers to 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 invention, one or more AAV vectors according to the present invention and/or one or more pharmaceutical compositions according to the present invention. Disclosed herein are methods and compositions for diagnosing, preventing and/or treating

5 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
10 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
15 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.

20 **[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

non-naturally occurring zinc finger or TALE proteins whose DNA binding domains (e.g., recognition helices or RVDs) have been altered (e.g., by selection and/or rational design) to bind to a pre-selected target site. Any of the zinc finger proteins described herein may include 1, 2, 3, 4, 5, 6 or more zinc fingers, each zinc finger having a recognition helix that binds to a target subsite in the selected sequence(s) (e.g., gene(s)). Similarly, any of the TALE proteins described herein may include any number of TALE RVDs. In some examples, at least one RVD has non-specific DNA binding. In some examples, at least one recognition helix (or RVD) is non-naturally occurring. In certain examples, the zinc finger proteins have the recognition helices in the proteins designated 45643 or 46025 (Table 1). In certain examples, the repressor comprises a DNA-binding domain (ZFP, TALE, single guide RNA) operably linked to a transcriptional repression domain. In some examples these ZFP-TFs, CRISPR/dCas-TFs or TALE-TFs include protein interaction domains (or “dimerization domains”) that allow multimerization when bound to DNA.

[0014] In certain examples, the zinc finger proteins (ZFPs), Cas protein of a CRISPR/Cas system or TALE proteins as described herein can be placed in operative linkage with a regulatory domain (or functional domain) as part of a fusion protein. The functional domain can be, for example, a transcriptional activation domain, a transcriptional repression domain and/or a nuclease (cleavage) domain. By selecting either an activation domain or repression domain for use with the DNA-binding domain, such molecules can be used either to activate or to repress gene expression. In some examples, a molecule comprising a ZFP, dCas or TALE targeted to a mutant Htt as described herein fused to a transcriptional repression domain that can be used to down-regulate mutant Htt expression is provided. In some examples, a fusion protein comprising a ZFP, CRISPR/Cas or TALE targeted to a wild-type Htt allele fused to a transcription activation domain that can up-regulate the wild type Htt allele is provided. In certain examples, the activity of the regulatory domain is regulated by an exogenous small molecule or ligand such that interaction with the cell’s transcription machinery will not take place in the absence of the exogenous ligand, while in other examples, the exogenous small molecule or ligand prevents the interaction. Such external ligands control the degree of interaction of the ZFP-TF, CRISPR/Cas-TF or TALE-TF with the transcription

machinery. The regulatory domain(s) may be operatively linked to any portion(s) of one or more of the ZFPs, dCas or TALEs, including between one or more ZFPs, dCas or TALEs, exterior to one or more ZFPs, dCas or TALEs and any combination thereof. Any of the fusion proteins described herein may be formulated into a pharmaceutical composition.

[0015] In some examples, the engineered DNA binding domains as described herein can be placed in operative linkage with nuclease (cleavage) domains as part of a fusion protein. In some examples, the nuclease comprises a Ttgo nuclease. In other examples, nuclease systems such as the CRISPR/Cas system may be utilized with a specific single guide RNA to target the nuclease to a target location in the DNA. In certain examples, such nucleases and nuclease fusions may be utilized for targeting mutant Htt alleles in stem cells such as induced pluripotent stem cells (iPSC), human embryonic stem cells (hESC), mesenchymal stem cells (MSC) or neuronal stem cells wherein the activity of the nuclease fusion will result in an Htt allele containing a wild type number of CAG repeats. Thus, any of the Htt repressors described herein can further comprise a dimerization domain and/or a functional domain (*e.g.*, transcriptional activation domain, a transcriptional repression domain or a nuclease domain). In certain examples, pharmaceutical compositions comprising the modified cells (*e.g.*, stem cells) are provided.

[0016] In yet another example, a polynucleotide encoding one or more of the DNA binding proteins described herein is provided. In certain examples, the polynucleotide is carried on a viral (*e.g.*, AAV or Ad) vector and/or a non-viral (*e.g.*, plasmid or mRNA vector). Host cells comprising these polynucleotides (*e.g.*, AAV vectors) and/or pharmaceutical compositions comprising the polynucleotides, proteins and/or host cells as described herein are also provided.

[0017] In other examples, the disclosure comprises delivery of a donor nucleic acid to a target cell. The donor may be delivered prior to, after, or along with the nucleic acid encoding the nuclease(s). The donor nucleic acid may comprise an exogenous sequence (transgene) to be integrated into the genome of the cell, for example, an endogenous locus. In some examples, the donor may comprise a full length gene or fragment thereof flanked by regions of homology with the targeted cleavage site. In some examples, the donor lacks homologous regions and is integrated into a target locus through homology independent mechanism (*i.e.* NHEJ).

The donor may comprise any nucleic acid sequence, for example a nucleic acid that, when used as a substrate for homology-directed repair of the nuclease-induced double-strand break, leads to a donor-specified deletion to be generated at the endogenous chromosomal locus or, alternatively (or in addition to), novel allelic forms of (*e.g.*, point mutations that ablate a transcription factor binding site) the endogenous locus to be created. In some examples, the donor nucleic acid is an oligonucleotide wherein integration leads to a gene correction event, or a targeted deletion.

[0018] In some examples, the polynucleotide encoding the DNA binding protein is an mRNA. In some examples, the mRNA may be chemically modified (*See e.g.* Kormann *et al*, (2011) *Nature Biotechnology* 29(2):154-157). In other examples, the mRNA may comprise an ARCA cap (see U.S. Patents 7,074,596 and 8,153,773). In further examples, the mRNA may comprise a mixture of unmodified and modified nucleotides (see U.S. Patent Publication 2012-0195936).

[0019] In yet another example, a gene delivery vector comprising any of the polynucleotides described herein is provided. In certain examples, the vector is an adenovirus vector (*e.g.*, an Ad5/F35 vector), a lentiviral vector (LV) including integration competent or integration-defective lentiviral vectors, or an adenovirus associated viral vector (AAV). In certain examples, the AAV vector is an AAV6 or AAV9 vector. Thus, also provided herein are adenovirus (Ad) vectors, LV or adenovirus associate viral vectors (AAV) comprising a sequence encoding at least one nuclease (ZFN or TALEN) and/or a donor sequence for targeted integration into a target gene. In certain examples, the Ad vector is a chimeric Ad vector, for example an Ad5/F35 vector. In certain examples, the lentiviral vector is an integrase-defective lentiviral vector (IDLV) or an integration competent lentiviral vector. In certain examples the vector is pseudo-typed with a VSV-G envelope, or with other envelopes.

[0020] Additionally, pharmaceutical compositions comprising the nucleic acids and/or proteins (*e.g.*, ZFPs, Cas or TALEs or fusion proteins comprising the ZFPs, Cas or TALEs) are also provided. For example, certain compositions include a nucleic acid comprising a sequence that encodes one of the ZFPs, Cas or TALEs described herein operably linked to a regulatory sequence, combined with a pharmaceutically acceptable carrier or diluent, wherein the regulatory sequence

allows for expression of the nucleic acid in a cell. In certain examples, the ZFPs, CRISPR/Cas or TALEs encoded are specific for a HD Htt allele. In some examples, pharmaceutical compositions comprise ZFPs, CRISPR/Cas or TALEs that modulate a HD Htt allele and ZFPs, CRISPR/Cas or TALEs that modulate a neurotrophic factor.

- 5 Protein based compositions include one of more ZFPs, CRISPR/Cas or TALEs as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[0021] In yet another example also provided is an isolated cell comprising any of the proteins, polynucleotides and/or compositions as described herein.

- [0022]** In another example, described herein are methods of modifying
10 expression of an Htt gene in a cell (*e.g.*, neuronal cell *in vitro* or *in vivo* in a brain of a subject, *e.g.*, the striatum), the method comprising administering to the cell one or more proteins, polynucleotides and/or cells as described herein. The Htt gene may comprise at least one wild-type and/or mutant Htt allele. In certain examples, Htt expression is repressed.

- 15 **[0023]** In another example, provided herein are methods for treating and/or preventing Huntington's Disease using the methods and compositions (proteins, polynucleotides and/or cells) described herein. In some examples, the methods involve compositions where the polynucleotides and/or proteins may be delivered using a viral vector, a non-viral vector (*e.g.*, plasmid) and/or combinations thereof. In some examples,
20 the methods involve compositions comprising stem cell populations comprising a ZFP or TALE, or altered with the ZFNs, TALENs, Ttago or the CRISPR/Cas nuclease system of the disclosure. The subject may comprise at least one mutant and/or wild-type Htt allele.

- [0024]** In a still further example, described here is a method of delivering a
25 repressor of Htt to the brain of the subject using an AAV (*e.g.*, AAV9) vector. Delivery may be to any brain region, for example, the striatum (*e.g.*, putamen) by any suitable means including via the use of a cannula. In some examples, delivery is through direct injection into the intrathecal space. In further examples, delivery in through intravenous injection. The AAV9 vector provides widespread delivery of the
30 repressor to brain of the subject, including via anterograde and retrograde axonal transport to brain regions not directly administered the vector (*e.g.*, delivery to the putamen results in delivery to other structures such as the cortex, substantia nigra,

thalamus, etc. In certain examples, the subject is a human and in other examples, the subject is a non-human primate.

[0025] Thus, in other examples, described herein is a method of preventing and/or treating HD in a subject, the method comprising administering a repressor of a mutant Htt allele to the subject. The repressor may be administered in polynucleotide form (*e.g.*, using a viral (*e.g.*, AAV) and/or non-viral vector (*e.g.*, plasmid and/or mRNA), in protein form and/or via a pharmaceutical composition as described herein (*e.g.*, a pharmaceutical compositions comprising a polynucleotide, AAV vector, protein and/or cell as described herein). In certain examples, the repressor is administered to the CNS (*e.g.*, putamen) of the subject. The repressor may provide therapeutic benefits, including, but not limited to, reducing the formation of Htt aggregates in HD neurons of a subject with HD; reducing cell death in a neuron or population of neurons (*e.g.*, an HD neuron or population of HD neurons); and/or reducing motor deficits (*e.g.*, claspings) in HD subjects.

[0026] In any of the methods described herein, the repressor of the mutant Htt allele may be a ZFP-TF, for example a fusion protein comprising a ZFP that binds specifically to a mutant Htt allele and a transcriptional repression domain (*e.g.*, KOX, KRAB, etc.). In other examples, the repressor of the mutant Htt allele may be a TALE-TF, for example a fusion protein comprising a TALE polypeptide that binds specifically to a mutant Htt allele and a transcriptional repression domain (*e.g.*, KOX, KRAB, etc.). In some examples, the mutant Htt allele repressor is a CRISPR/Cas-TF where the nuclease domains in the Cas protein have been inactivated such that the protein no longer cleaves DNA. The resultant Cas RNA-guided DNA binding domain is fused to a transcription repressor (*e.g.* KOX, KRAB etc.) to repress the mutant Htt allele. In still further examples, the repressor may comprise a nuclease (*e.g.*, ZFN, TALEN and/or CRISPR/Cas system) that represses the mutant Htt allele by cleaving and thereby inactivating the mutant Htt allele. In certain examples, the nuclease introduces an insertion and/or deletion (“indel”) via non-homologous end joining (NHEJ) following cleavage by the nuclease. In other examples, the nuclease introduces a donor sequence (by homology or non-homology directed methods), in which the donor integration inactivates the mutant Htt allele.

[0027] In any of the methods described herein, the repressor may be delivered to the subject (*e.g.*, brain) as a protein, polynucleotide or any combination of protein and polynucleotide. In certain examples, the repressor(s) is(are) delivered using an AAV (*e.g.*, AAV9) vector. In other examples, at least one component of the repressor (*e.g.*, sgRNA of a CRISPR/Cas system) is delivered as in RNA form. In other examples, the repressor(s) is(are) delivered using a combination of any of the expression constructs described herein, for example one repressor (or portion thereof) on one expression construct (*e.g.*, AAV such as AAV9) and one repressor (or portion thereof) on a separate expression construct (AAV or other viral or non-viral construct).

[0028] Furthermore, in any of the methods described herein, the repressors can be delivered at any concentration (dose) that provides the desired effect. In preferred examples, the repressor is delivered using an adeno-associated virus vector at 10,000 - 500,000 vector genome/cell (or any value therebetween). In certain examples, the repressor is delivered using a lentiviral vector at MOI between 250 and 1,000 (or any value therebetween). In other examples, the repressor is delivered using a plasmid vector at 150-1,500 ng/100,000 cells (or any value therebetween). In other examples, the repressor is delivered as mRNA at 150-1,500 ng/100,000 cells (or any value therebetween).

[0029] In any of the methods described herein, the method can yield about 70% or greater, about 75% or greater, about 85% or greater, about 90% or greater, about 92% or greater, or about 95% or greater repression of the mutant Htt alleles in one or more HD neurons of the subject.

[0030] In further examples, the disclosure described herein comprises one or more Htt-modulating transcription factors, such as a Htt-modulating transcription factors comprising one or more of a zinc finger protein (ZFP TFs), a TALEs (TALE-TF), and a CRISPR/Cas-TFs for example, ZFP-TFs, TALE-TFs or CRISPR/Cas-TFs. In certain examples, the Htt-modulating transcription factor can repress expression of a mutant Htt allele in one or more HD neurons of a subject. The repression can be about 70% or greater, about 75% or greater, about 85% or greater, about 90% or greater, about 92% or greater, or about 95% or greater repression of the mutant Htt alleles in the one or more HD neurons of the subject as compared to untreated (wild-type) neurons of the subject. In certain examples, the Htt-

modulating transcription factor can be used to achieve one or more of the methods described herein.

[0031] In some examples, therapeutic efficacy is measured using the Unified

Huntington's Disease Rating Scale (UHDRS) (Huntington Study Group (1996) *Mov*

Disord 11(2):136-142) for analysis of overt clinical symptoms. In other examples,

efficacy in patients is measured using PET and MRI imaging. In some examples,

treatment with the mutant Htt modulating transcription factor prevents any further

development of overt clinical symptoms and prevents any further loss of neuron

functionality. In other examples, treatment with the mutant Htt modulating

transcription factor improves clinical symptoms and improves neuron function.

[0032] Also provided is a kit comprising one or more of the AAV9 Htt-

modulators (*e.g.*, repressors) and/or polynucleotides comprising components of and/or

encoding the Htt-modulators (or components thereof) as described herein. The kits

may further comprise cells (*e.g.*, neurons), reagents (*e.g.*, for detecting and/or

quantifying mHtt protein, for example in CSF) and/or instructions for use, including

the methods as described herein.

[0033] Thus, the present disclosure encompasses, but is not limited to, the

following numbered examples:

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 shown in a

single row of Table 1.

2. The Htt repressor of 1, further comprising a dimerization domain that allows multimerization of zinc finger proteins when bound to DNA.

3. A fusion protein comprising a zinc finger protein of 1 or 2 and a functional domain.

4. The fusion protein of 3, wherein the functional domain is selected from the group consisting of a transcriptional activation domain, a transcriptional repression domain, and a nuclease domain.

5. A polynucleotide encoding one or more zinc finger proteins of 1 to 2 or one or more fusion proteins of 3 or 4.

6. An AAV vector comprising the polynucleotide of 5.

25. A method of treating and/or preventing Huntington's Disease in a subject in need thereof, the method comprising administering to the subject in need thereof one or more AAV9 vectors, the AAV9 vectors encoding one or more Htt repressors. [0034] These and other examples will be readily apparent to the skilled artisan in light of disclosure as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **Figures 1A through 1J** are images depicting results following infusion of AAV9 vectors into NHP putamen. Comparative panels for the MRI-guided infusion of AAV9-GFP (100 μ L) into the bilateral putamen of 2 normal nonhuman primates (designated NHP-1 and NHP-2) are shown in Figures 1A and 1B and 3 dimensional reconstructs of each subject are shown in Figures 1C and 1D. Reconstructed volumes for each putamen (n=4), labeled in blue measured approximately 850 μ L for NHP-1 and 625 μ L of NHP-2. The infusate containing AAV9-GFT particles and chelated gadolinium imaging reagent, labeled in orange, distributed into approximately 350 μ L for each infusion. Figures 1E, 1F and 1G show coronal, sagittal left and sagittal right views (respectively) of cannula trajectory paths of NHP-1 and Figures 1H, 1I and 1J show coronal, sagittal left and sagittal right views (respectively) of cannula trajectory paths for NHP-2.

[0036] **Figures 2A through 2H** show AAV9-mediated GFP expression in the putamen of NHPs and innate immune status. Figures 2A and 2B show GFP staining within the putamen. High magnification images of the boxed areas are shown below and depict NeuN-positive cells in putamen administered either a high dose ("HD", left hemisphere 1.5×10^{13} vg/mL) or low dose ("LD", right hemisphere, 1.5 and 10^{12} vg/mL) of vector. Figure 2C and 2D are graphs depicting the primary area of transduction (PAT) shown in white and the area within the putamen "outside" the GFP-positive signal is "oPAT" and shown in grey. Counts of NeuN-positive cells in three coronal plants at PAT and oPAT, performed alongside naive control NHPs within the putamen showed no detectable difference in NeuN signal when the low and high dose hemispheres were compared. Counts of co-localized GFP-positive/NeuN-positive cells revealed transduction is about 74% efficient in PAT with low to null efficiency in oPAT. Data are represented as mean number of cells $\pm$ SD. P-values (*) of <0.01 for transduced cells within PAT and oPAT, Wilcoxon sign-ranked S test.

9. The method of claim 8, wherein the Htt gene comprises at least wild-type and/or one mutant allele.

5 10. The method of any claim 8 or claim 9, wherein the fusion protein comprises a repression domain and expression of the Htt gene is repressed.

11. The method of any of claims 8 to 10, wherein the cell is a neuronal cell.

12. The method of claim 11, wherein the neuronal cell is in a brain.

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13. The method of claim 12, wherein the neuronal cell is in the striatum of the brain.

15 14. 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 claim 4, one or more AAV vectors according to claim 5 and/or one or more pharmaceutical compositions according to claim 7.