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(71) Demandeur/Applicant:
GALAPAGOS NV, BE
(72) Inventeurs/Inventors:
FISCHER, DAVID FREDERIK, NL;
JANSSEN, RICHARD ANTONIUS JOZEF, NL;
DE PRIL, REMKO, NL;
VAN STEENHOVEN, DESIRE MARIA PETRONELLA
CATHARINA, NL;
KWAK, SEUNG, US;
HOWLAND, DAVID S., US;
SIGNER, ETHAN, US
(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,S.R.L.

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DANS LE TRAITEMENT DES MALADIES NEURODEGENERATIVES
(54) Title: MOLECULAR TARGETS AND COMPOUNDS, AND METHODS TO IDENTIFY THE SAME, USEFUL IN THE
TREATMENT OF NEURODEGENERATIVE DISEASES

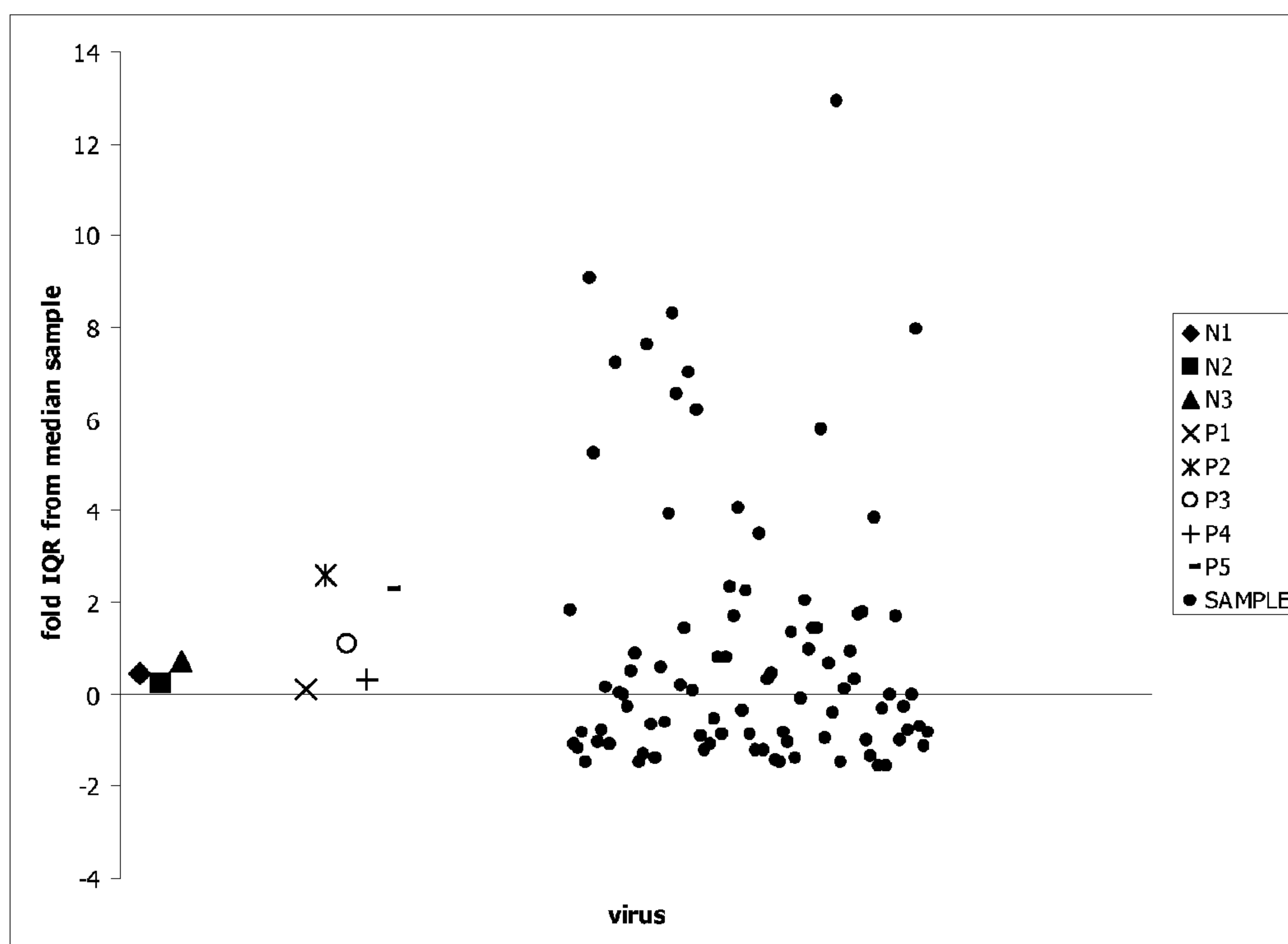


FIGURE 1
Example of a plate in the Ad-siRNA huntingtin conformation assay

(57) **Abrégé/Abstract:**

The present invention relates to methods and assays for identifying agents capable of inhibiting the mutant huntingtin protein, inhibiting or reducing polyglutamine-induced protein aggregation, and/or altering huntingtin protein conformation, which inhibition is useful in the prevention, amelioration and / or treatment of neurodegenerative diseases, and protein aggregation diseases more generally. In particular, the present invention provides methods and assays for identifying agents for use in the prevention and / or treatment of Huntingtons disease. The invention provides polypeptide and nucleic acid TARGETs and siRNA sequences based on these TARGETs.



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61/063,537 4 February 2008 (04.02.2008) US(71) Applicant (for all designated States except US): **BIOFOCUS DPI B.V.** [NL/NL]; Darwinweg 24, NL-2333CR Leiden (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FISCHER, David, Frederik** [NL/NL]; Darwinweg 24, NL-2333CR Leiden (NL). **JANSSEN, Richard, Antonius, Jozef** [NL/NL]; Darwinweg 24, NL-2333CR Leiden (NL). **DE PRIL, Remko** [NL/NL]; Darwinweg 24, NL-2333CR Leiden (NL). **VAN STEENHOVEN, Desire, Maria, Petronella, Catharina** [NL/NL]; Darwinweg 24, NL-2333CR Leiden (NL). **KWAK, Seung** [US/US]; CHDI Management Inc., 350 Seventh Avenue, Suite 601, New York, New York 10001 (US). **HOWLAND, David, S.** [US/US]; CHDI Management Inc., 350 Seventh Avenue, suite 301, NewYork, New York 10001 (US). **SIGNER, Ethan** [US/US]; CHDI Management Inc., 350 Seventh Avenue, Suite 601, New York, New York 10001 (US).(74) Agent: **NICHOL, Maria**; Galapagos NV, Chesterford Research Park, Saffron Walden Essex CB10 1XL (GB).

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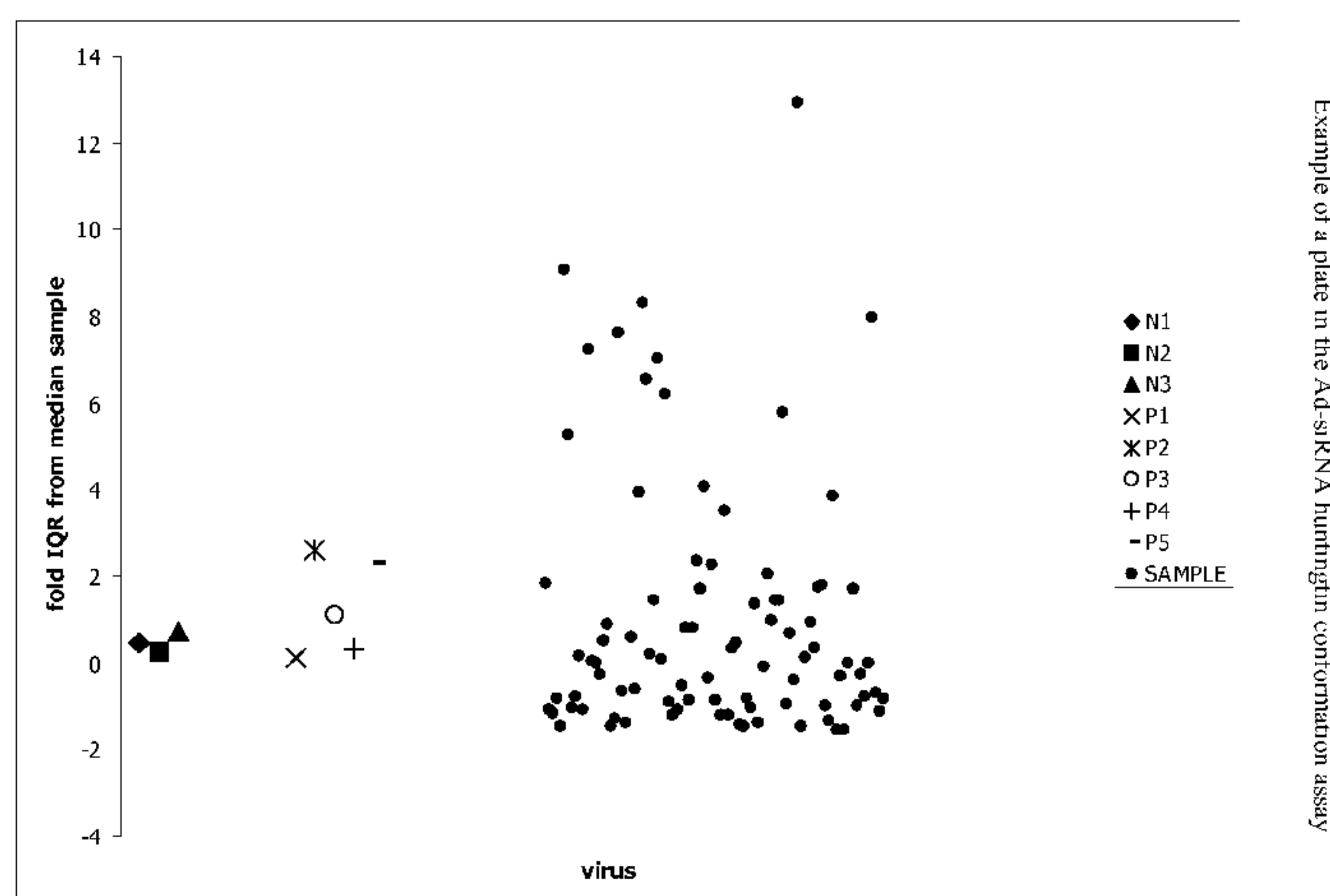
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(54) Title: MOLECULAR TARGETS AND COMPOUNDS, METHODS TO IDENTIFY THE SAME, USEFUL IN THE TREATMENT OF NEURODEGENERATIVE DISEASES.

FIGURE 1



Example of a plate in the Ad-siRNA huntingtin conformation assay

(57) Abstract: The present invention relates to methods and assays for identifying agents capable of inhibiting the mutant huntingtin protein, inhibiting or reducing polyglutamine-induced protein aggregation, and/or altering huntingtin protein conformation, which inhibition is useful in the prevention, amelioration and / or treatment of neurodegenerative diseases, and protein aggregation diseases more generally. In particular, the present invention provides methods and assays for identifying agents for use in the prevention and / or treatment of Huntingtons disease. The invention provides polypeptide and nucleic acid TARGETs and siRNA sequences based on these TARGETs.

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**MOLECULAR TARGETS AND COMPOUNDS, AND METHODS TO IDENTIFY THE SAME,
USEFUL IN THE TREATMENT OF NEURODEGENERATIVE DISEASES**

BACKGROUND OF THE INVENTION

[001] The present invention relates to methods for identifying agents capable of modulating the expression or activity of proteins involved in the processes leading to Huntington's Disease (HD) pathology. Inhibition of these processes is useful in the prevention and / or treatment of Huntington's Disease and other diseases involving neurodegeneration. In particular, the present invention provides methods for identifying agents for use in the prevention and / or treatment of HD.

FIELD OF THE INVENTION

[002] Huntington's Disease (HD) is an autosomal-dominant genetic neurodegenerative disease, characterized by neuropathology in the striatum and cortex. HD gives rise to progressive, selective (localized) neural cell death associated with choreic movements and dementia. No treatment exists for HD, and this disease leads to premature death usually within a decade from the onset of clinical signs. For reviews on HD, we refer to (Bates, 2005; Tobin and Signer, 2000; Vonsattel *et al.*, 1985; Zoghbi and Orr, 2000).

[003] Neuropathological analysis of the brains of HD patients clearly evidences the regions of the brain involved in the neurodegenerative processes (Vonsattel *et al.*, 1985). The striatum (caudate nucleus) and cortex are most severely affected, explaining the motor and cognitive deficits observed during the disease process.

[004] HD is associated with increases in the length of a CAG triplet repeat present in a gene called 'huntingtin' or HD, located on chromosome 4p16.3. The Huntington's Disease Collaborative Research Group (The Huntington's Disease Collaborative Research Group, 1993) found that a 'new' gene, designated IT15 (important transcript 15) and later called huntingtin, which was isolated using cloned trapped exons from the target area, contains a polymorphic trinucleotide repeat that is expanded and unstable on HD chromosomes. A (CAG)_n repeat longer than the normal range was observed on HD chromosomes from all 75 disease families examined. The families came from a variety of ethnic backgrounds and demonstrated a variety of 4p16.3 haplotypes. The (CAG)_n repeat appeared to be located within the coding sequence of a predicted protein of about 348 kD that is widely expressed but unrelated to any known gene. Thus it turned out that the HD mutation involves an unstable DNA segment similar to those previously observed in several disorders, including the fragile X syndrome, Kennedy syndrome, and myotonic dystrophy. The fact that the phenotype of HD is completely dominant suggests that the disorder results from a gain-of-function mutation in which either the mRNA product or the protein product of the disease allele has some new property or is expressed inappropriately.

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[005] DiFiglia *et al.* (DiFiglia *et al.*, 1997) contributed to the understanding of the mechanism of neurodegeneration in HD. They demonstrated that an amino-terminal fragment of mutant huntingtin localizes to neuronal intranuclear inclusions (NIIs) and dystrophic neurites (DNs) in the HD cortex and striatum, which are affected in HD, and that polyglutamine length influences the extent of huntingtin accumulation in these structures. Ubiquitin, which is thought to be involved in labeling proteins for disposal by intracellular proteolysis, was also found in NIIs and DNPs, suggesting (DiFiglia *et al.*, 1997) that abnormal huntingtin is targeted for proteolysis but is resistant to removal. The aggregation of mutant huntingtin may be part of the pathogenic mechanism in HD.

[006] Saudou *et al.* (Saudou *et al.*, 1998) investigated the mechanisms by which mutant huntingtin induces neurodegeneration by use of a cellular model that recapitulates features of neurodegeneration seen in Huntington disease. When transfected into cultured striatal neurons, mutant huntingtin induced neurodegeneration by an apoptotic mechanism. Antiapoptotic compounds or neurotrophic factors protected neurons against mutant huntingtin. Blocking nuclear localization of mutant huntingtin suppressed its ability to form intranuclear inclusions and to induce neurodegeneration. However, the presence of inclusions did not correlate with huntingtin-induced death. The exposure of mutant huntingtin-transfected striatal neurons to conditions that suppress the formation of inclusions resulted in an increase in mutant huntingtin-induced death. These findings suggested that mutant huntingtin acts within the nucleus to induce neurodegeneration. Altogether, intranuclear inclusions may reflect a cellular mechanism to protect against huntingtin-induced cell death.

[007] A method to reduce the levels of the cell death in neurons in the striatum and cortex observed in HD is likely to confer clinical benefit to HD patients.

[008] A remarkable threshold exists, where polyglutamine stretches of 35 repeats or more in the HD gene cause HD, whereas stretches of polyglutamine repeats fewer than 35 do not cause disease. A robust correlation between the threshold for disease and the propensity of the huntingtin protein to aggregate *in vitro*, suggests that aggregation is related to pathogenesis (Davies *et al.*, 1997; Scherzinger *et al.*, 1999).

[009] Protein aggregation follows a series of intermediate steps including an abnormal conformation of the protein, a globular intermediate, protofibrils, fibers and microscopic inclusions (Ross and Poirier, 2004). It is commonly believed that one or more of these molecular species confers toxicity in HD.

[0010] A method to reduce the expression levels of the toxic intermediates of the mutant HD protein would likely confer clinical benefit to HD patients.

Reported Developments

[0011] Neural and stem cell transplantation is a potential treatment for neurodegenerative diseases, e.g., transplantation of specific committed neuroblasts (fetal neurons) to the adult brain. Encouraged by animal

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studies, a clinical trial of human fetal striatal tissue transplantation for the treatment of Huntington disease was initially undertaken at the University of South Florida. In this series, 1 patient died 18 months after transplantation from causes unrelated to surgery.

[0012] The fact that activation of mechanisms mediating cell death may be involved in neurologic diseases makes apoptosis and caspases attractive therapeutic targets. Clinical trials of an inhibitor of apoptosis (minocycline) for HD are in progress.

[0013] A variety of growth factors had been shown to induce cell proliferation and neurogenesis, which could counter-act cell loss in HD (Strand *et al.*, 2007).

[0014] Inhibition of polyglutamine-induced protein aggregation could provide treatment options for polyglutamine diseases such as HD. Tanaka *et al.* (Tanaka *et al.*, 2004) showed through in vitro screening studies that various disaccharides can inhibit polyglutamine-mediated protein aggregation. They also found that various disaccharides reduced polyglutamine aggregates and increased survival in a cellular model of HD. Oral administration of trehalose, the most effective of these disaccharides, decreased polyglutamine aggregates in cerebrum and liver, improved motor dysfunction, and extended life span in a transgenic mouse model of HD. Tanaka *et al.* (Tanaka *et al.*, 2004) suggested that these beneficial effects are the result of trehalose binding to expanded polyglutamines and stabilizing the partially unfolded polyglutamine-containing protein. Lack of toxicity and high solubility, coupled with efficacy upon oral administration, made trehalose promising as a therapeutic drug or lead component for the treatment of polyglutamine diseases. The saccharide-polyglutamine interaction identified by Tanaka *et al.* (Tanaka *et al.*, 2004) thus provided a possible new therapeutic strategy for polyglutamine diseases.

[0015] Ravikumar *et al.* (Ravikumar *et al.*, 2004) presented data that provided proof of principle for the potential of inducing autophagy to treat HD. They showed that mammalian target of rapamycin (mTOR) is sequestered in polyglutamine aggregates in cell models, transgenic mice, and human brains. Such sequestration impairs the kinase activity of mTOR and induces autophagy, a key clearance pathway for mutant huntingtin fragments. This protects against polyglutamine toxicity.

[0016] There still exists a need in the art for compounds and agents for amelioration of symptoms, prevention, and treatment of Huntington's Disease and other diseases associated with or exacerbated by altered protein conformations, including polyglutamine-induced protein aggregation.

SUMMARY OF THE INVENTION

[0017] The present invention is based on the discovery that agents which inhibit or enhance the expression and / or activity of the TARGETs disclosed herein are able to modulate expression levels of a toxic conformation of the mutant (expanded) huntingtin protein in neuronal cells. In a particular aspect the agents inhibit the

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expression and / or activity of the TARGETs disclosed herein. The present invention therefore provides TARGETS which are involved in the pathways involved in HD pathogenesis, methods for screening for agents capable of inhibiting the expression and / or activity of TARGETS and uses of these agents in the prevention and / or treatment of neurodegenerative diseases such as HD. The present invention provides TARGETS which are involved in or otherwise associated with polyglutamine-induced protein conformation and aggregation and huntingtin protein conformation. Modulation of the TARGETS of the invention provides modulation of protein aggregation, particularly including polyglutamine-induced protein aggregation and huntingtin protein conformation.

[0018] The present invention relates to a method for identifying compounds that are able to modulate the expression or activity of the mutant huntingtin protein in neuronal cells, comprising contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52 (hereinafter "TARGETS") and fragments thereof, under conditions that allow said polypeptide to bind to said compound, and measuring a compound-polypeptide property related to huntingtin expression or activity. In a specific embodiment the compound-polypeptide property measured is huntingtin protein expression levels. In a specific embodiment, the property measured is huntingtin protein conformation and aggregation mediated by polyglutamine repeats. More generally, the method relates to identifying compounds which modulate protein conformation and protein aggregation, particularly as associated with polyglutamine repeats.

[0019] Aspects of the present method include the *in vitro* assay of compounds using a polypeptide corresponding to a TARGET, or fragments thereof, such fragments being fragments of the amino acid sequences described by SEQ ID NO: 27-52 and cellular assays wherein TARGET inhibition is followed by observing indicators of efficacy including, for example, TARGET expression levels, TARGET enzymatic activity and/or huntingtin protein levels.

[0020] The present invention also relates to

- (1) expression inhibitory agents comprising a polynucleotide selected from the group of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said polynucleotide comprises a nucleic acid sequence complementary to, or engineered from, a naturally occurring polynucleotide sequence encoding a TARGET polypeptide said polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO: 1-26 and
- (2) pharmaceutical compositions comprising said agent(s), useful in the treatment, or prevention, of neurodegenerative diseases such as Huntington's disease.

[0021] Another aspect of the invention is a method of treatment, or prevention, of a condition related to neurodegeneration, in a subject suffering or susceptible thereto, by administering a pharmaceutical composition

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comprising an effective TARGET-expression inhibiting amount of a expression-inhibitory agent or an effective TARGET activity inhibiting amount of a activity-inhibitory agent.

[0022] Another aspect of this invention relates to the use of agents which inhibit a TARGET as disclosed herein in a therapeutic method, a pharmaceutical composition, and the manufacture of such composition, useful for the treatment of a disease involving neurodegeneration. In particular, the present method relates to the use of the agents which inhibit a TARGET in the treatment of a disease characterized by neuronal cell death, and in particular, a disease characterized by abnormal aggregations of huntingtin protein. The agents are useful for amelioration or treatment of neurodegenerative conditions, particularly wherein it is desired to reduce or control protein aggregation, in particular huntingtin aggregation. Suitable neurodegenerative conditions include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Spinocerebellar Ataxia. In a particular embodiment the disease is a polyglutamine disease for example, but without limitation, Huntington's disease, Spinal and bulbar muscular atrophy (SBMA), - Dentatorubral-pallidoluysian atrophy (DRPLA), Spinocerebellar ataxia 1 (SCA1), Spinocerebellar ataxia 2 (SCA2), Spinocerebellar ataxia 3 (SCA3), Spinocerebellar ataxia 7 (SCA7) and Spinocerebellar ataxia 17 (SCA17). In particular the disease is Huntington's disease. Other objects and advantages will become apparent from a consideration of the ensuing description taken in conjunction with the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1: Example of a plate in the Ad-siRNA huntingtin conformation assay

[0024] Figure 2: Primary screening data of 11584 Ad-siRNAs in the huntingtin conformation assay

DETAILED DESCRIPTION

[0025] The following terms are intended to have the meanings presented therewith below and are useful in understanding the description and intended scope of the present invention.

[0026] The term 'agent' means any molecule, including polypeptides, polynucleotides, chemical compounds and small molecules. In particular the term agent includes compounds such as test compounds or drug candidate compounds.

[0027] The term 'agonist' refers to a ligand that stimulates the receptor the ligand binds to in the broadest sense.

[0028] As used herein, the term 'antagonist' is used to describe a compound that does not provoke a biological response itself upon binding to a receptor, but blocks or dampens agonist-mediated responses, or prevents or reduces agonist binding and, thereby, agonist-mediated responses.

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[0029] The term 'assay' means any process used to measure a specific property of an agent, including a compound. A 'screening assay' means a process used to characterize or select compounds based upon their activity from a collection of compounds.

[0030] The term 'binding affinity' is a property that describes how strongly two or more compounds associate with each other in a non-covalent relationship. Binding affinities can be characterized qualitatively, (such as 'strong', 'weak', 'high', or 'low') or quantitatively (such as measuring the K_D).

[0031] The term 'carrier' means a non-toxic material used in the formulation of pharmaceutical compositions to provide a medium, bulk and/or useable form to a pharmaceutical composition. A carrier may comprise one or more of such materials such as an excipient, stabilizer, or an aqueous pH buffered solution. Examples of physiologically acceptable carriers include aqueous or solid buffer ingredients including phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®.

[0032] The term 'complex' means the entity created when two or more compounds bind to, contact, or associate with each other.

[0033] The term 'compound' is used herein in the context of a 'test compound' or a 'drug candidate compound' described in connection with the assays of the present invention. As such, these compounds comprise organic or inorganic compounds, derived synthetically or from natural sources. The compounds include inorganic or organic compounds such as polynucleotides (e.g. siRNA or cDNA), lipids or hormone analogs. Other biopolymeric organic test compounds include peptides comprising from about 2 to about 40 amino acids and larger polypeptides comprising from about 40 to about 500 amino acids, including polypeptide ligands, enzymes, receptors, channels, antibodies or antibody conjugates.

[0034] The term 'condition' or 'disease' means the overt presentation of symptoms (i.e., illness) or the manifestation of abnormal clinical indicators (for example, biochemical indicators). Alternatively, the term 'disease' refers to a genetic or environmental risk of or propensity for developing such symptoms or abnormal clinical indicators.

[0035] The term 'contact' or 'contacting' means bringing at least two moieties together, whether in an *in vitro* system or an *in vivo* system.

[0036] The term 'derivatives of a polypeptide' relates to those peptides, oligopeptides, polypeptides, proteins and enzymes that comprise a stretch of contiguous amino acid residues of the polypeptide and that retain a biological activity of the protein, for example, polypeptides that have amino acid mutations compared

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to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may further comprise additional naturally occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally occurring form of the polypeptide. It may also contain one or more non-amino acid substituents, or heterologous amino acid substituents, compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

[0037] The term 'derivatives of a polynucleotide' relates to DNA-molecules, RNA-molecules, and oligonucleotides that comprise a stretch of nucleic acid residues of the polynucleotide, for example, polynucleotides that may have nucleic acid mutations as compared to the nucleic acid sequence of a naturally occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as PNA, polysiloxane, and 2'-O-(2-methoxy) ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

[0038] The term 'endogenous' shall mean a material that a mammal naturally produces. Endogenous in reference to the term 'enzyme', 'protease', 'kinase', or G-Protein Coupled Receptor ('GPCR') shall mean that which is naturally produced by a mammal (for example, and not limitation, a human). In contrast, the term non-endogenous in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human). Both terms can be utilized to describe both *in vivo* and *in vitro* systems. For example, and without limitation, in a screening approach, the endogenous or non-endogenous TARGET may be in reference to an *in vitro* screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous TARGET, screening of a candidate compound by means of an *in vivo* system is viable.

[0039] The term 'expressible nucleic acid' means a nucleic acid coding for a proteinaceous molecule, an RNA molecule, or a DNA molecule.

[0040] The term 'expression' comprises both endogenous expression and non-endogenous expression, including overexpression by transduction.

[0041] The term 'expression inhibitory agent' means a polynucleotide designed to interfere selectively with the transcription, translation and/or expression of a specific polypeptide or protein normally expressed within a cell. More particularly, 'expression inhibitory agent' comprises a DNA or RNA molecule that contains a nucleotide sequence identical to or complementary to at least about 15-30, particularly at least 17, sequential nucleotides within the polyribonucleotide sequence coding for a specific polypeptide or protein. Exemplary expression inhibitory molecules include ribozymes, double stranded siRNA molecules, self-

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complementary single-stranded siRNA molecules, genetic antisense constructs, and synthetic RNA antisense molecules with modified stabilized backbones.

[0042] The term ‘fragment of a polynucleotide’ relates to oligonucleotides that comprise a stretch of contiguous nucleic acid residues that exhibit substantially a similar, but not necessarily identical, activity as the complete sequence. In a particular aspect, ‘fragment’ may refer to a oligonucleotide comprising a nucleic acid sequence of at least 5 nucleic acid residues (preferably, at least 10 nucleic acid residues, at least 15 nucleic acid residues, at least 20 nucleic acid residues, at least 25 nucleic acid residues, at least 40 nucleic acid residues, at least 50 nucleic acid residues, at least 60 nucleic residues, at least 70 nucleic acid residues, at least 80 nucleic acid residues, at least 90 nucleic acid residues, at least 100 nucleic acid residues, at least 125 nucleic acid residues, at least 150 nucleic acid residues, at least 175 nucleic acid residues, at least 200 nucleic acid residues, or at least 250 nucleic acid residues) of the nucleic acid sequence of said complete sequence.

[0043] The term ‘fragment of a polypeptide’ relates to peptides, oligopeptides, polypeptides, proteins, monomers, subunits and enzymes that comprise a stretch of contiguous amino acid residues, and exhibit substantially a similar, but not necessarily identical, functional or expression activity as the complete sequence. In a particular aspect, ‘fragment’ may refer to a peptide or polypeptide comprising an amino acid sequence of at least 5 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of said complete sequence.

[0044] The term ‘hybridization’ means any process by which a strand of nucleic acid binds with a complementary strand through base pairing. The term ‘hybridization complex’ refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (for example, C_{0t} or R_{0t} analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (for example, paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed). The term “stringent conditions” refers to conditions that permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, for example, formamide, temperature, and other conditions well known in the art. In particular, reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature can increase stringency. The term ‘standard hybridization conditions’ refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such

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‘standard hybridization conditions’ are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20^NC below the predicted or determined T_m with washes of higher stringency, if desired.

[0045] The term ‘inhibit’ or ‘inhibiting’, in relationship to the term ‘response’ means that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

[0046] The term ‘inhibition’ refers to the reduction, down regulation of a process or the elimination of a stimulus for a process, which results in the absence or minimization of the expression of a protein or polypeptide.

[0047] The term ‘induction’ refers to the inducing, up-regulation, or stimulation of a process, which results in the expression of a protein or polypeptide.

[0048] The term ‘ligand’ means an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

[0049] The term ‘pharmaceutically acceptable salts’ refers to the non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds which inhibit the expression or activity of TARGETS as disclosed herein. These salts can be prepared *in situ* during the final isolation and purification of compounds useful in the present invention.

[0050] The term ‘polypeptide’ relates to proteins (such as TARGETS), proteinaceous molecules, fragments of proteins, monomers or portions of polymeric proteins, peptides, oligopeptides and enzymes (such as kinases, proteases, GPCR’s etc.).

[0051] The term ‘polynucleotide’ means a polynucleic acid, in single or double stranded form, and in the sense or antisense orientation, complementary polynucleic acids that hybridize to a particular polynucleic acid under stringent conditions, and polynucleotides that are homologous in at least about 60 percent of its base pairs, and more particularly 70 percent of its base pairs are in common, most particularly 90 per cent, and in a special embodiment 100 percent of its base pairs. The polynucleotides include polyribonucleic acids, polydeoxyribonucleic acids, and synthetic analogues thereof. It also includes nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate. The polynucleotides are described by sequences that vary in length, that range from about 10 to about 5000 bases, particularly about 100 to about 4000 bases, more particularly about 250 to about 2500 bases. One polynucleotide embodiment comprises from about 10 to about 30 bases in length. A special embodiment of polynucleotide is the polyribonucleotide of from about 17 to about 22 nucleotides, more commonly described

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as small interfering RNAs (siRNAs - double stranded siRNA molecules or self-complementary single-stranded siRNA molecules (shRNA)). Another special embodiment are nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate, or including non-naturally occurring nucleic acid residues, or one or more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection. Polynucleotides herein are selected to be 'substantially' complementary to different strands of a particular target DNA sequence. This means that the polynucleotides must be sufficiently complementary to hybridize with their respective strands. Therefore, the polynucleotide sequence need not reflect the exact sequence of the target sequence. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the polynucleotide, with the remainder of the polynucleotide sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the polynucleotide, provided that the polynucleotide sequence has sufficient complementarity with the sequence of the strand to hybridize therewith under stringent conditions or to form the template for the synthesis of an extension product.

[0052] The term 'preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop) in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset.

[0053] The term 'prophylaxis' is related to and encompassed in the term 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine, in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

[0054] The term 'solvate' means a physical association of a compound useful in this invention with one or more solvent molecules. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanolates and methanolates.

[0055] The term 'subject' includes humans and other mammals.

[0056] The term 'TARGET' or 'TARGETS' means the protein(s) identified in accordance with the assays described herein and determined to be involved in the modulation of a Huntington Disease phenotype.

[0057] 'Therapeutically effective amount' or 'effective amount' means that amount of a compound or agent that will elicit the biological or medical response of a subject that is being sought by a medical doctor or other clinician.

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[0058] The term ‘treating’ means an intervention performed with the intention of preventing the development or altering the pathology of, and thereby ameliorating a disorder, disease or condition, including one or more symptoms of such disorder or condition. Accordingly, ‘treating’ refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treating include those already with the disorder as well as those in which the disorder is to be prevented. The related term ‘treatment,’ as used herein, refers to the act of treating a disorder, symptom, disease or condition, as the term ‘treating’ is defined above.

[0059] The term ‘treating’ or ‘treatment’ of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (i.e., arresting the disease or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment ‘treating’ or ‘treatment’ refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In yet another embodiment, ‘treating’ or ‘treatment’ refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In a further embodiment, ‘treating’ or ‘treatment’ relates to slowing the progression of the disease.

[0060] The term “vectors” also relates to plasmids as well as to viral vectors, such as recombinant viruses, or the nucleic acid encoding the recombinant virus.

[0061] The term “vertebrate cells” means cells derived from animals having vertera structure, including fish, avian, reptilian, amphibian, marsupial, and mammalian species. Preferred cells are derived from mammalian species, and most preferred cells are human cells. Mammalian cells include feline, canine, bovine, equine, caprine, ovine, porcine murine, such as mice and rats, and rabbits.

[0062] The term ‘TARGET’ or ‘TARGETS’ means the protein(s) identified in accordance with the assays described herein and determined to be involved in the modulation of mast cell activation . The term TARGET or TARGETS includes and contemplates alternative species forms, isoforms, and variants, such as splice variants, allelic variants, alternate in frame exons, and alternative or premature termination or start sites, including known or recognized isoforms or variants thereof such as indicated in Table 1.

[0063] The term ‘neurodegenerative condition’ or ‘neurodegenerative disease’ refers to a disorder caused by the deterioration of neurons. The exact location and type of neurons that are lost may vary between conditions. It is changes in these cells which cause them to function abnormally, eventually bringing about their death. Neurodegenerative diseases include, without limitation, Huntington’s disease and other polyglutamine diseases, Alzheimer’s disease, Parkinson’s disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia.

[0064] The term ‘polyglutamine disease’ refers to a family of dominantly inherited neurodegenerative conditions that are caused by CAG triplet repeat expansions within genes. CAG encodes the amino acid glutamine, and the affected proteins have enlarged tracts of this amino acid. This family includes (without limitation) Huntington’s disease, Spinal and bulbar muscular atrophy (SBMA), - Dentatorubral-pallidoluysian

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atrophy (DRPLA), Spinocerebellar ataxia 1 (SCA1), Spinocerebellar ataxia 2 (SCA2), Spinocerebellar ataxia 3 (SCA3), Spinocerebellar ataxia 7 (SCA7) and Spinocerebellar ataxia 17 (SCA17).

TARGETS

[0065] Applicants invention is relevant to the treatment, prevention and alleviation of neurodegeneration, neural cell death, including for such diseases as Huntington's disease and other polyglutamine diseases, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Progressive Supranuclear Palsy, Frontotemporal Dementia and Vascular Dementia. Applicant's invention further and particularly relates to inhibition of polyglutamine-induced protein aggregation and cell death. The invention also relates to modulation of huntingtin protein expression, conformation, and/or aggregation. Applicant's invention is in part based on the TARGETs relationship to polyglutamine-induced protein aggregation and huntingtin protein conformation. The TARGETs are relevant, in particular, to neurodegeneration and HD.

[0066] The present invention provides methods for assaying for drug candidate compounds that modulate protein aggregation, particularly including polyglutamine-induced protein aggregation or aberrant conformation, comprising contacting a compound with a cell expressing an aggregating form of a protein, such as mutant huntingtin protein or such other protein comprising polyglutamine, and determining the degree, extent or amount of aggregation, or an aggregation-mediated activity or phenomenon such as aberrant conformation, in the presence and/or absence of the compound. Such methods may be used to identify target proteins that may play a role in protein aggregation, alternatively such methods may be used to identify compounds that are able to modulate protein aggregation or aberrant conformation. Exemplary such methods can be designed and determined by the skilled artisan. Particular such exemplary methods are provided herein.

[0067] The present invention is based on the inventor's discovery that the TARGET polypeptides and their encoding nucleic acids, identified as a result of screens described below in the Examples, are factors in polyglutamine-induced protein aggregation and huntingtin protein conformation. A reduced activity or expression of the TARGET polypeptides and/or their encoding polynucleotides is causative, correlative or associated with reduced or inhibited polyglutamine-induced protein aggregation and reduced huntingtin protein aggregation and polyglutamine-induced altered huntingtin protein conformation. Alternatively, a reduced activity or expression of the TARGET polypeptides and/or their encoding polynucleotides is causative, correlative or associated with enhanced polyglutamine-induced protein aggregation and increased huntingtin protein aggregation and polyglutamine-induced altered huntingtin protein conformation.

[0068] In a particular embodiment of the invention, the TARGET polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID 27-52 as listed in Table 1.

[0069]

Table 1

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
SLC7A5	NM_003486	1	NP_003477	27	Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 (SLC7A5), mRNA.	Transporter
HSD17B14	NM_016246	2	NP_057330	28	Homo sapiens dehydrogenase/reductase (SDR family) member 10 (DHR10), mRNA.	Enzyme
USP9X	NM_004652	3	NP_004643	29	Homo sapiens ubiquitin specific peptidase 9, X-linked (fat facets-like, Drosophila) (USP9X), transcript variant 1, mRNA.	Protease
CASP1	NM_033295	4	NP_150637	30	Homo sapiens caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase) (CASP1), transcript variant epsilon, mRNA.	Protease
CYB5R2	NM_016229	5	NP_057313	31	Homo sapiens cytochrome b5 reductase b5R.2 (CYB5R2), transcript variant 1, mRNA.	Enzyme
NOS1	NM_000620	6	NP_000611	32	Homo sapiens nitric oxide synthase 1 (neuronal) (NOS1), mRNA.	Enzyme
SPHK2	NM_020126	7	NP_064511	33	Homo sapiens sphingosine kinase 2 (SPHK2), mRNA.	Kinase
P2RY1	NM_002563	8	NP_002554	34	Homo sapiens purinergic receptor P2Y, G-protein coupled, 1 (P2RY1), mRNA.	GPCR
LRP11	NM_032832	9	NP_116221	35	Homo sapiens low density lipoprotein receptor-related protein 11 (LRP11), mRNA.	Receptor

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
PCSK6	NM_138325	10	NP_612198	36	Homo sapiens proprotein convertase subtilisin/kexin type 6 (PCSK6), transcript variant 6, mRNA.	Protease
DHCR7	NM_001360	11	NP_001351	37	Homo sapiens 7-dehydrocholesterol reductase (DHCR7), mRNA.	Enzyme
ENPP5	NM_021572	12	NP_067547	38	Homo sapiens ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function) (ENPP5), mRNA	PDE
ARHGEF15	NM_173728	13	NP_776089	39	Homo sapiens Rho guanine nucleotide exchange factor (GEF) 15 (ARHGEF15), mRNA.	Exchange Factor
PSMA2	NM_002787	14	NP_002778	40	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2 (PSMA2), mRNA.	Protease
ABCG2	NM_004827	15	NP_004818	41	Homo sapiens ATP-binding cassette, subfamily G (WHITE), member 2 (ABCG2), mRNA.	Transporter
CCR10	NM_016602	16	NP_057686	42	Homo sapiens chemokine (C-C motif) receptor 10 (CCR10), mRNA.	GPCR
KLKB1	NM_000892	17	NP_000883	43	Homo sapiens kallikrein B, plasma (Fletcher factor) 1 (KLKB1), mRNA.	Protease
EPOR	NM_000121	18	NP_000112	44	Homo sapiens erythropoietin receptor (EPOR), mRNA.	Receptor
CREBBP	NM_004380	19	NP_004371	45	Homo sapiens CREB binding protein (Rubinstein-Taybi syndrome) (CREBBP), mRNA.	Enzyme
APLP2	NM_001642	20	NP_001633	46	Homo sapiens amyloid	

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Target Gene Symbol	GenBank Nucleic Acid Acc #:	SEQ ID NO: DNA	GenBank Protein Acc #	SEQ ID NO: Protein	NAME	Class
					beta (A4) precursor-like protein 2 (APLP2), mRNA.	
MAP3K11	NM_002419	21	NP_002410	47	Homo sapiens mitogen-activated protein kinase kinase kinase 11 (MAP3K11), mRNA.	Kinase
TNFRSF10A	NM_003844	22	NP_003835	48	Homo sapiens tumor necrosis factor receptor superfamily, member 10a (TNFRSF10A), mRNA.	Receptor
HIF1A	NM_181054	23	NP_851397	49	Homo sapiens hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) (HIF1A), transcript variant 2, mRNA.	Transcription Factor
NOS2A	NM_153292	24	NP_695024	50	Homo sapiens nitric oxide synthase 2A (inducible, hepatocytes) (NOS2A), transcript variant 2, mRNA.	Enzyme
DAPK2	NM_014326	25	NP_055141	51	Homo sapiens death-associated protein kinase 2 (DAPK2), mRNA.	Kinase
NRG1	NM_013961	26	NP_039255	52	Homo sapiens neuregulin 1 (NRG1), transcript variant GGF, mRNA.	Secreted

[0070] A particular embodiment of the invention comprises the transporter TARGETs identified as SEQ ID NOs: 27 and 41. A particular embodiment of the invention comprises the TARGET identified as SEQ ID NO: 20. A further particular embodiment of the invention comprises the enzyme TARGETs identified as SEQ ID NOs: 28, 31, 32, 37, 45 and 50. A further particular embodiment of the invention comprises the protease TARGETs identified as SEQ ID NOs: 29, 30, 36, 40 and 43. A further particular embodiment of the invention comprises the kinase TARGETs identified as SEQ ID NOs: 33, 47 and 51. A further particular embodiment of the invention comprises the GPCR TARGETs identified as SEQ ID NOs: 34 and 42. A further particular

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embodiment of the invention comprises the receptor TARGETs identified as SEQ ID NOs: 35, 44 and 48. A further particular embodiment of the invention comprises the phosphodiesterase (PDE) TARGET identified as SEQ ID NOs: 38. A further particular embodiment of the invention comprises the secreted TARGETs identified as SEQ ID NOs: 52. A further particular embodiment of the invention comprises the exchange factor TARGET identified as SEQ ID NOs: 39. A further particular embodiment of the invention comprises the transcription factor TARGET identified as SEQ ID NOs: 49.

[0071] In one aspect, the present invention relates to a method for assaying for drug candidate compounds that inhibit polyglutamine-induced protein aggregation or altered huntingtin protein conformation, comprising contacting the compound with a polypeptide comprising an amino acid sequence of SEQ ID NO: 27-52, or fragment thereof, under conditions that allow said polypeptide to bind to the compound, and detecting the formation of a complex between the polypeptide and the compound. One particular means of measuring the complex formation is to determine the binding affinity of said compound to said polypeptide.

[0072] More particularly, the invention relates to a method for identifying an agent that inhibits polyglutamine-induced protein aggregation or altered huntingtin protein conformation, the method comprising further:

- (a) contacting a population of mammalian cells with one or more compound that exhibits binding affinity for a TARGET polypeptide, or fragment thereof, and
- (b) measuring a compound-polypeptide property related to polyglutamine-induced protein aggregation or altered huntingtin protein conformation.

[0073] In a further aspect, the present invention relates to a method for assaying for drug candidate compounds that inhibit polyglutamine-induced protein aggregation or altered huntingtin protein conformation, comprising contacting the compound with a polypeptide comprising an amino acid sequence of SEQ ID NO: 27-52, or fragment thereof, under conditions that allow said compound to modulate the activity or expression of the polypeptide, and determining the activity or expression of the polypeptide. One particular means of measuring the activity or expression of the polypeptide is to determine the amount of said polypeptide using a polypeptide binding agent, such as an antibody, or to determine the activity of said polypeptide in a biological or biochemical measure, for instance the amount of phosphorylation of a target of a kinase polypeptide.

[0074] The compound-polypeptide property referred to above is related to the expression and/or activity of the TARGET, and is a measurable phenomenon chosen by the person of ordinary skill in the art. The measurable property may be, for example, the binding affinity for a peptide domain of the polypeptide TARGET or the enzyme activity of the polypeptide TARGET or the level of any one of a number of biochemical markers including polyglutamine-induced protein aggregation or altered huntingtin protein conformation.

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[0075] Depending on the choice of the skilled artisan, the present assay method may be designed to function as a series of measurements, each of which is designed to determine whether the drug candidate compound is indeed acting on or mediating the activity or expression of the polypeptide to thereby modulate the HD phenotype. For example, an assay designed to determine the binding affinity of a compound to the polypeptide, or fragment thereof, may be necessary, but may be one exemplary assay or one assay among additional or more particular and specific assays to ascertain whether the test compound would be useful for modulating protein aggregation, including particularly polyglutamine-mediated protein aggregation and the HD phenotype, when administered to a subject.

[0076] Suitable controls should always be in place to insure against false positive readings. In a particular embodiment of the present invention the screening method comprises the additional step of comparing the compound to a suitable control. In one embodiment, the control may be a cell or a sample that has not been in contact with the test compound. In an alternative embodiment, the control may be a cell that does not express the TARGET; for example in one aspect of such an embodiment the test cell may naturally express the TARGET and the control cell may have been contacted with an agent, e.g. an siRNA, which inhibits or prevents expression of the TARGET. Alternatively, in another aspect of such an embodiment, the cell in its native state does not express the TARGET and the test cell has been engineered so as to express the TARGET, so that in this embodiment, the control could be the untransformed native cell. The control may also or alternatively utilize a known mediator of neurodegeneration and/or protein aggregation. Whilst exemplary controls are described herein, this should not be taken as limiting; it is within the scope of a person of skill in the art to select appropriate controls for the experimental conditions being used.

[0077] The order of taking these measurements is not believed to be critical to the practice of the present invention, which may be practiced in any order. For example, one may first perform a screening assay of a set of compounds for which no information is known respecting the compounds' binding affinity for the polypeptide. Alternatively, one may screen a set of compounds identified as having binding affinity for a polypeptide domain, or a class of compounds identified as being an inhibitor of the polypeptide. However, for the present assay to be meaningful to the ultimate use of the drug candidate compounds, a measurement of modulation of protein aggregation, including particularly polyglutamine-mediated protein aggregation and aberrant conformation and the HD phenotype is preferred. The means by which to measure, assess, or determine protein aggregation and the HD phenotype may be selected or determined by the skilled artisan. Validation studies including controls and measurements of binding affinity to the polypeptides or modulation of activity or expression of the polypeptides of the invention are nonetheless useful in identifying a compound useful in any therapeutic or diagnostic application.

[0078] Analogous approaches based on art-recognized methods and assays may be applicable with respect to the TARGETS and compounds in any of various disease(s) characterized by neurodegeneration and / or neural

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cell death, in particular due to abnormal protein aggregation. An assay or assays may be designed to confirm that the test compound, having binding affinity for the TARGET, inhibits neurodegeneration and / or neural cell death and/or polyglutamine-induced protein aggregation and / or altered huntingtin protein conformation. In one such method polyglutamine conformation is measured.

[0079] The present assay method may be practiced *in vitro*, using one or more of the TARGET proteins, or fragments thereof, including monomers, portions or subunits of polymeric proteins, peptides, oligopeptides and enzymatically active portions thereof.

[0080] The binding affinity of a compound with the polypeptide TARGET can be measured by methods known in the art, such as using surface plasmon resonance biosensors (Biacore[®]), by saturation binding analysis with a labeled compound (for example, Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarization assay, Fluorometric Imaging Plate Reader (FLIPR[®]) system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer. The binding affinity of compounds can also be expressed in dissociation constant (Kd) or as IC₅₀ or EC₅₀. The IC₅₀ represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypeptide. The EC₅₀ represents the concentration required for obtaining 50% of the maximum effect in any assay that measures TARGET function. The dissociation constant, Kd, is a measure of how well a ligand binds to the polypeptide, it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low Kd, IC₅₀ and EC₅₀ values, for example, in the range of 100 nM to 1 pM; a moderate- to low-affinity binding relates to high Kd, IC₅₀ and EC₅₀ values, for example in the micromolar range.

[0081] The present assay method may also be practiced in a cellular assay. A host cell expressing the TARGET, or fragment(s) thereof, can be a cell with endogenous expression or a cell modified to express or over-expressing the TARGET, for example, by transduction. When the endogenous expression of the polypeptide is not sufficient to determine a baseline that can easily be measured, one may use host cells that over-express TARGET. Over-expression has the advantage that the level of the TARGET substrate end-products is higher than the activity level by endogenous expression. Accordingly, measuring such levels using presently available techniques is easier. Alternatively, a non-endogenous form of TARGET may be expressed or overexpressed in a cell and utilized in screening.

[0082] The assay method may be based on the particular expression or activity of the TARGET polypeptide, including but not limited to an enzyme activity. Thus, assays for the enzyme TARGETs identified as SEQ ID NOs: 28, 31, 32, 37, 45 and 50 may be based on enzymatic activity or enzyme expression. Assays for the protease TARGETs identified as SEQ ID NOs: 29, 30, 36, 40 and 43 may be based on protease activity or expression. Assays for the kinase TARGETs identified as SEQ ID NOs: 33, 47 and 51 may be based on protease activity or expression, including but not limited to cleavage or alteration of a protease target. Assays

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for the GPCR TARGETs identified as SEQ ID NOs: 34 and 42 may be based on GPCR activity or expression, including downstream mediators or activators. In the case of the receptor TARGETs identified as SEQ ID NOs: 35, 44 and 48, assays may be based on receptor binding or activity. Assays for the phosphodiesterase (PDE) TARGET identified as SEQ ID NOs: 38 may be based on PDE activity or expression. Assays for the transcription factor TARGET identified as SEQ ID NO: 49 may utilize transcriptional reporter activity or expression of the TARGET. Assays for the nucleotide exchange factor TARGET identified as SEQ ID NOs: 39 may utilize exchange activity. Assays for the secreted TARGET identified as SEQ ID NO: 52 may utilize activity or expression in soluble culture media or secreted activity. The measurable phenomenon, activity or property may be selected or chosen by the skilled artisan. The person of ordinary skill in the art may select from any of a number of assay formats, systems or design one using his knowledge and expertise in the art.

[0083] The present inventors have identified certain target proteins and their encoding nucleic acids by screening recombinant adenoviruses mediating the expression of a library of shRNAs, referred to herein as ‘Ad-siRNAs’. This type of library is a screen in which siRNA molecules are transduced into cells by recombinant adenoviruses, which siRNA molecules inhibit or repress the expression of a specific gene as well as expression and activity of the corresponding gene product in a cell. Each siRNA in a viral vector corresponds to a specific natural gene. By identifying a siRNA or shRNA that regulates mutant huntingtin conformation, as measured using antibodies that recognise particular huntingtin conformations, for example as described in the examples herein, a direct correlation can be drawn between the specific gene expression and the pathway for modulating mutant huntingtin conformation. The TARGET genes identified using the knock-down library (the protein expression products thereof herein referred to as “TARGET” polypeptides) are then used in the present inventive method for identifying compounds that can be used to in the treatment of diseases associated with the abnormal protein aggregation. The knock down (KD) target sequences, identified in the Ad-siRNA screens more particularly described herein, include those set out below in Table 2 (SEQ ID NOs: 53-78) and shRNA compounds comprising the sequences listed in Table 2 have been shown herein to inhibit the expression and/or activity of these TARGET genes and the examples herein confirm the role of the TARGETS in the pathway modulating the aberrant conformation or aggregation or expression of mutant proteins, including huntingtin.

[0084]**Table 2**

Exemplary KD target sequences useful in the practice of the present expression-inhibitory agent invention

HIT REF	GeneSymbol	19-mer	SEQ ID No:
1	SLC7A5	AACAAGCCCAAGTGGCTCCTC	53
2	HSD17B14	ACGTACACCTTGACCAAGCTC	54

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3	USP9X	ACAGAATCAGACTTCATCGCC	55
4	CASP1	AAGATGTTTCTACCTCTTCCC	56
5	CYB5R2	ACGGAATCTTGGAATCAGACC	57
6	NOS1	TGATCATCTCTGACCTGATTC	58
7	SPHK2	ACTTCTGCATCTACACCTACC	59
8	P2RY1	AAGAGTGAAGACATGACCCTC	60
9	LRP11	AAAGTCTCAGAAAGCCACTGC	61
10	PCSK6	AAGAGAGGTTTCGTTTCCACAC	62
11	DHCR7	ACCATTGACATCTGCCATGAC	63
12	ENPP5	ACAGTCAAATACCTGCCTTAC	64
13	ARHGEF15	AAGCTCCTCAGAATACTCCTC	65
14	PSMA2	AAGCTTTGAAGGGCAAATGAC	66
15	ABCG2	ACCTCCTTCTGTCATCAACTC	67
16	CCR10	CCTCAATCCCGTTCTCTACGC	68
17	KLKB1	ACTGCTTTGATGGGCTTCCCC	69
18	EPOR	AAGCAGAAGATCTGGCCTGGC	70
19	CREBBP	CTGTACCGGGTGAACATCAAC	71
20	APLP2	AAGTGATGTCCTGCTAGTTCC	72
21	MAP3K11	AACAAGCTCACACTGCCCATC	73
22	TNFRSF10A	ACAATTCTGCTGAGATGTGCC	74
23	HIF1A	AGCCGAGGAAGAACTATGAAC	75
24	NOS2A	AGCGGGATGACTTTCCAAGAC	76
25	DAPK2	AAATTGTGAAC TACGAGCCCC	77
26	NRG1	AGTGCTTCATGGTGAAAGACC	78

[0085] Table 1 lists the TARGETS identified using applicants' knock-down library in the assays described in the examples herein, including the class of polypeptides identified. TARGETS have been identified in polypeptide classes including transporter, kinase, protease, enzyme, receptor, GPCR (as a subclass of receptors), phosphodiesterase and drugable/secreted proteins, for instance.

[0086] Specific methods to determine the activity of a kinase, such as the TARGETs represented by SEQ ID NOs: 33, 47 and 51, by measuring the phosphorylation of a substrate by the kinase, which measurements are performed in the presence or absence of a compound, are well known in the art.

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[0087] Specific methods to determine the inhibition by the compound by measuring the cleavage of the substrate by the polypeptide, which is a protease, are well known in the art. The TARGETS represented by SEQ ID NO: 29, 30, 36, 40 and 43 are proteases. Classically, substrates are used in which a fluorescent group is linked to a quencher through a peptide sequence that is a substrate that can be cleaved by the target protease. Cleavage of the linker separates the fluorescent group and quencher, giving rise to an increase in fluorescence.

[0088] G-protein coupled receptors (GPCR) are capable of activating an effector protein, resulting in changes in second messenger levels in the cell. The TARGETs represented by SEQ ID NOs: 34 and 42 are GPCRs. The activity of a GPCR can be measured by measuring the activity level of such second messengers. Two important and useful second messengers in the cell are cyclic AMP (cAMP) and Ca^{2+} . The activity levels can be measured by methods known to persons skilled in the art, either directly by ELISA or radioactive technologies or by using substrates that generate a fluorescent or luminescent signal when contacted with Ca^{2+} or indirectly by reporter gene analysis. The activity level of the one or more secondary messengers may typically be determined with a reporter gene controlled by a promoter, wherein the promoter is responsive to the second messenger. Promoters known and used in the art for such purposes are the cyclic-AMP responsive promoter that is responsive for the cyclic-AMP levels in the cell, and the NF-AT responsive promoter that is sensitive to cytoplasmic Ca^{2+} -levels in the cell. The reporter gene typically has a gene product that is easily detectable. The reporter gene can either be stably infected or transiently transfected in the host cell. Useful reporter genes are alkaline phosphatase, enhanced green fluorescent protein, destabilized green fluorescent protein, luciferase and β -galactosidase.

[0089] It should be understood that the cells expressing the polypeptides, may be cells naturally expressing the polypeptides, or the cells may be may be transfected to express the polypeptides, as described above. Also, the cells may be transduced to overexpress the polypeptide, or may be transfected to express a non-endogenous form of the polypeptide, which can be differentially assayed or assessed. In one particular embodiment the methods of the present invention further comprise the step of contacting the population of cells with an agonist of the polypeptide. This is useful in methods wherein the expression of the polypeptide in a certain chosen population of cells is too low for a proper detection of its activity. By using an agonist the polypeptide may be triggered, enabling a proper read-out if the compound inhibits the polypeptide

[0090] The population of cells may be exposed to the compound or the mixture of compounds through different means, for instance by direct incubation in the medium, or by nucleic acid transfer into the cells. Such transfer may be achieved by a wide variety of means, for instance by direct transfection of naked isolated DNA, or RNA, or by means of delivery systems, such as recombinant vectors. Other delivery means such as liposomes, or other lipid-based vectors may also be used. Particularly, the nucleic acid compound is delivered by means of a (recombinant) vector such as a recombinant virus.

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[0091] For high-throughput purposes, libraries of compounds may be used such as antibody fragment libraries, peptide phage display libraries, peptide libraries (for example, LOPAP™, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (for example, LOPAC™, Sigma Aldrich, BioFocus DPI) or natural compound libraries (Specs, TimTec, BioFocus DPI).

[0092] Particular drug candidate compounds are low molecular weight compounds. Low molecular weight compounds, for example with a molecular weight of 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently more likely to be successful drug candidates than compounds with a molecular weight above 500 Dalton (Lipinski *et al.*, 2001)). Peptides comprise another particular class of drug candidate compounds. Peptides may be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors. Natural compounds are another particular class of drug candidate compound. Such compounds are found in and extracted from natural sources, and which may thereafter be synthesized. The lipids are another particular class of drug candidate compound.

[0093] Another particular class of drug candidate compounds is an antibody. The present invention also provides antibodies directed against a TARGET. These antibodies may be endogenously produced to bind to the TARGET within the cell, or added to the tissue to bind to TARGET polypeptide present outside the cell. These antibodies may be monoclonal antibodies or polyclonal antibodies. The present invention includes chimeric, single chain, and humanized antibodies, as well as Fab fragments and the products of a Fab expression library, and Fv fragments and the products of an Fv expression library. In another embodiment, the compound may be a nanobody, the smallest functional fragment of naturally occurring single-domain antibodies (Cortez-Retamozo *et al.* 2004).

[0094] In certain embodiments, polyclonal antibodies may be used in the practice of the invention. The skilled artisan knows methods of preparing polyclonal antibodies. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. Antibodies may also be generated against the intact TARGET protein or polypeptide, or against a fragment, derivatives including conjugates, or other epitope of the TARGET protein or polypeptide, such as the TARGET embedded in a cellular membrane, or a library of antibody variable regions, such as a phage display library.

[0095] It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant

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(monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). One skilled in the art without undue experimentation may select the immunization protocol.

[0096] In some embodiments, the antibodies may be monoclonal antibodies. Monoclonal antibodies may be prepared using methods known in the art. The monoclonal antibodies of the present invention may be “humanized” to prevent the host from mounting an immune response to the antibodies. A “humanized antibody” is one in which the complementarity determining regions (CDRs) and/or other portions of the light and/or heavy variable domain framework are derived from a non-human immunoglobulin, but the remaining portions of the molecule are derived from one or more human immunoglobulins. Humanized antibodies also include antibodies characterized by a humanized heavy chain associated with a donor or acceptor unmodified light chain or a chimeric light chain, or vice versa. The humanization of antibodies may be accomplished by methods known in the art (see, for example, Mark and Padlan, (1994) “Chapter 4. Humanization of Monoclonal Antibodies”, The Handbook of Experimental Pharmacology Vol. 113, Springer-Verlag, New York). Transgenic animals may be used to express humanized antibodies.

[0097] Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, (1991) J. Mol. Biol. 227:381-8; Marks et al. (1991). J. Mol. Biol. 222:581-97). The techniques of Cole, *et al.* and Boerner, *et al.* are also available for the preparation of human monoclonal antibodies (Cole, *et al.* (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77; Boerner, *et al.* (1991). J. Immunol., 147(1):86-95).

[0098] Techniques known in the art for the production of single chain antibodies can be adapted to produce single chain antibodies to the TARGET polypeptides and proteins of the present invention. The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

[0099] Bispecific antibodies are monoclonal, particularly human or humanized, antibodies that have binding specificities for at least two different antigens and particularly for a cell-surface protein or receptor or receptor subunit. In the present case, one of the binding specificities is for one domain of the TARGET, while the other one is for another domain of the same or different TARGET.

[0100] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, (1983) Nature 305:537-9). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas

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(quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Affinity chromatography steps usually accomplish the purification of the correct molecule. Similar procedures are disclosed in Traunecker, *et al.* (1991) EMBO J. 10:3655-9.

[0101] Therefore, in a further embodiment the present invention relates to a method for identifying a compound that modulates the expression of the mutant huntingtin protein comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
- b) determining the binding affinity of the compound to the polypeptide;
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
- d) identifying the compound that modulates the expression of mutant huntingtin protein.

[0102] In one embodiment, the method relates to means for identifying compounds that are able to modulate the aggregation of Huntingtin protein.

[0103] The present invention further relates to a method for identifying a compound that modulates polyglutamine conformation, comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
- b) determining the binding affinity of the compound to the polypeptide;
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
- d) identifying the compound that modulates polyglutamine conformation.

[0104] The present invention further relates to a method for identifying a compound that modulates the expression of the mutant huntingtin protein, comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
- b) determining the ability of the compound inhibit the expression or activity of the polypeptide;
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ; and
- d) identifying the compound that modulates the expression of the mutant huntingtin protein.

[0105] The present invention further relates to a method for identifying a compound that modulates polyglutamine conformation, comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
- b) determining the ability of the compound inhibit the expression or activity of the polypeptide;

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- c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ; and
- d) identifying the compound that modulates polyglutamine conformation.

[0106] In particular aspects of the invention, the expression of the mutant huntingtin protein may be measured using an antibody that recognizes the protein by binding to a region outside the polyglutamine stretch. Exemplary such antibodies are well known in the art and publicly available including N18 (Santa Cruz, USA), MW7 (Ko *et al.*, 2001) and 4C8 (Trottier *et al.*, 1995a).

[0107] In particular aspects of the invention, the expression of the mutant huntingtin protein may be measured using an antibody that recognizes the protein by binding the polyglutamine repeat. Exemplary such antibodies are well known in the art and publicly available including 3B5H10, EM48 (Li *et al.*, 1999), MW1, MW2, MW3, MW4 and MW5 (Ko *et al.*, 2001) and 1C2 (Trottier *et al.*, 1995b).

[0108] In particular aspects of the invention, the mutant huntingtin protein conformation may be measured using an antibody that recognizes the protein by binding the polyglutamine repeat. In specific aspects of the invention, the antibody used may recognize the polyglutamine repeat in an abnormal conformation. Suitable antibodies are known to a person of skill in the art and include, without limitation 3B5H10 antibody described in US 6,291,652, 1C2 antibody described in WO 97/17445, which is directed against huntingtin protein polyglutamine repeat. Further information regarding huntingtin antibodies is provided and detailed in such references as (Brooks *et al.*, 2004; Imbert *et al.*, 1996; Trottier *et al.*, 1995b).

[0109] Alternatively, inclusion bodies indicative of protein aggregation may be identified using labeled huntingtin protein or other protein for which aggregation is being tested, and the inclusion bodies recognized by visual scanning in a microscope or other such system.

[0110] According to another particular embodiment, the assay method uses a drug candidate compound identified as having a binding affinity for a TARGET, and/or has already been identified as having down-regulating activity such as antagonist activity vis-à-vis one or more TARGET.

[0111] Candidate compound or agents may be validated or rescreened in the huntingtin protein conformation assay. Other assays for confirming activity in ameliorating, preventing or treating HD or other neurodegenerative diseases include neural cell death assays, assays for apoptosis, and animal models for HD or neurodegenerative diseases such as R6/2 (Mangiarini *et al.*, 1996) and YAC128 (Slow *et al.*, 2003)

[0112] The present invention further relates to a method for modulating the Huntington Disease phenotype comprising contacting mammalian cells with an expression inhibitory agent comprising a polyribonucleotide sequence that complements at least about 15 to about 30, particularly at least 17 to about 30, most particularly at least 17 to about 25 contiguous nucleotides of the nucleotide sequence selected from the group consisting of SEQ ID NO: 53-78.

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[0113] Another aspect of the present invention relates to a method for modulating the Huntington Disease phenotype, comprising by contacting mammalian cells with an expression-inhibiting agent that inhibits the translation in the cell of a polyribonucleotide encoding a TARGET polypeptide. A particular embodiment relates to a composition comprising a polynucleotide including at least one antisense strand that functions to pair the agent with the TARGET mRNA, and thereby down-regulate or block the expression of TARGET polypeptide. The inhibitory agent particularly comprises antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence selected from the group consisting of SEQ ID NO: 53-78.

[0114] A special embodiment of the present invention relates to a method wherein the expression-inhibiting agent is selected from the group consisting of antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide coding for SEQ ID NO: 27-52, a small interfering RNA (siRNA, particularly shRNA,) that is sufficiently homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1-26, such that antisense RNA, ODN, ribozyme, particularly the siRNA, particularly shRNA, interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide.

[0115] In one embodiment, the TARGET is a transporter, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 27 or 41 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1 or 15, exemplary oligonucleotide sequences include SEQ ID NO: 53 and 67. In a further embodiment, the TARGET is an enzyme, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 28, 31, 32, 37, 45 or 50 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 2, 5, 6, 11, 19, or 24, exemplary oligonucleotide sequences include SEQ ID NO: 54, 57, 58, 63, 71 and 76. In a further embodiment, the TARGET is a protease, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 29, 30, 36, 40 or 43 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 3, 4, 10, 14 or 17, exemplary oligonucleotide sequences include SEQ ID NO: 55, 56, 62, 66 and 69. In a further embodiment, the TARGET is a kinase, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 33, 47 or 51 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 7, 21 or 25, exemplary oligonucleotide sequences include SEQ ID NO: 59, 73 or 77. In a further embodiment, the TARGET is a GPCR, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 34 or 42 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 8 or 16, exemplary oligonucleotide sequences include SEQ ID NO: 60 and 68. In a further embodiment, the TARGET is a receptor, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 35, 44 or 48 or the siRNA or shRNA is homologous to a portion of the

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polyribonucleotide corresponding to SEQ ID NO: 9, 18 or 22, exemplary oligonucleotide sequences include SEQ ID NO: 61, 70 and 74. In a further embodiment, the TARGET is a phosphodiesterase (PDE), therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 38 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 12, exemplary oligonucleotide sequences include SEQ ID NO: 64. In a further embodiment, the TARGET is a drugable protein, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 39 or 52 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 13 or 26, exemplary oligonucleotide sequences include SEQ ID NO: 65 and 78. In a further embodiment, the TARGET is a transcription factor, therefore the ribozyme may cleave a polynucleotide coding for SEQ ID NO: 49 or the siRNA or shRNA is homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 23, exemplary oligonucleotide sequences include SEQ ID NO: 75.

[0116] Another embodiment of the present invention relates to a method wherein the expression-inhibiting agent is a nucleic acid expressing the antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide corresponding to SEQ ID 53-78, a small interfering RNA (siRNA, particularly shRNA,) that is sufficiently complementary to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1-26, such that the antisense RNA, ODN, ribozyme, particularly siRNA, particularly shRNA, interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide. Particularly the expression-inhibiting agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA, particularly shRNA, comprising a polyribonucleotide sequence that complements at least about 17 to about 30 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1-26. More particularly, the expression-inhibiting agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA, particularly shRNA, comprising a polyribonucleotide sequence that complements at least 15 to about 30, particularly at least 17 to about 30, most particularly at least 17 to about 25 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1-26. A special embodiment comprises a polyribonucleotide sequence that complements a polynucleotide sequence selected from the group consisting of SEQ ID NO: 53-78.

[0117] The down regulation of gene expression using antisense nucleic acids can be achieved at the translational or transcriptional level. Antisense nucleic acids of the invention are particularly nucleic acid fragments capable of specifically hybridizing with all or part of a nucleic acid encoding a TARGET polypeptide or the corresponding messenger RNA. In addition, antisense nucleic acids may be designed which decrease expression of the nucleic acid sequence capable of encoding a TARGET polypeptide by inhibiting splicing of its primary transcript. Any length of antisense sequence is suitable for practice of the invention so long as it is capable of down-regulating or blocking expression of a nucleic acid coding for a TARGET. Particularly, the antisense sequence is at least about 15-30, and particularly at least 17 nucleotides in length.

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The preparation and use of antisense nucleic acids, DNA encoding antisense RNAs and the use of oligo and genetic antisense is known in the art.

[0118] One embodiment of expression-inhibitory agent is a nucleic acid that is antisense to a nucleic acid comprising SEQ ID NO: 1-26, for example, an antisense nucleic acid (for example, DNA) may be introduced into cells *in vitro*, or administered to a subject *in vivo*, as gene therapy to inhibit cellular expression of nucleic acids comprising SEQ ID NO: 1-26. Antisense oligonucleotides may comprise a sequence containing from about 15 to about 100 nucleotides, more particularly from about 15 to about 30 nucleotides, and most particularly, from about 17 to about 25 nucleotides. Antisense nucleic acids may be prepared from about 15 to about 30 contiguous nucleotides selected from the sequences of SEQ ID NO: 1-26, expressed in the opposite orientation.

[0119] The skilled artisan can readily utilize any of several strategies to facilitate and simplify the selection process for antisense nucleic acids and oligonucleotides effective in inhibition of TARGET and/or Huntington Disease phenotype modulation. Predictions of the binding energy or calculation of thermodynamic indices between an oligonucleotide and a complementary sequence in an mRNA molecule may be utilized (Chiang et al. (1991) J. Biol. Chem. 266:18162-18171; Stull et al. (1992) Nucl. Acids Res. 20:3501-3508). Antisense oligonucleotides may be selected on the basis of secondary structure (Wickstrom et al (1991) in Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS, Wickstrom, ed., Wiley-Liss, Inc., New York, pp. 7-24; Lima et al. (1992) Biochem. 31:12055-12061). Schmidt and Thompson (U.S. Patent 6,416,951) describe a method for identifying a functional antisense agent comprising hybridizing an RNA with an oligonucleotide and measuring in real time the kinetics of hybridization by hybridizing in the presence of an intercalation dye or incorporating a label and measuring the spectroscopic properties of the dye or the label's signal in the presence of unlabelled oligonucleotide. In addition, any of a variety of computer programs may be utilized which predict suitable antisense oligonucleotide sequences or antisense targets utilizing various criteria recognized by the skilled artisan, including for example the absence of self-complementarity, the absence hairpin loops, the absence of stable homodimer and duplex formation (stability being assessed by predicted energy in kcal/mol). Examples of such computer programs are readily available and known to the skilled artisan and include the OLIGO 4 or OLIGO 6 program (Molecular Biology Insights, Inc., Cascade, CO) and the Oligo Tech program (Oligo Therapeutics Inc., Wilsonville, OR). In addition, antisense oligonucleotides suitable in the present invention may be identified by screening an oligonucleotide library, or a library of nucleic acid molecules, under hybridization conditions and selecting for those which hybridize to the target RNA or nucleic acid (see for example U.S. Patent 6,500,615). Mishra and Toulme have also developed a selection procedure based on selective amplification of oligonucleotides that bind target (Mishra et al (1994) Life Sciences 317:977-982). Oligonucleotides may also be selected by their ability to mediate cleavage of target RNA by RNase H, by selection and characterization of the cleavage fragments (Ho et al (1996) Nucl

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Acids Res 24:1901-1907; Ho et al (1998) Nature Biotechnology 16:59-630). Generation and targeting of oligonucleotides to GGGA motifs of RNA molecules has also been described (U.S. Patent 6,277,981).

[0120] The antisense nucleic acids are particularly oligonucleotides and may consist entirely of deoxyribonucleotides, modified deoxyribonucleotides, or some combination of both. The antisense nucleic acids can be synthetic oligonucleotides. The oligonucleotides may be chemically modified, if desired, to improve stability and/or selectivity. Specific examples of some particular oligonucleotides envisioned for this invention include those containing modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Since oligonucleotides are susceptible to degradation by intracellular nucleases, the modifications can include, for example, the use of a sulfur group to replace the free oxygen of the phosphodiester bond. This modification is called a phosphorothioate linkage. Phosphorothioate antisense oligonucleotides are water soluble, polyanionic, and resistant to endogenous nucleases. In addition, when a phosphorothioate antisense oligonucleotide hybridizes to its TARGET site, the RNA-DNA duplex activates the endogenous enzyme ribonuclease (RNase) H, which cleaves the mRNA component of the hybrid molecule. Oligonucleotides may also contain one or more substituted sugar moieties. Particular oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH₃, F, OCN, heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide.

[0121] In addition, antisense oligonucleotides with phosphoramidite and polyamide (peptide) linkages can be synthesized. These molecules should be very resistant to nuclease degradation. Furthermore, chemical groups can be added to the 2' carbon of the sugar moiety and the 5 carbon (C-5) of pyrimidines to enhance stability and facilitate the binding of the antisense oligonucleotide to its TARGET site. Modifications may include 2'-deoxy, O-pentoxy, O-propoxy, O-methoxy, fluoro, methoxyethoxy phosphorothioates, modified bases, as well as other modifications known to those of skill in the art.

[0122] Another type of expression-inhibitory agent that reduces the levels of TARGETS is the ribozyme. Ribozymes are catalytic RNA molecules (RNA enzymes) that have separate catalytic and substrate binding domains. The substrate binding sequence combines by nucleotide complementarity and, possibly, non-hydrogen bond interactions with its TARGET sequence. The catalytic portion cleaves the TARGET RNA at a specific site. The substrate domain of a ribozyme can be engineered to direct it to a specified mRNA sequence. The ribozyme recognizes and then binds a TARGET mRNA through complementary base pairing. Once it is bound to the correct TARGET site, the ribozyme acts enzymatically to cut the TARGET mRNA. Cleavage of

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the mRNA by a ribozyme destroys its ability to direct synthesis of the corresponding polypeptide. Once the ribozyme has cleaved its TARGET sequence, it is released and can repeatedly bind and cleave at other mRNAs.

[0123] Ribozyme forms include a hammerhead motif, a hairpin motif, a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) motif or Neurospora VS RNA motif. Ribozymes possessing a hammerhead or hairpin structure are readily prepared since these catalytic RNA molecules can be expressed within cells from eukaryotic promoters (Chen, et al. (1992) Nucleic Acids Res. 20:4581-9). A ribozyme of the present invention can be expressed in eukaryotic cells from the appropriate DNA vector. If desired, the activity of the ribozyme may be augmented by its release from the primary transcript by a second ribozyme (Ventura, *et al.* (1993) Nucleic Acids Res. 21:3249-55).

[0124] Ribozymes may be chemically synthesized by combining an oligodeoxyribonucleotide with a ribozyme catalytic domain (20 nucleotides) flanked by sequences that hybridize to the TARGET mRNA after transcription. The oligodeoxyribonucleotide is amplified by using the substrate binding sequences as primers. The amplification product is cloned into a eukaryotic expression vector.

[0125] Ribozymes are expressed from transcription units inserted into DNA, RNA, or viral vectors. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on nearby gene regulatory sequences. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Gao and Huang, (1993) Nucleic Acids Res. 21:2867-72). It has been demonstrated that ribozymes expressed from these promoters can function in mammalian cells (Kashani-Sabet, et al. (1992) Antisense Res. Dev. 2:3-15).

[0126] A particular inhibitory agent is a small interfering RNA (siRNA, particularly small hairpin RNA, "shRNA"). siRNA, particularly shRNA, mediate the post-transcriptional process of gene silencing by double stranded RNA (dsRNA) that is homologous in sequence to the silenced RNA. siRNA according to the present invention comprises a sense strand of 15-30, particularly 17-30, most particularly 17-25 nucleotides complementary or homologous to a contiguous 17-25 nucleotide sequence selected from the group of sequences described in SEQ ID NO: 1-26, particularly from the group of sequences described in SEQ ID No: 53-78, and an antisense strand of 15-30, particularly 17-30, most particularly 17-25 nucleotides complementary to the sense strand. The most particular siRNA comprises sense and anti-sense strands that are 100 per cent complementary to each other and the TARGET polynucleotide sequence. Particularly the siRNA further comprises a loop region linking the sense and the antisense strand.

[0127] A self-complementing single stranded shRNA molecule polynucleotide according to the present invention comprises a sense portion and an antisense portion connected by a loop region linker. Particularly, the loop region sequence is 4-30 nucleotides long, more particularly 5-15 nucleotides long and most

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particularly 8 or 12 nucleotides long. In a most particular embodiment the linker sequence is UUGCUAUA or GUUUGCUAUAAC (SEQ ID NO: 79). Self-complementary single stranded siRNAs form hairpin loops and are more stable than ordinary dsRNA. In addition, they are more easily produced from vectors.

[0128] Analogous to antisense RNA, the siRNA can be modified to confer resistance to nucleolytic degradation, or to enhance activity, or to enhance cellular distribution, or to enhance cellular uptake, such modifications may consist of modified internucleoside linkages, modified nucleic acid bases, modified sugars and/or chemical linkage the siRNA to one or more moieties or conjugates. The nucleotide sequences are selected according to siRNA designing rules that give an improved reduction of the TARGET sequences compared to nucleotide sequences that do not comply with these siRNA designing rules (For a discussion of these rules and examples of the preparation of siRNA, WO 2004/094636 and US 2003/0198627, are hereby incorporated by reference).

[0129] The present invention also relates to compositions, and methods using said compositions, comprising a DNA expression vector capable of expressing a polynucleotide capable of modulating a Huntington Disease phenotype and described hereinabove as an expression inhibition agent.

[0130] A special aspect of these compositions and methods relates to the down-regulation or blocking of the expression of a TARGET polypeptide by the induced expression of a polynucleotide encoding an intracellular binding protein that is capable of selectively interacting with the TARGET polypeptide. An intracellular binding protein includes any protein capable of selectively interacting, or binding, with the polypeptide in the cell in which it is expressed and neutralizing the function of the polypeptide. Particularly, the intracellular binding protein is a neutralizing antibody or a fragment of a neutralizing antibody having binding affinity to an epitope of the TARGET polypeptide of SEQ ID NO:27-52. More particularly, the intracellular binding protein is a single chain antibody.

[0131] A special embodiment of this composition comprises the expression-inhibiting agent selected from the group consisting of antisense RNA, antisense oligodeoxynucleotide (ODN), a ribozyme that cleaves the polyribonucleotide coding for SEQ ID NO: 27-52, and a small interfering RNA (siRNA) that is sufficiently homologous to a portion of the polyribonucleotide corresponding to SEQ ID NO: 1-26, such that the siRNA interferes with the translation of the TARGET polyribonucleotide to the TARGET polypeptide.

[0132] The polynucleotide expressing the expression-inhibiting agent, or a polynucleotide expressing the TARGET polypeptide in cells, is particularly included within a vector. The polynucleic acid is operably linked to signals enabling expression of the nucleic acid sequence and is introduced into a cell utilizing, particularly, recombinant vector constructs, which will express the nucleic acid or antisense nucleic acid once the vector is introduced into the cell. A variety of viral-based systems are available, including adenoviral, retroviral, adeno-associated viral, lentiviral, herpes simplex viral or a sendaviral vector systems. All may be used to introduce and express polynucleotide sequence for the expression-inhibiting agents in TARGET cells.

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[0133] Particularly, the viral vectors used in the methods of the present invention are replication defective. Such replication defective vectors will usually pack at least one region that is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution, partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed in vitro (on the isolated DNA) or in situ, using the techniques of genetic manipulation or by treatment with mutagenic agents. Particularly, the replication defective virus retains the sequences of its genome, which are necessary for encapsidating, the viral particles.

[0134] In a particular embodiment, the viral element is derived from an adenovirus. Particularly, the vehicle includes an adenoviral vector packaged into an adenoviral capsid, or a functional part, derivative, and/or analogue thereof. Adenovirus biology is also comparatively well known on the molecular level. Many tools for adenoviral vectors have been and continue to be developed, thus making an adenoviral capsid a particular vehicle for incorporating in a library of the invention. An adenovirus is capable of infecting a wide variety of cells. However, different adenoviral serotypes have different preferences for cells. To combine and widen the TARGET cell population that an adenoviral capsid of the invention can enter in a particular embodiment, the vehicle includes adenoviral fiber proteins from at least two adenoviruses. Particular adenoviral fiber protein sequences are serotype 17, 45 and 51. Techniques for construction and expression of these chimeric vectors are disclosed in US 2003/0180258 and US 2004/0071660, hereby incorporated by reference.

[0135] In a particular embodiment, the nucleic acid derived from an adenovirus includes the nucleic acid encoding an adenoviral late protein or a functional part, derivative, and/or analogue thereof. An adenoviral late protein, for instance an adenoviral fiber protein, may be favorably used to TARGET the vehicle to a certain cell or to induce enhanced delivery of the vehicle to the cell. Particularly, the nucleic acid derived from an adenovirus encodes for essentially all adenoviral late proteins, enabling the formation of entire adenoviral capsids or functional parts, analogues, and/or derivatives thereof. Particularly, the nucleic acid derived from an adenovirus includes the nucleic acid encoding adenovirus E2A or a functional part, derivative, and/or analogue thereof. Particularly, the nucleic acid derived from an adenovirus includes the nucleic acid encoding at least one E4-region protein or a functional part, derivative, and/or analogue thereof, which facilitates, at least in part, replication of an adenoviral derived nucleic acid in a cell. The adenoviral vectors used in the examples of this application are exemplary of the vectors useful in the present method of treatment invention.

[0136] Certain embodiments of the present invention use retroviral vector systems. Retroviruses are integrating viruses that infect dividing cells, and their construction is known in the art. Retroviral vectors can be constructed from different types of retrovirus, such as, MoMuLV (“murine Moloney leukemia virus” MSV (“murine Moloney sarcoma virus”), HaSV (“Harvey sarcoma virus”); SNV (“spleen necrosis virus”); RSV

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("Rous sarcoma virus") and Friend virus. Lentiviral vector systems may also be used in the practice of the present invention. Retroviral systems and herpes virus system may be particular vehicles for transfection of neuronal cells.

[0137] In other embodiments of the present invention, adeno-associated viruses ("AAV") are utilized. The AAV viruses are DNA viruses of relatively small size that integrate, in a stable and site-specific manner, into the genome of the infected cells. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies.

[0138] In the vector construction, the polynucleotide agents of the present invention may be linked to one or more regulatory regions. Selection of the appropriate regulatory region or regions is a routine matter, within the level of ordinary skill in the art. Regulatory regions include promoters, and may include enhancers, suppressors, etc.

[0139] Promoters that may be used in the expression vectors of the present invention include both constitutive promoters and regulated (inducible) promoters. The promoters may be prokaryotic or eukaryotic depending on the host. Among the prokaryotic (including bacteriophage) promoters useful for practice of this invention are lac, lacZ, T3, T7, lambda P.sub.r, P.sub.l, and trp promoters. Among the eukaryotic (including viral) promoters useful for practice of this invention are ubiquitous promoters (for example, HPRT, vimentin, actin, tubulin), intermediate filament promoters (for example, desmin, neurofilaments, keratin, GFAP), therapeutic gene promoters (for example, MDR type, CFTR, factor VIII), tissue-specific promoters (for example, actin promoter in smooth muscle cells, or Flt and Flk promoters active in endothelial cells), including animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift, *et al.* (1984) *Cell* 38:639-46; Ornitz, *et al.* (1986) *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, (1987) *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, (1985) *Nature* 315:115-22), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl, *et al.* (1984) *Cell* 38:647-58; Adames, *et al.* (1985) *Nature* 318:533-8; Alexander, *et al.* (1987) *Mol. Cell. Biol.* 7:1436-44), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder, *et al.* (1986) *Cell* 45:485-95), albumin gene control region which is active in liver (Pinkert, *et al.* (1987) *Genes and Devel.* 1:268-76), alpha-fetoprotein gene control region which is active in liver (Krumlauf, *et al.* (1985) *Mol. Cell. Biol.*, 5:1639-48; Hammer, *et al.* (1987) *Science* 235:53-8), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey, *et al.* (1987) *Genes and Devel.*, 1: 161-71), beta-globin gene control region which is active in myeloid cells (Mogam, *et al.* (1985) *Nature* 315:338-40; Kollias, *et al.* (1986) *Cell* 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead, *et al.* (1987) *Cell* 48:703-12), myosin light chain-2 gene control

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region which is active in skeletal muscle (Sani, (1985) Nature 314:283-6), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason, *et al.* (1986) Science 234:1372-8).

[0140] Other promoters which may be used in the practice of the invention include promoters which are preferentially activated in dividing cells, promoters which respond to a stimulus (for example, steroid hormone receptor, retinoic acid receptor), tetracycline-regulated transcriptional modulators, cytomegalovirus immediate-early, retroviral LTR, metallothionein, SV-40, E1a, and MLP promoters.

[0141] Additional vector systems include the non-viral systems that facilitate introduction of polynucleotide agents into a patient, for example, a DNA vector encoding a desired sequence can be introduced *in vivo* by lipofection. Synthetic cationic lipids designed to limit the difficulties encountered with liposome-mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner, *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84:7413-7); see Mackey, *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:8027-31; Ulmer, *et al.* (1993) Science 259:1745-8). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, (1989) Nature 337:387-8). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO 95/18863 and WO 96/17823, and in U.S. Pat. No. 5,459,127. The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages and directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, for example, pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides, for example, hormones or neurotransmitters, and proteins, for example, antibodies, or non-peptide molecules could be coupled to liposomes chemically. Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, for example, a cationic oligopeptide (for example, WO 95/21931), peptides derived from DNA binding proteins (for example, WO 96/25508), or a cationic polymer (for example, WO 95/21931).

[0142] It is also possible to introduce a DNA vector *in vivo* as a naked DNA plasmid (see U.S. Pat. Nos. 5,693,622; 5,589,466; and 5,580,859). Naked DNA vectors for therapeutic purposes can be introduced into the desired host cells by methods known in the art, for example, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, for example, Wilson, *et al.* (1992) J. Biol. Chem. 267:963-7; Wu and Wu, (1988) J. Biol. Chem. 263:14621-4; Hartmut, *et al.* Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990; Williams, *et al.* (1991). Proc. Natl. Acad. Sci. USA 88:2726-30). Receptor-mediated DNA delivery approaches can also be used (Curiel, *et al.* (1992) Hum. Gene Ther. 3:147-54; Wu and Wu, (1987) J. Biol. Chem. 262:4429-32).

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[0143] A biologically compatible composition is a composition, that may be solid, liquid, gel, or other form, in which the compound, polynucleotide, vector, or antibody of the invention is maintained in an active form, for example, in a form able to effect a biological activity. For example, a compound of the invention would have inverse agonist or antagonist activity on the TARGET; a nucleic acid would be able to replicate, translate a message, or hybridize to a complementary mRNA of a TARGET; a vector would be able to transfect a TARGET cell and express the antisense, antibody, ribozyme or siRNA as described hereinabove; an antibody would bind a TARGET polypeptide domain.

[0144] A particular biologically compatible composition is an aqueous solution that is buffered using, for example, Tris, phosphate, or HEPES buffer, containing salt ions. Usually the concentration of salt ions will be similar to physiological levels. Biologically compatible solutions may include stabilizing agents and preservatives. In a more particular embodiment, the biocompatible composition is a pharmaceutically acceptable composition. Such compositions can be formulated for administration by topical, oral, parenteral, intranasal, subcutaneous, and intraocular, routes. Parenteral administration is meant to include intravenous injection, intramuscular injection, intraarterial injection or infusion techniques. The composition may be administered parenterally in dosage unit formulations containing standard, well-known non-toxic physiologically acceptable carriers, adjuvants and vehicles as desired.

[0145] A particular embodiment of the present composition invention is a modulation of the Huntington Disease phenotype inhibiting pharmaceutical composition comprising a therapeutically effective amount of an expression-inhibiting agent as described hereinabove, in admixture with a pharmaceutically acceptable carrier. Another particular embodiment is a pharmaceutical composition for the treatment or prevention of a condition involving neurodegeneration, or a susceptibility to the condition, comprising an effective polyglutamine-induced protein aggregation and/or mutant huntingtin protein expression/activity inhibiting amount of a TARGET antagonist or inverse agonist, its pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof in admixture with a pharmaceutically acceptable carrier. Another embodiment of the present compositions include compositions comprising therapeutically effective amounts of two or more expression-inhibiting agents or two or more polyglutamine-induced protein aggregation and/or mutant huntingtin protein expression/activity inhibiting agents in combination.

[0146] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient. Pharmaceutical compositions for oral use can be prepared by combining active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or

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sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl-cellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinyl-pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

[0147] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

[0148] Particular sterile injectable preparations can be a solution or suspension in a non-toxic parenterally acceptable solvent or diluent. Examples of pharmaceutically acceptable carriers are saline, buffered saline, isotonic saline (for example, monosodium or disodium phosphate, sodium, potassium; calcium or magnesium chloride, or mixtures of such salts), Ringer's solution, dextrose, water, sterile water, glycerol, ethanol, and combinations thereof 1,3-butanediol and sterile fixed oils are conveniently employed as solvents or suspending media. Any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid also find use in the preparation of injectables.

[0149] The compounds or compositions of the invention may be combined for administration with or embedded in polymeric carrier(s), biodegradable or biomimetic matrices or in a scaffold. The carrier, matrix or scaffold may be of any material that will allow composition to be incorporated and expressed and will be compatible with the addition of cells or in the presence of cells. Particularly, the carrier matrix or scaffold is predominantly non-immunogenic and is biodegradable. Examples of biodegradable materials include, but are not limited to, polyglycolic acid (PGA), polylactic acid (PLA), hyaluronic acid, catgut suture material, gelatin, cellulose, nitrocellulose, collagen, albumin, fibrin, alginate, cotton, or other naturally-occurring biodegradable materials. It may be preferable to sterilize the matrix or scaffold material prior to administration or implantation, e.g., by treatment with ethylene oxide or by gamma irradiation or irradiation with an electron beam. In addition, a number of other materials may be used to form the scaffold or framework structure, including but not limited to: nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluorethylene (PTFE, teflon), thermanox (TPX), polymers of hydroxy acids such as polylactic acid (PLA), polyglycolic acid

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(PGA), and polylactic acid-glycolic acid (PLGA), polyorthoesters, polyanhydrides, polyphosphazenes, and a variety of polyhydroxyalkanoates, and combinations thereof. Matrices suitable include a polymeric mesh or sponge and a polymeric hydrogel. In the particular embodiment, the matrix is biodegradable over a time period of less than a year, more particularly less than six months, most particularly over two to ten weeks. The polymer composition, as well as method of manufacture, can be used to determine the rate of degradation. For example, mixing increasing amounts of polylactic acid with polyglycolic acid decreases the degradation time. Meshes of polyglycolic acid that can be used can be obtained commercially, for instance, from surgical supply companies (e.g., Ethicon, N.J.). In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof.

[0150] The composition medium can also be a hydrogel, which is prepared from any biocompatible or non-cytotoxic homo- or hetero-polymer, such as a hydrophilic polyacrylic acid polymer that can act as a drug absorbing sponge. Certain of them, such as, in particular, those obtained from ethylene and/or propylene oxide are commercially available. A hydrogel can be deposited directly onto the surface of the tissue to be treated, for example during surgical intervention.

[0151] Embodiments of pharmaceutical compositions of the present invention comprise a replication defective recombinant viral vector encoding the agent of the present invention and a transfection enhancer, such as poloxamer. An example of a poloxamer is Poloxamer 407, which is commercially available (BASF, Parsippany, N.J.) and is a non-toxic, biocompatible polyol. A poloxamer impregnated with recombinant viruses may be deposited directly on the surface of the tissue to be treated, for example during a surgical intervention. Poloxamer possesses essentially the same advantages as hydrogel while having a lower viscosity.

[0152] The active agents may also be entrapped in microcapsules prepared, for example, by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.

[0153] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, for example, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and

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lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0154] As defined above, therapeutically effective dose means that amount of protein, polynucleotide, peptide, or its antibodies, agonists or antagonists, which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions that exhibit large therapeutic indices are particular. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for human use. The dosage of such compounds lies particularly within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0155] For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, age, weight and gender of the patient; diet, desired duration of treatment, method of administration, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0156] The pharmaceutical compositions according to this invention may be administered to a subject by a variety of methods. They may be added directly to targeted tissues, complexed with cationic lipids, packaged within liposomes, or delivered to targeted cells by other methods known in the art. Localized administration to the desired tissues may be done by direct injection, transdermal absorption, catheter, infusion pump or stent. The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of

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treatment. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. Examples of ribozyme delivery and administration are provided in Sullivan *et al.* WO 94/02595.

[0157] Antibodies according to the invention may be delivered as a bolus only, infused over time or both administered as a bolus and infused over time. Those skilled in the art may employ different formulations for polynucleotides than for proteins. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

[0158] As discussed hereinabove, recombinant viruses may be used to introduce DNA encoding polynucleotide agents useful in the present invention. Recombinant viruses according to the invention are generally formulated and administered in the form of doses of between about 10^4 and about 10^{14} pfu. In the case of AAVs and adenoviruses, doses of from about 10^6 to about 10^{11} pfu are particularly used. The term pfu (“plaque-forming unit”) corresponds to the infective power of a suspension of virions and is determined by infecting an appropriate cell culture and measuring the number of plaques formed. The techniques for determining the pfu titre of a viral solution are well documented in the prior art.

[0159] Administration of the expression-inhibiting agent of the present invention to the subject patient includes both self-administration and administration by another person. The patient may be in need of treatment for an existing disease or medical condition, or may desire prophylactic treatment to prevent or reduce the risk for diseases and medical conditions affected by a disturbance in bone metabolism. The expression-inhibiting agent of the present invention may be delivered to the subject patient orally, transdermally, via inhalation, injection, nasally, rectally or via a sustained release formulation.

[0160] The polypeptides and polynucleotides useful in the practice of the present invention described herein may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. To perform the methods it is feasible to immobilize either the TARGET polypeptide or the compound to facilitate separation of complexes from uncomplexed forms of the polypeptide, as well as to accommodate automation of the assay. Interaction (for example, binding of) of the TARGET polypeptide with a compound can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the polypeptide to be bound to a matrix. For example, the TARGET polypeptide can be “His” tagged, and subsequently adsorbed onto Ni-NTA microtitre plates, or ProtA fusions with the TARGET polypeptides can be adsorbed to IgG, which are then combined with the cell lysates (for example, $(35)^S$ -labeled) and the candidate compound, and the mixture incubated under conditions favorable for complex formation (for example, at physiological conditions for salt and pH). Following incubation, the plates are washed to remove any unbound label, and the matrix is immobilized. The amount of radioactivity can be

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determined directly, or in the supernatant after dissociation of the complexes. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of the protein binding to the TARGET protein quantified from the gel using standard electrophoretic techniques.

[0161] Other techniques for immobilizing protein on matrices can also be used in the method of identifying compounds. For example, either the TARGET or the compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TARGET protein molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (for example, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the TARGETS but which do not interfere with binding of the TARGET to the compound can be derivatized to the wells of the plate, and the TARGET can be trapped in the wells by antibody conjugation. As described above, preparations of a labeled candidate compound are incubated in the wells of the plate presenting the TARGETS, and the amount of complex trapped in the well can be quantitated.

[0162] The invention is further illustrated in the following figures and examples.

Examples

[0163] As described in the introduction, both cell death caused by expression of mutant huntingtin and the abnormal conformation of the expanded huntingtin protein are phenotypes that serve as an entry-point for development of a drug that prevents or stops the neurodegeneration observed in HD and similar neurodegenerative diseases. The following assays, when used in combination with arrayed adenoviral shRNA (small hairpin RNA), adenoviral cDNA expression libraries (the production and use of which are described in WO99/64582), compounds, or compound libraries are useful for the discovery of factors that modulate the aggregation of neural proteins and the survival of neurons in neurodegenerative diseases.

[0164] **Example 1** describes the design and setup of a high-throughput screening method for the identification of regulators or modulators of mutant huntingtin conformation and is referred to herein as the “huntingtin conformation assay”.

[0165] **Example 2** describes the screening of 11584 “Ad-siRNA’s” in the huntingtin conformation assay and its results. This assay can be readily utilized for assays based on overexpressed proteins, such as Ad-cDNAs, wherein regulators or modulators of mutant huntingtin conformation or polyglutamine-induced aggregation are identified as overexpressed TARGET polypeptides. Alternatively and additionally, compounds/agents identified in the assay methods based on the TARGETS of the present invention may be further screened and assessed in the huntingtin conformation assay, in validation of any such compounds/agents.

[0166] **Example 3** describes the rescreen of the primary hits using independently repropagated material.

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[0167] **Example 4** describes gene expression analysis of the TARGETS

[0168] **Example 5** describes further “on target analysis” which may be used to further validate a hit.

[0169] **Example 6** describes a cell based assay which may be used for further confirmation of the hits.

Example 1. Design and setup of a high-throughput screening method for the identification of regulators of mutant huntingtin conformation

Background and principle of the polyglutamine conformation assay.

[0170] The pathological expansion (>35 glutamine) of the polyglutamine tract in the HD gene results in a huntingtin protein with an abnormal conformation. Various abnormal conformation-specific antibodies against mutant huntingtin exist, and can be used to detect changes in levels of the abnormal conformation of mutant huntingtin.

[0171] The 3B5H10 antibody is described in US 6,291,652. The 1C2 antibody is described in WO 97/17445. The 4C8 antibody is described in (Trottier *et al.*, 1995a). Relevant literature to these antibodies is in: (Brooks *et al.*, 2004; Imbert *et al.*, 1996; Trottier *et al.*, 1995b).

[0172] Detection of specific changes in levels of 3B5H10 immunoreactive mutant huntingtin protein are used to identify modulators of mutant huntingtin conformation.

[0173] The polyglutamine conformation assay that has been developed for the screening of the SilenceSelect[®] collection has following distinctive features:

- 1) The assay is run with neuronally differentiated SH-SY5Y neuroblastoma cells (Biedler *et al.*, 1973), but could be used for any other source of primary neuronal cells.
- 2) The assay has been optimized for the use with arrayed adenoviral collections for functional genomics purposes.
- 3) The assay can also be adapted for use to screen compounds or compound collections.
- 4) The assay can be run in high throughput mode.
- 5) The assay can also be adapted to screen other RNA or DNA collections for functional genomics purposes, for example but without limitation dominant negative (DN), cDNA or RNAi collections.

Selection of a readout for the polyglutamine conformation assay.

[0174] Antibody-based detection methods are amenable to high throughput screening (HTS) development. Therefore, we aimed at evaluating a cELISA detection method for mutant huntingtin using the 3B5H10 antibody.

[0175] Human Neuroblastoma cell line SH-SY5Y is obtained from ATCC. SH-SY5Y cells are cultured on cell culture grade plastic. SH-SY5Y cells are cultured in DMEM with glutamax containing 10% heat inactivated and filtered FBS, 100 units/mL Penicillin, 100 µg/mL Streptomycin and 10 mM Hepes Buffer at

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37°C, 5% CO₂ in a humidified chamber. For High-Throughput screening, 96-well plates are seeded with 10 000 cells per well in 100 µL/well.

[0176] After 1 day cells are differentiated with 10 µM retinoic acid, followed after 4 hours by transduction with 4 µL/well shRNA library viruses.

[0177] Cells were cultured overnight and refreshed with medium containing 10 µM all-trans retinoic acid (tRA). Four hours after medium refreshment the cells were transduced with 4 µL of the SilenceSelect[®] library (BioFocus DPI).

[0178] Toxic conformations are measured by using a expanded huntingtin protein Q100-HTT-3kb (Kim *et al.*, 1999). To efficiently express the Q100-HTT-3kb protein in SH-SY5Y cells, the reporter cDNA is synthesized and cloned in adenoviral adapter plasmids. dE1/dE2A (deleted for adenoviral genes E1 and E2A). Adenoviruses are generated from these adapter plasmids by co-transfection of the helper plasmid pWEAd5AflIII-rITR.dE2A in PERC6.E2A packaging cells, as described in WO99/64582.

[0179] To determine the optimal conditions for adenoviral transduction, several conditions for the expression of the Q100-HTT-3kb protein are tested. An experiment is performed where increasing amounts of adenoviral vectors as defined by virus particles per cell (VPU) are used to transduce SH-SY5Y cells. VPU is determined by quantitative PCR, and is defined as adenoviral particles per mL according to (Ma *et al.*, 2001). Four days after transduction of the cells with the Q100-HTT-3kb protein, transduction efficiency is tested according to the assay described here.

[0180] Three days after shRNA transduction of the cells with library viruses, medium was removed and SH-SY5Y cells are transduced with Huntingtin virus (Q100-HTT-3kb, VPU 2000). The virus is suspended in fresh medium supplemented with 10 µM Retinoic Acid.

[0181] To capture all Huntingtin protein conformations in the assay, Huntingtin N18 antibody (Santa Cruz, USA) is used to coat plates 3 days after knock-in Huntingtin virus transduction. White maxisorp Nunc plates are coated with 50 µL/well Huntingtin N18 antibody solution (antibody diluted to 400 ng/mL in phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) and the plates were stored at +4°C with seal for 16 hours.

[0182] One day after the coating of the plates, the plates are washed once with 100 µL/well phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) and blocked with 100 µL/well blocking solution (phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4), 1% Non fat dry milk, 3% Bovine Serum Albumin and 0.2% Tween-20) for one hour at room temperature. At the same time cells are lysed with 100 µL/well lysis buffer (phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) with 0.2% EDTA, 10mM Tris-HCl, 100 mM NaCl, and 1% NP40 with protease inhibitors (0.03 mg/mL pancreas extract,

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0.003 mg/mL pronase, 0.0008 mg/mL thermolysin, 0.0015 mg/mL chemotrypsin, 0.0002 mg/mL trypsin, 1.0 mg/mL papain)). Plates are sealed and incubated at +4°C for 30 minutes.

[0183] After 30 minutes, blocking solution is removed from the plates and all of the lysed cells are transferred to the plates. Plates are then sealed and incubated at +4°C for 16 hours.

[0184] Subsequently, plates are washed three times: 100 µL/well phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) for 15 minutes after incubation time. Specific toxic Huntingtin conformations are detected by using the anti-polyglutamines clone 3B5H10 antibody, diluted to 400 ng/mL in blocking solution (phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4), 1% Non fat dry milk, 3% Bovine Serum Albumine and 0.2% Tween-20). Plates are incubated with 50 µL/well 3B5H10 antibody solution for 1 hour at room temperature.

[0185] For this assay, horseradish peroxide labeled anti-mouse secondary antibody, is used for the detection system. Plates are washed three times with 100 µL/well phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) for 15 minutes. Goat anti-mouse IgG/IgM HRP labeled antibody is diluted to 800 ng/mL in blocking solution (phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4), 1% Non fat dry milk, 3% Bovine Serum Albumine and 0.2% Tween-20). Incubation with the antibody is performed at room temperature using 50 µL/well. After one hour incubation, the plates are washed with 100 µL/well phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄ at pH 7.4) for 15 minutes.

[0186] BM Chemiluminescence ELISA Substrate [POD, Roche] (luminol) is used as the detection reagent for the ELISA readout. Reagent B is diluted 100 times in Reagent A, 15 minutes in advance and set to mix until further use. The substrate is added (50 µL/well) to the plates and after an incubation time of 2 minutes, luminescence is measured by a multilabel plate reader (Perkin-Elmer Envision 2102). Each well is read for 1 second at 400-700 nm by using a luminescence filter.

Example 2. Screening of 11584 “Ad-siRNA’s” in the huntingtin conformation assay.

[0187] The huntingtin conformation assay, the development of which is described in Example 1, may be used to screen an arrayed collection of 11584 different recombinant adenoviruses mediating the expression of shRNAs in retinoic acid-differentiated neuroblastoma cells. These shRNAs cause a reduction in expression levels of genes that contain homologous sequences by a mechanism known as RNA interference (RNAi). The 11584 Ad-siRNAs contained in the arrayed collection target 5119 different transcripts. On average, every transcript is targeted by 2 to 3 independent Ad-siRNAs. .

[0188] Every Ad-siRNA plate contains control viruses that are produced under the same conditions as the SilenceSelect[®] adenoviral collection. The viruses include three sets of negative control viruses (N₁ (Ad5-empty_KD)), N₂ (Ad5-Luc_v13_KD), N₃ (Ad5-mmSrc_v2_KD)), together with positive control viruses (P₁

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(Ad5- AHSA2_v2_KD), P₂ (Ad5-NOS2A_v1_KD), P₃ (Ad5- HIF1A_v2_KD), P₄ (Ad5-HSPCB_v15_KD) and P₅ (Ad5-HDAC9_v3_KD)). Every well of a virus plates contains 150 µL of virus crude lysate. A representative example of the performance of a plate tested with the screening protocol described above is shown in Figure 1. In this figure, the 3B5H10 ELISA signal detected upon performing the assay for every recombinant adenovirus on the plate is shown in fold inter-quartile range of the sample over the median of the sample. The use of inter quartile range (IQR) is chosen over standard deviations to allow better comparison of duplicate samples in an assay with a very large dynamic range (approximately 100-fold). When the value for the 3B5H10 ELISA signal exceeds the cutoff value (defined as 1.5 fold the inter-quartile range of the sample over the median of the sample for Ad-siRNA repressors, +3 for Ad-siRNA activators), an Ad-siRNA virus is marked as a hit. A total of 222 Ad-siRNA hits were isolated that scored below the threshold for repressors. A total of 331 Ad-siRNA hits were isolated that scored above the threshold for activators.

[0189] In Figure 2, all datapoints obtained in the screening of the SilenceSelect® collection in the polyglutamine conformation assay are shown (Ad-siRNAs).

Example 3: Rescreen of the primary hits using independent repropagation material

[0190] To confirm the results of the identified Ad-siRNA in the polyglutamine conformation assay the following approach may be taken: the Ad-siRNA hits are repropagated using PerC6 cells (Crucell, Leiden, The Netherlands) at a 96-well plate level, followed by retesting in the polyglutamine conformation assay. First, tubes containing the crude lysates of the identified hit Ad-siRNA's samples are picked from the SilenceSelect® collection and rearranged in 96 well plates together with negative/positive controls. As the tubes are labeled with a barcode (Screenmates™, Matrix technologies), quality checks are performed on the rearranged plates. To propagate the rearranged hit viruses, 40.000 PerC6.E2A cells are seeded in 200 µL of DMEM containing 10% non-heat inactivated FBS into each well of a 96 well plate and incubated overnight at 39°C in a humidified incubator at 10% CO₂. Subsequently, 2 µL of crude lysate from the hit Ad-siRNA's rearranged in the 96 well plates as indicated above is added to the PerC6.E2A cells using a 96 well dispenser. The plates may then be incubated at 34°C in a humidified incubator at 10% CO₂ for 5 to 10 days. After this period, the repropagation plates are frozen at -80°C, provided that complete CPE (cytopathic effect) could be seen. The propagated Ad-siRNAs are rescreened in the huntingtin conformation assay.

[0191] Data analysis for the rescreen is performed as follows. For every plate the average and standard deviation is calculated for the negative controls and may be used to convert each data point into a "cutoff value" that indicates the difference between the sample and the average of all negatives in terms of standard deviation of all negatives. Threshold settings for the huntingtin conformation repressor rescreen were -3. At this cut-off, 228 Ad-siRNAs are positive in the huntingtin conformation assay.

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[0192] Threshold settings for the huntingtin conformation activator rescreen were for Ad-siRNAs a cutoff of greater than 2. At this cut-off, 208 Ad-siRNAs are positive in the huntingtin conformation assay.

[0193] A quality control of target Ad-siRNAs was performed as follows: Target Ad-siRNAs are propagated using derivatives of PER.C6[®] cells (Crucell, Leiden, The Netherlands) in 96-well plates, followed by sequencing the siRNAs encoded by the target Ad-siRNA viruses. PERC6.E2A cells are seeded in 96 well plates at a density of 40,000 cells/well in 180 μ L PERC6.E2A medium. Cells are then incubated overnight at 39°C in a 10% CO₂ humidified incubator. One day later, cells are infected with 1 μ L of crude cell lysate from SilenceSelect[®] stocks containing target Ad-siRNAs. Cells are incubated further at 34°C, 10% CO₂ until appearance of cytopathic effect (as revealed by the swelling and rounding up of the cells, typically 7 days post infection). The supernatant is collected, and the virus crude lysate is treated with proteinase K by adding 4 μ L Lysis buffer (4x Expand High Fidelity buffer with MgCl₂ (Roche Molecular Biochemicals, Cat. No 1332465) supplemented with 1 mg/mL proteinase K (Roche Molecular Biochemicals, Cat No 745 723) and 0.45% Tween-20 (Roche Molecular Biochemicals, Cat No 1335465) to 12 μ L crude lysate in sterile PCR tubes. These tubes are incubated at 55°C for 2 hours followed by a 15 minutes inactivation step at 95°C. For the PCR reaction, 1 μ L lysate is added to a PCR master mix composed of 5 μ L 10x Expand High Fidelity buffer with MgCl₂, 0.5 μ L of dNTP mix (10 mM for each dNTP), 1 μ L of "Forward primer" (10 mM stock, sequence: 5' CCG TTT ACG TGG AGA CTC GCC 3') (SEQ. ID NO: 80), 1 μ L of "Reverse Primer" (10 mM stock, sequence: 5' CCC CCA CCT TAT ATA TAT TCT TTC C) (SEQ. ID NO: 81), 0.2 μ L of Expand High Fidelity DNA polymerase (3.5 U/ μ L, Roche Molecular Biochemicals) and 41.3 μ L of H₂O. PCR is performed in a PE Biosystems GeneAmp PCR system 9700 as follows: the PCR mixture (50 μ L in total) is incubated at 95°C for 5 minutes; each cycle runs at 95°C for 15 sec., 55°C for 30 sec., 68°C for 4 minutes, and is repeated for 35 cycles. A final incubation at 68°C is performed for 7 minutes. For sequencing analysis, the siRNA constructs expressed by the target adenoviruses are amplified by PCR using primers complementary to vector sequences flanking the SapI site of the pIPspAdapt6-U6 plasmid. The sequence of the PCR fragments is determined and compared with the expected sequence. All sequences are found to be identical to the expected sequence.

[0194]

Table 4

[0195] Summary of the data obtained for the rescreen for all huntingtin conformation hits. The activity of each hit is presented in fold standard deviation in 3B5H10 signal of the 96-well plate from the average in 3B5H10 signal of the 96-well plate. In the primary screen, standard deviation and average were calculated on the library viruses. In the re-screen, standard deviation and average were calculated on the negative control viruses.

HIT REF	SYMBOL	primary screen		re-screen	
		RUN A	RUN B	RUN A	RUN B
		score	score	score	score

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		primary screen		re-screen	
		RUN A	RUN B	RUN A	RUN B
HIT REF	SYMBOL	score	score	score	score
1	SLC7A5	-1.83	-1.71	-6.02	-12.2
2	HSD17B14	-1.21	-1.22	-9.63	-7.17
3	USP9X	-1.16	-1.3	-5.23	-11.1
4	CASP1	-1.73	-1.59	-4.99	-10.9
5	CYB5R2	-1.39	-1.28	-4.83	-9.27
6	NOS1	-1.67	-2.2	-7.75	-10.37
7	SPHK2	-1.12	-0.96	-4.41	-9.03
8	P2RY1	-0.84	-0.68	-7.15	-5.85
9	LRP11	-1.37	-1.45	-6.49	-6.42
10	PCSK6	-1.62	-1.16	-7.29	-5.45
11	DHCR7	-1.37	-1.13	-7.45	-5.28
12	ENPP5	-1.13	-1.48	-7.46	-5.24
13	ARHGEF15	-1.27	-1.15	-4.53	-7.91
14	PSMA2	-1.71	-1.89	-5.34	-6.43
15	ABCG2	-1.68	-1.51	-4.01	-7.59
16	CCR10	-1.39	-1.02	-7.06	-4.07
17	KLKB1	-1.16	-0.96	-5.88	-4.41
18	EPOR	-1.22	-1.06	-5.71	-3.99
19	CREBBP	-1.03	-1.34	-4.8	-5.52
20	APLP2	-0.01	-0.23	-3.81	-5.35
21	MAP3K11	-0.47	-0.23	-5.03	-4.09
22	TNFRSF10A	-0.97	-0.79	-4.19	-3.69
23	HIF1A	-2.55	-2.89	-1.87	-1.55
24	NOS2A	-0.64	-0	1.52	0.9
25	DAPK2	-0.35	-0.54	-4.36	-3.4
26	NRG1	-1.69	-1.69	-8.74	-6.42

Example 4: Gene expression analysis

[0196] To validate these targets as actively expressed in the human brain, particularly the striatum and cortex, areas which are affected in HD (Vonsattel *et al.*, 1985), the gene expression in the human brain of the transcripts represented by the hit viruses may measured by either one of two methods.

4.1

[0197] A publicly (Hodges *et al.*, 2006) available microarray data-set may be analyzed (NCBI Gene Expression Omnibus entry GSE3790).The arrays with good quality RNA are used (Table 5).

Table 5 Microarrays analyzed

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Sample	No. of arrays
Caudate Nucleus - control	26
Caudate Nucleus – Vonsattel grade 1&2	32
Cortex Brodman Area 9 - control	12
Cortex Brodman Area 9 - Vonsattel grade 4	4

[0198] The hybridization levels are reported as p-values (statistical significance that the gene is expressed with a cut-off at $p=0.05$). Genes expressed on more than 50% of the arrays are ranked as expressed genes. The median p-value of expression across the striatum and cortex is presented in Table 7. Furthermore, a ratio between the $-\log$ of the median p-values from the striatum of HD patients with Vonsattel grade 1 or 2 and from the striatum of control subjects may be used to indicate disease-specific expression.

4.2

[0199] For genes not analyzed in this (Hodges *et al.*, 2006) data-set, RNA may be isolated from fresh frozen brain tissue from control subjects and from HD patients, both from the striatum and from the cortex. The gene expression is analyzed using Real-time TaqMan analysis of gene expression mRNA expression data (quantitative RT-PCR).

[0200] Total RNA may be isolated from these samples using the Qiagen RNeasy kit and the quality of RNA may be assessed using an Agilent 2100 Bioanalyzer Pico chip. RNAs are selected on the basis of quality (28S and 18S peaks rRNA). cDNA is prepared from the RNA and pools of cDNA are made if appropriate (Table 6).

Table 6 Clinical status of RNA samples used in TaqMan analysis.

Some cDNA samples are pooled cDNAs from 2 or 3 samples (indicated by multiple entries in the fields).

RNA sample	Clinical status	Area of the brain	Sex	Age	CAG repeat
1	control	striatum	m	48	N/A
2	control	parietal cortex	m	51	N/A
		frontal cortex	m	46	N/A
3	HD Vonsattel II	striatum	m	55	21-43
		striatum	m	81	19-41
4	HD Vonsattel II	frontal cortex	f	52	17-47

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		frontal cortex	m	55	21-43
		frontal cortex	m	81	19-41
5	HD Vonsattel IV	striatum	f	52	16-53
6	HD Vonsattel IV	frontal cortex	f	52	16-53

[#N/A = not applicable – no CAG repeat]

[0201] Each sample is measured in duplicate on different plates. The gene expression is calculated in cycle thresholds (Ct) (Applied Biosystems manual). A low cycle threshold indicates high expression, a Ct of 35 or greater indicates no expression. A differential gene expression in the striatum of HD patients with Vonsattel grade 1 or 2 and from the striatum of control subjects is calculated with $2^{(\Delta Ct)}$. Targets showing a ratio greater than 1 are over-expressed in HD striatum, and therefore of increased value as a drug target.

Table 7: Results of gene expression analysis.

Target Gene Symbol	SEQ ID NO: DNA	Expression array (p value)	Expression TaqMan (Ct)	Relative expression HD (ratio –logP or $2^{\Delta Ct}$)
SLC7A5	1	0.0506		1.46
HSD17B14	2	0.0279		1.07
USP9X	3	0.0124		1.00
CASP1	4	0.0383		0.96
CYB5R2	5	0.0163		1.05
NOS1	6	#N/A		#N/A
SPHK2	7		27.66	2.02
P2RY1	8	0.0564		0.91
LRP11	9	0.0017		1.00
PCSK6	10		26.45	0.90
DHCR7	11	0.0478		1.07
ENPP5	12	0.0022		1.00
ARHGEF15	13		30.21	6.80
PSMA2	14	0.0022		1.00
ABCG2	15	0.0019		0.98
CCR10	16		33.04	4.68
KLKB1	17	0.0847		1.04

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Target Gene Symbol	SEQ ID NO: DNA	Expression array (p value)	Expression TaqMan (Ct)	Relative expression HD (ratio $-\log P$ or $2^{\Delta Ct}$)
EPOR	18		26.87	3.88
CREBBP	19	#N/A		#N/A
APLP2	20	0.0038		1.00
MAP3K11	21	0.0227		1.11
TNFRSF10A	22		30.26	1.78
HIF1A	23	#N/A		#N/A
NOS2A	24	#N/A		#N/A
DAPK2	25		30.61	1.48
NRG1	26		30.91	0.57

Example 5: “On target analysis” using KD viruses

[0202] To strengthen the validation of a hit, it is helpful to recapitulate its effect using a completely independent siRNA targeting the same target gene through a different sequence. This analysis is called the “on target analysis”. In practice, this will be done by designing multiple new shRNA oligonucleotides against the target using a specialised algorithm previously described, and incorporating these into adenoviruses, according to WO 03/020931. After virus production, these viruses will be arrayed in 96 well plates, together with positive and negative control viruses. On average, 6 new independent Ad-siRNA’s will be produced for a set of targets. One independent repropagation of these virus plates will then be performed as described above for the rescreen in Example 3. The plates produced in this repropagation will be tested in biological duplicate in the primary screening assay at 3 MOIS according to the protocol described (Example 1). Ad-siRNA’s mediating a functional effect above the set cutoff value in at least 1 MOI will be nominated as hits scoring in the “on target analysis”. The cutoff value in these experiments will be defined as the average over the negative controls + 2 times the standard deviation over the negative controls. These hits are considered “on target”, and proceed to the next validation experiment.

Example 6: Primary cell based assay confirmation

[0203] A cell model with increased clinical relevance for Huntington’s Disease will have a phenotype similar to the population of neurons most severely affected in Huntington’s Disease. Neuropathological analysis of the brains of HD patients clearly evidences the regions of the brain involved in the

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neurodegenerative processes (Vonsattel *et al.*, 1985). The striatum (caudate nucleus) and cortex are most severely affected, explaining the motor and cognitive deficits observed during the disease process. A conditionally immortalized cell line derived from the human fetal striatum will be used to replicate the assay described in Example 1. Such a cell line may be cultured under the conditions that allow active proliferation, but upon turning off the immortalization gene such as c-myc, cells will terminally differentiate to a striatal neuron phenotype. The response of such neurons to the assay described in example 1 will be more relevant to the sensitivity of the striatal neuron population in the HD patient. Hit Ad-siRNAs active in the human striatal neuron assay will represent genes with increased validation as a drug target compared to Ad-siRNAs that fail to show an effect in the human striatal neuron assay. An example of a human striatal neuron cell line is the STROCO5 cell line described in Usbat application 20060067918 (Sinden *et al.*, ReNeuron Ltd.).

[0204] REFERENCES

- Bates, G.P. 2005. History of genetic disease: The molecular genetics of Huntington disease - a history. *Nat Rev Genet.*
- Biedler, J.L., L. Helson, and B.A. Spengler. 1973. Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Res.* 33:2643-2652.
- Brooks, E., M. Arrasate, K. Cheung, and S.M. Finkbeiner. 2004. Using antibodies to analyze polyglutamine stretches. *Methods Mol Biol.* 277:103-28.
- Davies, S.W., M. Turmaine, B.A. Cozens, M. DiFiglia, A.H. Sharp, C.A. Ross, E. Scherzinger, E.E. Wanker, L. Mangiarini, and G.P. Bates. 1997. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell.* 90:537-48.
- DiFiglia, M., E. Sapp, K.O. Chase, S.W. Davies, G.P. Bates, J.P. Vonsattel, and N. Aronin. 1997. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science.* 277:1990-1993.
- Hodges, A., A.D. Strand, A.K. Aragaki, A. Kuhn, T. Sengstag, G. Hughes, L.A. Elliston, C. Hartog, D.R. Goldstein, D. Thu, Z.R. Hollingsworth, F. Collin, B. Synek, P.A. Holmans, A.B. Young, N.S. Wexler, M. Delorenzi, C. Kooperberg, S.J. Augood, R.L. Faull, J.M. Olson, L. Jones, and R. Luthi-Carter. 2006. Regional and cellular gene expression changes in human Huntington's disease brain. *Hum Mol Genet.* 15:965-77.
- Imbert, G., F. Saudou, G. Yvert, D. Devys, Y. Trottier, J.M. Garnier, C. Weber, J.L. Mandel, G. Cancel, N. Abbas, A. Durr, O. Didierjean, G. Stevanin, Y. Agid, and A. Brice. 1996. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat Genet.* 14:285-91.

GAL-123-WO-PCT

- Kim, M., H.S. Lee, G. LaForet, C. McIntyre, E.J. Martin, P. Chang, T.W. Kim, M. Williams, P.H. Reddy, D. Tagle, F.M. Boyce, L. Won, A. Heller, N. Aronin, and M. DiFiglia. 1999. Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J Neurosci.* 19:964-973.
- Ko, J., S. Ou, and P.H. Patterson. 2001. New anti-huntingtin monoclonal antibodies: implications for huntingtin conformation and its binding proteins. *Brain Res Bull.* 56:319-29.
- Li, H., S.H. Li, A.L. Cheng, L. Mangiarini, G.P. Bates, and X.J. Li. 1999. Ultrastructural localization and progressive formation of neuropil aggregates in Huntington's disease transgenic mice. *Hum Mol Genet.* 8:1227-1236.
- Lipinski, C.A., F. Lombardo, B.W. Dominy, and P.J. Feeney. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 46:3-26.
- Ravikumar, B., C. Vacher, Z. Berger, J.E. Davies, S. Luo, L.G. Oroz, F. Scaravilli, D.F. Easton, R. Duden, C.J. O'Kane, and D.C. Rubinsztein. 2004. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet.* 36:585-95.
- Ross, C.A., and M.A. Poirier. 2004. Protein aggregation and neurodegenerative disease. *Nat Rev Neurosci.* 5:S10-S17.
- Saudou, F., S. Finkbeiner, D. Devys, and M.E. Greenberg. 1998. Huntingtin Acts in the Nucleus to Induce Apoptosis but Death Does Not Correlate with the Formation of Intranuclear Inclusions. *Cell.* 95:55-66.
- Scherzinger, E., A. Sittler, K. Schweiger, V. Heiser, R. Lurz, R. Hasenbank, G.P. Bates, H. Lehrach, and E.E. Wanker. 1999. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: Implications for Huntington's disease pathology. *Proc Natl Acad Sci USA.* 96:4604-4609.
- Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Hum Mol Genet* 12:1555–1567.
- Strand, A.D., Z.C. Baquet, A.K. Aragaki, P. Holmans, L. Yang, C. Cleren, M.F. Beal, L. Jones, C. Kooperberg, J.M. Olson, and K.R. Jones. 2007. Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. *J Neurosci.* 27:11758-68.
- Tanaka, M., Y. Machida, S. Niu, T. Ikeda, N.R. Jana, H. Doi, M. Kurosawa, M. Nekooki, and N. Nukina. 2004. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat Med.* 10:148-54.
- The Huntington's Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell.* 72:971-983.

GAL-123-WO-PCT

- Tobin, A.J., and E.R. Signer. 2000. Huntington's disease: the challenge for cell biologists. *Trends Cell Biol.* 10:531-6.
- Trottier, Y., D. Devys, G. Imbert, F. Saudou, I. An, Y. Lutz, C. Weber, Y. Agid, E.C. Hirsch, and J.L. Mandel. 1995a. Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nat Genet.* 10:104-10.
- Trottier, Y., Y. Lutz, G. Stevanin, G. Imbert, D. Devys, G. Cancel, F. Saudou, C. Weber, G. David, L. Tora, Y. Agid, A. Brice, and J.L. Mandel. 1995b. Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature.* 378:403-6.
- Vonsattel, J.P., R.H. Myers, T.J. Stevens, R.J. Ferrante, E.D. Bird, and E.P. Richardson, Jr. 1985. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol.* 44:559-77.
- Zoghbi, H.Y., and H.T. Orr. 2000. Glutamine Repeats and Neurodegeneration. *Annu Rev Neurosci.* 23:217-247.

[0205] From the foregoing description, various modifications and changes in the compositions and methods of this invention will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

[0206] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

WE CLAIM:

1. A method for identifying a compound that modulates the aberrant conformation or aggregation or expression of mutant huntingtin protein comprising:
 - a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
 - b) determining the binding affinity of the compound to the polypeptide;
2. The method according to claim 1 which additionally comprises the steps of
 - c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
 - d) identifying the compound that modulates the expression of mutant huntingtin protein.
3. A method for identifying a compound that modulates polyglutamine conformation, said method comprising:
 - a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
 - b) determining the binding affinity of the compound to the polypeptide;
4. The method according to claim 3 which additionally comprises the steps of
 - c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
 - d) identifying the compound that modulates polyglutamine conformation.
5. A method for identifying a compound that modulates the expression or activity of the mutant huntingtin protein comprising:
 - a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
 - b) determining the ability of the compound inhibit the expression or activity of the polypeptide;
6. The method according to claim 5 which additionally comprises the steps of
 - c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ; and
 - d) identifying the compound that modulates the expression of mutant huntingtin protein.
7. A method for identifying a compound that modulates polyglutamine conformation, said method comprising:

- a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52;
 - b) determining the ability of the compound inhibit the expression or activity of the polypeptide;
8. The method according to claim 7 which additionally comprises the steps of
- c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide ;
 - and
 - d) identifying the compound that modulates polyglutamine conformation.
9. The method according to any one of claims 1-8, wherein said polypeptide is in an *in vitro* cell-free preparation.
10. The method according to any one of claims 1-8, wherein said polypeptide is present in a cell.
11. The method according to claim 10, wherein the cell is a mammalian cell
12. The method according to claim 10 or 11, wherein the cell naturally expresses said polypeptide.
13. The method according to claim 10 or 11, wherein the cell has been engineered so as to express the target.
14. The method according to any one of claims 1-8, wherein said compound is selected from the group consisting of compounds of a commercially available screening library and compounds having binding affinity for a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27-52.
15. The method according to any one of claims 1-8, wherein said compound is a peptide in a phage display library or an antibody fragment library.
16. An agent effective in modulating polyglutamine conformation or huntingtin protein expression, selected from the group consisting of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence of about 17 to about 30 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1-26.
17. The agent according to claim 16, wherein a vector in a mammalian cell expresses said agent.
18. The agent according to claim 16, which is effective in modulating polyglutamine confirmation in a polyglutamine conformation assay.

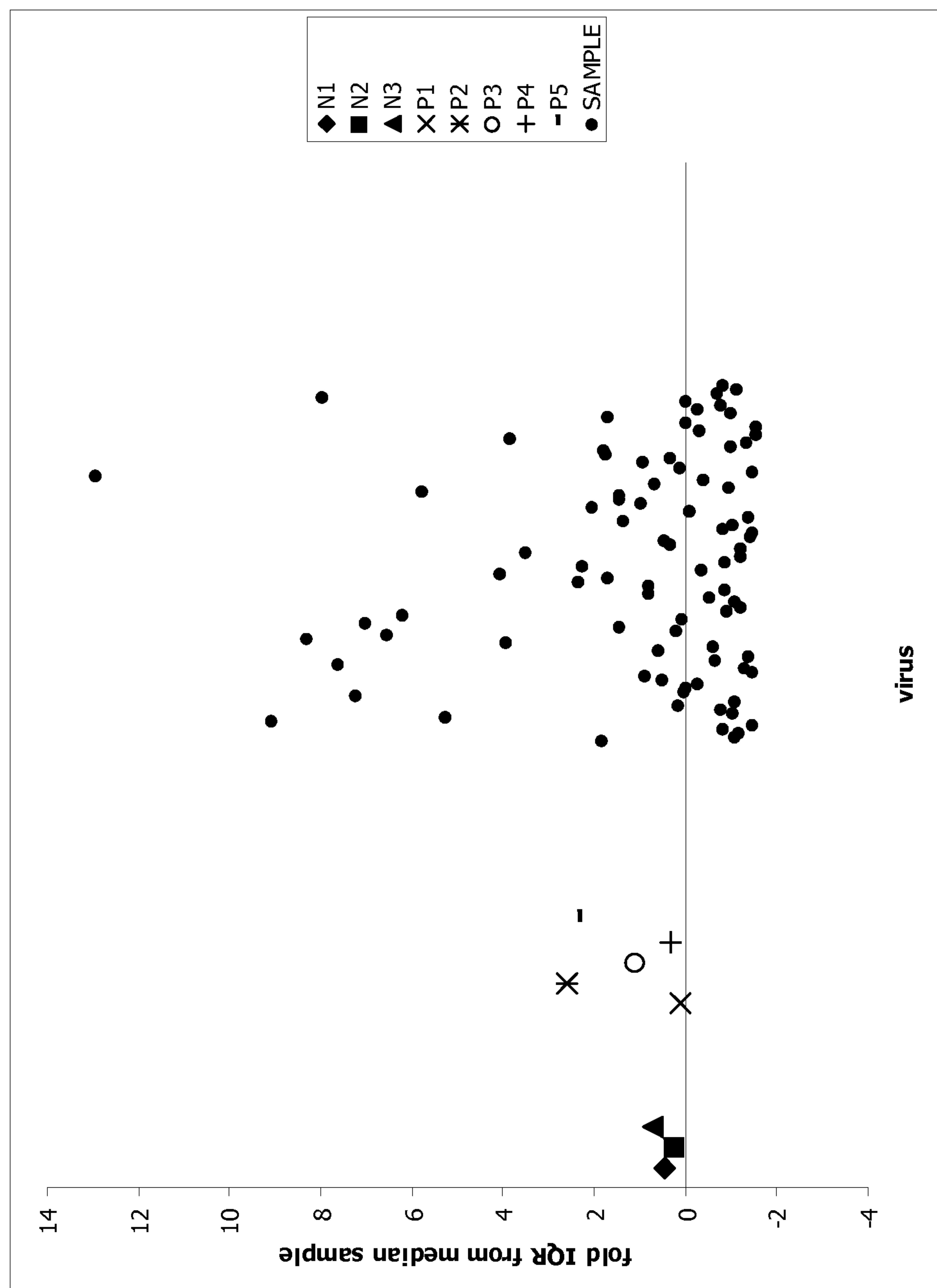
19. The agent according to claim 17, wherein said vector is an adenoviral, retroviral, adeno-associated viral, lentiviral, a herpes simplex viral or a sendai viral vector.
20. The agent according to claim 16, wherein said antisense polynucleotide and said siRNA comprise an antisense strand of 17-25 nucleotides complementary to a sense strand, wherein said sense strand is selected from 17-25 continuous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1-26.
21. The agent according to claims 20, wherein said siRNA further comprises said sense strand.
22. The agent according to claim 21, wherein said sense strand is selected from the group consisting of SEQ ID NO: 53-78.
23. The agent according to claim 22, wherein said siRNA further comprises a loop region connecting said sense and said antisense strand.
24. The agent according to claim 23, wherein said loop region comprises a nucleic acid sequence selected from the group consisting of UUGCUAUA and GUUUGCUAUAAC (SEQ ID NO: 79).
25. The agent according to claim 23, wherein said agent is an antisense polynucleotide, ribozyme, or siRNA comprising a nucleic acid sequence complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 53-78.
26. A huntingtin protein modulating pharmaceutical composition comprising a therapeutically effective amount of an agent of claim 16 in admixture with a pharmaceutically acceptable carrier.
27. A polyglutamine conformation modulating pharmaceutical composition comprising a therapeutically effective amount of an agent of claim 16 in admixture with a pharmaceutically acceptable carrier.
28. A method of treating and/or preventing a disease involving neurodegeneration, comprising administering to said subject a pharmaceutical composition according to claim 26 or 27.
29. The method according to claim 28 wherein the disease is a polyglutamine disease.
30. The method according to claim 29, wherein the disease is Huntington's disease.
31. Use of an agent according to claims 16-25 in the manufacture of a medicament for the treatment and/or prevention of a disease involving neurodegeneration.
32. Use according to claim 31, wherein the disease is a polyglutamine disease.
33. Use according to claim 31 or 32, wherein the disease is Huntington's disease.

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FIGURE 1

Example of a plate in the Ad-siRNA huntingtin conformation assay



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FIGURE 2

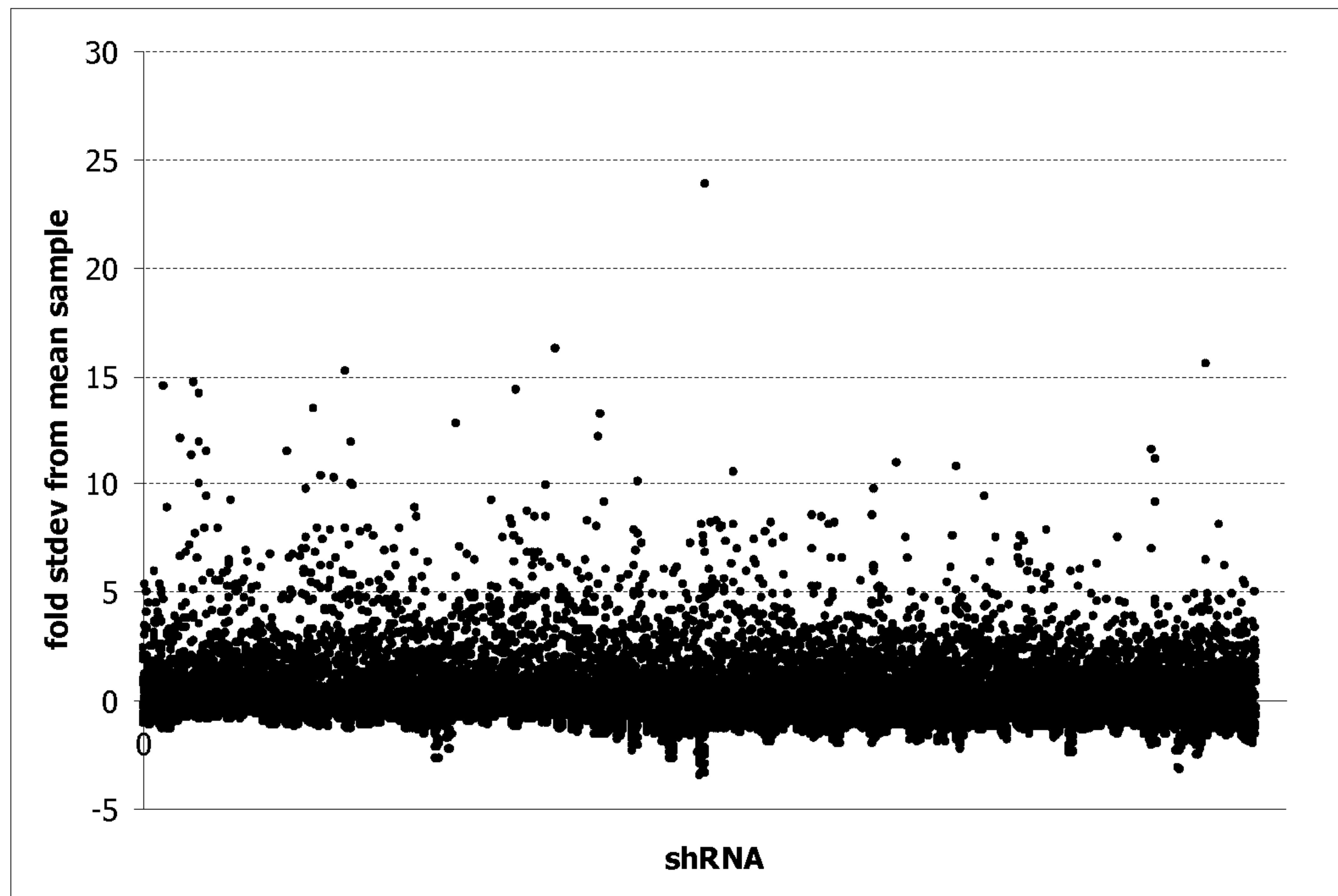


FIGURE 1
Example of a plate in the Ad-siRNA huntingtin conformation assay

