TREATMENT AND DIAGNOSIS OF HUNTINGTON'S DISEASE

FIGURE 1

(54) Title: TREATMENT AND DIAGNOSIS OF HUNTINGTON'S DISEASE


(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on nextpage]
Published: with international search report (Art. 21(3))
TREATMENT AND DIAGNOSIS OF HUNTINGTON'S DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS


GOVERNMENT RIGHTS

[0002] This invention was made with Government support under Grant No. NIH 1SC3GM096945, awarded by the National Institutes of Health and under a CSUPERB New Investigator S12 Grant, awarded by the California State University.

FIELD OF THE INVENTION

[0003] This invention relates to the discovery of novel methods for the treatment and diagnosis of Huntington's disease. Specifically, novel methods for detecting disease progression, determining disease progression, and treating the physiological symptoms of the disease are disclosed herein.

BACKGROUND

[0004] Huntington's Disease (HD) is a fatal autosomal-dominant genetic degenerative disease, characterized by debilitating motor and cognitive defects. Generally, HD is considered a neuropathy; specifically, HD is 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 effective treatment exists for HD, and the disease typically leads to death within a decade from the onset of clinical signs.

[0005] 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)
isolated the I T 1 5 (important transcript 15) gene, commonly referred to as the huntingtin gene, using cloned trapped exons from the target area and found that it 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 in the study. 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. Based on these studies it was determined 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.

[0006] The cognitive and motor deficits associated with HD have led the majority of the scientific research to focus on the neurological component of the disease. For example, Vonsattel et al. performed neuropathological analysis of the brains of HD patients that clearly evidences the regions of the brain involved in the neurodegenerative processes in 1985. They determined that the striatum (caudate nucleus) and cortex are most severely affected, explaining the motor and cognitive deficits observed during the disease process.

[0007] In 1997 DiFiglia et al. demonstrated that an amino-terminal fragment of mutant huntingtin localizes to neuronal intranuclear inclusions (Nils) 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 (DiFiglia et al., 1997). Ubiquitin, which is thought to be involved in labeling proteins for disposal by intracellular proteolysis, was also found in Nils and DNs, suggesting that abnormal huntingtin is targeted for proteolysis but is resistant to removal. Accordingly, the aggregation of mutant huntingtin may be part of the pathogenic mechanism in HD.

[0008] Following DiFiglia et al., Saudou et al. 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.

Accordingly, in view of these and other studies, clinical treatment of HD typically focuses on neuro-based tratement. Recently, neural and stem cell transplantation has been suggested as a potential treatment for neurodegenerative diseases such as HD. Specifically, the transplantation of specific committed neuroblasts (fetal neurons) to the adult brain has been proposed as a treatment for neurodegenerative diseases.

A second approach for the treatment of neurodegenerative diseases focuses on preventing cell death because it has been proposed that the activation of mechanisms mediating cell death may be involved in neurologic diseases. Accordingly, therapeutic agents that modulate apoptosis such as minocycline and growth factors are also being used in treatment studies of neurodegenerative diseases.

Others have also suggested that inhibition of polyglutamine-induced protein aggregation could provide treatment options for polyglutamine diseases such as HD (Tanaka et al., 2004). Tanaka et al. 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. further suggested that these beneficial effects are the result of trehalose binding to expanded polyglutamines and stabilizing the partially unfolded polyglutamine-containing protein.

However, these representative studies in the field of HD treatment are limited because they solely focus on neurodegeneration. Specifically, HD is characterized by motor defects such
as chorea, rigidity, dystonia, and muscle weakness which such studies have misattributed solely to neurodegeneration (Waters et al. PNAS (2013)).

[0013] As described herein, this invention discloses and focuses on the muscular component of HD. Specifically, this invention describes the physiological properties of HD muscle and provides novel methods for the treatment, diagnosis, and prognosis of HD.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0014] **FIG. 1A-FIG. 1D** depict action potentials from wild-type (WT) and HD muscle fibers. Figure 1A shows an overlay of an action potential of a WT muscle fiber compared to an action potential of a HD muscle fiber. Specifically, the rising and falling (repolarizing) phases of the action potentials are shown and a clear slowing of the falling phase of the HD muscle fibers can be seen. Figure 1B provides a detailed plot of the repolarization phase of the WT and HD muscle fibers from Figure 1A. Figure 1C shows the minimum 0.5-ms depolarizing stimulus current (nA) that is needed to trigger an action potion in WT and HD fibers. The average (mean ± SEM) current needed to trigger an action potential was significantly less in HD (n=12) compared to WT (n=17) fibers. Figure 1D shows a representative spontaneous action potential that self-triggered after an apparent subthreshold stimulus in a HD muscle fiber.

[0015] **FIG. 2A-FIG. 2F** depict averaged chloride currents in WT (n=14) and HD (n=9) muscle fibers. All WT and HD open probability data was fitted using a Boltzmann curve. Figure 2A depicts a voltage clamp protocol used for the experiments presented in Figures 2B-2D. The protocol uses a holding potential of -20mV, a first pulse to +60mV, a second test pulse that increased in 20mV steps from -140mV to +120mV for 200ms, and a third test pulse to -100mV for 50ms. Figures 2B-2D are current records that were normalized to muscle fiber surface area (cm²). Figure 2B shows the recorded current acquired using the protocols of Figure 2A. The recorded currents are composed the chloride current, the leak current, and the capacitive current. The full capacitive transients are not shown. Figure 2C is a record of the current acquired using the protocols of Figure 2A in the presence of the chloride channel blocker 9AC. Accordingly, with the chloride currents blocked, the traces shown in Figure 2C are the leak and capacitive currents only. The full capacitive transients are not shown. Figure 2D shows the specific chloride current (I_{ci}) by subtracting the leak and capacitive currents from Figure 2C from the
recorded current of Figure 2B. Figure 2E shows the Current-Voltage (IV) relationship of the peak chloride current (I_{Cl}) recorded from the second test pulse (20mV steps from -140mV to +120mV for 200ms) acquired in WT and HD muscle fibers. Figure 2F shows the relative open probability of the chloride channels in WT and HD muscle fibers. The open probability was calculated by plotting the normalized peak currents recorded at the third, -100mV pulse, as a function of steady-state voltage from the second test pulse (20mV steps from -140mV to +120mV for 200ms).

[0016] FIG. 3A- FIG. 3C depict the average inward rectifying potassium (I_{K,i}) currents recorded in WT (n=12) and HD (n=11) muscle fibers. Figure 3A shows the voltage protocol used in the voltage clamp experiments performed in Figure 3B and plotted in Figure 3C. The protocol was: holding potential of OmV, a 700ms test pulse stepping from -60mV to +20mV in +10mV increments. Figure 3B shows the I_{K,i} currents recorded using the protocol of Figure 3A and subtracting out the leak and capacitive current (leak and capacitive currents were recorded by performing a second round of recordings in the presence of 5mM Ba^{2+} to block the potassium currents and then subtracted from the total current recorded in the absence of 5mM Ba^{2+}). Figure 3C shows the Current-Voltage relationship of the peak potassium current from Figure 3B for WT and HD muscle fibers.

[0017] FIG. 4A- FIG. 4D show the reduced expression of mature Clcnl mRNA and the atypical splicing of Clcnl pre-mRNA in HD muscle. Figure 4A shows the splicing of Clcnl mRNA in WT and HD interosseous muscle. Figure 4B shows exon 7a+ is expressed at a higher level in HD (n=2) interosseous muscle than in WT (n=3) interosseous muscle, indicating abberant mRNA splicing in HD muscle. Figures 4C and 4D show the expression of total mature Clcnl mRNA in WT and HD muscle as determined by RT-PCR using two different primer sets. Both sets of primers showed significantly reduced levels of mature Clcnl mRNA in HD muscle.

[0018] FIG. 5 is an image of a muscle fiber that is impaled with two intracellular microelectrodes, as used to obtain the electrophysiological results described in this application. The black bar at the bottom right corner of the image is a 50µm scale bar. The two electrodes are used to 1) administer voltage and 2) measure current.

[0019] FIG. 6A- FIG. 6E shows chloride channel currents recorded using the protocol of Figure 2D. Figure 6A shows the steady-state Current-Voltage relationship recorded during test pulse 2
in WT (n=14) muscle fibers. Figure 6B shows the steady-state Current-Voltage relationship recorded during test pulse 2 in HD (n=9) muscle fibers. Other than magnitude, the steady-state Current-Voltage relationships of WT (Figure 6A) and HD (Figure 6B) are very similar, other than a slightly greater relative inhibition of the chloride current steady-state at positive voltages in HD compared to WT fibers. Figure 6C shows the time course of chloride current deactivation from a test pulse (20mV steps from -140mV to -80mV). As shown, there was no obvious difference in the kinetics of the chloride deactivation in the HD compared to WT muscle fibers. Figure 6D shows the outward movement of chloride ions measured in nC/cm² during the deactivation of the chloride current in HD and WT muscle fibers. The calculations were obtained by integrating individual recordings of the chloride current. It can be seen that there is a faster rate of chloride current deactivation in WT compared to HD muscle fibers. Figure 6E shows the faster time-to peak of the chloride current in HD compared to WT muscle fibers at -140mV during the second test pulse according to the protocol of Figure 2D.

[0020] FIG. 7A- FIG. 7F show the kinetics of the average inward rectifying potassium currents of WT (n=12) and HD (n=11) muscle fibers. Figure 7A shows the voltage protocol used in Figures 7B-7D: holding voltage of 0mV followed by a series of +10mV steps from -60mV to +20mV for 700ms each. Figure 7B shows the total currents measured in a WT and HD muscle fiber. These recordings were measured in the absence of any channel blockers; accordingly, the traces are made up of the $I_{k_{f}}$, leak, and capacitive currents. Figure 7C shows recordings from the WT and HD muscle fibers in the presence of 5mM Ba²⁺ to block the potassium channels and provide a clean recording of the capacitive and leak currents only. Figure 7D shows the $I_{k_{f}}$ current obtained by subtracting the recordings of Figure 7C from the recordings of Figure 7B. Figure 7E shows provides a comparative time course of the $I_{k_{f}}$ current measured at -60mV in WT and HD muscle fibers. Figure 7F shows that the HD muscle fibers exhibited a faster time-to-peak $I_{k_{f}}$ at -60mV compared with WT muscle fibers.

[0021] FIG. 8A and FIG. 8B show the capacitive transients from voltage clamped WT and HD muscle fibers. The measurements were taken under blocking conditions, in the presence of anthracene-9-carboxylic acid (9AC). Figure 8A shows the voltage protocol. Figure 8B shows a representative recording of the capacitive transients acquired using the protocol of Figure 8A. The capacitance of the WT muscle fiber was 5.9µF/cm² and the HD muscle fiber was 3^F/cm².
Capacitive transients at the onset and termination of the voltage steps were integrated and specific capacitance ($C_m$) was obtained.

[0022] FIG. 9A and FIG. 9B show the atypical splicing of the Clcnl gene product. mRNA was extracted from the extensor digitorum longus muscle. Figure 9A shows the spliced Clcnl mRNA of WT and HD muscle. Figure 9B shows the quantification of mRNA expression. Specifically, it can be seen that exon 7a was expressed at a higher proportional level in HD (n=2) compared to WT (n=3) in the extensor digitorum longus muscle. The "*" indicates that the difference between HD and WT is statistically significant (p<0.05).

[0023] FIG. 10 shows the development of Clc-1 current defects in HD muscle, $G_{Clc-1}$ (mS/cm) of individual WT (x) and HD (®) muscle fibers plotted as function of time, with linear regressions (WT - and HD -). 

[0024] FIG. 11A and FIG. 11B Figure 11A shows the development of Clc-1 current defects in HD muscle, $G_{Clc-1}$ (mS/cm) of individual WT (x) and HD (®) muscle fibers plotted as function of time, with linear regressions (WT — and HD —). Figure 11B shows the mean WT and HD Gcic-i values in mS/cm² at early-, middle-, and late-stages. An * indicates a significant difference between WT and HD (P<0.05)

[0025] FIG. 12A and FIG. 12B Figure 12A shows development of Kir current defects in HD muscle. $G_{Kir}$ (mS/cm²) of individual WT (x) and HD (®) muscle fibers plotted as function of time, with linear regressions (WT — and HD —). Figure 12B shows the mean WT and HD $G_{Kir}$ values in mS/cm² at early-, middle-, and late-stages. An * indicates a significant difference between WT and HD (P<0.05).

[0026] FIG. 13 shows the quantification of the Clcnl mRNA mis-spliced transcript that includes exon 7a in HD mice relative to WT as a function of age. There was a significant difference in the relative proportion of this specific mis-spliced transcript only in late-stage HD mice relative to WT. These findings show a decrease in this specific transcript in WT as age progresses. Whereas, in HD mice the relative proportion of this specific transcript remained fairly constant.
BRIEF SUMMARY OF THE INVENTION

[0027] In certain embodiments, this invention is directed at a method of diagnosing Huntington's disease by detecting a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a with a reagent that binds nucleic acids in diseased muscle compared to normal muscle. In a specific embodiment, the expression of exon 7a is indicative of disease progression. In a specific embodiment, the expression of 7a is detected before a patient manifests any physical symptoms of Huntington's disease.

[0028] In certain embodiments, this invention is directed at a method of treating Huntington's disease by administering a muscle relaxant to a patient in need thereof. In specific embodiments the muscle relaxant is selected from the group consisting of succinylcholine, gallamine, and dantrolene. In specific embodiments two or more muscle relaxants are administered. In specific embodiments succinylcholine and dantrolene are co-administered. In specific embodiments gallamine and dantrolene are co-administered. In specific embodiments the method further comprises the administration of a CIC-1 or Kir modulating agent. In specific embodiments the patient has CAG repeats indicative of Huntington's disease but does not exhibit any physical symptoms of Huntington's disease. In specific embodiments the patient has juvenile on-set Huntington's disease. In specific embodiments the patient has late on-set Huntington's disease.

[0029] In certain embodiments, this invention is directed at a method of determining the prognosis of a patient suffering from Huntington's disease by detecting a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a in diseased muscle compared to normal muscle, wherein a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a is indicative of disease progression. In specific embodiments the expression of 7a is detected before a patient manifests any physical symptoms of Huntington's disease.

[0030] In certain embodiments, this invention is directed at a method the method comprising: (a) Taking a first measurement to detect the expression of Clcnl mRNA or Clcnl exon 7a in a muscle sample (b) Administering a muscle relaxant and (c) Taking a second measurement to detect the expression of Clcnl mRNA or Clcnl exon 7a in a muscle sample, wherein, a higher dose of the muscle relaxant is administered if a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a is detected.
In certain embodiments, this invention is directed at a method of treating Huntington's disease by administering a combination treatment comprising a muscle relaxant and a traditional Huntington's disease therapy to a patient in need thereof. In specific embodiments the traditional therapy is tetrabenazine. In specific embodiments the traditional therapy is an antipsychotic drug. In specific embodiments the traditional therapy is a drug that targets the brain and suppresses chorea. In specific embodiments the traditional therapy is a drug that targets the brain and suppresses dystonia. In specific embodiments the traditional therapy is a drug that targets the brain and reduces muscle rigidity. In specific embodiments the traditional therapy is an antidepressant. In specific embodiments the traditional therapy is a mood-stabilizing drug. In specific embodiments the traditional therapy is selected from the group consisting of psychotherapy, speech therapy, physical therapy, and occupational therapy. In specific embodiments the traditional therapy is administered at a lower dose in the combination treatment than it would be if administered alone. In specific embodiments the combination treatment reduces the severity of Huntington's disease symptoms. In specific embodiments the combination treatment reduces the number of Huntington's disease symptoms. In specific embodiments the combination treatment slows the progression of Huntington's disease symptoms. In specific embodiments the combination treatment reduces the severity of Huntington's disease symptoms. In specific embodiments the combination treatment prevents the development of Huntington's disease symptoms.

The above-described embodiments are non-limiting.
DETAILED DESCRIPTION OF THE INVENTION

Overview

[H0033] Huntington's disease (HD) is a progressive and fatal degenerative disorder with devastating cognitive and motor defects. Characteristics symptoms of HD include but are not limited to behavioral changes, abnormal and unusual movements, and neurological changes. Behavioral changes include behavioral disturbances, hallucinations, irritability, moodiness, restlessness, fidgeting, paranoia, and psychosis. Examples of abnormal movements include unusual facial movements such as grimaces, head turning to shift eye position, quick and sudden (e.g., jerking) movements of the arms, legs, face, and other body parts, slow and uncontrolled movements, and an unsteady gait. Examples of neurological changes include progressive dementia (e.g., disorientation, confusion, loss of judgment, loss of memory, personal changes, speech changes), anxiety, stress, tension, difficulty swallowing, speech impairment, rigidity, slow movements, and tremors. Accordingly, the traditional view is that HD is a neurological disease wherein the changes in behavior, movement, and dementia are attributed to neurological deficits and loss of function.

[H0034] In addition to the symptoms described above, depression and suicide are common among persons afflicted with HD, manifested side effects of an afflicted person cognitively being aware of his/her progressive loss of control of his/her own body. Furthermore, as the disease progresses, the person will need assistance and supervision, and may eventually need 24-hour care.

[H0035] Based on the assumption that HD is a neurological disease, diagnosis often includes a physical examination which includes neurological tests. The neurological tests are aimed at detecting dementia, abnormal movements, abnormal reflexes, "prancing" and wide walk, hesitant speech of poor enunciation. Additional tests such as a CT scan of the head, an MRI scan of the head, and/or a PET scan of the brain are also performed to detect loss of brain tissue. Additional genetic tests are also performed to determine if a person carries the gene for HD.

[H0036] The disease is caused by an expanded CAG repeat in the huntingtin gene and is one of several trinucleotide repeat disorders. Although the huntingtin gene is widely expressed throughout the body, it is generally believed that the defects of the disease are the result of neurodegeneration. Accordingly, genetic tests can detect the number of CAG repeats that, in combination with the neurological tests described above, can be used to definitively diagnose HD.
The cause of HD is a genetic defect on chromosome 4. Specifically, the defect is a triplicate repeat defect wherein a CAG sequence repeats many more times than it is supposed to. In a normal individual, the section of DNA found on chromosome 4 has 10 to 28 CAG repeats; however, an individual with HD has 36 to 120 CAG repeats.

HD is a hereditary disease and as the gene is passed down through families, the number of CAG repeats generally increases with each generation. Furthermore, the disease tends to become tragic as it is passed through generations because the larger the number of repeats, the greater your chance of developing symptoms at an earlier age. Therefore, as the disease is inherited over multiple generations, symptoms develop at younger and younger ages within each successive generation. Furthermore, there is some evidence that the number of CAG repeats is also linked to the severity of the disease, and in turn morbidity. Nevertheless, there are no available tests to track the pre-clinical symptom progression of the disease or to assess the disease's progress prior to manifestation of physical symptoms.

As an inherited disease, there is a 50% chance a child will inherit HD if one of his/her parents has HD. Accordingly, if a parent has the CAG repeats and HD, there is a 50% chance that the parent's child will develop HD at some point in his or her life.

There are two forms of Huntington's disease: 1) adult onset, and 2) early onset. Adult onset is the most common form of HD. Persons with adult onset HD usually develop symptoms in their mid 30s and 40s, a period in peoples' lives when they typically are having or have young children. The early-onset form of HD accounts for a small number of cases and begins in childhood or adolescence.

However, determining the number of CAG repeats via genetic testing cannot predict with any certainty when symptoms will begin to surface or when sub-clinical physiological changes begin to occur, prior to any physiological (e.g., neurological) symptoms. Accordingly, there is a need to develop methods for tracking the progression of the disease, before and after clinical symptoms are detected.

There is no cure for HD and no way to prevent the disease from progressing. Accordingly, current treatment regimes are aimed at delaying the progression and severity of the symptoms in order to allow the patient to live and function for as long and as comfortably as possible.
While treatments may vary, they are typically aimed at addressing the symptoms from a neurological perspective. For example, dopamine blockers are often prescribed to reduce the frequency and severity of abnormal behaviors and movements.

Amantadine (a weak antagonist of the NMDA type glutamate receptor) and tetrabenazine (promotes the early metabolic degradation of monoamines, in particular the neurotransmitter dopamine) are often frequently prescribed as well to aid in controlling abnormal movements.

Antipsychotic drugs such as alopexol and clozapine are often prescribed as well because they often exhibit the side effect of suppressing unwanted movements; however, also have a primary sedative effect that is less than desirable for most HD patients. Furthermore, antipsychotic drugs also often worsen the involuntary contractions (dystonia) and muscle rigidity. Accordingly, use of antipsychotic drugs is not an ideal solution for improving an HD patient's quality of life in view of the side effects.

Drugs that suppress chorea, dystonia, and other muscle rigidity are also used; however, they are not specific for symptoms. For example, antiseizure drugs such as clonazepan may be prescribed. Antianxiety drugs such as diazepam are also often prescribed. However, both classes of drugs were designed to treat neurological disease, not muscle disease. These medications also significantly alter consciousness and have a high risk of dependence and abuse.

Additional treatments include medications to treat psychiatric disorders. These medications include antidepressants (e.g., escitalopram, fluoxetine, and sertraline), antipsychotic drugs, and mood-stabilizing drugs (e.g., lithium and anticonvulsants). Commonly prescribed anticonvulsants include but are not limited to valproic acid, divalproex, and lamotrigine.

However, antidepressants often cause nausea, diarrhea, insomnia, and sexual dysfunction. Similarly, mood-stabilizing drugs also have severe side effects such as weight gain, tremors, and gastrointestinal problems. Lithium is particularly problematic for some HD patients because it can cause thyroid and kidney failure, thus requiring periodic blood tests to monitor the drug's toxic effects.

Coenzyme Q10 (co-Q10) has also been suggested to have a beneficial effect at slowing down the course of the HD progression; however, there is little evidence to support or dismiss this theory.
As a progressive disease, HD causes disability that gets worse over time regardless of medications prescribed. The typical lifespan for an HD patient is 15-20 years after disease onset. While the disease itself may eventually lead to death, infection and suicide are leading indirect causes of morbidity.

Accordingly there is a need to develop diagnostic tests that can be used track the progression of the disease, before and after the first manifestation of physical symptoms. There is also an unmet need to develop treatment regimens targeted at the muscular defect component of the disease rather than using neurological drugs to indirectly treat abnormal movement disorders.

As described herein, contrary to the existing belief that HD is purely a neurological disease rooted in neurodegeneration, the inventors herein show that HD is also a muscle disease. Specifically, it is demonstrated herein that skeletal muscle from R6/2 HD mice is hyperexcitable because of chloride and potassium channel dysfunction and disruptions in chloride channel mRNA processing.

Chloride and potassium currents are key to normal skeletal muscle physiology. Specifically, chloride and potassium currents in skeletal muscle maintain the resting membrane potential, help determine the responsiveness to neuronal stimulation, and govern action potential propagation and frequency. The defects in the chloride and potassium currents in HD skeletal muscle disclosed herein explain the movement disorders of HD and offer novel therapeutic targets.

Definitions

As used herein, HD refers to Huntington's disease and all symptoms related to and arising from the underlying disease. Specifically, the focus of the inventions described herein are the muscular disease components of HD.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition. Specifically, the treatments described herein are aimed at muscular disorders/symptoms associated with HD.
[0056] As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0057] As used herein, the terms "therapy," "therapeutic," "treating," "treat," "treatment," "treatment regimen," or "treatment regime" can be used interchangeably and refer broadly to treating a disease, arresting, or reducing the development of the disease or its clinical symptoms, and/or relieving the disease, causing regression of the disease or its clinical symptoms. Therapy encompasses prophylaxis, treatment, remedy, reduction, alleviation, and/or providing relief from a disease, signs, and/or symptoms of a disease. Therapy encompasses an alleviation of signs and/or symptoms in patients with ongoing disease signs and/or symptoms (e.g., movement disorders such as involuntary jerking or writhing movements (i.e., chorea), involuntary and sustained contracture of muscles (i.e., dystonia), muscle rigidity, slow and uncoordinated fine movements, slow or abnormal eye movements, impaired gait, impaired posture, impaired balance, difficulty with the physical production of speech, and difficulty swallowing). Therapy also encompasses "prophylaxis".

[0058] The term "reduced", for purpose of therapy, refers broadly to the clinical significant reduction in signs and/or symptoms.

[0059] Therapy includes treating stable and progressive symptoms of HD as well as preemptively administering an agent to delay the onset of one or more symptoms of HD.

[0060] Therapy encompasses but is not limited to precluding the appearance of signs and/or symptoms anytime as well as reducing existing signs and/or symptoms and reducing or eliminating existing signs and/or symptoms.

[0061] Therapies include but are not limited to ClC-1 and Kir modulators and muscle relaxants.

[0062] As used herein, the term "a pharmaceutical composition" refers to a composition suitable for administration to a patient in need thereof.
[0063] As used herein, "symptoms" of a disease refers broadly to any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the patient and indicative of disease.

[0064] As used herein, "solid support," "support," and "substrate" refer broadly to any material that provides a solid or semi-solid structure with which another material can be attached including but not limited to smooth supports (e.g., metal, glass, plastic, silicon, and ceramic surfaces) as well as textured and porous materials.

[0065] As used herein "diagnostic" refers broadly to identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of "true positives"). While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[0066] As used herein, "diagnosing" refers broadly to classifying a disease or a symptom, determining a severity of the disease, monitoring disease progression, forecasting an outcome of a disease and/or prospects of recovery. The term "detecting" may also optionally encompass any of the foregoing. Diagnosis of a disease according to the present invention may, in some embodiments, be affected by determining a level of exon 7a mRNA expression and more generally the processing of HD gene-associated mRNA from muscle samples. In alternate embodiments, the samples may come from blood or CSF.

[0067] As used herein, "predisposition" or "predispose" refers to the increased likelihood or susceptibility of a patient acquiring or developing a disease. For example, a patient with a high number of CAG repeats may be considered to be predisposed to HD and/or HD symptoms. Accordingly, a patient who is predisposed to HD and/or HD symptoms may receive frequent testing to determine the pre-clinical symptom progression of the disease.

[0068] As used herein, "patient" or "subject" refers broadly to any animal who is in need of treatment either to alleviate a disease state or to prevent the occurrence or reoccurrence of a disease state. Also, "Patient" as used herein, refers broadly to any animal who has risk factors, a history of disease, susceptibility, symptoms, signs, was previously diagnosed, is at risk for, or is a member of a patient population for a disease. The patient may be a clinical patient such as a
human or a veterinary patient such as a companion, domesticated, livestock, exotic, or zoo animal. The term "subject" may be used interchangeably with the term "patient." In preferred embodiments, a patient is a human.

[0069] As used herein, "muscle relaxants" refer to a family of drugs that inhibit action potential generation. Non limiting examples of muscle relaxants include succinylcholine, gallamine, dantrolene, and derivatives thereof. In specific embodiments the muscle relaxants can be administered in combination with each other. For example, combinations of succinylcholine or gallamine with dantrolene may be administered together. Additional examples of muscle relaxants include, but are not limited to atacurium, rocuronium, vecuronium, pancuronium, cisatracurium, and derivatives thereof. Furthermore, one of skill in the art will know additional muscle relaxants, specifically those muscle relaxants that act directly on skeletal muscle or neuromuscular junctions. In specific embodiments, the muscle relaxants act directly on skeletal muscle or neuromuscular junctions and exhibit a non-depolarizing effect.

Physiology of HD Muscle

[0070] The majority of studies of HD have focused on the central nervous system rather than the motor defects under the assumption that the motor defects are merely the result of neurodegeneration (Bett JS, Bates GP, Hockly E (2006) in Genetic Instabilities and Neurological Diseases, Molecular pathogenesis and therapeutic targets in Huntington's disease, eds WellsRD, AshizawaT (Elsevier/Academic Press, Boston), 2nd Ed, pp 223-249; Lo DC, Hughes RE (2010) Neurobiology of Huntington's Disease: Applications to Drug Discovery Neurol 43(3):397-400; Sasson et al. (2009) Huntington's disease: the current state of research with peripheral tissues. Exp. Neurol. 219(2):285-391). Accordingly, no previous study has examined the basic membrane properties in HD muscle (i.e., the membrane properties that control action potential initiation and propagation).

[0071] A normal skeletal muscle contraction requires that action potentials initiated at the neuromuscular junction propagate along the surface membrane and into the interior of the muscle fiber through the transverse tubular system. The responsiveness of the muscle to neuronal stimulation and the shape of each action potential repolarization are determined by potassium and chloride currents, which set and maintain the resting membrane potential of skeletal muscle (Hodgkin et al., (1959) The influence of potassium and chloride ions on the membrane potential
of single muscle fibres. J. Physiol. 148:127-160). In effect, the inward rectifying potassium (Kir) and chloride conductances buffer the membrane potential at rest such that if they are reduced, the membrane becomes hyperexcitable and normally subthreshold events can initiate action potentials and involuntary contractions. Most of the resting conductance in skeletal muscle is mediated by chloride through the muscle chloride channel (CIC-1), and a large decrease in this conductance results in the hyperexcitability, involuntary contractions, rigidity, and persistent contractions that characterize general myotonia at the cellular level.

In 2004, Ribchester et al. showed that the membrane time constant under resting conditions was prolonged in the transgenic R6/2 Huntington's disease compared to wild type muscle fibers (Ribchester et al. (2004) in the European Journal of Neuroscience, Vol. 20, pp. 3092-3114). However, the authors failed to properly analyze the source of the prolonged membrane time constant. The authors attributed this result to the decreased fiber diameter of the R6/2 muscle fibers. However, the membrane time constant of nerve and muscle is independent of fiber diameter, a property derived from the basic equation for the time constant. Specifically, the authors did not consider the chloride or potassium current contributions to the membrane time constant. The applicants correctly hypothesized that the prolonged voltage responses in the diseased fibers were caused by dysfunctional chloride and/or potassium channels (defects in these channels could cause an increase in the membrane resistance that would explain the longer time constant).

The basic equation for measuring the membrane time constant, is \( \tau_m = R_m C_m \) where, \( \tau_m \) is the membrane time constant, \( R_m \) is the specific membrane resistance, and \( C_m \) is the specific membrane capacitance. This equation makes clear that the time constant should be independent of fiber diameter. Furthermore, a detailed analysis of membrane time constants and calculations in skeletal muscle was prepared by Hodgkin and Nakajima in 1972 ("The effect of diameter on the electrical constants of frog skeletal muscle fibres"; Journal of Physiology - London, 221, pp. 105-120). Hodgkin and Nakajima empirically demonstrated that membrane time constant in frog skeletal muscle was independent of fiber diameter. Thus, the prolonged membrane time constant in the R6/2 was likely the result of an increased resting membrane resistance (an increased membrane capacitance did not seem likely). An increased resting membrane resistance in muscle most likely results from a loss or inhibition of chloride channels. Accordingly, this discovery

17
suggests that Huntington's disease muscle is a myotonic disease rather than a purely neurological disease as previously proposed.

As described herein, action potentials, as well as CIC-1 and Kir conductances were measured in dissociated adult skeletal muscle fibers from WT and HD mice (from the same litter, age-matched) to study the muscular components of HD. The HD muscle came from the R6/2 transgenic mouse line, which carries the human HD gene and exhibits many of the motor and cognitive defects found in HD patients. As described above, HD is caused by an expanded CAG trinucleotide repeat in the huntingtin gene (IT15) in humans. Patients with >40 CAG repeats develop the disease during middle age; those with >50 CAG repeats develop a more extreme juvenile form of the disease.

The R6/2 line most closely resemble the juvenile form of HD because of the rapid onset of reduced activity at 4.5 wk of age and overt motor defects at 8 wk of age. Accordingly, the R6/2 line is often considered the model of choice for preclinical trials of potential HD therapeutics owing to its rapidly developing and well-described phenotype.

Furthermore, in consideration of the fact that the expanded trinucleotide repeat has been shown to disrupt Clcn1 (gene for CIC-1) mRNA processing in myotonic dystrophy type 1, the studies presented herein were also aimed at determining whether there was a disruption in Clcn1 mRNA processing in HD muscle. Surprisingly, the studies presented herein reveal defects in HD muscle that cause hyperexcitability and contribute to the severe involuntary and prolonged contractions that are hallmarks of the disease.

As described in the examples herein, fundamental membrane properties were examined in order to study the responsiveness of HD muscle to neuronal stimulation. There were significant decreases in GQC-1 and Gk, - In normal muscle, GQC-1 and Gk, accounts for most of the resting conductance in the muscle which keeps the membrane potential near resting levels. For example, an action potential will not be triggered unless the excitatory stimulus overcomes the Gcic- and Gkir. Thus, when Gcic and Gkir are reduced, action potentials will be triggered at a lower threshold. In HD muscle, GQC-1 and Gk were found to be reduced. This decrease in conductances explains the prolonged falling phase of the action potentials in HD fibers and the increased firing of action potentials due to the lower threshold for firing. These findings as
presented herein prove that Gcic.i and Gi decreases lead to an increased input resistance and membrane time constant.

[0078] It was previously found that reductions in the muscle CIC-1 occurred 2 days after denervation and is likely mediated by myogenic factors. Additional studies were performed and showed that neuromuscular innervation was normal throughout the life of R6/2 mice, occurring despite significant, uniform, and progressive muscle atrophy. Furthermore, R6/2 mice maintain a normal ability to regenerate motor axons and functional neuromuscular junctions after crushing the tibial nerve. Thus, the normal innervation of HD muscle indicates that the defects reported in this study were independent of denervation and motor neuron degeneration.

[0079] The studies described herein how greater percent decreases in Gcic-i and Gi decreases when normalized to surface area (rather than to capacitance). This is because some of the CIC-1 and Kir channels were lost because of a decrease in the transverse tubular system (either due to disease and/or muscle dissociation). This is supported by our studies which show a partial detubulation in the HD fibers as evidenced by a decrease in Cm and the faster time-to-peak of the CIC-1 and Kir channel currents. This is further supported by the known fact that Kir channels are expressed on the transverse tubular system. One of skill in the art would know that additional confirmatory studies could be performed using optical methods such as electron microscopy.

[0080] Accordingly, key aspects of this invention include the discovery of the decreased resting conductances cause hyperexcitability in HD muscle. The degree of hyperexcitability was high enough to cause self-triggering action potentials that occurred after apparent subthreshold stimuli. Also disclosed herein is the minimal disruption of CIC-1 and Kir function in HD fibers, suggesting that the decreased conductances were caused by a reduced expression of CIC-1 and Kir channels. Consistent with this, it was found that reduced levels of normal mature Clcn1 and Kenj2 mRNA. A disruption of mRNA splicing has been shown to cause a decrease in CIC-1 expression in myotonic dystrophy type 1, another trinucleotide repeat disorder. Accordingly, measurements were taken and elevated levels of aberrant Clcn1 mRNA containing exon 7a was detected in HD muscle, which indicates a similar disruption in Clcn1 pre-mRNA splicing. Surprisingly and unexpectedly, the studies herein describe biophysical and molecular similarities
between HD and myotonic dystrophy muscle, suggesting, that there is a common pathophysiology that involves a disruption in RNA processing.

[0081] Accordingly, detection of pre-mRNA splicing can be used as a diagnostic tool and modulation of the hyperexcitability and decreased conductances in HD muscle provide a novel pathway for treating the motor deficits associated with HD.

**Diagnostic Methods**

[0082] In some embodiments, this disclosure relates to diagnostic methods and products. In one embodiment, the diagnostic method and products can be used to monitor the treatment of HD, as described herein. In some embodiments, the diagnostic methods and products include molecular assays to measure levels of proteins, genes or specific genetic mutations. Such measurements can be used, for example, (1) to predict whether ClC-1 modulator, Kir modulator, and/or muscle relaxant therapy will benefit a specific individual, (2) to predict the effective dosage of ClC-1 modulator, Kir modulator, and/or muscle relaxant therapy, (3) to monitor ClC-1 modulator, Kir modulator, and/or muscle relaxant therapy, (4) to adjust ClC-1 modulator, Kir modulator, and/or muscle relaxant therapy, (5) to tailor the ClC-1 modulator, Kir modulator, and/or muscle relaxant therapy to an individual, and (6) to track HD progression.

[0083] In specific embodiments, levels of Clcn1 mRNA are measured and used to diagnose the onset of HD. In specific embodiments, levels of Clcn1 mRNA are measured and used to detect the progression of HD. In specific embodiments, levels of Clcn1 mRNA are measured and used to predict the prognosis of a HD patient. In specific embodiments, levels of Clcn1 mRNA are measured and used to determine the efficacy of treatment regimes. In specific embodiments, levels of Clcn1 mRNA are measured and used to adjust treatment regimes.

[0084] In specific embodiments, levels of Clcn1 pre-mRNA are measured and used to diagnose the onset of HD. In specific embodiments, levels of Clcn1 pre-mRNA are measured and used to detect the progression of HD. In specific embodiments, levels of Clcn1 pre-mRNA are measured and used to predict the prognosis of a HD patient. In specific embodiments, levels of Clcn1 pre-mRNA are measured and used to determine the efficacy of treatment regimes. In specific embodiments, levels of pre-mRNA are measured and used to adjust treatment regimes.
[0085] In specific embodiments, levels of exon 7a mRNA are measured and used to diagnose the onset of HD. In specific embodiments, levels of exon 7a mRNA are measured and used to detect the progression of HD. In specific embodiments, levels of exon 7a mRNA are measured and used to predict the prognosis of a HD patient. In specific embodiments, levels of exon 7a mRNA are measured and used to determine the efficacy of treatment regimes. In specific embodiments, levels of exon 7a mRNA are measured and used to adjust treatment regimes.

[0086] In specific embodiments, levels of CIC-1 and/or Kir are measured and used to diagnose the onset of HD. In specific embodiments, levels of CIC-1 and/or Kir are measured and used to detect the progression of HD. In specific embodiments, levels of CIC-1 and/or Kir are measured and used to predict the prognosis of a HD patient. In specific embodiments, levels of CIC-1 and/or Kir are measured and used to determine the efficacy of treatment regimes. In specific embodiments, levels of CIC-1 and/or Kir are measured and used to adjust treatment regimes. In certain embodiments the levels of CIC-1 and/or Kir are DNA levels. In certain embodiments the levels of CIC-1 and/or Kir are protein levels. In certain embodiments the levels of CIC-1 and/or Kir are mRNA levels.

[0087] In some embodiments, the diagnostic method or product is a companion diagnostic or product. In certain embodiments the companion diagnostic or product can be used to monitor a combination therapy.

**Administration and Treatment Modalities using Muscle Relaxants**

[0088] Muscle relaxants can be administered to a subject, in accord with known methods, such as intracolonic, intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. In certain aspects, the modulators are administered to a subject who has an increased expression of exon 7a mRNA expression. In certain aspects, the modulators are administered to a subject prior to any physiological symptoms of HD. In certain aspects, the modulators are administered to a subject at the onset of physiological symptoms of HD. In certain aspects, the modulators are administered to a subject after the onset of physiological symptoms of HD.

[0089] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an
improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) decreased or elimination of chorea, (2) decreased or elimination of dystonia, (3) decreased muscle rigidity, (4) improved or normal eye movements, (5) improved gait, (6) improved posture, (7) improved balance, (8) improved speech, (9) improved swallowing, (10) fewer involuntary movements, (11) improved quality of life, and (12) improved coordination.

[0090] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[0091] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[0092] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "therapeutically effective amount" for treatment of HD disease may also be measured by its ability to stabilize the progression of the muscular component of the disease.

[0093] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0094] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.
The efficient dosages and the dosage regimens for the muscle relaxants used in the present invention depend on the severity of the disease, the progression of the disease, and overall health of the patient. Specific dosages will optimally effective can be determined by the persons skilled in the art.

A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In one embodiment, the muscle relaxants of this invention are administered in a combination therapy. For example, the muscle relaxants can be administered in combination with tetrabenzine, antipsychotic drugs, drugs that suppress chorea, drugs that suppress dystonia, drugs that reduce muscle rigidity, antidepressants, mood-stabilizing drugs, psychotherapy, speech therapy, physical therapy, and occupational therapy.

In one embodiment, only a muscle relaxant is administered in combination with one or more drugs that modulate Kir and/or CIC-1.

In specific embodiments, the combination therapy comprises one or more muscle relaxants and 1, 2, 3, 4, 5, 6, 7, 8, or 9 additional agents. In specific embodiments the combination treatment is administered such that the Muscle relaxants are administered at the same time as the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent. In specific embodiments the combination treatment is administered such that the muscle relaxants are administered before the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent. In specific embodiments the combination treatment is administered such that the muscle relaxants are administered after the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent.

In specific embodiments, the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent that upregulates CIC-1 and/or Kir expression. In specific embodiments, the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent that downregulates CIC-1 and/or Kir expression.
In specific embodiments, the muscle relaxant is succinylcholine, gallamine, dantrolene, or a derivative thereof. In specific embodiments, one or more muscle relaxant is administered concurrently. In specific embodiments, one or more muscle relaxant is administered consecutively. In specific embodiments, one or more muscle relaxant is administered one or more days after the first muscle relaxant is administered. In specific embodiments, succinylcholine or gallamine are administered with dantrolene.

In other embodiments, this disclosure relates to companion diagnostic methods and products. In one embodiment, the companion diagnostic method and products can be used to monitor the treatment of HD disease. In some embodiments, the companion diagnostic methods and products include molecular assays to measure levels of proteins, genes or specific genetic mutations. Such measurements can be used, for example, to predict whether the muscle relaxants therapy will benefit a specific individual, to predict the effective dosage of muscle relaxants therapy, to monitor muscle relaxants therapy, adjust muscle relaxants therapy, tailor the muscle relaxants therapy to an individual, and track the progression symptoms of HD.

In some embodiments, the companion diagnostic can be used to monitor a combination therapy.

In some embodiments, the companion diagnostic can include muscle relaxants.

In some embodiments, the companion diagnostic can be used before, during, or after muscle relaxants therapy.

In certain embodiments, the muscle relaxant is administered in combination with a traditional HD therapy that targets the brain. Traditional HD therapies include but are not limited to tetrabenazine, antipsychotic drugs, drugs that suppress chorea, drugs that suppress dystonia, drugs that reduce muscle rigidity, antidepressants, mood-stabilizing drugs, psychotherapy, speech therapy, physical therapy, and occupational therapy. For example, muscle relaxants may be administered in combination with one or more current drug regimes that target the brain.

In certain embodiments, the muscle relaxers can be administered in combination with a traditional HD therapy in order to reduce the dosage of the traditional HD therapy. For example, a patient who is prescribed an antipsychotic drug to treat HD but who suffers side
effects of the treatment, may be administered a muscle relaxant wherein the combination therapy allows the reduced dosage of the antipsychotic drug and a reduction in the number and severity of the side effects associated with the antipsychotic drug.

[00108] In certain embodiments, the combination treatment of a traditional HD therapy and a muscle relaxant will reduce the number of HD symptoms. In certain embodiments, the combination treatment of a traditional HD therapy and a muscle relaxant will reduce the severity of HD symptoms. In certain embodiments, the combination treatment of a traditional HD therapy and a muscle relaxant will slow the progression of HD symptoms. In certain embodiments, the combination treatment of a traditional HD therapy and a muscle relaxant will prevent the development of HD symptoms. In certain embodiments, the combination treatment of a traditional HD therapy and a muscle relaxant will improve the prognosis for HD patients.

**Administration and Treatment Modalities using Modulators of CLC-1 and Kir**

[00109] Modulators of CLC-1 and Kir can be administered to a subject, in accord with known methods, such as intracolonic, intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. In certain aspects, the modulators are administered to a subject who has an increased expression of exon 7a mRNA expression. In certain aspects, the modulators are administered to a subject prior to any physiological symptoms of HD. In certain aspects, the modulators are administered to a subject at the onset of physiological symptoms of HD. In certain aspects, the modulators are administered to a subject after the onset of physiological symptoms of HD.

[00110] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) decreased or elimination of chorea, (2) decreased or elimination of dystonia, (3) decreased muscle rigidity, (4) improved or normal eye movements, (5) improved gait, (6) improved posture, (7) improved balance, (8) improved speech, (9) improved swallowing, (10) fewer involuntary movements, (11) improved quality of life, and (12) improved coordination.
In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "therapeutically effective amount" for treatment of HD disease may also be measured by its ability to stabilize the progression of the muscular component of the disease.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

The efficient dosages and the dosage regimens for the CIC-1 and Kir modulators used in the present invention depend on the severity of the disease, the progression of the disease, and overall health of the patient. Specific dosages will optimally effective can be determined by the persons skilled in the art.
A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In one embodiment, the CIC-1 and Kir modulators of this invention are administered in a combination therapy. For example, the CIC-1 and Kir modulators can be administered in combination with tetrabenzine, antipsychotic drugs, drugs that suppress chorea, drugs that suppress dystonia, drugs that reduce muscle rigidity, antidepressants, mood-stabilizing drugs, psychotherapy, speech therapy, physical therapy, and occupational therapy.

In one embodiment, only a CIC-1 modulator is administered alone or in combination with one or more drugs that do not modulate Kir.

In one embodiment, only a Kir modulator is administered alone or in combination with one or more drugs that do not modulate CIC-1.

In specific embodiments, the combination therapy comprises one or more CIC-1 and/or Kir modulators and 1, 2, 3, 4, 5, 6, 7, 8, or 9 additional agents. In specific embodiments the combination treatment is administered such that the CIC-1 and/or Kir modulator are administered at the same time as the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent. In specific embodiments the combination treatment is administered such that the CIC-1 and/or Kir modulator are administered before the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent. In specific embodiments the combination treatment is administered such that the CIC-1 and/or Kir modulator are administered after the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent.

In specific embodiments, the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent that upregulates CIC-1 and/or Kir expression. In specific embodiments, the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth agent that downregulates CIC-1 and/or Kir expression.

In other embodiments, this disclosure relates to companion diagnostic methods and products. In one embodiment, the companion diagnostic method and products can be used to
monitor the treatment of HD disease. In some embodiments, the companion diagnostic methods and products include molecular assays to measure levels of proteins, genes or specific genetic mutations. Such measurements can be used, for example, to predict whether the CIC-1 and/or Kir modulator therapy will benefit a specific individual, to predict the effective dosage of CIC-1 and/or Kir modulator therapy, to monitor CIC-1 and/or Kir modulator therapy, adjust CIC-1 and/or Kir modulator therapy, tailor the CIC-1 and/or Kir modulator therapy to an individual, and track the progression symptoms of HD.

[00124] In some embodiments, the companion diagnostic can be used to monitor a combination therapy.

[00125] In some embodiments, the companion diagnostic can include a CIC-1 and/or Kir modulator.

[00126] In some embodiments, the companion diagnostic can be used before, during, or after CIC-1 and/or Kir modulator therapy.

[00127] Modulators of CLC-1 and Kir include but are not limited to small molecules, antibodies, and siRNA. One of skill in the art will know all possible modulators of CIC-1 and Kir.

Articles of Manufacture

[00128] In other embodiments, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the antibody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a
commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[00129] Also within the scope of the invention are kits.

[00130] In certain embodiments, the kits contain necessary reagents and the method for diagnosis, monitoring or assessing chronic inflammatory disease (e.g., Crohn's disease) using an immunoassay such as an ELISA, a western blot, a protein array, a reverse phase protein array, a single cell barcode chip, flow cytometry, a single cell cytokine analysis assay, immunofluorescent staining, or any other means of detecting cytokine expression in cells known to one of skill in the art. In preferred embodiments, the kit contains the necessary reagents for mRNA extraction and analysis.

[00131] In certain embodiments the kit comprises probe(s), wherein the probe(s) are attached to a solid support. This solid support preferably comprises beads (more preferably, immunobeads), a gel (e.g., agarose or polyacrylamide gel), or any array-type solid matrix such as a slide made of distinct materials, such as glass with or without a gold-covered surface.

[00132] As described herein, the kits may include instructions, directions, labels, marketing information, warnings, or information pamphlets.

EXAMPLES

[00133] All electrophysiological experiments described herein were performed on individual dissociated HD and WT flexor digitorum brevis or interosseous muscle fibers using two intracellular microelectrodes (FIGURE 5). All HD mice averaged 12 wks of age and all WT mice averaged 13 wks of age.

Example 1. Action Potentials

[00134] An action potential is the basis of excitability and the physiological signal that initiates muscle contraction. We measured action potentials in HD (n = 12) and WT fibers (n = 17) by injecting a series of depolarizing current pulses through a current-passing electrode and measuring the membrane potential with a voltage-sensing electrode (FIGURE 1). This simulates
the physiological condition whereby an inward current through acetylcholine receptors at the
neuromuscular junction triggers an action potential. Our series of 0.5-ms current pulses ranged in
amplitude from subthreshold to suprathreshold. There was a striking prolongation of the
repolarization phase in HD compared with WT fibers (FIGURE 1A). The slower time constant of
the repolarization to the resting membrane potential of HD fibers (15.0 ± 2.1 ms) was nearly
double that of WT (7.7 ± 0.4 ms) (FIGURE 1B and TABLE 1). The HD fibers were also
hyperexcitable compared with WT: the minimum current pulse required to trigger an action
potential was significantly less in HD than in WT fibers (FIGURE 1C). We also observed an
after-depolarization in four of the HD fibers following an apparent subthreshold current pulse,
which resulted in a spontaneous action potential (FIGURE 1D). The spontaneous action
potentials occurred at an average of 6.4 ± 1.7 ms after the termination of the stimulating current
pulse. In all of the action potentials, there were no significant differences in the maximum rate-
of-rise or peak amplitude, suggesting that the effects we measured were not due to changes in the
function and/or density of the fast voltage-gated sodium channels in HD fibers (Table 1).

Table 1. Action potential properties of HD and WT fibers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Resting membrane potential (mV)</th>
<th>Max rate-of-rise (mV/ms)</th>
<th>Max Δvoltage (mV)</th>
<th>Decay τ1 (ms)</th>
<th>Decay τ2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 17)</td>
<td>87 ± 1</td>
<td>420 ± 33</td>
<td>127 ± 3</td>
<td>0.62 ± 0.04</td>
<td>17.7 ± 0.4</td>
</tr>
<tr>
<td>HD (n = 12)</td>
<td>86 ± 1</td>
<td>465 ± 47</td>
<td>125 ± 2</td>
<td>0.82 ± 0.06*</td>
<td>15.0 ± 2.1*</td>
</tr>
</tbody>
</table>

[00135] As shown in Table 1, Average values (±SEM) of the resting or baseline
membrane potential, the maximum rate-of-rise of the depolarization, the peak change in
membrane potential, the initial repolarization time constant (τ₁), and the slower repolarization
time constant (τ₂). * indicates a significant difference compared to WT fibers (P < 0.05).

[00136] The prolonged repolarization, hyperexcitability, and spontaneous action potentials
would be expected if there was a reduction in the resting muscle chloride conductance through
CIC-1 (Gcic-i)- The presence of self-triggered action potentials after only subthreshold stimuli
indicates a pronounced hyperexcitability in HD muscle. This could occur if there was also a
reduction in the inward rectifying potassium conductance (G Kir). The slight but significant
increase in the initial repolarization time constant of the HD action potentials could also be the
result of decreases in Gcic-i and G Kir (Table 1).
Example 2. Chloride Channels

We measured chloride currents ($i_{ci}$) from 9 HD and 14 WT fibers using a three-pulse voltage clamp protocol (FIGURE 2A) that accounted for the voltage- and time-dependent deactivation of ClC-1. To ensure large inward currents, we used high intracellular chloride (70 mM). From a holding potential of -20 mV, the chloride equilibrium potential, we applied a depolarizing conditioning pulse (PI) that was followed by two consecutive test pulses (P2 and P3). PI fully activated the chloride channels. We then used P2 to determine the voltage dependence of the instantaneous or peak currents through the open channels. After the channels deactivated, we used the currents at the onset of P3 to determine the relative open probability of the chloride channels. The resulting average specific currents ($\mu$A/cm$^2$) are shown in FIGURE 2B-2D. To isolate the chloride currents, we blocked the major Na$^+$, K$^+$, and Ca$^{2+}$ channels with tetrodotoxin, Cs$^+$ substitution, and nifedipine, respectively. The specific $i_{ci}$ (FIGURE 2D) was the difference between the currents recorded before (FIGURE 2B) and during (FIGURE 2D) exposure to the chloride channel blocker anthracene-9-carboxylic acid (9AC).

There was a clear reduction in the specific $I_{Cl}$ and $G_{ci}$ of HD compared with WT fibers (FIGURE 2D). The peak $j_{Cl}$ at -140 mV during P2 in HD fibers (-360 ± 48 $\mu$A/cm$^2$) was significantly smaller than the mean value in WT (-1,133 ± 72 $\mu$A/cm$^2$) ($P < 0.001$). To determine $G_{ci}$ we examined the current-voltage (IV) relationship of the peak $I_{cl}$ values during P2 (FIGURE 2E). The peak $G_{ci}$, the slope of the IV relationship from -100 to -140 mV, was significantly reduced in HD fibers (4.0 ± 0.5 mS/cm$^2$) compared with WT (11.9 ± 0.8 mS/cm$^2$) ($P < 0.001$).

We also examined the average ClC-1 steady-state values, deactivation kinetics and the corresponding outward Cl$^-$ movement, and the peak $I_{cl}$ kinetics (FIGURE 6). Generally the results indicate that any change in ClC-1 function in HD fibers was minor compared with the marked reduction total ClC-1 currents. This was also demonstrated by the chloride channel relative open probability, which we determined by plotting the peak currents at the onset of P3 (normalized to the maximum current) as a function of the steady-state voltages from P2 (FIGURE 2F). The relative open probability data were fitted with a Boltzmann curve. The resulting mean 0.5 values for the HD (-41 ± 4 mV) and WT (-41 ± 3 mV) fibers were not significantly different ($P = 0.88$). The small difference in slope factors ($k$ values) for HD (27 ± 1
mV) and WT (24 ± 1 mV) fibers (P = 0.02) suggests a minor change in ClC-1 function in HD fibers.

**Example 3. Inward Rectifying Potassium Channels**

We measured the specific Kir currents (I_Kr) from HD (n = 11) and WT (n = 12) fibers (FIGURE 3). Kir channels are unique because their opening depends on the membrane potential and the extracellular [K⁺]. At potentials negative to the potassium equilibrium potential (E_Kr), the Kir channels open and generate inward currents; whereas, at potentials positive to E_Kr, the channels are mostly closed. To generate large inward currents, we used high extracellular K⁺ (130 mM). The E_K was -9.5 mV. We measured I_Kr from a holding potential of 0 mV by applying large negative and small positive pulses (FIGURE 3A). To isolate the potassium currents, we blocked the major Na⁺, CF, and Ca²⁺ channels with tetrodotoxin, 9AC, and nifedipine, respectively. The specific I_Kr (FIGURE 3B) was the difference between the currents recorded before and during exposure to the Kir channel blocker, Ba²⁺ (FIGURE 7).

The IV relationship of the peak I_Kr values is shown in FIGURE 3C. The peak I_Kr at -60 mV in HD (-87 ± 11 μA/cm²) was significantly less than in WT fibers (-216 ± 12 μA/cm²) (P < 0.001). The peak G_Kr, the slope of the peak I_Kr values from -40 to -60 mV, was significantly less in HD (1.8 ± 0.2 mS/cm²) than in WT fibers (4.3 ± 0.2 mS/cm²) (P < 0.001).

The decline in I_Kr at large negative voltage pulses (FIGURE 3B) was likely the result of K⁺ depletion in the transverse tubular system. A decrease of G_Kr should slow the rate at which the I_Kr declines. Accordingly, I_Kr declines at a slower rate in HD than WT fibers (FIGURE 7E).

Our results are consistent with a previous study showing a reduced Kir current density in striatal medium-sized spiny neurons in R6/2 HD mice.

To more fully assess the decreases in G_cl and G_Kr as well as the membrane properties, we analyzed the capacitance. The amount of plasma membrane is generally considered to be directly proportional to the capacitance of the fiber. Thus, current levels are often normalized to total fiber capacitance and reported as current densities.

**Example 4. Specific Capacitance and Current Densities**

We measured capacitance by integrating capacitive transients in the same fibers used to study C_cl and G_Kr under conditions whereby the chloride and potassium channels were
blocked (FIGURE 8). This minimized the errors due to voltage-activated channels. The capacitance of HD and WT fibers were compared by normalizing to total fiber surface area to obtain the specific capacitance (C_m). The specific chloride and K currents listed above were obtained the same way. For each fiber, we estimated the surface area using an image that included the fiber length and diameter (FIGURE 5) and assuming the fiber was cylindrical. If the surface membrane accounted for all of the plasma membrane, the C_m of muscle would be ~ 1 \mu F/cm^2. In muscle, the C_m is higher because of the transverse tubular system, a series of invaginations of the surface membrane that spread radially into the interior of the fiber.

C_m was significantly lower in HD (3.4 ± 0.2 \mu F/cm^2, n = 20) than in WT fibers (5.1 ± 0.2 \mu F/cm^2, n = 26) (P < 0.001). A smaller average diameter of the HD fibers may contribute to, but does not likely explain, the full decrease in C_m (Table 2). The decreased C_m of HD fibers suggests there was a reduction of the transverse tubular system (detubulation) in the diseased muscle. Because detubulation could cause decreases in membrane conductance, we also normalized the CIC-1 and Kir results to total fiber capacitance. The HD G_{CIC-i} (1-0 ± 0.1 mS/\mu F) and G_K (0.6 ± 0.1 mS/\mu P) values normalized to capacitance were significantly less than the WT G_{CIC-i} (2.4 ± 0.2 mS/\mu P, P < 0.001) and G_K (0.9 ± 0.1 mS/\mu P, P = 0.008). The decreases in G_{CIC-i} and G_K (when normalized to capacitance) should be independent of fiber diameter. Thus, the density of functional CIC-1 and Kir channels in HD fibers was reduced.

Table 2. Fiber Capacitance and Dimensions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Capacitance per surface area C_m (\mu F/cm^2)</th>
<th>Diameter (\mu m)</th>
<th>Length (\mu m)</th>
<th>Surface area (x10^-4 cm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n=26)</td>
<td>5.1±0.2</td>
<td>52±2</td>
<td>527±9</td>
<td>9.1±0.3</td>
</tr>
<tr>
<td>HD (n=20)</td>
<td>3.4±0.2*</td>
<td>43±2*</td>
<td>519±13</td>
<td>7.3±0.4*</td>
</tr>
</tbody>
</table>

*Significant difference compared with WT fibers (p<0.05).

Example 5. Exon 7a mRNA analysis

The loss of chloride channels in myotonic dystrophy is thought to be due to an accumulation of RNA with CUG or CCUG repeats in the nucleus that disrupt the function of RNA binding proteins, such as muscleblind-like 1 and 2; consequently, aberrantly spliced Clcn1 mRNA that contains exon 7a is degraded via nonsense-mediated decay. We tested for this mechanism in HD interosseous muscle (FIGURE 4). We found a nearly threefold increase in the
proportion of aberrantly spliced Clcn1 mRNA (containing exon 7a) in HD compared with WT muscle (FIGURE 4A-4B). A similar increase was found in extensor digitorum longus muscle (FIGURE 5). Additionally, the total level of normal ClcR1 mature mRNA, determined using two separate primer sets, was significantly reduced in HD compared with WT muscle (FIGURE 4C-D). Similarly, the level of mRNA for the gene that encodes the Kir 2.1 potassium channel (Kcnj2) was significantly reduced in the HD interosseous muscle (0.186 ± 0.005) (n = 2) relative to WT (1.000 ± 0.181) (n = 3) (P = 0.04) and in the HD extensor digitorum longus muscle (0.495 ± 0.074) (n = 2) relative to WT (1.000 ± 0.171) (n = 3) (P = 0.02).

Example 6. Time course for development of muscle defect

[00146] The time course over which the muscle defects develop can be determined. To address this, changes in skeletal muscle chloride and potassium channels, as well as mRNA processing throughout the lifetime of the Huntington’s R6/2 and wild-type mice can be studied. The progression of the disease in the R6/2 mice is rapid, with death occurring at -12 weeks of age, which most closely models the juvenile-onset form of Huntington's disease. The disease onset can also be examined in the more slowly developing Q175 mice, which more closely model the adult-onset form of the disease. Q175 mice can be examined at up to 1 year of age. Examining the time course over which defects in skeletal muscle develop in models of the juvenile- and adult-onset forms of the disease will discern the contribution of the muscle defects to the motor symptoms. For example, motor deficits have been reported for R6/2 mice as early as 4.5 weeks of age and our preliminary data show a clear reduction in muscle chloride currents from 4 week-old R6/2 mice compared to wild-type (FIGURE 1). This highlights that muscle specific defects could underlie the motor symptoms of the disease. It also highlights that the muscle defects could serve as very useful biomarker of the disease.

Example 7. Neuromuscular transmission

[00147] The neuromuscular transmission in R6/2 and Q175 skeletal muscle can be assessed by determining if myotonic phenotypes are present and the contributions of pre- and post-synaptic defects. The neuromuscular experiments can be completed on tissue from late-stage R6/2 and Q175 mice. For example, the nerve can be stimulated and visually monitored to observe the resulting contractions. After a train of nerve-evoked contractions, it can be determine if there are any unstimulated, post-triggered contractions, as would be expected if the decreased chloride
conductance results in myotonia. It can also be determine if any contractions occur that are completely independent of nerve stimulation. Measurements of action potentials resulting from nerve stimulation or direct stimulation of the muscle can be measured and this action potential data will be correlated to the contraction data. Miniature endplate currents (frequency and amplitude) and nerve-evoked currents can also be measured, which can be used to determine the quantal content.

Example 8. Muscle relaxants

[00148] The ability of muscle relaxants to treat the motor symptoms of Huntington's disease can be assessed. Because of the hyperexcitability, action potentials are generated too easily in the Huntington's disease muscle. Accordingly, muscle relaxants, particularly those that inhibit action potential generation, will improve the motor function of Huntington's disease mice. The effects of therapeutics that directly target the muscle and/or neuromuscular junction on the muscle properties and whole animal motor function can be studied in WT and HD muscle. Non-limiting examples of muscle relaxants include succinylcholine, gallamine, and dantrolene. Succinylcholine is a depolarizing muscle relaxant that binds cholinergic receptors and inhibits neuromuscular transmission. Gallamine is a non-depolarizing muscle relaxant that also binds cholinergic receptors and inhibits neuromuscular transmission. Both succinylcholine and gallamine will reduce action potential generation. Dantrolene acts as a muscle relaxant by inhibiting Ca\textsuperscript{2+} release in muscle. Interestingly, dantrolene has been shown to improve motor function in Huntington's disease mice; however, dantrolene was considered to be neuroprotective in the study. Using a combination of succinylcholine or gallamine with dantrolene may be more effective in treating the motor symptoms than using only one muscle relaxant at a time.

[00149] The clearest way to assess the efficacy of the muscle relaxants is to examine motor function in whole animals. To examine whole animal behavior, video-based behavior-recognition software can be used. The motor abnormalities in R6/2 mice manifested as decreased hanging, jumping, stretching, and rearing. This method can also be used to assess the behavior of the Q175 mice. Accordingly, muscle relaxants should lessen the decreases in hanging, jumping, stretching, and rearing. The effects of the muscle relaxants on electrical and contractile
properties of innervated muscle as described above can also be studied and should generate reduce action potential generation and nerve-evoked contractions.


Summary

Huntington's disease is a progressive and fatal degenerative disorder with devastating cognitive and motor defects. The disease is caused by an expanded CAG repeat in the huntingtin gene and is one of several trinucleotide repeat disorders. Although the huntingtin gene is widely expressed throughout the body, it is generally believed that the defects of the disease are the result of neurodegeneration. It was recently demonstrated that skeletal muscle from R6/2 Huntington's disease mice is hyperexcitable because of chloride channel (ClC-1) and inward rectifying potassium channel (Kir) dysfunction. These defects correlated with reduced levels of normal mature Clcn1 (ClC-1 gene) and Kcnj2 (Kir2.1 gene) mRNA, as well as elevated levels of aberrantly spliced Clcn1 (1). ClC-1 and Kir currents in skeletal muscle maintain the resting membrane potential, help determine the responsiveness to neuronal stimulation, and govern action potential propagation and frequency. The defects discovered may help explain the dystonia, rigidity and delayed muscle relaxation associated with HD. Moreover, the ClC-1 defects found also occur in myotonic dystrophy type 1, another trinucleotide repeat disorder. These muscle defects discovered may thus serve as novel therapeutic targets for the treatment of motor dysfunction in Huntington's disease and perhaps other trinucleotide repeat disorders.

An important issue is the time course over which the muscle defects develop. To address this, the changes in skeletal muscle ClC-1 and Kir channels, as well as mRNA processing, throughout the lifetime of the R6/2 and wild type mice were examined. The progression of the disease in the R6/2 mice is rapid, with death occurring at 12 weeks of age. Overt motor defects in the R6/2 line are typically observed at 8 weeks of age and deficits have been reported as early as 4.5 weeks of age (2-6). Figures 11 (at end of document) illustrates that the ClC-1 conductance in R6/2 muscle fibers (HD) begin to significantly diverge from WT at an early age. A similar trend was observed in the Kir conductance of R6/2 muscle fibers (Fig. 12). The processing of Clcn1 mRNA has also been examined. There is progressive increase in the amount of abnormal Clcn1 splice variants in HD compared to WT muscle (Fig. 13). This divergence in mRNA splice variants correlates well with the relative decrease in ClC-1 currents.
Interestingly, the relative amount of Clcnl splice variants in HD muscle is roughly stable, whereas the level in WT muscle decreases from 25- to 90-day-old mice. The early onset and progression of CIC-1 and Kir channel defects as well as Clcnl mRNA mis-splicing in HD muscle supports a primary muscle defect in Huntington's disease.

[00152] As a first step to examining neuronal vs. muscle influences in the disease, compare neuromuscular transmission in late-stage R6/2 muscle to wild type has begun. Spontaneous neurotransmitter release (miniature endplate potentials), nerve-evoked neurotransmitter release (evoked endplate potential), and the amount of synaptic vesicle released per nerve action potential (quantal content) have been measured. Thus far, there is a significant increase in the amplitude of miniature endplate potentials and evoked endplate potentials, which could be explained by the decreases in CIC-1 and Kir currents. There is no significant difference in the quantal content of HD muscle compared to WT; however, a larger sample size is needed and the data is trending toward a slight increase in the quantal content in HD muscle. If a difference is found, this could provide an important therapeutic target as more pharmacological agents have been identified that target the neuromuscular junction than target the muscle itself.

[00153] Ultimately, there will be a need to test the effect of potential therapeutics on the motor function of whole animals. To this end, assessment of the motor behavior of R6/2 HD mice compared to WT using video-based behavior-recognition software has begun. The results of this analysis will provide a baseline to assess the efficacy of potential therapeutics.
Last, movement of the research toward clinical trials has begun. The human symptoms may in part be explained by hyperexcitable skeletal muscle. The symptoms best described by the muscle defects include the dystonia and rigidity that occur in nearly all late-stage patients and juvenile HD, as well as the "hung-up knee jerk". The hung-up knee jerk is a sustained contraction of the quadriceps that is triggered after the initial knee jerk reflex, which keeps the lower leg extended. This altered reflex is present in many HD patients. The delayed muscle relaxations that would result from hyperexcitability could even contribute to the characteristic bradykinesia. Supporting the muscle defects in human patients is a paper in *Parkinsonism and Related Disorders* on the hung-up knee jerk in human HD patients (7). The paper shows EMG traces of the quadriceps during the hung-up knee jerk. The records show low amplitude activity following the initial nerve-evoked contraction. Although the paper proposed a long loop reflex to the subcortical/basal ganglia, this is type of EMG recording is very myotonic-like and is most easily explained by skeletal muscle hyperexcitability. Further research will focus on preparing to use EMG on patients to look for muscle hyperexcitability in dystonic/rigid muscle and in the hung-up knee jerk reflex.

### References


[00155] All references, publications, patent applications, issued patents, accession records and databases cited herein, including in any appendices, are incorporated by reference in their entirety for all purposes.
CLAIMS

WHAT I CLAIMED IS:

1. A method of diagnosing Huntington's disease by detecting a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a with a reagent that binds nucleic acids in diseased muscle compared to normal muscle.
2. The method of claim 1 wherein expression of exon 7a is indicative of disease progression.
3. The method of claim 1 wherein the expression of 7a is detected before a patient manifests any physical symptoms of Huntington's disease.
4. A method of treating Huntington's disease by administering a muscle relaxant to a patient in need thereof.
5. The method of claim 4 wherein the muscle relaxant is selected from the group consisting of succinylcholine, gallamine, and dantrolene.
6. The method of claim 4 or 5 wherein two or more muscle relaxants are administered.
7. The method of claim 4 or 5 wherein succinylcholine and dantrolene are co-administered.
8. The method of claim 4 or 5 wherein gallamine and dantrolene are co-administered.
9. The method of any of claims 4-8 further comprising the administration of a CIC-1 or Kir modulating agent.
10. The method of any of claims 4-9 wherein the patient has CAG repeats indicative of Huntington's disease but does not exhibit any physical symptoms of Huntington's disease.
11. The method of any of claims 4-10 wherein the patient has juvenile on-set Huntington's disease.
12. The method of any of claims 4-11 wherein the patient has late on-set Huntington's disease.
13. A method of determining the prognosis of a patient suffering from Huntington's disease by detecting a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a in diseased muscle compared to normal muscle, wherein a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a is indicative of disease progression.
14. The method of claim 1 wherein the expression of 7a is detected before a patient manifests any physical symptoms of Huntington's disease.

15. A method of slowing the progression of Huntington's disease, the method comprising:
   a. Taking a first measurement to detect the expression of Clcnl mRNA or Clcnl exon 7a in a muscle sample
   b. Administering a muscle relaxant
   c. Taking a second measurement to detect the expression of Clcnl mRNA or Clcnl exon 7a in a muscle sample

Wherein, a higher dose of the muscle relaxant is administered if a reduced expression of Clcnl mRNA or an increase in Clcnl exon 7a is detected.

16. A method treating Huntington's disease by administering a combination treatment comprising a muscle relaxant and a traditional Huntington's disease therapy to a patient in need thereof.

17. The method of claim 16 wherein the traditional therapy is tetrabenzine.

18. The method of claim 16 wherein the traditional therapy is an antipsychotic drug.

19. The method of claim 16 wherein the traditional therapy is a drug that targets the brain and suppresses chorea.

20. The method of claim 16 wherein the traditional therapy is a drug that targets the brain and suppresses dystonia.

21. The method of claim 16 wherein the traditional therapy is a drug that targets the brain and reduces muscle rigidity.

22. The method of claim 16 wherein the traditional therapy is an antidepressant.

23. The method of claim 16 wherein the traditional therapy is a mood-stabilizing drug.

24. The method of claim 16 wherein the traditional therapy is selected from the group consisting of psychotherapy, speech therapy, physical therapy, and occupational therapy.

25. The method of any of claims 16-24 wherein the traditional therapy is administered at a lower dose in the combination treatment than it would be if administered alone.

26. The method of any of claims 16-25 wherein the combination treatment reduces the severity of Huntington's disease symptoms.

27. The method of any of claims 16-25 wherein the combination treatment reduces the number of Huntington's disease symptoms.
28. The method of any of claims 16-25 wherein the combination treatment slows the progression of Huntington's disease symptoms.

29. The method of any of claims 16-25 wherein the combination treatment reduces the severity of Huntington's disease symptoms.

30. The method of any of claims 16-25 wherein the combination treatment prevents the development of Huntington's disease symptoms.
FIGURE 1

A

Membrane Potential (mV)

HD

WT

Time (ms)

0

10

20

30

B

Membrane Potential (mV)

HD

WT

Time (ms)

0

10

20

30

C

Minimum AP Stimulus (nA)

WT

HD

0

100

200

300

D

Membrane Potential (mV)

HD

Time (ms)

0

10

20

30

40
FIGURE 2

A

WT

HD

mV

100

-100

B

Total Current

0

0

C

Plus CIC-1 Inhibitor

0

0

D

Specific CIC-1 Current (I_out)

0

0

E

mV

-100

100

peak I_out (µA/cm²)

WT

HD

F

Open Probability (P_o)

1.0

0.5

0.0

0.0

Membrane Potential (mV)

-100

0

100

500 µA/cm²

50 ms
FIGURE 3

A

B

C

Specific
Kir
Current
(\(i_{K}\))

100
\(\mu A/cm^2\)

300 ms

mV

-60
-20
20

-50
-150

-250

peak \(i_{K}\) (\(\mu A/cm^2\))

WT

HD
FIGURE 10

Age-Dependence of Chloride Conductance

Peak CIC-1 Conductance (mS/cm²)

WT Mice
HD Mice

Age (Days)
FIGURE 11A

A

$G_{CIC-1}$ (mS/cm$^2$) vs. Age (days) for WT and HD groups.
FIGURE 11B

B

![Bar graph showing data for WT and HD groups across different stages: Early-Stage, Middle-Stage, Late-Stage. Asterisks indicate significant differences.](image-url)
FIGURE 12B

B

![Bar graph showing data for early, middle, and late stages for WT and HD groups. The graph includes error bars and asterisks (*) indicating significance.](image-url)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (201 5.01 )
CPC - G01 N 2800/2835 (201 5.01 )

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 9/08, 49/14; A61P 21/00, 21/02, 25/28; C12Q 1/68 (2015.01)
USPC - 435/21; 5141/1, 11, 291, 461, 520

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12N 15/1 137, 2310/14; G01N 33/6896, 2333/916, 2500/02, 2800/2835 (2015.01) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Pubmed, Google Patents, Google
Search terms used: chloride channel CLC T chloride channel voltage sensitive "t" CLCN1 CLCN "t" CLC1 Huntington' triplet repeat' neurodegenerative gallamine succinylcholine dantrolene muscle'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Y</td>
<td>WO 201 1/01956 A2 (BIOVAIL LABORATORIES INTERNATIONAL (BARBADOS) S.R.L.) 17 February 201 1 (17.02.201 1) entire document</td>
<td>6-8</td>
</tr>
<tr>
<td>Y</td>
<td>EP 1 364 069 B1 (EPGENOMICS AG) 22 March 2009 (22.03.2009) entire document</td>
<td>16-20, 22, 23, 25</td>
</tr>
<tr>
<td>Y</td>
<td>WO 201 1/21361 A1 (SANOMUNE INC.) 28 October 201 0 (28.10.201 0) entire document</td>
<td>6, 7</td>
</tr>
<tr>
<td>Y</td>
<td>WO 2013/034669 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 28 March 201 3 (28.03.201 3) entire document</td>
<td>8</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "Z" document member of the same patent family

Date of the actual completion of the international search 11 March 2015

Date of mailing of the international search report 06 MAY 2015

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA-US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer: Blaine R. Copenhaver
PCT hjelpdesk: 571-272-4300
PCT GSP: 571-272-3774

Form PCT/ISA/210 (second sheet) (July 2009)
**INTERNATIONAL SEARCH REPORT**

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-12, 26-30
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (July 2009)