Bench to Bedside: Huntington's Disease Genomic Causes, Molecular Mechanisms, and Clinical Trials and Treatment

[1]Tanya Gracias and [2]Arianna Broad
[1]The Cathedral and John Connon School
[2]Weill Institute for Cell and Molecular Biology, Cornell University

I. INTRODUCTION

Huntington's Disease (HD) is a progressive, fatal, neurodegenerative disorder defined by motor dysfunction, cognitive decline, and psychiatric disturbance.[1] It is an autosomal dominant inherited disorder that has a prevalence of ~0.01%. HD's mean adult onset is 35 years and can be identified by predictive genetic testing, using PCR-based tests.[1] HD is caused by a trinucleotide CAG/CAA repeat in exon 1 of the HTT gene. This trinucleotide repeat encodes for a polyglutamine repeat within the N-terminal domain of the Huntingtin protein.[1,2] The function of the Huntingtin protein is not fully known but is associated with vesicle transport, transcriptional, and regulation of RNA trafficking. [2] There is currently no cure for HD; however, treatments for the various symptoms associated with HD exists. The disease is fatal approximately 15-20 years after the age of onset.[1]

II. GENOMIC CAUSES

Huntington's Disease is an autosomal dominant inherited disorder.[3,4] It is caused by a trinucleotide, CAG repeat in exon 1 of the HTT gene on chromosome 4 in *Homo sapiens*. A CAG repeat range of 6-39 is associated with a normal or functional huntingtin protein with no HD symptoms. Whereas a repeat range of 36-180 is associated with the mutant HTT gene and with the pathological presence of HD in individuals. Repeats in the range of 36-39 are associated with reduced penetrance, that is only a certain proportion of people who carry the pathological gene actually express it to create the mutant HTT protein. Penetrance is 100% in the range of 40 repeats and higher. [5]

There is some evidence that CAG repeat length is associated with the progression and severity of HD.[6] Trinucleotide repeat lengths in the range 27-35 have been described as intermediate alleles (IA). Intermediate alleles are not associated with HD expression. It does not cause HD in the carrier. However, emerging evidence states that these intermediate allele repeats can expand into the HD range (through somatic expansion), which in some cases can be inherited by their descendants. [4] On the other hand, it is also hypothesized that individuals with intermediate alleles would develop HD if they were to live long enough. A main piece of evidence that supports this hypothesis is the observation that individuals with IAs also have subtle motor, cognitive and behavioural changes compared to normal controls. [7]

Age of Onset – The CAG repeat size has a strong inverse correlation with the age of onset.[1] A singular additional CAG repeat can reduce age of onset by ~2 years.[5] There exists much contention amongst different studies and clinical trials, but 50%-80% of the age of onset variance can be attributed to CAG repeat length.[2] Variability in age of onset is particularly evident in carriers of reduced penetrance alleles, those with 36-39 trinucleotide repeats.[8] Apart from CAG repeat length, modifier genes play a role in the determination of age of onset. Genome-wide association studies (GWAS) have identified multiple genes that influence the rate at which motor signs emerge.[9]

Trinucleotide CAG repeat sequence variants in HTT exon one directly affect HD outcomes.[10] The loss of interruption (LOI) variant does not have the penultimate CAA codon (i.e. CAA-CAG to CAG-CAG) and thus has an increased length of uninterrupted CAG repeats. However the length of the polyglutamine tract remains the same in both cases. [8] Loss of penultimate CAA codons hastens age of onset in Huntington's Disease. The absence of the CAA-CAG tract is associated with an incorrect predicted age of onset of ~16 years, with a median age of onset of 32 years, relative median value of 48 years for the typical HD allele. Whereas, the duplication of the CAA-CAG cassette is associated with an ~3 year later predicted age of onset with a median age of onset of 51 years. [10]

The LOI variant is associated with increased somatic instability. The LOI variant has particular importance for reduced penetrance HD carriers and is the cause of earlier than predicted onset for such individuals.[8] The glutamine-encoding CAG/CAA tract is followed downstream by a proline-encoding CCA/CCG/CCT repeat tract, although polymorphic. It has no apparent impact on HD age of onset. [10]

Late Onset of Huntington's Disease – Late onset of HD is defined as the onset of HD related symptoms over the age of 60 years old. A minor percentage of individuals with HD (4.4-11.5%) have

late onset. The increased prevalence of late onset Huntington's Disease (Late onset HD), is due to increased life expectancy and more accurate genetic testing. Due to the large number of people born in the baby boomer generation, now reaching their 60s, there has been a subsequent increase in the number of peoples in their 60s. This is thought to have contributed to the rise of Late onset HD cases.[7] Late onset HD is largely underreported since individuals attribute their decline in cognitive and motor skills to ageing. Individuals with late onset HD are also more likely (compared to other individuals with regular onset of HD) to have no family history of the disease, and their expression of HD could be a result of repeat expansion from an unaffected parent.[7] As documented by various studies, when age of onset was more than or equal to 60 years, the CAG repeat length was 40.9 (range 36-47, n = 145) with an average age of onset of 65.5 years.[7] The strength of correlation between CAG repeat length and age of onset decreases as the age of onset increases.[7] Individuals with late onset HD report motor manifestations, usually chorea, as the primary clinical feature that presents at onset. However, cognitive and psychiatric symptoms are also seen. [7] Studies on the survival of individuals with late onset HD have conflicting results. It has not been decided whether the prognosis of individuals with late onset HD is the same, better or worse than those with regular HD. The co-morbidities associated with ageing, seen in individuals with late onset HD, make it difficult to get a definitive result. [7]

Juvenile Huntington's Disease – Juvenile HD is defined as the onset of HD related symptoms at the age of 20 or younger. It is seen in individuals with more than 60 CAG trinucleotide repeats, which can be a result of expansions via intergenerational repeat instability. Juvenile HD is a rare variant accounting for only 4-10% of all HD cases.[11]

Intergenerational Repeat Instability – HD CAG repeats are unstable in the male germline and often increase in length between generations.[5] 24% of new diagnosis of HD are seen in individuals with no HD history.[4] Thus, the frequency of new mutation resulting in HD and/or intergenerational instability is quite high. Parental sex is a major determinant of intergenerational repeat instability.[3] Paternal transmissions have a higher occurrence of expansions, whereas maternal transmissions have a higher occurrence of contractions. There is no significant difference in stable transmissions between both sexes.[8] Longer CAG repeats are also associated with a higher frequency of repeat expansions.[3] Male expansion may be related to the large number of mitotic cell divisions of premeiotic speratogonia.[3] The largest somatic expansions are seen in sperm cells, skin cells and brain cells.

Intergenerational instability has been seen to arise from nuances in paternal transmission, wherein descendants of men with a repeat range between 27-35 have inherited repeats of 40 or more.[4] Paternal age has a modest, but statistically significant positive correlation with the magnitude of expansions. However, paternal age did not show any correlation with the magnitude of repeat contractions. [3] This could be attributed to progressive somatic expansion of the CAG repeat in a HD individual with time. Postzygotic mechanisms also may affect triplet repeat instability. In a maternal transmission, the daughter often carries the contractions of the trinucleotide repeat; whereas, the sons more often carry the expansions. While with paternal transmission expansions are equally frequent in male and female offspring, the CAG repeat increases in length significantly more in sons than in daughters.12

III. MOLECULAR MECHANISMS

Huntingtin Protein – The Huntingtin protein consists of ~60 amino acids. It has two antiparallel alpha-helices with a hairpin configuration that assembles a super helical structure with a continuous hydrophobic core. [2] The Huntingtin protein is expressed in both central and peripheral tissues. The protein is highest expressed in the brain tissue. [6] The function of the Huntingtin protein is not fully known but is associated with vesicle transport, transcriptional, and regulation of RNA trafficking.[2] The Huntingtin protein also plays a role in embryonic development and neurogenesis. It has also been hypothesized that Huntington protein has a role in energy metabolism. [6]

Increasing experimental evidence from genetic model systems like mice, zebrafish, and *Drosophila* indicate that polyglutamine expansion within the Huntingtin protein disrupts its normal function.[1] Mutant Huntingtin (seen in HD patients) forms abnormal conformations, including beta-sheet structures. This results in toxic gain-of-function.[2] This gain of function is assumed to be either overactivity of the normal function or the introduction of a novel function.[6] Large aggregates of abnormal Huntingtin are found in neuronal nuclei, dendrites, and axon terminals of the neuron. The rate of aggregation is proportional to the length of polyglutamine expansion.[1]

Huntington's Disease Pathology – Huntington's disease primarily results in central nervous system (CNS) degradation, because of neuronal cell death. [2]

Specifically, the death of medium-sized spiny neurons of the striatum that utilize gamma-aminobutyric acid (GABA) is the most highly associated with HD pathogenesis. When spiny neurons degrade, cortical pyramidal neurons that project to the striatum are soon to follow. Striatal neurons projecting to the substantia nigra also degenerate in pre-symptomatic patients. [1]
Another interesting correlation in HD patients is their below average brain volumes before disease symptoms appear. [1] Huntington's disease is classified into five pathological grades based on the condition of the brain (specifically the striatum) at the time of autopsy. Form grade 0 that indicates no gross/microscopic changes to grade 4 which indicates severe atrophy and concave caudate/ventricular interface.[6] Post-mortem morphometric analysis of HD patients revealed a 21-29% area loss of the cerebral cortex, 29-34% loss of telencephalic white matter, 64% loss in the putamen, and 57% loss in the caudate nucleus, compared individuals of the same age used as a control. [1]

Mutant Huntingtin Protein (mHTT) and Neurons – The presence of mHTT is deleterious to many types of neurons, but the medium spiny neurons (MSNs) found in the striatum are most vulnerable. In the presence of mHTT, there is increased cell death of medium spiny neurons. 13 Brain-derived neurotrophic factor (BDNF), a crucial neuronal differentiation factor, is decreased in individuals with HD. More than 70% of the cells found in the striatum are astrocytes. 14 mHTT is known to decrease levels of both EAAT2 (GLT-1) and Kir4.1 in astrocytes, resulting in neuronal cell death by excitotoxity. mHTT reduces the transcription of the neurotransmitter transporter, glutamate transport (GLT-1) in astrocytes, this is hypothesised to increase glutamate levels and cause excitotoxic neuronal death. 14 This is detrimental to the patient's motor function as astrocytes have a wide range of functions including supporting neuronal cells and reuptake of neurotransmitters. 14 Neuronal mitochondria from HD patients exhibit impaired respiratory chain function. [6]

Mitochondrial Dysfunction by Mutant Huntingtin (mHTT) – The mHTT disrupts the electron transport chain (ETC), causing the depletion of intracellular adenosine triphosphate (ATP) and increase in reactive oxygen species (ROS) is observed. The mitochondrial tricarboxylic acid (TCA) cycle enzyme, aconitase, is susceptible to superoxide-mediated inactivation. Thus, the generation of ROS further reduces ATP production by inhibiting the TCA cycle.13 Furthermore, retrograde and anterograde mitochondrial trafficking along axons is impeded by mHTT. This causes disruption of mitochondrial maintenance and reduced number of mitochondria at high energy demand sites like synapses. 13 In HD individuals, mHTT has been shown to impair peroxisome proliferator-activated receptor-g (PPAR-g) coactivator-1a (PGC-1a) mediated expression of genes that regulate mitochondrial biogenesis. Early mitochondrial fragmentation is hypothesized to occur through GTPase dynamin related protein-1. 13 The dysregulation of the mitochondrial function suggests that mHTT causes neuronal dysfunction by affecting energy metabolism and causing oxidative damage. [13]

Transcriptional Dysregulation by Mutant Huntingtin (mHTT) – Mutant Huntingtin interacts and disrupts major factors of transcription, affecting both general promoter accessibility and recruitment of RNA polymerase II. In studies of pre-symptomatic HD brains, it was seen that soluble mHTT affects important mediators of general promoter accessibility and transcription initiation, including - specificity protein 1 (SP1), TATA box binding protein (TBP), the TFIID subunit TAFII130, the RAP30 subunit of the TFIIF complex, and the CAAT box transcription factor NF-Y. 13 The expression of mHTT also disrupts the activity of histone acetyl transferases (HATs), such as CBP/p300 and p300/CBP associated factor (PCAF), which results in histone hypoacetylation and increased heterochromatin formation. [13]

Impaired protein homeostasis by Mutant Huntingtin (mTT) – Chronic expression of expanded polyglutamine peptides results in an age-dependent disruption of the proteome. This is seen due to increased aggregation and mis-localization of metastable proteins, and a reduction of chaperones. [13]

Somatic Expansion – In individuals with Huntington's Disease, the CAG repeat is unstable and increases progressively in length. It was analysed that the repeat expansion was seen in all individuals, but the repeat mosaicism has the highest levels in the sperm and in the brain. 12 Somatic CAG instability is drastically reduced in transgenic HD mouse models lacking either the mismatch pair enzyme MSH2 or the base excision repair enzyme OCC1. The role of MSH1 in CAG repeat expansion is not currently known. However, it is known that OCC1 is responsible for initiating an escalating oxidation-excision cycle that leads to progressive age-dependent expansion of the CAG repeat in post-mitotic neurons in organisms affected with HD. This is based on evidence seen from analysis of mice and cell lines lacking OGG1. Thus, at least one mechanism of CAG expansion appears to involve

oxidative DNA damage and single-strand break repair. [12]

IV. PHYSIOLOGICAL SYMPTOMS AND CLINICAL TREATMENT

Current Treatment for Physiological Symptoms:

Diagnosis of HD is based on the manifestation of motor disorders; despite being preceded by cognitive and behavioral symptoms.

Other disorders include - sleep disorders, urinary incontinence, pain/discomfort, dental pain, excessive perspiration, weight loss, hypersalivation, reduced lung function and respiratory muscle strength.

Table 1. Motor impairment symptoms in HD diagnosis[15]

Symptom	Characteristics	Treatment
Chorea	Characterized by abnormal, involuntary, spontaneous, uncontrollable, irregular, non-rhythmic and aimless movements affecting the trunk, the face and the limbs.	Tetrabenazine (Grade A) is used if the patient does not suffer from depression or suicidal thoughts. Second generation neuroleptics are used when patients have personality and/or behavioral and/or psychotic disorders. Monotherapy is preferred.
Dystonia	Characterized by abnormal postures that may affect all body segments and is frequently associated with rigidity.	Active and passive physiotherapy is used as a preventive measure.
Rigidity	-	Can be induced by the use of neuroleptics or tetrabenazine. Thus, the reduction/withdrawal of this medication should be considered in accordance with the severity of the chorea. Physiotherapy is recommended to improve and/or maintain mobility. Levodopa may provide partial and temporary relief to the akinetic symptoms.
Akathisia	Characterised by the inability to stay still.	Neuroleptics or tetrabenazine may cause akathisia, and reduction/withdrawal of medication should be considered.
Swallowing Disorders	These disorders appear early in the progression of HD, and eventually lead to choking, secondary bronchopulmonary infections, and cardiac arrest	Referral to a speech and language therapist is recommended. In severe cases, the use of a gastrostomy device Percutaneous Endoscopic Gastrostomy (PEG) may be considered.
Myoclonus	Characterized by brief, involuntary, sudden muscle contractions, similar to spasms and jerks in epileptic seizures.	When affecting daily activities, treatment with valproate or clonazepam, in escalating doses is used.
Gait and Balance Disorders	Characterized by disruption of cadence regulation, increased variability in step width and length, disturbed initiation and postural sway	Physiotherapy is used, along with the introduction of fall prevention programs, gait, core stability and balance interventions.
Bruxism	Involuntary clenching of the jaw muscles with excessive contractions, and can cause tooth damage. Custom mouth guards can reduce the effects of bruxism.	Injecting botulinum A into the masseter muscles is also done for treatment. Bruxism is also a possible side effect of neuroleptics, and serotonin reuptake inhibitors.
Manual Dexterity	Can be impaired from chorea, or dystonia, or akinesia, or rigidity but can also occur in their absence by abnormal motor planning and sequencing	Neuroleptics and tetrabenazine can help in maintaining manual dexterity by reducing chorea.

Table 2. Cognitive impairment symptoms in HD diagnosis[15]

Symptom	Characteristics	Treatment
Bradyphrenia	Characterized by a decline in speed of cognitive information processing and prolongation of reaction time.	-
Language and Communication Disorders	Consists of slurred and slowed speech and progressive reduction in verbal fluency. After which, reduction in lexical capacities appears.	Referral to a speech and language therapist is recommended at an early stage.
Memory Disorders	Characterized by difficulties in learning new information and	-

	retrieving previously acquired information. Sedative drugs, neuroleptics and tetrabenazine can have a negative impact on memory	
Disorientation	Disorientation, in terms of time and space, are seen in HD progression.	Establishing a regular routine, and environment can be helpful.
Visuospatial and Visual Perceptual Disorders	Appear late in the progression of HD, by interference with the integration and understanding of visual information.	-

Table 3. Psychiatric impairments in HD diagnosis[15]

Symptom	Characteristics	Treatment
Depression & Suicidal Ideation or Attempts	Depression and suicidal ideation or attempts are common in individuals with HD, and often correlate with a family history of the same.	-
Irritability	Common symptom characterized by impatience, tendency to become angry in response to minimal provocation.	. Selective serotonin reuptake inhibitors (SSRIs) are used at near maximum recommended dosage to be effective. Individuals who do not benefit from SSRI alone, can use combination theory with mianserine or mirtazapine.
Apathy	Is clinically characterized as a reduction in interest, spontaneity, motivation and drive.	-
Anxiety	Characterized by the uncomfortable feeling of nervousness or worry about something happening currently or in the future.	Selective serotonin reuptake inhibitors (SSRIs) are used to treat anxiety.
Obsessions	Characterized by the recurrent and persistent thoughts, ideas or images, that do not let the mind rest causing anxiety.	-
Sexual Disorders	Are common in HD, with decreased libido being most common while hypersexuality or disinhibited behaviour being more rare.	-
Hallucinations	Defined as a perception without an object.	Second generation neuroleptics are the first line treatment for hallucinations and delusions. Clozapine should be proposed as the first-line treatment in the case of akinetic forms of HD with debilitating Parkinsonian symptoms.

V. DISCUSSION

Huntington's Disease is a fatal disorder which results in and progressive motor, cognitive and psychiatric decline. [1] It is an autosomal inherited condition; however, individuals with no generational history of the disease may develop the condition, most often through somatic expansion in the male germline. [3,4,5]

HD is caused by a trinucleotide; CAG repeat in exon 1 of the HTT gene. This gene encodes for the huntingtin protein. A CAG repeat length of 6-39 is associated with a normal or functional huntingtin protein, while a repeat range of 36-180 is associated with the mutant HTT gene. [5]

The CAG repeat size has a strong inverse correlation with the age of onset. [1] Mean age of onset for HD is 35 years. [1] However, HD does have cases of juvenile onset as well as late onset. Both juvenile onset and late onset are rare accounting for 4.4-11.5% and 4-10% of all HD cases respectively.[7,11]

Huntington's disease primarily results in central nervous system (CNS) degradation. [2] Mutant huntingtin is associated with neuronal cell death, mitochondrial dysfunction, and impaired protein homeostasis. [13]

Currently there is no treatment for HD. However there are treatments available to manage the symptoms associated with the condition. Diagnosis of manifest HD is usually based on motor disorders; despite being preceded by cognitive and behavioral symptoms. [15]

Expansion of a CAG/glutamine repeat in different genes is itself responsible for at least 8 neurodegenerative diseases, excluding HD.[16] Research conducted on the genomic causes, molecular mechanisms, and clinical trials and treatment of Huntington's Disease can be applicable in understanding other similar diseases.

Future of Huntington's Disease – A method used to treat gain-of function neurodegenerative diseases is reducing the pathogenic protein. An experimental study showed that shutting off expression of mutant

HTT in an inducible transgenic mouse system led to the partial recovery of behavioral and pathological features. [2] Mutant HTT can also be reduced in the brain by small interfering RNA (siRNA). siRNA can decrease mutant HTT expression and improve the phenotype of mouse models with HD. Promising results have been seen with mHTT siRNA injected directly into the lateral ventricles of mouse models with HD.[2] Allele-specific suppression of the expression of the mutant Huntington(mHTT) protein has been explored as a method to cure HD. However, in mice the loss of Huntingtin protein led to embryonic fatality.[16]

Research done by a study shows that permanent suppression of endogenous mHTT expression in the striatum of adult HD1400-knockin mice, done using non-allele-specific CRISPR or Cas9-mediated inactivation, effectively depletes mHTT aggregates and decreased early neuropathology. This reduction in mHTT expression did not affect viability, but alleviated motor deficits and neurological symptoms. With such positive outcomes, CRISPR seems like a promising cure to HD.[16]

ACKNOWLEDGEMENTS

Thank you for the guidance of Ari Broad from the Weill Institute for Cell and Molecular Biology at Cornell University in the development of this research paper. Also thank Lumiere support staff.

REFERENCES

[1] Schulte J, Littleton JT. *The Biological Function of the Huntingtin Protein and Its Relevance to Huntington's Disease Pathology*.

[2] Ross CA, Tabrizi SJ. *Huntington's Disease: From Molecular Pathogenesis to Clinical Treatment*. Vol 10.; (2011)

[3] Neto JL, Lee JM, Afridi A, et al. Genetic contributors to intergenerational CAG repeat instability in Huntington's disease knock-in mice. *Genetics*. 2017;205(2):503-516. doi:10.1534/genetics.116.195578

[4] Myers RH. *Huntington's Disease Genetics*.; 2004. Accessed July 10, 2021. https://link.springer.com/article/10.1602/neurorx.1.2.255

[5] Ciosi M, Maxwell A, Cumming SA, et al. A genetic association study of glutamine-encoding DNA sequence structures, somatic CAG expansion, and DNA repair gene variants, with Huntington disease clinical outcomes. *EBioMedicine*. 2019;48:568-580. doi:10.1016/j.ebiom.2019.09.020

[6] Walling HW, Baldassare JJ, Westfall TC. *Mini-Review Molecular Aspects of Huntington's Disease*. Vol 54.; 1998.

[7] Chaganti SS, McCusker EA, Loy CT. What do we know about late onset Huntington's disease? *Journal of Huntington's Disease*. 2017;6(2):95-103. doi:10.3233/JHD-170247

[8] Wright GEB, Collins JA, Kay C, et al. Length of Uninterrupted CAG, Independent of Polyglutamine Size, Results in Increased Somatic Instability, Hastening Onset of Huntington Disease. *American Journal of Human Genetics*. 2019;104(6):1116-1126. doi:10.1016/j.ajhg.2019.04.007

[9] Loupe JM, Pinto RM, Kim KH, et al. Promotion of somatic CAG repeat expansion by Fan1 knock-out in Huntington's disease knock-in mice is blocked by Mlh1 knock-out. *Human Molecular Genetics*. 2020;29(18):3044-3053. doi:10.1093/hmg/ddaa196

[10] Ciosi M, Maxwell A, Cumming SA, et al. A genetic association study of glutamine-encoding DNA sequence structures, somatic CAG expansion, and DNA repair gene variants, with Huntington disease clinical outcomes. *EBioMedicine*. 2019;48:568-580. doi:10.1016/j.ebiom.2019.09.020

[11] Fusilli C, Migliore S, Mazza T, et al. Biological and clinical manifestations of juvenile Huntington's disease: a retrospective analysis. *The Lancet Neurology*. 2018;17(11):986-993. doi:10.1016/S1474-4422(18)30294-1

[12] Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of huntington's disease. In: *International Review of Neurobiology*. Vol 98. Academic Press Inc.; 2011:325-372. doi:10.1016/B978-0-12-381328-2.00014-6

[13] Labbadia J, Morimoto RI. Huntington's disease: Underlying molecular mechanisms and emerging concepts. *Trends in Biochemical Sciences*. 2013;38(8):378-385. doi:10.1016/j.tibs.2013.05.003

[14] Hong Y, Zhao T, Li XJ, Li S. Mutant huntingtin impairs BDNF release from astrocytes by disrupting conversion of Rab3a-GTP into Rab3a-GDP. *Journal of Neuroscience*. 2016;36(34):8790-8801. doi:10.1523/JNEUROSCI.0168-16.2016

[15] Bachoud-Lévi AC, Ferreira J, Massart R, et al. International guidelines for the treatment of Huntington's disease. *Frontiers in Neurology*. 2019;10(JUL). doi:10.3389/fneur.2019.00710

[16] Yang S, Chang R, Yang H, et al. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *Journal of Clinical Investigation*. 2017;127(7):2719-2724. doi:10.1172/JCI92087