

# Huntington's Disease: Pathophysiology and Clinical Prospects

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Huntington's disease (HD) is a monogenic neurodegenerative disorder with autosomal dominance. Progressive brain degeneration is characterized by the prevalent loss of GABAergic medium spiny neurons (MSN) in the striatum. Clinical features include progressive motor dysfunctions and cognitive impairments.<sup>1</sup> HD is caused by an extended repeat in the Huntingtin (HTT) gene, which then encodes for an elongated glutamine stretch in the protein.<sup>2</sup> Induced pluripotent stem cell (iPSC) technology has also been used for the pathological modeling of HD.<sup>3</sup>

## Genetic and Clinical Manifestations

The genetic defect responsible for HD is a CAG triplet repeat expansion on exon 1 in the IT15 gene on chromosome 4, encoding an expanded polyglutamine (PolyQ) region of the protein Huntingtin (Htt), which forms mutant Htt (mHtt).<sup>2</sup> The presence of more than 40 CAGs consistently causes disease within normal lifespan, whereas longer repeats predict early disease onset.<sup>1</sup> Therefore, the number of CAG repeats correlates directly with the phenotypic severity.<sup>1</sup> As the preclinical stage of HD is defined by subtle changes in personality and cognitive ability without neuropathological features, diagnosis can only occur at a later stage when specific histopathological hallmarks and advanced symptoms are present. Early neuropathological hallmarks include striatal atrophy, while progressed stages show degeneration in the basal ganglia and the cerebral cortex.<sup>4</sup>

## Huntington's Disease Pathways

### Introduction

The presence of mHtt results in the dysregulation of MSN signaling pathways and gene expression, causing the neural cell death, and subsequent motor and cognitive impairments exhibited during the progression of HD.

### Htt Functions

Htt interacts with various organelles (including, the nucleus, endoplasmic reticulum and mitochondria), and is involved in mediating:

- Chromatin remodeling and gene transcription<sup>9</sup>
- Neuroprotection (BDNF transcription and trafficking)<sup>9</sup>
- Cell survival (Htt is anti-apoptotic)
- Calcium homeostasis<sup>7,9</sup>
- Mitochondrial function and trafficking<sup>8,9</sup>
- Intracellular trafficking<sup>2,9</sup> and synaptic vesicle recycling<sup>2</sup>

The reduction of Htt and coexistence of mHtt results in the disruption of these cellular processes, culminating in cell death.<sup>8</sup>

### Gene Transcription

Htt interacts with the transcription machinery and enzymes involved in chromatin remodeling to influence transcription. Investigations based on microarray assays show a change in expression of a large number of genes, even before the onset of symptoms.<sup>8,9</sup> mHtt represses the transcription of SP-1 dependent genes by interacting with SP-1, TFIIF and TFIID. It also interacts with cAMP-responsive binding protein (CREB) and TFIID resulting in a reduced transcription of PGC1- $\alpha$ , which is involved in mitochondrial function and energy metabolism. mHtt also causes a general transcriptional repression by disrupting the balance between histone acetyltransferases (HATs) and histone deacetylases (HDACs).<sup>8,9</sup>

## Products available from Tocris

### Trk Receptors

ANA 12, BDNF (human), 7,8-Dihydroxyflavone, LM 22A4

### NMDA Receptors

D-AP5, DL-AP5, (R)-CPP, (S)-CPP, Homoquinolinic acid, Ibotenic acid, Memantine, (+)-MK 801, NMDA, PDZ1 Domain inhibitor peptide, Quinolinic acid

### Kainate Receptors

CNQG, DNQG, Kainic acid, Topiramate

### Glutamate Transporters

Ceftriaxone disodium salt, Dihydrokainic acid, DL-TBOA, LDN 212320, TFB-TBOA

### Glutamate (Metabotropic) Receptors

CDPPB, CHPG Sodium salt, (R,S)-3,5-DHPG, (S)-3,5-DHPG, L-Quisqualic acid, LY 379268, (S)-MCPG, MPEP, MTEP, VU 0360172

### Neural Stem Cell Differentiation

DAPT, DMH-1, Dorsomorphin, Fluoxetine, Forskolin, IBMX, Purmorphamine, SAG, SB 431542, SU 5402

### iPSCs

CHIR 99021, Valproic acid sodium salt

### Histone Deacetylases

SAHA, Trichostatin A

### SIRT

EX 527, Nicotinamide, Resveratrol

### Histone Acetyltransferases

Anacardic acid, C 646, Garcinol

### Caspases

Cisplatin, Z-DEVD-FMK, Z-VAD-FMK

### Calpains

Acetyl-Calpastatin (184-210) (human), Calpeptin, PD 150606

### Cathepsin

CA 074, E 64, E 64d, MDL 28170

### Heat Shock Proteins

17-AAG, Geldanamycin, VER 155008

### IP<sub>3</sub> Receptor

2-APB, (-)-Xestospingon C

### Mitochondrial Permeability Transition Pore

Cyclosporin A, TRO 19622

### Proteasome

Lactacystin, MG 132, PSI

### Autophagy

Bafilomycin A1, (±)-Bay K 8644, Dexamethasone, LY 294002, Rapamycin, Taxol, Thapsigargin, Torin 1, Tunicamycin

### Dopamine Receptors

Dopamine, Olanzapine, Risperidone, SCH 23390, SKF 38393, Tetrabenazine

### Adenosine A<sub>2A</sub> Receptors

CGS 21680, Istradefylline, SCH 58261, ZM 241385

### Neuroprotective Compounds

Minocycline, N-Acetylcysteine, Riluzole

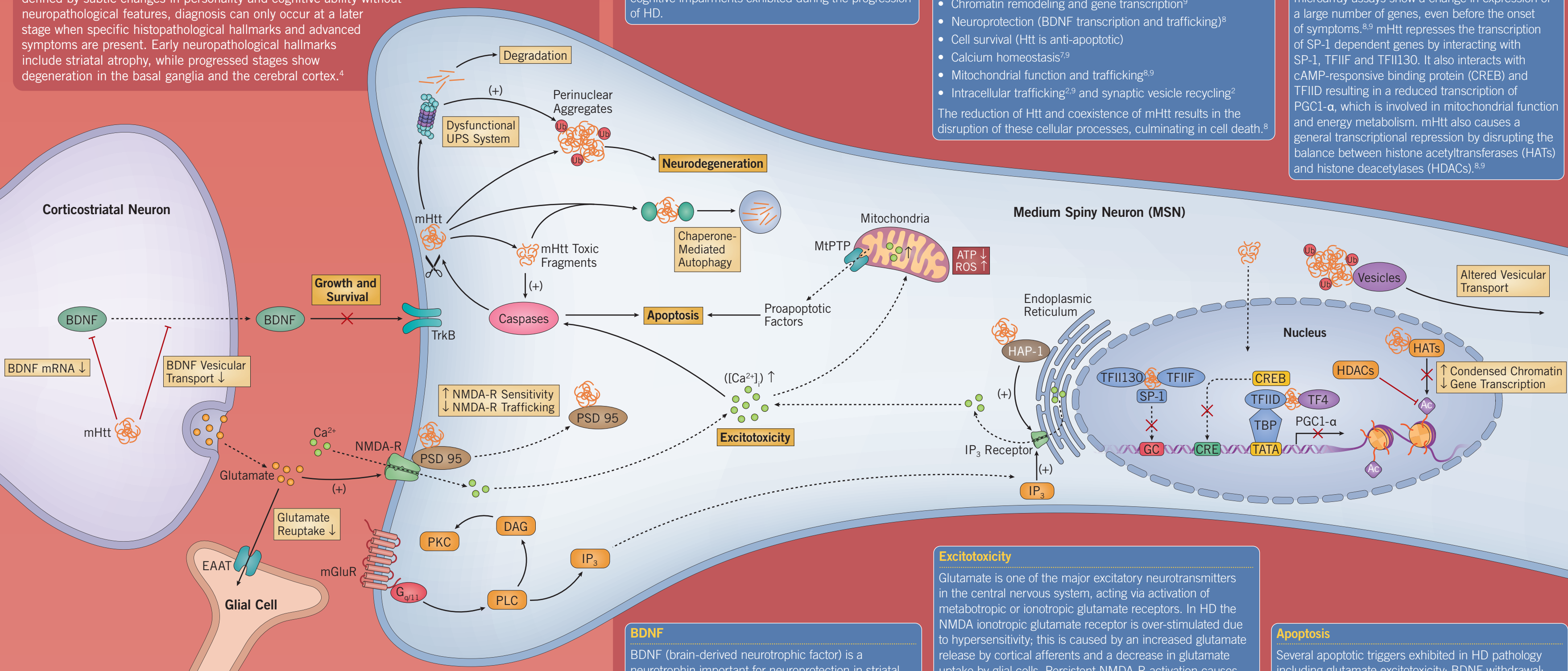
### Caged Compounds

MNI caged kainic acid, MNI-caged-D-aspartate, MNI-caged-L-glutamate, MNI-caged-NMDA, NPEC-caged-dopamine, RuBi-Dopa, RuBi-Glutamate

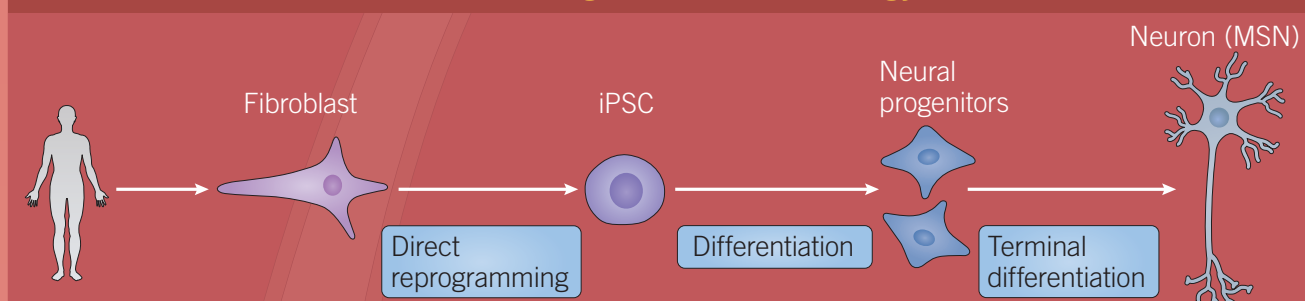
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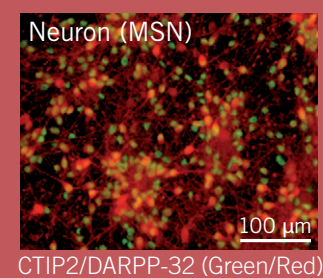
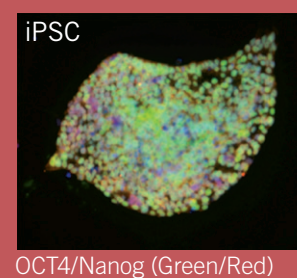


## HD Modeling with iPSC Technology



Somatic cells can be epigenetically reprogrammed to a pluripotent state by the enforced expression of specific transcription factors. These so-called human induced pluripotent stem cells (hiPSCs) can then be differentiated into any cell type.<sup>5</sup>

The creation of disease models using cells carrying the genotype of interest has taken research one step further and opened new frontiers for drug discovery.<sup>6</sup> Based on *in vivo* striatal development, hiPSCs can be differentiated into mature MSN populations modeling the HD phenotype.<sup>7</sup>



### BDNF

BDNF (brain-derived neurotrophic factor) is a neurotrophin important for neuroprotection in striatal neurons. It is synthesized in the cortex and transported to MSNs via the cortico-striatal tract. BDNF binds to tropomyosin-related kinase B receptors (TrkB), which activate multiple signaling cascades such as anti-apoptotic enzyme production, glutamate receptor transcription and calcium binding protein expression. mHtt disrupts BDNF transcription and trafficking, resulting in neuronal vulnerability.<sup>2,8,9</sup>

### Proteasomal Degradation and Autophagy

Studies have shown that both autophagy and proteasomal mechanisms of degradation are impaired in HD. The presence of mHtt impairs the rate of macroautophagy<sup>10</sup>, whilst mHtt degradation is inhibited due to a dysfunctional ubiquitin-proteasome system (UPS). Failure to remove mHtt results in accumulation of toxic, insoluble mHtt aggregates in the cytoplasm, which induces neuronal cell death and earlier disease onset.

### Excitotoxicity

Glutamate is one of the major excitatory neurotransmitters in the central nervous system, acting via activation of metabotropic or ionotropic glutamate receptors. In HD the NMDA ionotropic glutamate receptor is over-stimulated due to hypersensitivity; this is caused by an increased glutamate release by cortical afferents and a decrease in glutamate uptake by glial cells. Persistent NMDA-R activation causes a prolonged  $Ca^{2+}$  influx, which further results in a chronic increase of intracellular  $Ca^{2+}$  via  $Ca^{2+}$ -induced  $Ca^{2+}$ -release (CICR). This results in caspase activation, impaired mitochondrial  $Ca^{2+}$  buffering, and thus neuronal toxicity. Enhanced NMDA-R function could also be a result of a distorted interaction with postsynaptic density protein 95 (PSD95).<sup>11</sup> The mHtt-HAP-1 complex may also contribute to  $Ca^{2+}$  excitotoxicity by enhancing IP<sub>3</sub> receptor activity.<sup>12</sup>

### Mitochondrial Dysfunction

HD patients exhibit impaired mitochondrial function and altered mitochondrial morphology, as a result of direct and indirect mHtt activity. mHtt binds mitochondria directly, altering metabolic activity and mitochondrial trafficking, while mitochondria are affected indirectly by mHtt-dependent transcriptional dysregulation<sup>8</sup>. mHtt was also shown to be associated with mitochondrial membrane potential lowering, resulting in reduced ATP production and increased reactive oxygen species (ROS) production, which eventually leads to cell death.<sup>9</sup>

### Apoptosis

Several apoptotic triggers exhibited in HD pathology including glutamate excitotoxicity; BDNF withdrawal; metabolic and oxidative stress; and mitochondrial dysfunction indicate that apoptosis is the main mechanism of cell death for HD neurons. The protease caspase is a key regulator of apoptosis and is activated by  $Ca^{2+}$  excitotoxicity and mHtt toxic fragments in HD neurons. Caspases also cleave mHtt aggregates into toxic fragments, which accumulate and reinforce neuronal vulnerability.<sup>13</sup>

### Vesicular Transport

HD neurons present abnormalities in neurotransmitter transmission. Axons containing mHtt aggregates have fewer synaptic vesicles than normal axons and also present a reduction in glutamate release. Furthermore, mHtt binds more tightly to vesicles, which reduces their association with HAP-1.<sup>11</sup>