Gene-Environment Interactions in Huntington's Disease

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Abstract

Referring to the debate about nature versus nurture, neuropsychologist Dr. Donald Hebb is said to have asked, "Which contributes more to the area of a rectangle, its length or its width?"

Huntington's Disease (HD) is a neurodegenerative disease characterized by selective loss of striatal GABAergic medium spiny neurons (MSNs). Despite the inverse relationship between CAG repeat number and age of onset, repeat number only accounts for 60% of the variability in HD. Interestingly, the residual variability is caused by other genetic and/or environmental factors, including single nucleotide polymorphisms, heavy metal toxicity, environmental enrichment, exercise, and diet that modify synaptic activity and neuroprotective functions. Importantly, heavy metals such as manganese (Mn) can accumulate in the striatum, which is the most vulnerable brain region in HD following excessive exposure. Unfortunately, the precise function of Mn in the striatum as a positive or negative modifier of age of onset, disease progression, and clinical symptomology is unknown. Our laboratory has previously reported a striatal specific gene-environment interaction between the mutant Huntingtin gene (HTT) and Mn in both in vitro and in vivo models of HD. Importantly, mutant HTT expression confers resistance against Mn toxicity partly by decreasing net Mn uptake and storage capabilities compared to wildtype. Thus, reduced physiological Mn levels in the brain may profoundly affect Mn-dependent neuronal enzyme function, downstream signaling pathways, and explain some of the aforementioned variability observed in HD. In essence, understanding the relationship between mutant HTT and Mn transport dynamics may elucidate additional in vivo functions of the huntingtin protein (HTT) and contribute to therapeutic interventions in HD.

Keywords

Huntington's
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Environment

Huntington's Disease: Onset, Genetics, Protein, and Models

In the 1980's, researchers studying an isolated community in Venezuela with a very high incidence of HD pinpointed the genetic cause to an increase in CAG repeats in exon 1 of the huntingtin gene (HTT). This excess of CAG repeats leads to an expansion in the polyglutamine (polyQ) tract in the huntingtin protein[1]. Despite the inverse relationship between prognosis and number of CAG repeats, CAG repeats only explain 60% of the large variability in age of onset, disease progression, and susceptibility. In addition, subsequent sibling studies have demonstrated that modifier genes explained only 13% more of this variance[2,3,4,5]. Furthermore, cases of identical twins discordant for onset and symptoms reveal the influence of other environmental factors on the phenotypic variability observed in HD patients[6,27] (Figure 1). Environmental influences have not been demonstrated to accelerate the pace of HD, but many factors described below (including enriched environments, exercise and diets) have been demonstrated to increase endogenous brain derived neurotrophic factor (BDNF) levels, with a concomitant delay in disease progression. Pollutants and heavy metals, such as copper (Cu), iron (Fe), and manganese (Mn), have been suggested to influence the pathology of many neurodegenerative diseases, via alterations in vesicular transport, mitochondrial dysfunction, protein aggregation, and induction of oxidative stress. The pace of neurodegeneration may be due to several mechanisms, including diminished neurotrophic support and deranged essential metal ion homeostasis in vivo (Figure 2).

Huntington's Disease is an autosomal dominant neurodegenerative disorder with a median age of onset at 39[8]. Although it has been two decades since the identification of the HD-associated mutation, there is growing debate about whether HD symptoms are caused by a: (i) loss of function of normal HTT; (ii) toxic gain of function in mutant HTT[9]; or both (i) and (ii)[10]. HTT knockout mouse models cannot survive past embryonic day 7[11,12],but the essential role for HTT in embryological development remains unclear[13]. Moreover, mouse models of HD develop disease[14]

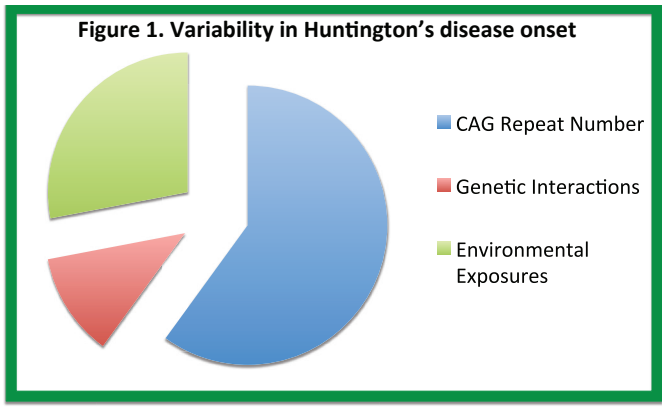

Figure 1: Proportion of the variability in Huntington's disease onset explained by CAG repeat number and modifier genes, with the remainder attributed to environmental influence.

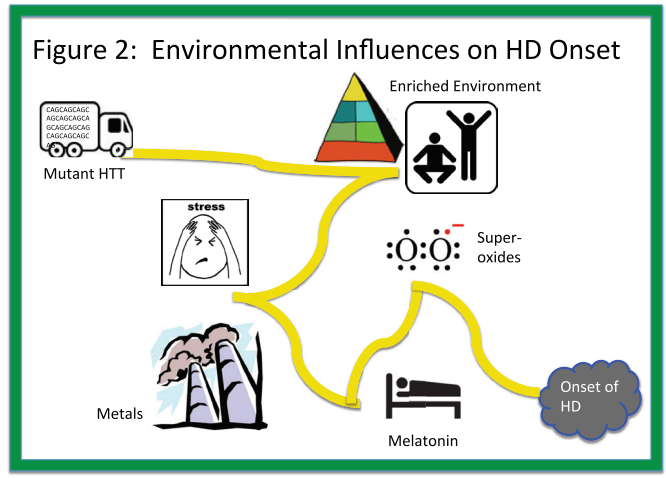

Figure 2: Selected purported environmental influences on age of HD onset, both positive and negative: enriched environment, stress, heavy metals, melatonin, and mitochondrial insults.

while normal HTT is still functional, which suggests that excess mutant HTT is the cause of HD neurodegeneration. However, a careful study of homozygous human patients controlled for CAG repeat number on both alleles demonstrated worsened disease progression compared to heterozygotes[15]. The results from this study suggest that loss of function of normal HTT may still play a role in disease pathology.

Although all of the functions of wildtype HTT are unknown, it has been shown to be involved in crucial neuronal pathways, including apoptosis, transcriptional regulation, axonal transport, and as a scaffold for protein-protein interactions[16]. The ubiquitously expressed HTT protein is large (348-kDa), with over 50 identified binding partners[9] and its unwieldy size has delayed a determination of its crystal structure. The polyQ tract is located in the first exon at the N-terminal of HTT and appears to have evolved relatively recently, adoptimg several different structures (helix, coil, loop) that enable it to function as a key regulator of binding interactions[17,18]. HTT is post-translationally modified by phosphorylation[19], sumoylation[20], ubiquitination[21], acetylation[22,23], palmitoylation[24] and is cleaved in a several different ways by caspases and calpain[25,26]. The N-terminal fragment is the main site of these modifications and the source of HD pathology, including aggregate formation and mitochondrial dysfunction[14,27].

There are at least nine commonly used mouse models of HD, including both transgenics and knock-ins (reviewed in Zuccato et al[14]). The R6/1 and R6/2 transgenic mice carry a fragment of exon 1 from the 5' end of human HTT with 113 and 144 CAG repeats, respectively[28]. The R6/2 mouse has a pronounced HD phenotype, developing weight loss, aggregates, brain atrophy, and motor symptoms by 12 weeks. The YAC128 mouse has a transgene expressing the full-length human HTT gene with 128 repeats[29]. Though it lives a normal lifespan and has increased weight, it develops motor symptoms and has increased n-methyl-D-aspartate (NMDA), AMPA, and metabotropic glutamate receptor (mGluR) binding, and reduced striatal and cortical volume. The BACHD transgenic mouse also expresses the full-length HTT gene and develops inclusions and brain atrophy, but demonstrates fewer motor symptoms than the YAC128[30]. Other models with knock-in polyQ sequences inserted into the mouse htt gene do not develop a robust phenotype[31,32], but do show some neuronal defects and motor abnormalities.

HD is described as having three phases, both in humans and mouse models: (i) pre-manifest, in which the gene mutation has been identified but there are no signs and symptoms; (ii) prodromal, during which there are cognitive and emotional signs, but no loss of function; and (iii) manifest, in which motor symptoms become obvious and there is sharp functional decline[33]. During the prodromal phase, there is loss of cortical mass followed by loss of striatal GABAergic MSNs, which suggests that disease processes in cortical neurons may lead to the subsequent excitotoxic postsynaptic deterioration of MSN's[34]. In fact, decortication in an HD mouse model has been reported to ameliorate HD symptomology[35]. Currently, there are at least nine diseases that are known to be caused by excess CAG repeats, all of which are neurodegenerative. Each of these nine diseases have neuronal loss restricted to specific brain regions[36] and

similar intracellular manifestations, which include alterations in metal processing, protein misfolding, inclusions and aggregates[37]. Protein misfolding and cellular metal mishandling is also present in non-CAG repeat neurodegenerative diseases, such as Alzheimer's Disease (AD), and Parkinson's Disease (PD), both of which have a greater environmental basis than genetic attribution[37]. Environmental factors such as environmental enrichment (EE), exercise, diet, and exposures to xenobiotics have been reported to worsen or ameliorate disease processes in all of these neurodegenerative diseases[38].

Environmental Influences in HD: Lifestyle Effects

Stimulation: Research mice are usually kept in small boxes with bedding, food and water. Under these standardized conditions, mice expressing full length or fragments of the mutant HTT protein develop motor and cognitive disease[28]. However, when allowed access to exercise wheels, stimulating toys and novel objects, their healthy phase is prolonged[39, 40,41]. Exercise alone prolongs the premanifest phase, as does EE alone[42]. Both exercise and EE have been shown to increase levels of striatal BDNF[43] as well as neurogenesis in wildtype mice[44]. This addition to the reserve pool of healthy neurons may explain the protective effects observed in neurodegenerative diseases in general[45]. With HD in particular, BDNF gene transcription[46], as well as serum and cortical BDNF levels are reduced in patients[47-48] compared to healthy controls. However, BDNF protein levels are increased via EE even in the HD murine model[49].

The general function of BDNF is to promote neurogenesis and neuronal survival[50] through binding to the tyrosine kinase B receptor (TrkB), thereby phosphorylating and activating neuroprotective pathways[50,51]. Striatal neurons express TrkB to receive BDNF transported from the cortex or substantia nigra, but have substantially less BDNF when compared to other brain regions[52]. One of the recognized roles of HTT is in the interaction with Huntingtin-associated protein 1 (HAP1) to facilitate transport of BDNF along cortical axons to synapses on MSN's[53]. In addition, wildtype HTT regulates transcription of BDNF by suppressing a key site in the BDNF promoter[54,55]. Emerging evidence has shown that increasing BDNF protein levels protects post-synaptic MSN's even in the presence of mutant HTT[56]. Furthermore, overexpression of cortical BDNF transcription ameliorates symptoms in HD model mice[57] and protects mitochondria[58]. Increasing BDNF in the brain, either directly or indirectly, has been suggested to improve the symptoms observed in HD, AD, PD and Amylotrophic Lateral Sclerosis (ALS)[14] as well.

Diet: There is also a role for diet in delaying the inevitable genetic destiny of HD. Glucose metabolism is altered in HD, with early weight gain followed by hyperglycemia and severe weight loss[59,60,61]. Leptin levels are normal in premanifest human patients[62], but levels do not increase appropriately with BMI[61,63], and leptin is high in murine models compared to wildtype[64,65]. The R6/2 mouse develops metabolic and motor symptoms similar to what is observed in HD human patients[66] . Treating these mice with dietary supplements of essential fatty acids (linoleic and a-linoleic acids) reduced motor signs such as foot clasping and locomotor deficits, but did not correct weight loss or reduction in dopamine receptors[67]. A randomized placebo-controlled double-blind study of fatty acid supplementation in humans with HD also showed a significant improvement compared to placebo[68]. Interestingly, restriction of a-linoleic acid reduces BDNF in a striatal specific manner in wildtype mice[69]. Other dietary manipulations such as dietary restriction (DR) (fasting on alternate days), have been shown to be neuroprotective in wildtype animals[70], delaying locomotor dysfunction, reducing oxidative stress, restoring BDNF levels and glucose metabolism, and increasing lifespan in mice[65]. The DR model has also been shown to increase longevity in C. elegans[71,72]. Ironically, both DR and fatty acid supplementation increases BDNF, which may be the protective mechanism of dietary manipulation in HD.

Oxidative Stress: In addition to the factors that prolong the premanifest phase, oxidative stress and mitochondrial insults[73] from either genetic and/or environmental factors hasten HD pathology. HD post-mortem tissue exhibits severe reductions in mitochondrial complexes II – IV in the striatum with no effect in the blood[74]. Furthermore, PET studies have revealed abnormalities in energy metabolism prior to striatal loss[75]. Systemic treatment with the complex II inhibitor, 3-nitropropionic acid (3NP), causes HD-like abnormal motor behavior[76], striatal-specific neurodegeneration[77] and reduced phosphorylation of DARPP-32[78] (a protein encoded by a modifier gene reported to affect HD onset). Interestingly, pre-treatment with BDNF protects neurons from the effects of 3NP[58].

Mitochondria are abnormal in HD with alterations in enzymatic complexes[27] and calcium (Ca^{2+}) kinetics in HD models[75]. In the YAC128 mouse, mutant HTT interacts with the NR2B subunit of the NMDA receptor, enhancing Ca^{2+} influx and increasing excitotoxicity in striatal MSN's, which carry the NR2B subunit longer in adulthood than most other neurons[27]. Mitochondria are both a source and target of reactive oxygen species[79], and oxidative stress further hastens pathology, increasing apoptosis

and aggregation in cultured cells expressing mutant HTT[80]. Overexpression of the mitochondrial enzyme, superoxide dismutase 1 (SOD1), which binds Cu/Zn, reverses oxidative stress in cultured murine cells[80]. Systemic supplementation with mitochondrial components, such as creatine and ubiquinone, also known as coenzymeQ10 (CoQ10), improved HD symptomology in both HD animal models and human clinical trials[81,82] . Perhaps diets highly enriched with creatine and CoQ10 may contribute to delay in onset of HD, while exposure to toxins that target or accumulate in mitochondria may hasten onset.

Environmental Influences: Metals

Pollutants and heavy metals, expecially Cu, Fe, Mg and Mn, have been implicated in many neurodegenerative diseases[27,83]. The brain appears to be more vulnerable to the toxic effects of metals than other organs, and the striatum appears to be especially vulnerable to mitochondrial toxins[84]. The presence of mutant HTT on mitochondrial membranes causes mitochondria to be even less resilient to excitotoxic insults than other tissues. Heavy metals selectively accumulate in specific brain regions; specifically, Mn^{2+} accumulates in the thalamus and substantia nigra, Fe^{2+} in globus pallidus and Cu^{2+} in the striatum and thalamus following excessive exposures in rats[85]. Emerging studies have demonstrated accumulation of Cu^{2+} and Fe^{2+} in HD[86] and decreased serum ferritin in the striatum of HD[87]. Cu^{2+} interacts with wildtype HTT and decreases its solubility[88]. Furthermore, nuclear inclusions of fragments of the mutant polyQ protein are associated with Fe-dependent oxidation[89]

HTT has been associated with both endocytic and microtubule-mediated vesicle transport, mechanisms which transport both BDNF and various metals into cells and organelles. HTT is closely associated with vesicles and endosomes[90,91,92,93], microtubles[91,94], and directly with the plasma membrane[95]. HTT interacts via HAP1 with an integral member of the microtubule transport system, the dynactin subunit p150Glued[96,97], and co-fractionates with the transferrin receptor (TfR)[98,99]. Knockdown of HTT expression in zebrafish causes increased transferrin receptor 1 transcription in the presence of hypochromic blood. Interestingly, this phenotype is reversed upon administration of bioavailable iron, demonstrating a functional role of normal HTT to make endocytosed iron accessible[100]. The authors theorize that the function of normal HTT must be related to the release of iron from endocytic vesicles.

Manganese: Overexposure to Mn causes preferential accumulation in the mitochondria of the brain and liver[101], especially in the basal ganglia of rats[102] and humans[103], the region also most affected in both HD and PD. Mn also appears to selectively accumulate in the mitochondria of these regions[101] and causes apoptosis from mitochondrial cytochrome c release[104,105] in a caspase-dependent pathway[106]. Subtoxic Mn exposure causes greater susceptibility to 1-methyl-4-phenylpyridinium (MPP), a mitochondrial toxin which targets nigral dopaminergic neurons and is used to create a common PD model. This MPP+ related apoptosis can be reversed by n-acetyl creatine[107]. In HD, it is possible that mutant HTT causes normal levels of bioactive agents to become neurotoxic to selected populations of neurons.

Overexposure to Mn increases risk of a Parkinsonian phenotype referred to as manganism[108,109]. This condition is similar to HD in that it is a progressive neurodegenerative condition which primarily affects the basal ganglia motor pathways. However, there is a loss of nigrostriatal dopaminergic pathways in PD, while there is deterioration of the striatal GABAergic MSN's in HD, and motor symptoms differ[110]. Surprisingly, in HD models, studies utilizing immortalized striatal cells and striatum of knock-in mouse models of HD have demonstrated a resistance to the toxic effects of Mn[111-113]. Emerging evidence from our laboratory aimed at examining Mn transport dynamics in the immortalized striatal cell line model of HD has revealed a significant decrease in instantaneous Mn uptake and storage capablilites in mutant HTT cells compared to wildtype following Mn exposure, though efflux rate appears to be equal in both[114]. It is possible that mutant HTT interacts with constituents of the neuronal Mn transport system and dysregulates Mn kinetics. This gene-environment interaction between mutant HTT and Mn may serve to explain how xenobiotics influence genetic functions. A reduction of Mn in neurons would alter the normal neuronal and glial functions of proteins that cannot function without sufficient Mn, and their byproducts would be reduced in HD. A review of studies on manganoproteins shows either directly or indirectly that they are all reduced in the presence of mutant HTT.

Manganoproteins: Enzymes which require Mn^{2+} to function include glutamine synthetase (GLN), superoxide dismutase 2 (SOD2), arginase 1 and 2 (ARG1, ARG2), pyruvate carboxylase (PC), and selected serine/threonine phosphatase (PPMs)[115].

GLN: The function of GLN is to convert glutamate to glutamine in astrocytes, and a reduction in its activity could partially explain the neurotoxic destruction of MSNs[103,116]. Indeed, Mn accumulates in astrocytes[117], and glial dysfunc-

tion has been shown to play a large a role in the pathogenesis of HD[118]. Interestingly, the glutamate-glutamine cycle is dysregulated in HD[119], as well as reduced GLN is found in HD animal models and patients[120,121].

SOD2: SOD2 is a mitochondrial protein that requires four Mn ions per tetramer, and it acts to convert toxic superoxide into harmless hydrogen peroxide and O_2[122]. A reduction in SOD2 worsens the severity of HD symptoms as confirmed in HD brain tissue[123].

ARG1 and ARG2: Arginase is part of the urea cycle, responsible for converting arginine into either ornithine and urea in somatic tissues or ornithine and nitric oxide in neurons. Arginase requires two Mn ions to function[124]. ARG1 has been found to prevent neuronal death in trophic factor-deprived cell cultures[125]. Patients with HD have an abnormal growth hormone response to arginine infusion[126], and an HD mouse model fed with diets high in arginine has an earlier onset[127]. However, there have been no studies directly examining arginase levels in HD to date.

PC: PC also depends on magnesium and thiamine pyrophosphate as cofactors. PC is essential in the urea cycle and in the metabolism of glucose, cleaving pyruvic acid into acetaldehyde and CO_2[128,129]. There have not been studies of PC in HD, but imaging studies confirm severe hypometabolism of glucose in basal ganglia, even in premanifest HD[130].

PPM: Finally, a subset of the serine/threonine phosphatases require both Mn^{2+} and Mg^{2+} ions to perform the vital function of dephosphorylation at the serine and threonine sites, which is a crucial regulatory step for myriad neuronal proteins[131]. There have not yet been published studies of PPMs in HD, however there has been a reported reduction in gene expression of PPMs after 3NP treatment[78].

Importantly, there is either direct or indirect evidence of reduced activity of all of these manganoproteins in HD models.

Transporters: The mechanism by which expression of mutant HTT reduces neuronal Mn uptake is unknown. Mn has been shown to be transported across neuronal cell membranes by at least 9 known metal transporters. Surprisingly, alterations in many of these transporters have been definitively linked to other neurodegenerative disorders[132], but not specifically to HD[115]. Among these transporters, PARK9 and HIP14 might be especially interesting in the context of HD. Wild type PARK9 (also known as ATP13a2) has been shown to prevent Mn toxicity in neuronal cells and yeast, but mutations in this gene cause Kufor-Rakeb syndrome, a form of parkinsonism[133,134]. Recent studies investigating the function of the metal transporters HIP14 and HIP14L has revealed the necessity for HIP14 to palmitoylate Htt for proper function of the protein[24,135] In addition, the expanded mutant polyQ tract reduces its palmitoylation[136]. In fact, mice with disrupted expression of HIP14 show reduced palmitoylation and display many of the manifestations of HD[137].

Conclusion

There is no evidence yet to show that Mn treatment or exposure will worsen or improve the condition of HD, but the molecular interactions between Mn and HTT may help explain the both the function of the gene and the role of metals in neurological processes. The expanded polyQ series on mutant HTT has an impact on myriad region-specific neuronal functions, including transcriptional regulation, protein interactions, neurogenesis, cell death, and glucose metabolism[138]. A lack or excess of Mn may potentially be involved with many neurodegenerative disorders, including PD, AD, ALS, HD and prion diseases that cause severe progressive encephalophathies in humans[139]. Recently, the prion protein was shown to bind Mn as well as Cu[140]. Moreover, cells expressing abnormal prion proteins are also resistant to Mn toxicity[141]. All of these neurodegenerative diseases share alterations in protein aggregation, mitochondrial damage, and oxidative stress, and all are improved with interventions that increase BDNF. In addition, it is possible that mutant HTT dysregulates both intracellular neurotrophin and metal transport dynamics, and impairs downstream signaling cascades that have been implicated in HD and other neurodegenerative diseases.

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