A Novel Pathophysiological Gene-Environment Interaction Suppresses the Neurotoxic Activities of (Mn) and Mutant *HD*

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Huntington's disease (HD) is a progressive autosomal dominant neurodegenerative disorder characterized by motor impairment, cognitive deterioration, emotional disturbance, and psychiatric disorder, which were first described by George Huntington in1872. Prevalence of HD is approximately 5 in 100,000 worldwide and 1 in 10,000 in the United States with a median age of onset at 39[1]. Surprisingly, approximately 5% of patients have a juvenile form of the disease characterized by increased repeat length in comparison with adult HD onset. Unfortunately, there is currently no effective treatment for the disease. HD is caused by an expansion of a glutamine-encoding triplet repeat (CAG) in the *huntingtin* gene (IT15)[1,2,3]. Interestingly, both genetic and environmental factors have been reported to contribute to variability in age of disease onset. In fact, monozygotic twin[4,5,6,7] and Venezuelan kindred studies[8] have revealed significant environmental influences on the age of onset and clinical presentation of HD. The neuropathology of HD is characterized by selective degeneration of medium spiny neurons (MSNs) in the corpus striatum. The clinical presentation of HD reflects this selective vulnerability with patients exhibiting choreatic hyperkinetic movements. However, as the disease progresses, subsets of neurons within the cortex are lost, whereas the brainstem, cerebellum and hippocampus remain unaffected[1]. Several potential cellular mechanisms of HD neurotoxicity are supported by experimental evidence including alterations in iron homeostasis, energy metabolism, transcriptional regulation, brain-derived neurotophic factor (BDNF) signaling, axonal transport and altered calcium signaling[9-14]. A significant challenge in HD neuropathology is segregating the cellular pathologies into direct and indirect effects of HD neurotoxicity. This review will provide insights into studies that examine how metal ions modulate HD neuropathology. A discussion on wild-type huntingtin protein function and its relation to HD will commence the review, followed by environmental factors and aggregates in HD. The review will conclude with metal ion (manganese) essentiality and how its transport mechanisms may play a role in HD pathology.

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HUNTINGTIN PROTEIN AND HD NEUROPATHOLOGY

The *huntingtin* gene encodes a large 350kDa protein called huntingtin, which when mutated in HD, causes progressive degeneration of the MSNs in the striatum. Huntingtin is a soluble cytoplasmic protein of 3,144 amino acids that is ubiquitously expressed in all regions of the brain and peripheral tissues[14]. The protein is enriched in neurons with similar expression patterns for wildtype and mutant huntingtin[1]. Despite its identification more than a decade ago, the function of wild-type huntingtin remains largely unclear. Huntingtin has many potential domains, boundaries and activities of which are not fully understood. One obviously significant portion of the mammalian protein is the polyglutamine (polyQ) region itself, which has been reported to be present in many transcription factors and aberrantly expanded in other disease-causing proteins[16]. Expansion of the polyQ tract alters the conformational state of the mutant protein and modifies fragmentation by proteolytic processing. Thus, the expanded polyQ tract is required for its subsequent aggregation and accumulation into inclusion bodies[17-21]. Although the relationship between polyQ aggregates and neurotoxicity is complex, recent data has demonstrated an inverse correlation between the polyQ protein inclusions neuropathology at the cellular level[17,22-23]. In unaffected individuals, the polyQ stretch in huntingtin begins at the eighteenth amino acid from the 5' N-terminal region and contains up to 34 glutamine residues[3]. Data from Perutz *et al* in1994 showed that this region forms a polar zipper structure and suggested physiological interactions with other transcriptional factors that contain a polyQ region[25]. Bioinformatics analyses

have reported 37 sequential HEAT repeat domains (named after similarity to domains found in the HD protein, Elongation factor 3, PR65/A subunit of protein phosphatase 2A, and TOR) spanning the entire protein, though other functional domains of the HD protein have also been identified[26-30]. The precise biological activity of HD is unknown, but many HEAT repeat containing proteins function as molecular scaffolds for stable interacting partners. While numerous interacting partners have been identified, the significance of these interactions to normal HD function is unclear. Huntingtin is found in multiple cellular compartments but localizes predominantly to the cytoplasm where it is found as both a soluble protein and in association with membranes including a variety of vesicles, organelles, and the plasma membrane. Subcellular localization may be important for HD pathogenesis, as nuclear localization of the mutant HD protein has been associated with increased neurotoxicity and correlates with neuropathology[31,32]. HD has been implicated in various cellular processes including iron metabolism, transcription, intracellular transport and membrane trafficking, axonal transport and mitochondrial function[33,34]. A major gap in our understanding of the disease mechanism is the absence of a known function for wild-type huntingtin. In 1995, three independent studies showed that the *huntingtin* gene is essential for normal embryonic development and neurogenesis: its complete inactivation in huntingtin-knockout mice results in embryonic lethality before day 8 (before gastrulation and the formation of the nervous system)[35-37]. Most intriguingly, the loss of *huntingtin* gene function in the adult brain results in motor dysfunction and a broad neurodegenerative phenotype, but not specifically for the neurons vulnerable to the polyglutamine-expanded disease protein[37-39]. The effects of polyglutamine expansion on HD function are unclear, although the mutant protein can rescue the embryonic lethal phenotype of the null mouse[40]. Evidence from mouse genetics and the dominant inheritance pattern of HD, indicates that HD is caused predominantly by a toxic gain-of-function, although there is also evidence for a dose-dependent neuroprotective loss-of-function. Thus, a pivotal question in HD research is aimed at understanding how mutant huntingtin causes selective neuronal pathology, especially in the MSNs of the striatum and pyramidal neurons in the motor cortex. One possibility is that environmental agents such as neurotoxic metal ions and toxins may modulate HD pathophysiology by promoting aberrant protein-protein interactions with mutant huntingtin to alter normal wild-type huntingtin physiological functions in striatal and cortical neurons. Thus, metal ions may facilitate mutant huntingtin's toxic gain-of-function processes in HD neuropathology.

ENVIRONMENTAL FACTORS IN HD PATHOPHYSIOLOGY

Over a decade after the identification of the HD mutation, there has been conflicting reports linking complete or incomplete penetrance of HD to triplet repeat expansion length. Fortunately, Rubinsztein and other researchers have provided data which shows that triplet repeat expansion at the HD locus beyond 35 glutamine-encoding CAG repeats is sufficient to cause HD, though repeats between 36-40 show incomplete penetrance[41,42]. Although longer repeat length has been associated with earlier onset, repeat length in general account for only 60% of the variability in age of onset[8]. Thus, it is rationale to link both genetic and environmental factors as likely partners in contributing to HD, specifically, environmental factors contributing to the largest share of residual variability[8,43]. Gómez-Esteban and other researchers in the HD field have revealed significant environmental influences on the age of onset and clinical presentation in monozygotic twin studies that have the same number of expanded repeats[45-48]. Unfortunately, the aforementioned monozygotic twin studies failed to reveal the nature of the environmental factors involved. Animal models of HD have provided further support for the influence of environmental factors on HD onset and progression[43,44]. Indeed, Rozengzweig, Bennet and colleagues since the 1960s have studied the effects of environmental enrichment on the neuroanatomy and neurochemistry in wildtype animals that may enhance memory[49]. With these clear indications that environmental factors can influence HD pathophysiology, it is compelling to probe the possible contributing environmental factors and how they modulate HD pathophysiology.

THE ROLE OF METALS IN NEURODEGENERATIVE DISEASES

In the past decade, there has been a growing interest to understand the metabolism of neurotoxic metals and their influence on various neurodegenerative diseases, such as Manganism, Wilson's, Parkinson's, and Alzheimer's diseases. Occupational and environmental exposures to these metals [Manganese (II) (Mn^{2+}) and other metal ions (e.g. Cu^{2+}, Zn^{2+}, Al^{3+}] have been suggested as a possible cause of neurodegenerative diseases. However, less attention has been focused on metals in HD neuropathology. Currently, there is evidence supporting amyloid fibrillogenesis and aggregation of proteins such as prion protein (PrP) and α-synuclein via Mn^{2+} and other metal ions (e.g. Cu, Al, Zn) interactions[50]. These proteins are metal ion binding proteins that interact with divalent metal ions to play a role in their altered conformational state, solubility, and aggregation[51-56]. However, *in vitro* analysis of

prion protein aggregates have shown that Mn can promote aggregation independent of the PrP metal binding site[55]. Uversky has proposed that polyvalent metal ions, such as Mn, may promote aggregation by cross-linking protein carboxylates[56]. Comparatively, Perutz and Green have also hypothesized that the mechanisms of neuronal intranuclear inclusions (NIIs) of mutant huntingtin (aggregates) in HD is either via polar zipper formation or covalent bonding by transglutaminase-catalyzed cross-linking[24,57]. Given the strong association between protein aggregation, metal ions and neurodegeneration, it is highly rationale to speculate that metal ions have the potential to modulate HD pathophysiology.

RELATIONSHIP BETWEEN ALTERED METAL ION HOMEOSTASIS, METAL TOXICITY, AND HD

The clinical progression of HD has been reported to be associated with altered metal ion homeostasis, wherein iron and copper are significantly elevated in the corpus striatum[58]. In addition, data from animal models have also shown that there is significant increase in the levels of microglia ferritin, an intracellular iron storage protein [59]. In fact, a common phenomenon in multiple neurodegenerative diseases is the alteration of various metal ion levels, and their obvious neurotoxic consequences. Although the distribution of metals throughout the brain is not uniform, metal ion accumulation in specific brain regions reflects neurotoxicity (example: manganese accumulation and neurotoxicity in the globus pallidus results in manganism). Interestingly, Fox *et al* have recently reported that huntingtin protein interacts with Cu ions, with this specific metal binding decreasing the solubility of wild-type huntingtin protein[60]. However, the cellular effects of Cu or other metal ions on HD function, proteolytic processing to generate N-terminal fragments, aggregation of fragments, and formation of mutant huntingtin inclusion bodies remain unknown. A recent study suggested that inclusion bodies formed by CAG expansion in mutant huntingtin protein fragments are associated with iron-dependent oxidative events, opening the possibility that other redox-reactive metal ions, such as Mn, may influence polyglutamine aggregation[61]. In essence, several studies have provided evidence that supports a role for oxidative stress, mitochondrial dysfunction, excitotoxicity, and alterations in iron homeostasis as critical steps in both Mn neurotoxicity and HD neuropathology. Importantly, chronic exposure of Mn in animal models shows significant accumulation in the striatum, providing any potential interaction between Mn and HD to occur within the neurons most vulnerable to HD pathology. Unfortunately, there are currently no reported studies examining the connection between metal exposure, including Mn and HD neuropathology. With the increasing evidence supporting strong association between metal ions and protein aggregation, similarities between metal ion cytotoxicity and cellular pathways of neurodegeneration, altered metal ion homeostasis, and the differential accumulation of various metals across neuronal subtypes, it is highly rationale to propose that metal ions with neurotoxic properties are the strongest candidates for the largest residual environmental variability that has been hypothesized to modulate selective neurodegenerative process in HD. In an attempt to identify the link between HD and metal ions, our lab has screened the impact of several neurotoxic metal ions on a striatal cell line model of HD and found striking interactions between mutant huntingtin expression and Mn exposure, wherein mutant huntingtin protein and Mn suppress the neurotoxic activities of each other. The remaining sections of this review will focus on manganese essentiality, neurotoxicity, mechanisms of transport and its possible link to HD.

MANGANESE: ESSENTIALITY AND NEUROTOXICITY

Mn is an essential ubiquitous trace element required for normal growth, development and functioning in all bodily tissues, and cellular homeostasis[62]. In humans and animals, manganese functions as a cofactor for several Mn-dependent enzymes that are appropriate for neuron or glial cell function, as well as enzymes involved in neurotransmitter synthesis and metabolism. These Mn-dependent enzymes include glutamine synthetase, pyruvate decarboxylase, superoxide dismutase 2 (SOD2), and arginase[63]. The idea of Mn involvement in HD stems from earlier studies by Butterworth in 1986 where it was shown that there are significant decreases in Mn-dependent enzymes, specifically, glutamine synthetase and pyruvate carboxylase in the caudate nucleus of HD patients[64]. Interestingly, given Mn essentiality, inadequate intake of Mn can result in abnormal glucose tolerance[65]. Despite its essentiality in multiple metabolic functions, Mn can be toxic at high concentrations. The brain in particular is highly susceptible to Mn neurotoxicity. Excessive dietary intake and environmental exposures to Mn for longer periods result in accumulation of Mn in the globus pallidus, striatum and subthalamic nucleus of the basal ganglia, which causes a clinical disorder referred to as manganism. This disorder causes extrapyramidal symptoms that resemble idiopathic Parkinson's disease (IPD). Although extensive studies have been conducted to link altered manganese levels to IPD, the connection between manganese and HD remain unknown. Mn exposure and increased brain Mn levels may modulate other neurodegenerative

diseases (example: Alzheimer's and polyglutamine diseases) in which protein aggregation and amyloid deposition are parts of the pathophysiology. Recent data shows that non-human primates exposed to Mn have diffuse amyloid-Beta plaques in the frontal cortex, similar to what is seen in Alzheimer's patients[66]. This is particularly interesting in light of the observation that Mn levels are elevated in Alzheimer disease brains[67]. Indeed alterations in various brain metals, including Mn, have been suggested to modulate sensitivity to oxidative stress, which likely plays a fundamental role in the pathophysiology of most neurodegenerative disease states. For example, a possible mechanism by which Mn exposure may modulate neurodegenerative conditions is through alterations in Mn-dependent antioxidant enzyme SOD2 level or activity. It is known that reduction of SOD2 levels enhances Alzheimer's disease pathology in a transgenic mouse model[68]. Preferentially enhanced NMDA (N-methyl-D-aspartic acid) receptor mediated excitotoxicity, mitochondrial dysfunction, and oxidative stress have also been implicated in HD[1,69]. Exposure of rats to Mn was found to decrease the levels of two manganese bound enzymes, SOD2 and glutamine synthetase, in the basal ganglia[70]. Furthermore, recent data from HD mouse models have also linked altered arginase activity resulting in urea cycle deficiency[71]. Thus, Mn exposure may diminish the activities of Mn-dependent enzymes, thereby contributing to the HD pathophysiology.

MECHANISMS OF MANGANESE TRANSPORT

Unpublished data from our lab shows that mutant HD striatal cell cultures are resistant to Mn toxicity relative to wild-type, over a broad Mn concentration range. This observation suggests a strong interaction between mutant huntingtin and Mn. It is highly rationale to speculate perturbations in Mn transport (import and export) and storage in the aforementioned mutant striatal cell line models of HD. The remainder of this review will focus on the mechanisms of manganese transport. Due to the delicate relationship between Mn's essentiality and toxicity, both the absorption and tissue levels of this metal are tightly regulated. Mn can cross the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB) on several carrier(s) and in different oxidation states[72,73]. Unfortunately, no unique mammalian Mn transporter has yet been identified. It appears that several potential modes of Mn transport across the BBB and BCB have been reported and others speculated to occur via facilitated diffusion, active transport, divalent metal transporter 1 (DMT-1)-mediated transport, voltage-regulated and stored operated Ca^{2+} channels, ZIP8, citrate, and transferrin (Tf)-dependent transporters[72]. Of all the above listed polyvalent transporters, Tf and DMT1 are the most extensively documented[74]. Approximately 80% of Mn in plasma is bound to $beta_1$-globulin and albumin and a smaller fraction of Mn is bound to transferrin (Tf), an iron-binding protein[75]. Mn binding to Tf is time-dependent and Tf receptors have been shown to be present on cerebral capillary surfaces[74]. When complexed with Tf for transport across the BBB, Mn is exclusively present in the trivalent oxidation-state[76]. Another critical regulator of brain Mn levels is the divalent metal transporter (DMT-1). The transporter belongs to the family of natural resistance-associated macrophage protein (NRAMP) and has also been referred to as the divalent cation transporter (DCT). Gene transcription of this protein is regulated by Fe concentration via a Fe-response element (IRE) located on the mRNA[77]. Orthologous mutations (glycine 185 to arginine) in the DMT-1 gene of the Belgrade (b) rat, and microcytic anemia (mk) mouse result in significantly lower Mn and Fe tissue levels, including the brain[78,79]. The reduction in brain Mn and Fe uptake in the above animal models suggests that defective DMT-1 allele alters the disposition of both metals and that Mn and Fe may utilize DMT-1 as a putative transporter across the BBB and BCB. These results support the notion that impaired DMT1 alters Mn transport due to a defect in Mn uptake, export and storage.

Given the above evidence of metal ions (example, Mn) essentiality and neurotoxicity in neurodegenerative diseases, specifically HD, and alterations in Mn transport and levels in HD animal models, it is highly likely that there exists a gene-environment interaction between mutant huntingtin and Mn that may modulate HD pathophysiology. However, the identification and functional characterization of both Mn transport pathways and Mn-bound proteins is not completely understood. Thus, further investigations would have to be conducted to support the gene-environment interaction hypothesis between mutant huntingtin and Mn in HD neuropathology.

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FURTHER INFORMATION

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