Review Article

The role of astrocytes in human Huntington's disease pathology

Journal of Huntington's Disease
2025, Vol. 14(3) 214–228

DOI: 10.1177/18796397251344173
journals.sagepub.com/home/hun

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Abstract

Huntington's disease (HD) is a neurodegenerative disorder caused by a repeat expansion in the *HTT* gene. The disease is well known for severe and progressive loss of neurons in the caudate and putamen, although other areas are also involved. Much of the attention on understanding the mechanisms underlying HD has focused on the neurons. The brain also contains large numbers of glial cells, such as astrocytes, oligodendrocytes, and microglia, which also become dysfunctional in HD. Astrocytes are one of the most abundant cell types in the central nervous system and are critical for regulating the brain environment and supporting neurons in many ways. In this review, we discuss the changes in astrocytes during the evolution of HD in the human brain. We detail the key phenotypes of astrocytes in human HD, which encompass reactive astrogliosis, loss of homeostatic function, gain of a neuroprotective function, changes in lipid metabolism, huntingtin protein aggregation, and limited somatic repeat expansion. We briefly discuss the conservation of these phenotypes in mouse models and propose a model of how astrocyte states change in human HD. Finally, we present open questions for astrocyte researchers in the HD field. Together, this review represents a valuable resource for readers interested in astrocytic changes in human HD.

Keywords

astrocytes, Huntington's disease, mHTT, glutamate transporters, metallothionines, transcriptomics

Received: 17 December 2024; accepted: 1 May 2025

Huntington's disease (HD) is a progressive neurodegenerative disease caused by a mutation in the *HTT* gene.[1] The neuropathologic substrate of the disease is classically described as degeneration of the medium spiny neurons in the caudate and putamen,[2–5] but neuronal loss is well-documented in the cortex[6–8] and thalamus,[9] and even in brain stem regions.[10] Atrophy in non-striatal areas does not correlate with the HD Vonsattel grade.[3,10,11] Accordingly, whether non-striatal neuronal loss occurs independently from MSN degeneration or concomitantly is not fully understood. Of note, neuronal loss is associated with the aggregation of mutant HTT protein as intranuclear inclusions and neuropil aggregates.[12] In addition, these neuropathologic processes are accompanied by reactive astrogliosis,[2,5] a process whereby astrocytes, the major glial cells in the brain, increase the expression of the intermediate filament glial fibrillary acidic protein (GFAP)[13,14] and exhibit a host of alterations, which we describe below. This is the main emphasis of this short review.

We will begin by introducing astrocytes, their functions, and dysfunctions in general terms, then detail recent evidence for their involvement in HD. We will not delve into the development of astrocytes or discuss work using pluripotent stem cells differentiated into astrocytes.

Astrocyte function: In brief

In the human brain, neurons and non-neuronal (glial) cells are roughly equal in proportion,[15] and estimates of astrocytic numbers in the central nervous system (CNS) suggest they range from 25–50% of all CNS cells.[16,17] Astrocytes perform several important homeostatic functions, including promoting, maintaining, and regulating synaptogenesis,[18–21] regulating neuronal morphology and

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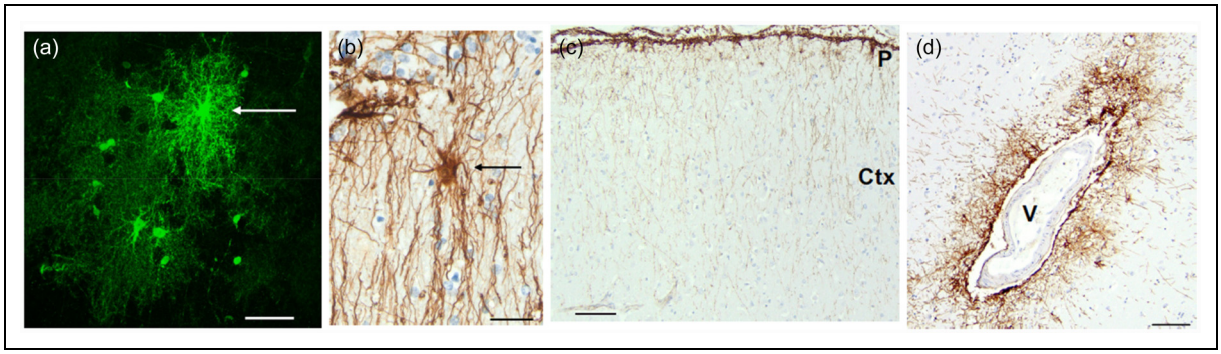

Figure 1. (a) human cortical, protoplasmic astrocyte (arrow) in a surgical specimen injected with Lucifer yellow. Note the many, highly branched, processes and also the dye traversing the gap junctions into neighboring astrocytes. (b) White matter fibrous astrocyte (arrow points to the cell body) in the corpus callosum, showing long processes oriented along the axon tract, GFAP immunostain. (c) Interlaminar astrocytes, whose processes begin at the pial surface (P) and move radially through the upper cortex (Ctx), CD44 immunostain. (d) Long process astrocytes attached and emanating from a large blood vessel (V) in the basal ganglia, CD44 immunostain. Scale bars: (a) 20 μm, (b) 20 μm, (c) 200 μm, (d) 50 μm. Panel A is courtesy of Xiaoping Wu and Alexander Sosunov, Department of Neurosurgery, Columbia University, NY, USA.

function,[22] providing trophic support for neurons,[23] taking up potassium[24] and glutamate,[25] coupling neuronal activity to cerebral blood flow,[26] releasing gliotransmitters,[27] and maintaining the blood-brain barrier.[28] For more in-depth reviews on astrocyte function, see.[13,29] Astrocytes are not passively integrated into the neuronal circuitry, since they exhibit electrophysiologic responses and increments in intracellular Ca++ in response to synaptic activity.[30] Neurotransmitters like glutamate, norepinephrine, and ATP released at synapses interact with astrocyte receptors and transporters to promote Ca++ entry, which in turn activates intracellular inositol-1,4,5-triphosphate receptors to release Ca++ from intracellular stores.[31,32] This increase in Ca++ can be propagated from astrocyte to neighboring astrocytes through gap junctions, thus increasing the spatial scope of astrocyte activation.[33] This functional and physiologic astrocytic attribute can be monitored *in vivo* and *in vitro* using calcium imaging.[30,34–36] The results of increased intracellular astrocyte Ca++ include the release of "gliotransmitters", such as ATP,[37] glutamate,[38,39] and D-serine,[40] which can modulate neuronal[41] and microglial[42] functions (see for review[31]).

Although murine and human astrocytes share functional similarities, there are significant differences between humans and mice. A few examples to note include, for instance, that human protoplasmic astrocytes are at least three times larger than their murine counterparts.[43] Also, astrocyte subtype heterogeneity differs between the two species. For example, the interlaminar astrocytes (discussed below) are more complex and abundant in humans and non-human primates compared to rodents.[44] Metabolically, human astrocytic mitochondria are more susceptible to oxidative stress, and compared to murine astrocytes, human astrocytes engage less in oxidative phosphorylation and rely more on glycolysis, which makes them less susceptible to hypoxia.[45] Functionally, human astrocytes respond more robustly to pro-inflammatory stimuli such as poly I:C (model of viral infection) and TNFα than murine astrocytes.[45] These differences in astrocytes across species are important to consider when modeling astrocytes in HD from functional and metabolic perspectives.

Astrocytes subtypes

Astrocytes can be divided into multiple subtypes. The classic distinction is that of "protoplasmic" vs. "fibrous".[46,47] Protoplasmic astrocytes (Figure 1) are the major type in gray matter structures, such as the cerebral cortex and the striatum. Roughly spherical in volume, they form only partially overlapping domains.[48] They are structurally complex with primary, secondary, and numerous tertiary branches or branchlets/leaflets, also referred to as peripheral astrocytic processes, which make contact with neuronal synapses[49] and participate in forming the so-called "tripartite synapse".[50,51] Other ends of the astrocyte processes contact blood vessels,[46] where they participate in stabilizing the blood-brain barrier and regulating vascular tone.[52]

In comparison, fibrous astrocytes exist in white matter and differ from protoplasmic astrocytes in a number of ways: 1) They emit longer and less branched processes, which often follow the axonal pathways in which they reside[46] (Figure 1). 2) They do not contact synapses; rather, they contact the nodes of Ranvier of myelinated axons, where they regulate neuronal action potentials.[53–56] 3) Fibrous astrocytes appear to express higher levels of GFAP compared to protoplasmic astrocytes, as detected by immunohistochemistry.[57] 4) Calcium waves, which propagate between protoplasmic astrocytes via gap junctions,[58] do not appear to be dependent on gap junction proteins in white matter fibrous astrocytes.[42,59] 5) Fibrous astrocytes are less capable of buffering extracellular glutamate than protoplasmic astrocytes, which is attributed to lower levels of the glutamate transporter SLC1A2.[60]

In addition to the classically defined protoplasmic and fibrous astrocytes, the human CNS contains astrocytes whose cell bodies are close to the pial surface and which send long processes deep into the cortex, orthogonal to the surface.[44,46,61,62] These astrocytes with long, radial processes have been termed "interlaminar", first shown by Andriezen (1893), and more recently in detail by Colombo et al. (1997), Falcone et al. (2019), Oberheim et al. (2009, 2012), Sosunov et al. (2014), and Al-Dalahmah et al. (2023). Interlaminar astrocytes express the extracellular matrix transmembrane receptor CD44, as do fibrous astrocytes.[63,64] There are also long-process astrocytes in the superficial white matter and the deep cortical layer that send long processes into the deep cortical layers.[64–66] In human and other large brains, the long process astrocytes in deep cortical layers can appear varicose, or beaded,[44,66] although it is not clear whether these are resting astrocyte types or whether they are responses to metabolic changes.[65] Other astrocyte types include the Bergmann glial cells, spanning the molecular layer of the cerebellar cortex from the Purkinje cell layer to the pial surface, velate astrocytes of the cerebellar granule cell layer, and tanycytes that project processes into the brain from the walls of the third ventricle.[29]

In addition to morphology and neuroanatomic localization, protein marker expression can be used to distinguish astrocytic subtypes. For example, interlaminar astrocytes show expression of pax6, hopx, and nestin, among others.[44] Interlaminar and fibrous astrocytes, but not protoplasmic astrocytes, express the cell membrane matrix receptor CD44.[63,64,67] Finally, *in situ* hybridization studies of the human cingulate cortex demonstrated that upper-layer astrocytes express higher levels of *CHRDL1* compared to deep-layer astrocytes.[68]

Another way to classify astrocytes is by examining their transcriptional states. Several studies have examined astrocytic transcriptional states in the human brain.[69–76] These studies have compared gene expressions in astrocytes in various regions in disease, such as Alzheimer's and Huntington's, with the same areas in "control" brains of individuals without significant neuropathology. At this point, comparing all studies in terms of the different areas that were analyzed and the different "types" or "states" of astrocytes described in each study is an endeavor we are interested in pursuing; however, it falls beyond the scope of this review.

The above data highlight the importance of considering astrocyte heterogeneity when examining the roles of astrocytes in disease. Across the spectrum of neurologic diseases, astrocytes of different subtypes are exposed to injurious stimuli. As an example, white matter fibrous astrocytes are involved in several facets of the pathology of demyelination (see for review[77]). Specifically, in human multiple sclerosis brain samples, it is the white matter—fibrous, and not grey matter, protoplasmic—astrocytes that exhibit a pro-inflammatory phenotype characterized by decreased NRF2-driven gene expression and increased MAFG and MAT2α signaling, which promote inflammation in the EAE model of autoimmune demyelination.[78] Work from our laboratory examining astrocytic responses to COVID-19 in human postmortem brains identified differences between white matter and parenchymal astrocytes in the brain stem, and linked the deficiency of upregulation of CHI3L1 (YKL-40) in fibrous astrocytes to the increased neuroinflammation seen in the brain stem of COVID-19 victims.[79] That said, studies that investigate fibrous astrocytes in HD are limited. While we provide a summary of what is known about fibrous astrocytes in HD in a separate section below, the majority of the data presented below relates to protoplasmic astrocytes.

Astrocyte pathology in HD

The major findings in HD astrocytes in postmortem brains are summarized in Figure 2 and include: 1) reactive astrogliosis; 2) loss of protoplasmic astrocyte functions; 3) changes in astrocyte lipid metabolism; 4) increases in neuro-protective functions; 5) the presence of HTT protein inclusions; and 6) the absence of significant CAG repeat expansion. For this review, we have limited ourselves to studies of human HD, rather than discussing the large and interesting sets of studies on many HD mouse models. Instead, we note examples of the conservation of each of the human astrocyte phenotypes in model animals when present in a separate section.

Reactive astrogliosis

Reactive astrogliosis is a general term that often appears in studies that find increased levels of *GFAP* expression and GFAP protein, or increased somal hypertrophy and eosinophilia on H&E stains, proliferation, as well as other characteristics.[14] In the caudate nucleus, the site of the most severe neurodegeneration in HD, a number of studies have found increases in GFAP + astrocytes in HD. In fact, the Vonsattel classification system of HD neuropathologic severity uses neuronal depletion and astrogliosis to determine the disease's severity (grade).[3] This landmark study documented the increase in GFAP + astrocyte densities in the caudate, putamen, and globus pallidus, which appears most prominent in the dorsal-medial caudate nucleus, starting in grade 1 cases, and increasing more caudally.[3] A study demonstrating increased PCNA+/GFAP + cells in the subependymal layer adjacent to the caudate nucleus in donors with HD (grades 1, 2, and 3) suggests that these astrocytes may proliferate in HD.[80] Whether proliferative astrocytes - or their precursors - migrate from the subependymal zone into the caudate is challenging to assess in human tissue. Despite this evidence, an increased density of astrocytes does not necessarily mean that there are more astrocytes.

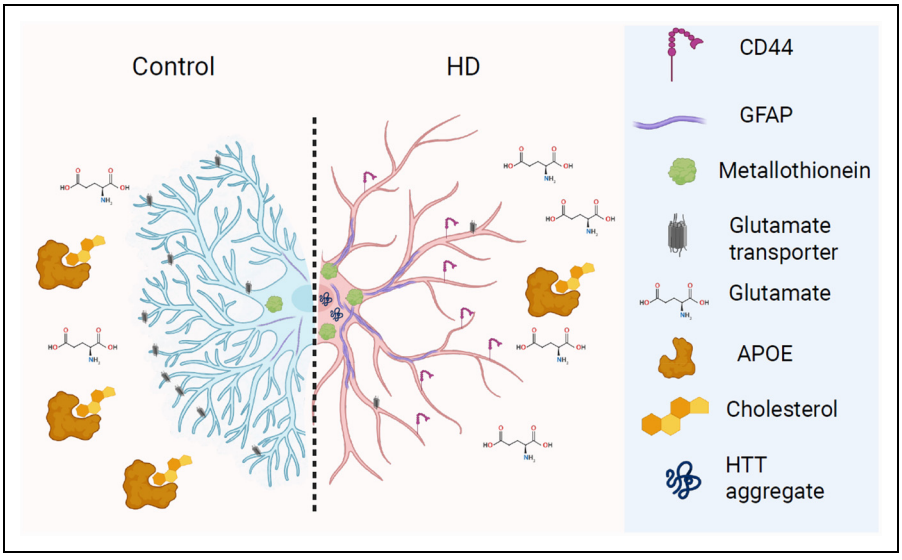

Figure 2. Summary of changes in human HD astrocytes.

Rather, since the volume of the striatum is decreased due to neuronal loss, the surviving cells, including astrocytes, will have a higher density. Furthermore, since GFAP levels are lower in protoplasmic astrocytes than in fibrous or reactive astrocytes, the increased density of GFAP + astrocytes may in part be due to the increase in GFAP levels in the protoplasmic astrocytes.

There is mixed evidence to support that astrocyte reactivity precedes the onset of neuronal loss. In three Grade 0 cases with no demonstrable neuronal loss, the authors did not show astrogliosis in that series when examined with GFAP immunostaining,[3] or when astrocytes were defined morphologically on Cresyl Violet stains.[4] The latter study did not leverage immunohistochemistry to label astrocytes, but the findings were consistent with Vonsattel's original work. In a different series, the dorsal caudate of two grade 0 HD donors demonstrated increased GFAP + astrocyte densities.[81] Given the small sample size for grade 0 cases, it is possible that heterogeneity within the donor cohorts may explain the discrepancy in the results. Nonetheless, across different cohorts, increased GFAP density was seen as early as Vonsattel grade 1, and increased with advancing grade.[3,4,81] Across cohorts, the authors note a dorsal (high) to ventral (low) gradient in GFAP density that was most apparent in Grade 3 cases. This dorsal-to-ventral gradient in astrogliosis, which mirrors neuronal loss, was also recently documented by a separate group.[82] Our data also support the increase in GFAP in HD caudate astrocytes when measured at the RNA level.[74] Interestingly, in Faideau et al. (2010), the authors documented altered morphologic features of astrocytes with somal hypertrophy and retraction of processes[81]—a finding we also confirmed in our cohort,[83] and was recapitulated in morphologic studies of iPSC-derived astrocytes.[84]

In addition to the striatum, there is an increase in the density of GFAP + astrocytes in cortical areas, including the cingulate cortex[69] and the middle temporal cortex.[85] Another study used immunohistochemistry to quantify the GFAP-covered area in the cingulate cortex of HD patients and did not find an increase in GFAP signal.[86] This may be related to differences in quantification, as the density of GFAP + cells was not measured as in our study.

Also relevant to reactive astrogliosis, we found that astrocytes increase the gene expression and protein level of the matrix protein receptor CD44 in the caudate nucleus of HD donors, but not in the relatively less severely affected cingulate cortex.[69,74] *CD44* expression was one of the genes most correlated with the modal CAG repeat length. Normally, CD44 is present in fibrous astrocytes,[63] and we found that it was increased in protoplasmic astrocytes of the caudate nucleus in HD,[74] in a manner similar to that which we observed in stroke and in epilepsy brains.[63] Other reactive astrocyte genes correlated with CAG repeat length include *LIFR* and *OSMR*. We will discuss this transition from protoplasmic astrocytes in a separate section.

Loss of protoplasmic astrocyte functions

As mentioned in the preceding section, protoplasmic and fibrous-type astrocytes differ in many ways, one of which is the presence of relatively higher levels of glutamate transporters in protoplasmic astrocytes.[60] Thus, a loss of glutamate transporters represents a major loss of protoplasmic astrocyte function. Indeed, a number of studies have found a loss of the EAAT2 transporter (*SLC1A2*), also known as glutamate transporter 1 (GLT-1), in the HD brain. RNA *in situ* hybridization studies in the caudate, putamen, claustrum, and insular cortex show this loss.[87]

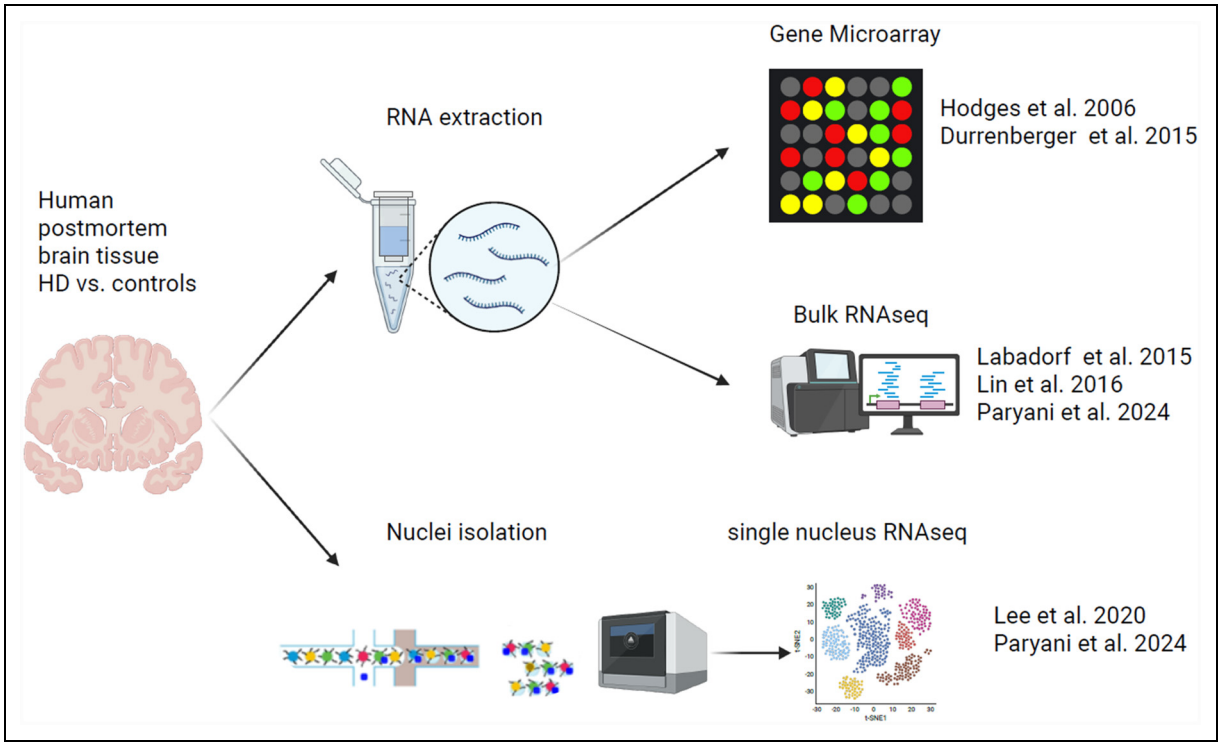

Figure 3. Transcriptomic datasets analyzing human HD brain samples. See main text for details.

Similarly, immunostaining of the human caudate showed a significant decrease in levels of GLT-1 as the HD grade increased from 0 to 4.[81] Significantly, the authors found the reduction in the two Grade 0 brains prior to the onset of neuronal loss. Another study confirmed the reduction of EAAT2 + cell density and area in the cingulate cortex, and when the authors stratified the cases by motor vs. mood symptomatology, the reduction was significant in donors with mood symptoms.[86] Our single-nucleus RNAseq results from the cingulate cortex,[69] caudate, and nucleus accumbens[74] also identified a reduction of *SLC1A2* RNA expression across the three brain regions, which is consistent with what Lee et al. described in the caudate and putamen.[88] Adding to this complexity, a tissue microarray study revealed no changes in the levels of GLT-1 in the middle temporal gyrus in HD brain donors[85]; however, limited sampling in tissue microarrays may limit the interpretation of this finding. Taken together, these data suggest that loss of protoplasmic function is not only progressive with advancing Vonsattel grade, but is also an early event that precedes the onset of neuronal loss in the caudate, and is seen in areas with relatively little neuronal loss in a manner correlated with patient symptoms.

Other proteins involved in maintaining protoplasmic astrocyte functions include the EAAT1 glutamate transporter (*SLC1A3*), also known as GLAST, glutamine synthetase (GS – *GLUL*), which converts glutamate to glutamine, and potassium channels such Kir4.1 (*KCNJ10*). These are variably dysregulated in HD astrocytes in studies that leveraged gene expression microarrays, bulk RNAseq, and single-nucleus RNAseq (Figure 3). We found that *SLC1A3* was decreased in cingulate and caudate protoplasmic astrocytes, and in cingulate, caudate, and accumbens fibrous-like astrocytes[74] (see Figure 4). *KCNJ10* was decreased in several bulk RNAseq/microarray datasets,[89–92] but the log fold changes in snRNAseq datasets were small.[74,88] Other genes encoding potassium channels and related proteins were also altered in both of the snRNAseq datasets, including *KCNIP1*, which was reduced in these snRNAseq datasets.[74,93]

Relevant to the loss of protoplasmic function is the loss of a core gene set of 62 genes that were concordantly reduced in HD postmortem caudate and mouse models of HD,[75] as measured in microarray studies.[75,90] These genes were enriched in pathways related to protein ubiquitination, GABA, and cAMP signaling.[75] The authors found that the downregulation of this core gene set was partially reversed when mutant HTT protein aggregates were reduced in mouse astrocytes. Accordingly, this gene set represents a conserved signature that can be measured in perturbative studies in animal models of HD.

We compared the scaled reported log fold changes in quiescent, protoplasmic, astrocyte genes[69] and the Diaz-Castro core genes[75] among bulk[69,74,89–92] and snRNAseq datasets,[69,74,88] comparing HD versus control across different brain regions (Figures 3 and 4). We downloaded the

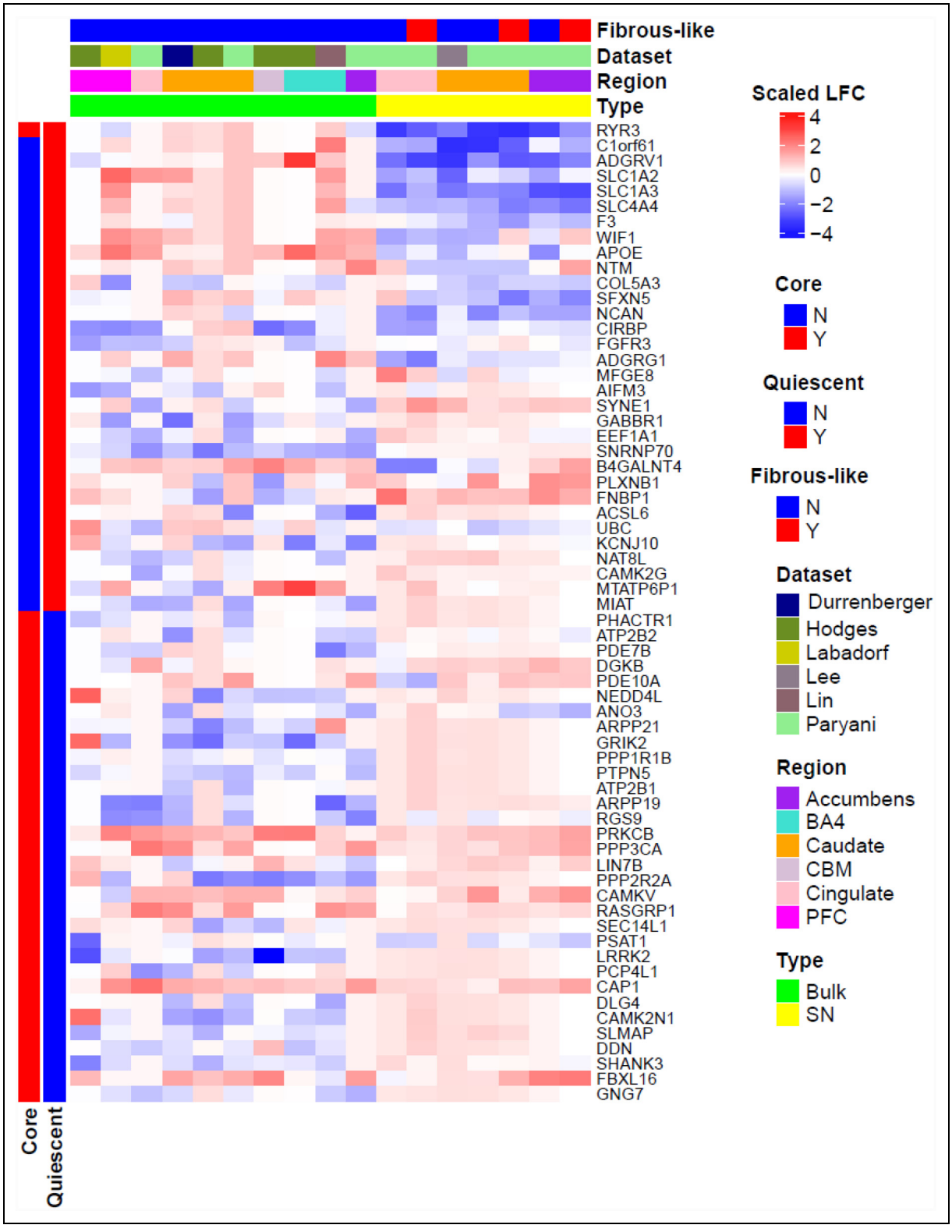

Figure 4. A heatmap showing the column-scaled log fold change (LFC) values (normalized within dataset). Select astrocyte genes are shown – see row side colors (Core genes and Quiescent protoplasmic genes). The dataset, brain region, dataset type (bulk vs. snRNAseq), and fibrous-like vs. protoplasmic for snRNAseq datasets are shown on the top. BA4: motor cortex; CBM: cerebellum; PFC: Prefrontal cortex; SN: single-nucleus RNAseq.

bulk transcriptomics datasets from www.hdinhd.org, and the supplementary results from Lee et al. (2020). Astrocyte DEG lists are not available in the supplementary results of more recently published datasets.[94–96] Importantly, the cohorts are diverse and include a broad range of samples that are not equally distributed across Vonsattel grade. Also, the differentially expressed genes we extracted from these datasets did not account for HD Vonsattel grade as a covariate. Regression analysis to extract gene expression changes in astrocytes that correlate with HD grade or CAG repeat length is needed.

In the snRNAseq datasets, *SLC1A2* and *SLC1A3* were decreased in most brain regions examined (caudate/putamen,[88] caudate, accumbens, and cingulate[74]). This effect was seen in both protoplasmic and fibrous-like astrocytes. Similarly, the ryanodine receptor 3 (*RYR3*), encoding a protein involved in releasing calcium from the sarcoplasmic reticulum, was decreased. Conversely, the gene encoding glutamine synthetase (*GLUL*) was decreased only in caudate astrocytes. Other genes we previously described in cortical, quiescent, protoplasmic astrocytes were also decreased across the two snRNAseq datasets in different regions, including *ADGRV1* and *UBC* (Figure 4). When comparing the snRNAseq changes to those in bulk tissue studies (bulk RNAseq and microarray studies), the cell-type-specific changes were not clearly seen. The reduction of subsets of the core gene set[75] was evident in the caudate nucleus, but the variability between studies (cohorts and the analysis modality) poses challenges in comparing datasets without additional analysis to regress out platform-related effects. It is noteworthy that bulk-level studies are influenced by cellular composition and represent the average expression across all cell types. Therefore, negative findings in these studies should be interpreted with caution.

The consistent reduction of glutamate transporter levels (*SL1A2* and *SLC1A3* – EAAT2 and EAAT1), coupled with the reduction of glutamine synthetase that converts glutamate to glutamine, implicate glutamate toxicity in the pathology of HD. However, results of several biomarker studies that quantified the levels of glutamate as well as other metabolites using magnetic resonance spectroscopy did not show consistent changes in glutamate levels in the caudate nucleus or the putamen.[97] That said, two studies found an increase in the ratio of glutamate to glutamine in presymptomatic HD patients compared to controls in the putamen. Other studies showed a reduction in this index when comparing manifest HD to pre-manifest HD in ~22% of cases. In the caudate nucleus, glutamate levels were largely unchanged in HD cases, including premanifest HD – please refer to Lozada et al. (2024) for details.[97] These differences may arise due to heterogeneity within the cohorts, sensitivity of the methods, and functional differences between the putamen and the caudate. Overall, additional work is needed to further explore glutamate excitotoxicity as a component of neurodegenerative mechanisms in HD.

Changes in astrocyte lipid metabolism

Over many years, a number of studies have examined levels of different lipids and biosynthetic lipid enzymes in the human HD brain. The most studied changes are in the levels of cholesterol and cholesterol esters. For original work and reviews, see.[98–107] That said, there is a significant level of variability in the findings of these studies. The sources of variability encompass the use of different brain regions and different sample sizes, not stratifying patients by clinical symptoms, examining patient tissue from different Vonsattel grades, and differences in the measurement platforms. In addition, these studies examine bulk-level human postmortem brain samples from the caudate, putamen, or cortex. Thus, it is difficult to ascribe specific changes to astrocytes or other cell types.

With these caveats, we will focus on specific changes relevant to astrocytes. First, several cholesterol metabolism enzymes are decreased in the human striatum in grade 1 and grade 2 HD donors.[104] For example, cholesterol 24-hydrolase (CYP46A1), an enzyme needed for the elimination of cholesterol, is decreased at the RNA level in striatal astrocytes as measured with snRNAseq[74,88] and with bulk RNAseq in the caudate.[74,89] Human astrocytes have higher levels of CYP46A1 RNA compared to neurons.[71] Protein studies confirm the reduction of CYP46A1 in HD using Western blots from grade 4 HD and control putamen tissue.[102] Interestingly, in mouse models, an increase in this enzyme is neuroprotective.[108] Together, the reduction of CYP46A1 may result in higher levels of cholesterol, and the inability to eliminate excess cholesterol in the HD striatum may represent a failure to mount a neuroprotective response.

Consistent with CYP46A1 reduction, two studies measured the levels of 24-hydroxycholesterol, the product of cholesterol hydroxylation by CYP46A1, and found them to decrease in the caudate and putamen tissue of grade 4 HD compared to controls.[102,103] 24-Hydroxycholesterol, measured in blood, decreases in parallel with caudate volume.[109] Interestingly, the changes in 24-hydroxycholesterol were not significant in the cerebellum or cortex.[102,103] This may reflect regional heterogeneity in lipidomic pathology in HD, which is an understudied area.

Second, cholesterol esterification, which depends on the activity of the enzyme cholesterol acyltransferase (ACAT1),[110] is also altered in HD brains.[103] Philips and colleagues did not find significant changes in the levels of ACAT1 protein or RNA, and snRNAseq studies also do not show significant changes in striatal astrocytes.[74,88] Nonetheless, the levels of cholesterol esters are increased in the caudate and putamen,[103] and were correlated with a gene signature associated with HD grade when measured in the cingulate cortex.[74] The increase in cholesterol esters may result from a functional increase in enzyme activity or available precursors and may not be reflected in protein

level changes on the bulk tissue level or gene expression in astrocytes. It is also possible that cholesterol esterification occurs in cells other than astrocytes. Additional work is needed to investigate these questions in human brain tissue. For a review of the role of lipids as biomarkers for disease progression and lipidomic changes in HD, see.[99]

Third, since astrocytes shuttle cholesterol to neurons and deliver it via apolipoprotein E (APOE),[111] astrocyte pathology may affect neuronal lipid states. In fact, HD mouse astrocytes display reduced APOE levels,[112,113] and HD astrocyte-derived media lacking APOE failed to rescue neuronal neurite growth impairment of HD neurons, compared to control astrocyte-derived media.[112] Other studies have also shown reduced astrocyte cholesterol transport to neurons via APOE, in a mechanism involving disruption of SREBP nuclear translocation.[114] Data directly focused on astrocytic APOE in human tissue are lacking. Nonetheless, our analysis of astrocytes in the cingulate cortex, nucleus accumbens, and the caudate nucleus, based on snRNAseq, revealed a decrease in the expression of *APOE*.[74] This was also seen in an independent study by Lee et al. (2020).[88] In our study, we performed targeted lipidomic analysis of the cingulate cortex, which is useful for quantifying changes in a limited, predetermined set of lipids. We did not find a significant change in the levels of cholesterol esters or free cholesterol, which may be due to sample heterogeneity (different Vonsattel grades) and differences between the cortex and striatum in HD. However, when combined with paired transcriptomic data, the levels of cholesterol esters were correlated with HD grade.[74]

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry is a relatively new technique that can spatially map and quantify metabolites[115] and lipids.[116] Hunter et al. (2018) used this approach in control and grade 3 HD tissue to measure changes in lipids in the astrocyte-rich subependymal zone adjacent to the caudate nucleus.[117] Note that the subependymal zone contains CD44 + astrocytes.[63] Hunter et al. (2018) identified loss of triglycerides and accumulation of sphingomyelins in HD subependymal zone tissue, which suggests that fatty acid metabolism is altered in astrocytes in HD. In a recent study, the authors found that the HD striatum demonstrates diminished levels of docosahexaenoic and adrenic acids, the ganglioside GM1, and glycerophospholipids with long polyunsaturated fatty acyls. Conversely, the abundance of sphingomyelins and glycerophospholipids with shorter monounsaturated fatty acyls was focally increased in HD.[118] Given that MALDI imaging mass spectrometry does not yet have cellular resolution, one cannot attribute any of the above findings to changes in astrocyte function.

Interestingly, in our work,[74] we found differences in other classes of lipids that astrocytes are intimately involved in metabolizing, the polyunsaturated fatty acids. We found that several polyunsaturated long-chain fatty acids, including ones with 20 and 22 carbons, with two and three unsaturated bonds, increased in HD across various grades. One of these fatty acids, di-homo-γ-lenolenic acid (22:3), sensitized neurons to death upon oxidative stress *in vitro*. In support of the role of astrocytes in this process, several genes involved in fatty acid elongation and desaturation, such as the desaturase *FADS2* and the elongase *ELOVL2* were altered in protoplasmic astrocytes in the cingulate in our dataset and the caudate in both our dataset and Heiman's group's data.[88] Additional validation results are needed to confirm if these changes are reflected in changes at the protein level. Together, these data may provide yet another pathway that may connect altered fatty acid metabolism with neurodegeneration in HD.

Gain of a compensatory function: upregulation of metallothioneins

The levels of a number of metals are increased in the striatum of the brains of individuals with HD. These include copper, iron, and zinc.[119] Copper accumulates in the human and mouse HD brain and has drawn attention for several reasons.[119,120] Copper can bind to huntingtin and to its N-terminal fragments, and promote its aggregation *in vitro* and its accumulation in mammalian cells.[120,121] Copper is reduced by its interaction with N-terminal huntingtin, and thus changes redox states in the cell.[120] Thus, excess copper may be in part responsible for the oxidative stress found in HD brains.[122]

Metallothioneins (MTs), are a highly-conserved class of small cysteine-rich molecules that bind heavy metals, particularly copper and zinc.[123,124] Thus, MTs can be thought of as neuroprotective in disorders in which metals accumulate in the brain. Indeed, MT3 is protective for HeLa cells against huntingtin toxicity.[121] Protoplasmic astrocytes in the cingulate cortex of HD brains increase their expression of metallothioneins.[69,74] These astrocytes appear to go through a number of "states" in the HD cingulate, beginning with quiescent protoplasmic astrocytes of the normal cortex, followed by an increase in MTs, followed by a loss of expression of normal protoplasmic genes and proteins and MTs, and finally reaching a disease associated-state that shares some properties with fibrous-like astrocytes, including the presence of CD44.[69,74] Astrocyte MT expression was different in the cingulate from that in the caudate, since the proportion of astrocytes positive for metallothioneins 1, 2, and 3 were increased in the relatively less severely affected cingulate, but were either unchanged (MT1/2 proteins) or even decreased (MT3) in the severely affected caudate. The findings from Lee et al. (2020) also support downregulation of MTs in human striatal HD astrocytes.[88] Co-culture studies of human astrocytes with HD patient-derived directly-converted medium spiny neurons and murine neurons exposed to the mitochondrial toxin Rotenone support that MT3-high astrocytes are neuroprotective.[74] Furthermore, increasing

MT3 expression in the human cultured astrocytes increased their ability to buffer glutamate, a key protoplasmic function.[74]

Finding increased MTs in cingulate astrocytes but not in caudate astrocytes, might mean that caudate astrocytes are not as protective in this way. Alternatively, it could mean that the caudate astrocytes have reached a more disease-associated state in their evolution during the progression of HD.

In addition to MTs, there are other potential neuroprotective pathways that astrocytes can engage in. One such pathway is JAK-STAT signaling. There is evidence in mouse models of HD that astrocytes also engage in a compensatory response centered around JAK2-STAT3 activation. This response induces a reactive state in which proteostasis is activated, thereby ameliorating the formation of huntingtin aggregates in astrocytes and potentially in neurons via chaperone-rich, astrocyte-derived exosomes.[125] This signaling axis has not been fully explored in human tissue, and additional work is needed to increase our understanding of this mechanism.

Accumulation of HTT aggregates in astrocytes

Colocalization studies that label HTT aggregates and astrocytes by GFAP immunostains in human tissue have consistently shown the presence of HTT protein aggregates in the nuclei and somata of astrocytes.[69,81,126,127] It is unclear whether these aggregates are endogenous astrocytic HTT or aggregates in the neuropil that are engulfed by astrocytes. Detailed quantification studies of HTT aggregates in astrocytes comparing the frequency of nuclear versus cytoplasmic aggregates, as well as the types of aggregates, are needed to further understand the nature of astrocytic aggregates, whether and how they differ from neuronal aggregates, and their localization in regard to vulnerable versus resilient brain regions and cells.

Absence of significant CAG repeat expansion in astrocytes

A somatic expansion of CAG repeats over the germ cell repeat length occurs in the brain, leading to a mosaicism.[128] These somatic expansions occur primarily in neurons. Thus, striatal MSNs and cholinergic interneurons, cortical pyramidal neurons in layers 5–6, and Purkinje cells show significant expansions above the modal length of the inherited mutant allele.[95,129] Astrocytes, however, do not show the profound repeat expansions seen in MSNs, gaining only a few units above the modal length, if any.[95,129] This is in contrast to the increases in repeat expansions observed in cultured, immortalized astrocytes and astrocytes derived from iPSCs from HD individuals.[130,131] In the latter study, HD astrocytes of 125Q and 180Q repeat lengths showed higher degrees of DNA damage than astrocytes from individuals with normal repeat lengths.[131] A recent study that used single-nucleus CAG repeat expansion measurement leveraging long-read RNAseq has proposed that all brain cells exhibit somatic repeat expansion to varying degrees, with most cell types showing CAG repeats within a few units of the modal length.[96] These data are consistent with the results from the Heintz group,[95,129] which measured CAG repeats from genomic DNA extracted from sorted nuclei at the bulk level, and could quantify 113 CAG at the upper end. Therefore, CAG repeat expansion appears to be mild, if at all present, in HD astrocytes *in vivo*.

Conservation of HD astrocyte phenotypes in HD mouse models

The constellation of phenotypes observed in human postmortem brain tissue astrocytes can be modeled in various systems, including mice. This review is not intended to summarize changes in astrocytes in murine models of HD—the reader is referred to several reviews.[132–136] Here, we cite examples from the literature that argue for the conservation of some of the phenotypes seen in human brain tissue. The loss of protoplasmic function, exemplified by the reduction of GLT-1 and GS, has been reproduced in both rapidly progressive HD mice (R6/2)[137] and more slowly progressive Q175 mice.[138] Astrogliosis, defined by an increase in GFAP, on the other hand, is mild and only seen in advanced ages in the Q175 mice,[138] the Q140 mice,[139] but not the R6/2 mice.[140] Astrocytic HTT aggregates can be readily seen in HD models.[127,141] Many of the findings on cholesterol and lipid abnormalities in HD have been discovered and described in HD mice (see above). Notably, in HD mice, most of the work has focused on somatic repeat expansion in neurons, and data on somatic repeat expansion in astrocytes in HD mouse models is sparse. Finally, whether murine astrocytes upregulate a neuroprotective response, including metallothioneins, as we have seen in the human HD cortex, remains to be investigated. As noted in earlier sections, there are inherent differences between human and mouse astrocytes in sensitivity to hypoxia and capacity to promote neuroinflammation, so modelling these aspects of astrocyte function may be limited. Still, mouse models can offer great insights into disease-relevant astrocyte phenotypes and are particularly useful for measuring functional changes.

Changes in fibrous-like astrocytes in HD

Limited research has been conducted to detail the pathology within fibrous-like astrocytes, which, as mentioned in the initial section of this review, differ from protoplasmic astrocytes and occupy distinct anatomical locations, including the white matter. This is significant because abnormalities in white matter detectable via magnetic resonance

imaging are among the earliest findings in HD patients years prior to the onset of motor symptoms.[142] In Paryani et al. (2024), we classified HD and control astrocytes into fibrous-like and protoplasmic categories, revealing shared gene expression changes between them alongside notable differences; for example, gene ontologies related to solute:sodium symporter activity and transmembrane transporter activity were enriched in genes decreased in HD fibrous-like astrocytes but not in protoplasmic astrocytes across different regions.[74] Likewise, gene ontologies associated with ERBB2 signaling in cancer, cell morphogenesis, and differentiation were enriched in genes increased in HD fibrous-like astrocytes but not in protoplasmic astrocytes across different regions. When analyzing the proportion of fibrous-like astrocytes with altered MT3 levels in HD (as a proxy for neuroprotective status), no differences were observed between controls and HD subjects, contrary to findings with CD44-negative, protoplasmic astrocytes. Together, these observations suggest that fibrous-like astrocytes are also affected in HD, and patterns of dysregulation of fibrous astrocytes may relate to the white matter abnormalities seen early on in HD.

In our work, a disease-severity associated signature that consists of genes positively correlated with CAG repeat length (e.g., *CD44*, *OSMR*, *CHI3L2*, and *SERPINA3*) had the highest scores in two striatal fibrous-like astrocyte subclusters.[74] Whether these clusters are fibrous astrocytes that become reactive in HD, or protoplasmic astrocytes that adopt a more fibrous-like phenotype remains unresolved. We hypothesize that both phenomena occur in HD.

Recently, work from the Heiman group identified a subset of vasculature-coupled astrocytes that were increased in HD postmortem tissue.[76] In this subset, the authors found several innate immune activation genes to be increased in HD, including *HSPH1, IL6R, IRF3, NFKBI,* and *NFKBIA*. The latter gene was also differentially increased in Paryani et al. (2024). Together, these findings point to the potential immune activation in vasculature-coupled and fibrous-like astrocytes in HD. Additional work is required to further investigate changes in these less well-studied astrocyte subtypes.

Astrocyte states in HD

We conceptualize astrocyte changes in HD, as the disease progresses, as a continuum of "States" rather than an abrupt change from a quiescent protoplasmic state to a disease-associated reactive state that resembles fibrous astrocytes. Furthermore, not all astrocytes enter this reactive continuum synchronously. That is, at a given stage in the course of the disease, there are astrocytes at different stages of reactivity. We hypothesize that the reduction of protoplasmic function and the gain of neuroprotective functions occur first, prior to the loss of neuroprotective function, and then the development of a severe, reactive, disease-associated state that resembles fibrous astrocytes. Thus, along this trajectory of transitions, the neuroprotective metallothioneins are eventually diminished. Subsequently, more and more astrocytes become closer to the disease-associated state, which resembles fibrous astrocytes.[74] In our snRNAseq datasets, protoplasmic astrocytes first expressed quiescent astrocyte genes, then co-expressed quiescent astrocyte genes with metallothioneins, and then diminished quiescent astrocyte genes as disease-severity-associated genes were upregulated.

It is worth noting that differences in astrocyte states in different brain regions in HD may be related to the severity of neurodegeneration in a correlative or even partially causative manner. This heterogeneity may also be influenced by the differences between cortical and striatal astrocytes. The latter question is fascinating to us and is the subject of active research in our laboratory.

Open questions

There are several critical questions related to astrocyte pathology in HD.

First, the spatial and causal relationships between the reactive astrocyte states and neuronal pathology are not known. For example, we do not know if neuroprotective astrocytes are spatially correlated with resilient or resistant neurons or if neuroprotective astrocyte states determine vulnerability to neurodegeneration. One confounding issue is that astrocytes express the *mHTT* and may undergo cell-autonomous transformations due to the accumulation of the mutant protein. However, astrocytes also respond to pathology in their local environments. Thus, the degeneration of medium spiny neurons in the striatum will produce secondary changes in astrocytes,[143] as does neuro degeneration in many neurological disorders. Thus, teasing out what is a primary astrocyte dysfunction from that which is reactive to neuronal degeneration is a challenge in human studies.

Second, we do not know if there is a direct lineage relationship between neuroprotective astrocytes and disease-associated astrocytes. Do the transitions from protoplasmic quiescent astrocytes to disease-associated reactive astrocytes necessarily go through a neuroprotective state? Although pseudotime analysis sheds light on this question, designing experiments to directly observe these transitions is necessary to resolve this question.

Third, although we know that disease-associated astrocytes are abundant in the caudate nucleus, where neurodegeneration is most severe, it is unclear whether this state is preceded by a neuroprotective astrocyte state that is no longer present at the time of postmortem examination. Studies into early-stage grade 0 HD are needed to examine this question.

Finally, it is unknown how HTT aggregate-bearing astrocytes differ transcriptionally or functionally from

ones that do not have aggregates. Highly multiplexed immunohistochemistry studies are needed to address this question.

Conclusions

Astrocytes in HD appear to have both neuroprotective and disease-associated states. Differences in the abundance of astrocyte states are correlated to HD severity as well as to regions that are differentially affected in HD. However, a general theme in HD astrocytes is that they downregulate genes and decrease proteins that buffer extracellular levels of glutamate. Astrocytes are closely connected with altered lipid metabolism and/or lipid-transport function, and changes in HD may affect neuronal lipid components, neuronal membranes, and process growth. Recent studies have attempted to disentangle how much of these changes are cell-autonomous, that is, due to the expression of mHTT in astrocytes themselves, and how much is due to a "reactive" state due to neuronal degeneration.[143] However, the fact that not all astrocyte phenotypes are modeled in HD mouse models, and that human astrocytes differ from their murine counterparts, leaves more to be learned.

Acknowledgements

We thank the patients and their families for their precious donations to the NY brain bank.

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Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Hereditary Disease Foundation.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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