Cell-intrinsic glial pathology is conserved across human and murine models of Huntington's disease

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Graphical abstract

Glial pathology across models of Huntington disease

Truncated mHTT
- mHTT-human striatal astrocytes
- R6/2

Full length mHTT
- HD-derived human ESC astrocytes
- zQ175

Astrocyte isolation
- Fluorescence Activated Cell Sort

Gene expression analysis

SREBF1/2
- TCF7L2
- RUNX1

SIRT2
- BMP4
- NRG

mTORC1
- ATF4
- PAX6

Both model-specific and shared dysregulated glial gene sets

Highlights

- Glial transcription differs between cells expressing full-length and exon1-only mHTT
- Truncated mHTT inhibits glial cholesterol pathway expression; full-length mHTT does not
- Astrocytic structural genes are dysregulated by mHTT-expressing glia in all models
- Mutant HTT-expressing mouse astrocytes manifest altered fiber distributions in vivo

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In brief

Benraiss et al. assess astrocytic and microglial gene expression across mouse and human models of Huntington’s disease, to define commonalities that may contribute to HD pathogenesis. They report differences between glia expressing full-length and exon 1-only mHTT and identify a core set of dysregulated pathways that predict glial pathology.
Cell-intrinsic glial pathology is conserved across human and murine models of Huntington’s disease

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SUMMARY

Glial pathology is a causal contributor to the striatal neuronal dysfunction of Huntington’s disease (HD). We investigate mutant HTT-associated changes in gene expression by mouse and human striatal astrocytes, as well as in mouse microglia, to identify commonalities in glial pathobiology across species and models. Mouse striatal astrocytes are fluorescence-activated cell sorted (FACS) from R6/2 and zQ175 mice, which respectively express exon1-only or full-length mHTT, and human astrocytes are generated either from human embryonic stem cells (hESCs) expressing full-length mHTT or from fetal striatal astrocytes transduced with exon1-only mHTT. Comparison of differential gene expression across these conditions, all with respect to normal HTT controls, reveals cell-type-specific changes in transcription common to both species, yet with differences that distinguish glia expressing truncated mHTT versus full-length mHTT. These data indicate that the differential gene expression of glia expressing truncated mHTT may differ from that of cells expressing full-length mHTT, while identifying a conserved set of dysregulated pathways in HD glia.

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive motor dysfunction, cognitive decline, dementia, and death with an invariably fatal outcome. The disease is caused by an expansion of the polyglutamine stretch encoded by the first exon of the gene huntingtin (HTT) (Andrew et al., 1993; Snell et al., 1993). Mutant HTT (mHTT) is ubiquitously expressed in all tissues; however, striatal medium spiny neurons (MSNs) of the basal ganglia and, to a lesser extent, cortical pyramidal neurons display an early and selective vulnerability (Hedreen et al., 1991; Mitchell et al., 1999). Several mechanisms including both toxic gain-of-function and trophic loss-of-function have been implicated in HD pathogenesis, however, the cellular mechanisms underlying the selective vulnerability of MSNs are still unclear (Zuccato et al., 2001; Clabough 2013). Yet, recent studies have implicated astrocytic pathology as contributory to neuronal dysfunction in HD (Benraiss et al., 2016; Jiang et al., 2016; Khakh et al., 2017; Osipovitch et al., 2019), and the regional heterogeneity of astrocytes—and hence of glial dysfunction—may contribute to the regional selectivity of neuronal loss in HD (Khakh 2019).

We have previously noted that mHTT-expressing human glial progenitor cells manifest delayed and deficient differentiation, and the astrocytes generated from these progenitors are themselves structurally abnormal and functionally deficient. They manifest the downregulated expression of a host of glutamate receptor components as well as of potassium channels and transporters, which together may account for the observed hyperexcitability of resident striatal neurons (Osipovitch et al., 2019). Yet, this analysis was performed on human embryonic stem cell-derived glial progenitors and their derived astrocytes in vitro. Such in vitro models of HD lack the paracrine interactions that modulate gene expression in vivo and which may do so selectively as a function of disease. More recent efforts have purified astrocytes from HD mouse models and correlated their differential gene expression to that of postmortem human brain, but these studies have lacked direct comparison to the expression signatures of mHTT-expressing human astrocytes (Diaz-Castro et al., 2019).

HD pathogenesis is also accompanied by the activation of microglia, critical mediators of the innate immune system of the CNS (Harry and Kraft, 2008; Politis et al., 2011). Microglial activation starts long before disease onset, suggesting that microglial pathology may appear early in disease progression, potentially so early as to be cell-autonomous (Björkqvist et al., 2008). At later stages of the disease, microglial activation tightly correlates with the degree of striatal and cortical neuronal loss, suggesting
the possibility of a causal linkage between microglial state and neuronal degeneration (Sapp et al., 2001). Previous studies indicated that restricted expression of mHTT in microglia was sufficient to induce both pro-inflammatory gene expression and frank inflammation, with an associated exacerbation of neurodegeneration (Crotti et al., 2014). Nonetheless, it has been unclear whether microglial transcription was differentially perturbed as a function of mHTT gene length, or whether commonalities between mouse and human HD microglial gene expression might be identified.

To address these issues, in this study, we defined the gene expression patterns of both human and mouse astrocytes, across both in vitro and in vivo models that expressed either full-length or truncated mHTT. We did so as a means of defining those shared features of astrocytic pathology that, by virtue of their commonality across species and models, might indicate causal relevance in HD pathogenesis. In mice, we first compared the RNA expression profiles of Glt1-sorted striatal astrocytes and CD11b-defined microglia separately extracted from two distinct HD mouse models, the R6/2 and zQ175 mice; these mice, respectively, harbor the truncate exon 1-only or full-length mHTT genes. To identify those mHTT-regulated glial genes and pathways similarly dysregulated in human glia, we then assessed gene expression by astrocytes differentiated from human embryonic stem cells (hESCs) derived from HD subjects expressing the full-length mHTT gene, as well as by fetal human striatal glia transduced to express truncated mHTT. We found that the transcriptional profile of mHTT-expressing astrocytes was altered in both mouse and human glia compared to their respective wild-type (WT) controls, but in both species, it was strikingly distinct between cells expressing the truncated exon 1-only and full-length versions of mHTT. At the same time, differentially regulated ontology classifications were shared across models and species (i.e., by both truncated and full-length mHTT forms in both species), revealing a predominance of shared terms associated with morphological structure. On that basis, we assessed astrocytic morphology in R6/2 mice and confirmed that they were indeed structurally aberrant relative to those of HTT WTs. These data provide insight into the transcriptional basis for cell autonomous glial pathology in HD. Furthermore, they serve to highlight that some mHTT-dependent dysregulation may be an artifactual product of the specific, exon 1-only truncated mHTT forms used in R6/2 mice and mHTT-transduced human glia, which as a result may not faithfully reflect the molecular concomitants of glial pathology in HD.

**RESULTS**

**Murine HD astrocytes manifested mHTT-length-dependent changes in gene expression**

Murine models of HD comprise either the full-length or truncated mHTT gene, with varying CAG repeat lengths leading to a spectrum of phenotypes. These murine models exhibit pronounced neuronal dysfunction that can be partially alleviated by glial replacement, suggesting a contributory role for glia in the neuronal pathology of HD (Bernaiss et al., 2016). To better define the molecular underpinnings of HD glial pathology, we sought to identify a conserved astrocytic transcriptional signature shared by different murine HD models in vivo. To this end, we analyzed the gene expression of mature HD-derived astrocytes relative to their WT littermates in vivo using cell marker-directed fluorescence-activated cell sorting (FACS) (Lovatt et al., 2007) to extract astrocytes from acutely dissociated striata of either 6- and 12-week-old R6/2 mice, or from 6- and 12-month-old zQ175 mice, along with their respective matched littermate controls (Figures 1A and S1). This difference in time points used between the two mouse models was based on the much later onset of symptoms in zQ175 mice (Peng et al., 2016). Disease-derived astrocytes for each model were compared to WT littermate controls at their respective time points (R6/2 and WT; n = 5-6/group for each, at both 6 and 12 weeks; zQ175 and WT: n = 4/group, at 6 and 12 months).

Dissociated striata were immediately processed for FACS based upon Glt1 and CD11b immunoreactivity. Glt1 (EAAT2/SLC1A2) is expressed by astrocytes (Rothstein et al., 1994), but also by activated microglia (López-Redondo et al., 2000), whereas within the CNS, CD11b is expressed only by microglia (Akiyama and McGeer 1990). As such, Glt1+/CD11b−-sorted cells could be defined as highly enriched for astrocytes and CD11b+ cells as microglial (although some parenchymal entry of peripheral CD11b+ leukocytes cannot be excluded). Overall, no significant difference was observed in the relative proportion of either astrocytes or microglia across genotypes and time points, except in 12-week-old R6/2 mice in which a lower fraction of Glt1+/CD11b−-defined astrocytes were noted compared to WT littermates (Figure S1C) (p < 0.05 by 2-way ANOVA, followed by Tukey’s multiple comparison tests). qRT-PCR confirmed the downregulation of Glt1/Slc1a2 gene expression by Glt1+/CD11b−-sorted R6/2 striatal astrocytes at 12 weeks, compared to their WT counterparts (0.51 ± 0.05 versus 0.9 ± 0.06, mean ± SEM; n = 3 each, p < 0.01, t test), whereas the expression of other astrocyte-specific markers, such as Gfap, Aqp4, or Glast (Eaat1/Slc1a3), was not affected (Figure S2); Glt1 was thus selectively downregulated with age in R6/2 mice, relative to other astrocytic markers. Because our sort purification was directed at Glt1, it is possible that a minor fraction of astrocytes in the R6/2 12-week group might have downregulated Glt1 so severely as to be excluded from this study. Yet, because any significant Glt1−/− gial subpopulations would have been represented in the Glt1+/−/CD11b− group and because the fraction of Glt1−/−/CD11b− cells did not differ significantly between R6/2 and WT mice (Figure S1C), it would seem unlikely that any significant fraction of astrocytes were excluded by virtue of Glt1−/− based sorting. To confirm this point, we used a second astrocytic cell surface marker, GLAST, to assess if there was any loss of astrocytes to the Glt1−/−/CD11b− pool. We found that despite the downregulation of Glt1, GLAST+ cells comprised less than 5% of all Glt1−/−/CD11b− cells in both R6/2 and WT striata, and this fraction did not differ between the R6/2 and WT mice (Figure S1E). Together, these data argue that the lower fraction of Glt1−/−/CD11− astrocytes in 12-week R6/2 striata was a function of disease-associated astrocytic loss, and the expression data derived from Glt1+/−/CD11− cells was reflective of most if not all striatal astrocytes in these mice.

To define the effect of mHTT expression on glial transcription in vivo, we then subjected these sorted astrocytes and microglia
from R6/2 and zQ175 mice to whole-genome microarray analysis using Affymetrix U430A chips (n = 4–6 per genotype, time point, and population). From both genotypes, Glt1+/CD11b− cells highly expressed AQP4, GFAP, Glt1/SLC1A2, and TNC, consistent with their astrocytic phenotype, when compared to both CD11b+ and Glt1−/CD11b− cells. These other fractions were non-astrocytic; the microglial markers CD68 and AIF1 were highly enriched in CD11b+ selected cells, while markers of other CNS cell types were enriched in the Glt1+/CD11b− cell fraction, which was profiled as a negative control (Figure 1B).

Principal component analysis (PCA) of R6/2 and control samples revealed tight clustering of biological replicates segregated by group, with larger differences emerging between WT and R6/2 astrocytes at the 12-week time point (Figure 1C). PCA of zQ175 astrocytes showed a similar, yet less drastic partitioning (Figure 1D). Scatterplots of HD probe intensities versus their matched controls reflected an increasing degree of transcriptional dysregulation in both R6/2 and zQ175 at later time points, mirroring the severity of their symptoms (Figures 1E and 1F). Differential expression (DE) analysis within these four groups identified 232 genes that were differentially expressed between R6/2 and WT astrocytes at 6 weeks (162 upregulated, 70 downregulated), and 4,526 genes at 12 weeks (2,121 upregulated, 2,405 downregulated). In contrast, in zQ175 astrocytes taken from 6-month-old mice, 47 genes were significantly differentially expressed (56 upregulated, 1 downregulated), while 801 genes were differentially expressed at 12 months (637 upregulated, 164 downregulated). Importantly, of the conserved differentially expressed genes between late time point R6/2 and zQ175 astrocytes, 92% were dysregulated in the same direction (Figure 1G; Table S1). Together, these data indicate that R6/2 astrocytes, bearing a long CAG repeat but a truncated HTT gene, suffer substantially more transcriptional dysregulation than do zQ175 astrocytes, which express an even longer CAG repeat but in the context of a full-length HTT gene, and that the different degrees

Figure 1. Gene expression by mouse astrocytes differs between cells with full-length versus truncated mHTT

(A) HD mouse and littermate control striata were dissociated, and Glt1+ astrocytes, CD11b+ microglia, and Glt1−/CD11b− cells isolated via FACS. RNA was extracted and profiled via microarray.

(B) Principal component analysis of R6/2 and control astrocytes showed segregation of R6/2 samples that intensified with age.

(C) zQ175 samples displayed similar but less drastic partitioning.

(D) Marker gene intensities confirmed enrichment of astrocytic genes.

(E and F) Time point-matched fold-changes of R6/2 (E) and zQ175 (F) astrocytes revealed increasingly dysregulated expression with age.

(G) Venn diagram illustration of differential expression within groups (adjusted p value <0.05, limma).
of mHTT-associated differential glial gene expression in these two models both mirror and portend the severity of each model’s clinical phenotype.

**Network analysis identifies distinct functional modules between mouse models**

We next asked whether there are shared molecular pathways underlying the common cellular pathology of mouse HD astrocytes, despite profound differences in the gene expression profiles of glia derived from the two HD murine models. To this end, we constructed a pathway enrichment network containing gene set comparison nodes, their differentially expressed genes, and significant Ingenuity Pathway Analysis (IPA) terms derived from each gene set (network in Figure 2A; module sorted heatmap in Figure 2B, pathway enrichment graphs in Figure 2C; complete network information in Table S2). Unsupervised modularity analysis was then utilized to aggregate closely related functions, genes, and comparison datasets (Subelj and Bajec, 2011). This approach identified four functionally distinct modules (M1–M4).

This approach highlighted striking divergence between R6/2 and zQ175 mice in their gene sets and derived modules. That is, components of M1 were well connected to the 6-week R6/2 comparison set, with near exclusive connectivity to the 12-week R6/2 and almost none with the zQ175 nodes, potentially highlighting a profound deregulation between models at any time point (Figure S3). IPA analysis revealed separation of control and diseased populations at the 6-week time point, that became markedly more distinct at 12 weeks (Figure S5A). Like the dimensional reduction behavior of zQ175 astrocytes, zQ175 microglia were very similar to their control counterparts at 6 months of age, with a larger effect noted at 1 year of age (Figure S5B). Differential expression within time points and models uncovered 47 significant genes between R6/2 and WT microglia at 6 weeks (39 upregulated, 8 downregulated) and 755 at the 12-week time point (330 upregulated, 425 downregulated) (Figure S5C). Differential expression was milder in zQ175 microglia, with 4 significant genes found between HD and control microglia at 6 months (all upregulated), increasing to 576 at 12 months (475 up, 101 down) (Table S1). Differences in microglial transcriptomes between models were very distinct, with only 72 differentially expressed genes shared between models at any time point (Figure S3). IPA analysis revealed a number of signaling families that were significantly and specifically associated with the induction of inflammation in R6/2 microglia (Table S3). These included IRF7, interferon-alpha (IFNA), STAT1, and STAT3 signaling, none of which were significantly enriched in zQ175 microglia (Figures SSD and SSE). Interestingly, these inflammatory terms were also noted in 6-week R6/2 astrocytes, although to a much lesser extent (IRF7 signaling, p = 1.9E−08; IFNA, 9.5E−05; STAT1, p = 6.7E−07; STAT3, p = 6.3E−05; Table S2). Significant functional

Figure 2. Network analysis reveals distinct dysregulation of R6/2 and zQ175 astrocytes

(A) A network was constructed from differentially expressed (DE) genes (circles) for each comparison (squares), along with their significantly dysregulated IPA terms (triangles). Unsupervised modularity determined four modules (M1–M4: M1: bronze; M2: pink; M3: teal; and M4: blue), each of closely related genes, annotations, and DE gene sets, which were then clustered. Each module’s percent of total IPA connectivity is listed. The sizes of the triangles (IPA terms) and squares (DE genes) are proportional to the node degree.

(B) Heatmap representation of module-associated DE genes, with their respective significance in each group.

(C) Significance of highly enriched IPA terms within each module and group.
terms associated with microglia derived from 12-month-old zQ175 mice principally included those associated with cell-cell contact and morphology, as well as with TCF7L2 signaling. These latter terms were also shared with astrocytic cohorts, with differential expression of cell-cell contact and TCF7L2 signaling genes appearing at only the late time points in both models (Table S2).

On the basis of these data, we next asked how conserved the differences across both models and time points were between astrocytes and microglia (Figure S5F; Table S4). These analyses recovered few shared genes at both early mouse model time points (12 in R6/2, 1 in zQ175) with larger cohorts in the later time points (295 in R6/2, 140 in zQ175). Examination of these later time-point gene sets revealed a large level of directional concordance between cell types in each model, with inflammatory genes (STAT1, IRF9, IFIT1, CSF1) conserved in R6/2 and signaling ligands BMP4 and FGF1 in zQ175 (Figure S5G). However, these shared microglial and astrocyte gene sets were not conserved across models in large numbers, with only 8 genes found in common in diseased R6/2 and zQ175 (Figure S5H). Thus, although the oldest R6/2 astrocytes expressed an inflammatory signature akin to that of microglia, younger R6/2 astrocytes, and zQ175 astrocytes of both young and older mice, did not do so, suggesting that the acquisition of an inflammatory phenotype by HD astrocytes might be especially prominent in animals expressing truncated, rather than the full-length, mHTT.

**Mutant HTT overexpression drives distinct expression profiles in human astrocytes**

We next asked if truncated mHTT could induce similar consequences in human astrocytes. To this end, we isolated glial cells from the lateral ganglionic eminence of 14–18 week gestational age (g.a.) human brain tissue (n = 3; Figure 3A). Cells were transduced with lentivirus constitutively expressing EGFP in tandem with the first exon of either WT HTT (LV-HTTQ23), mHTT (LV-HTTQ73), or control expressing reporter only (LV-EGFP). EGFP was used together with antigenic selection of mHTT (LV-HTTQ73), or control expressing reporter only (LV-EGFP). EGFP was used together with antigenic selection of mHTT RNA sequencing (RNA-seq) dataset that we had previously generated (Ospovitch et al., 2019). These data were generated from five hESC lines (3 mutant, 2 control) that were differentiated in vitro to astrocytes. A differentially expressed gene set comparing mRNA expression by the pooled mutant lines relative to their pooled controls was obtained from our publicly available database of glial gene expression (https://cnogoldmanlab.genialis.com/rochester/visual_analysis/gene_view?s=dc851949-5a55-4999-b21b-f770f8e08338) for inclusion in IPA network analyses (Figure 4A; Table S5). Construction of this network was carried out as in Figure 2, with unsupervised modularity analysis identifying four modules (M1–M4). M1 primarily housed IPA terms contributing to cholesterol biosynthesis, as enriched by genes MVD, MVK, HMGR, INSIG1, ACAT2, SQLE, LDLR, and DHCR7. Interestingly, strong association with this module was only observed in the truncated mHTT cohorts LV-HTTQ73 versus LV-EGFP and LV-HTTQ73 versus LV-HTTQ23. M1 also contained terms strongly associated with stress, including ATF4 and NUPR1 signaling, along with endoplasmic reticulum stress and the unfolded protein response (Pakos-Zebrucka et al., 2016). The selective expression of these functions by the LV-HTTQ73 transduced glia, rather than by the mHTT-expressing hESC-derived cells, suggested their preferential association with the truncated mHTT exon1, rather than with mHTT per se.

The pathway components of M2 consisted primarily of terms modulating neuronal signaling, including neurotransmission and BDNF signaling, but it also included the histone-lysine H3K9 methyltransferase SETDB1 and terms associated with progressive neurological disorders. M3 was enriched for a number of pathways previously associated with HD, including IFNG, TGFβ1, and SNCA signaling, whereas M4 possessed IPA terms modulating migration, along with both TNF and CTNBB1 signaling. Notably, M2 and M4 were primarily associated with full-length hESC-derived astrocytes and the LV-HTTQ73-transduced human glial cohort; the pathology evidenced in each of these modules tracked the presence of CAG repeat expansions.

**Transcriptional patterns common to mouse and human HD astroglia highlight structural pathology**

We next compared across all four of these models, in an effort to parse out potential artifacts of gene length, while defining commonalities across both models and species. To accomplish this, differentially expressed gene sets derived from both zQ175 and R6/2 mice, each at both early and late time points, as well as from LV-HTTQ73 versus LV-EGFP human glia and Genea HD versus normal (Ctr) hESC-derived astrocytes, were all filtered for one-to-one mouse to human orthologs, and their intersections observed (Figure 5A). Notable intersections that were pursued further for GO analysis were the overlap of Genea HD and zQ175, signifying cross species conservation of genes specific to full-length mHTT only (Figure 5A, red, 43 genes), R6/2 and LV-HTTQ73, representing functions driven by the presence of a truncated mHTT (blue, 118 genes), and genes common...
Figure 3. Forced mHTT expression drives transcriptional dysregulation in normal human astrocytes

(A) Fetal human lateral ganglionic eminence (LGE) (14–18 weeks g.a.; n = 3) was dissociated and transduced with either lentivirus expressing only EGFP (LV-EGFP), or EGFP in tandem with HTT-Q23 (LV-HTTQ23), or mHTT-Q73 (LV-HTTQ73). The latter two included only the truncated exon1 of the HTT gene. The transduced cells were then isolated via EGFP-directed FACS, and further apportioned as neurons or glia by the presence or absence, respectively, of neuronal PSA-NCAM. RNA extracted from each sample was then profiled via microarray.

(B) EGFP+/PSA-NCAM− cells largely developed as astrocytes. Cells expressing WT HTT-Q23 displayed diffuse cellular immunostaining of HTT, whereas mHTTQ73 displayed both clear cytoplasmic and nuclear HTT aggregates.

(C) Principal component analysis displayed distinct clustering of groups.

(D) Venn diagram of DE gene sets in astrocytic groups (adjusted p < 0.1, limma with SVA-determined batches regressed) (Leek and Storey 2007; Leek et al., 2012).

(E) Relative probe intensities of LV-HTTQ23 and LV-HTTQ73, each versus LV-EGFP controls, shows that dysregulated expression was primarily a function of the CAG repeat expansion. Scale, 50 μm.
**Gene Z-Score**

<table>
<thead>
<tr>
<th>LV-HTTQ23 vs LV-EGFP</th>
<th>HD vs CTR GENEA Astrocytes</th>
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**Genea**

- Astrocytes-Ctr
- Astrocytes-HD

**Gene Symbols**

- SCD
- ACAT2
- FASN
- MVK
- INSIG1
- SQLE
- HMGCR
- HMGCS1
- ID1
- LDLR
- FDFT1
- DHCR7
- DHCR24
- NSDH1
- APC
- MSMO1
- SC5D
- TOP2A
- GAS1
- MVD
- LRP1
- CHAC1
- EIF4EBP1
- VLDLR
- DDR2
- ALDH1L2
- VEGFA
- LAMP3
- NUPR1
- CTF1
- PPARD
- ICAM1
- BCL3
- OSMR
- SOCS3
- CD44
- GFAP
- KCNA2
- NNAT
- ARC
- PCDH17
- NFAT
- KCNA2
- Module 1 (43.3%)
- Module 2 (14.3%)
- Module 3 (1.9%)
- Module 4 (40.5%)

**Module 1 (43.3%)**

- LV-HTTQ23 vs LV-EGFP

**Module 2 (14.3%)**

- HD vs CTR GENEA Astrocytes

**Module 3 (1.9%)**

- LV-HTTQ73 vs LV-HTTQ23

**Module 4 (40.5%)**

- HD vs CTR GENEA Astrocytes

**Pathways**

- CTNNB1 Signaling
- Organization of extracellular matrix
- TNF Signaling
- Unfolded protein response
- Endoplasmic reticulum stress response
- Metabolism of cholesterol
- Synthesis of cholesterol
- Superpathway of Cholesterol Biosynthesis
- SREBF1 Signaling
- SREBF2 Signaling
- SREBF1 Signaling
- Neurotransmission
- SETDB1 Signaling
- BDNF Signaling
- IFNG Signaling
- TGFBI Signaling
- SNACA Signaling
- Migration of cells
- Organismal death
- ATF4 Signaling

**Gene Expression**

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(legend on next page)
across all models (green, 786 genes) (Table S6). This final intersection gene set was crafted by inclusion of genes that intersected in at least one truncated and full-length mHTT model, and ideally defines conserved artifact-independent gene dysregulation across models and species.

Pathway enrichment analysis (IPA) of the exclusively full-length mHTT dataset yielded few terms, due to its small size (Figure 5B). Terms of note, however, included abnormal morphology of neurons, which was enriched in all datasets of interest, as well as MITF, RUNX1, PAX6, and neuregulin signaling, along with stratification of cerebral cortex. Enrichment analysis of the exclusively truncated mHTT dataset agreed with our previous analyses, showing a vast enrichment of pathways concerning cholesterol biosynthesis, cell stress, as well as apoptosis and organismal death (Figure 5C). Given the profound dysregulation of cholesterol synthetic pathways in particular, we investigated these terms and their shared genes across both species and models (Figure 6A; Table S7). Of 20 selected IPA terms associated with cholesterol metabolism, 19 were significantly dysregulated only in the truncated HTT models, both in R6/2 mice in vivo and in human LV-HTTQ73-transduced glia in vitro. In contrast, only a single IPA term, HDL-cholesterol signaling, was identified as dysregulated in astrocytes derived from either of the full-length models, z0175 mice in vivo or hESC-derived glia in vitro (Figures 6B and 6C). Thus, although glial cholesterol synthetic genes were prominent targets of mHTT in model systems expressing a truncated, exon1-only mHTT gene, our data suggest that cholesterol pathway dysregulation is not as salient a feature in HD astrocytes expressing the CAG repeat expansion of mHTT in the context of the full-length HTT gene.

Our analysis of those gene sets shared across gene length models yielded a number of prominent shared functional terms (Figures 5D, 5E, and 5F). Among these, functional enrichment of genes modulating the morphology and differentiation of astroglia was highlighted by the mHTT-associated dysregulation of GFAP, S100B, BMP4, PALLD, TRIO, and FLNC (Boukhelifa et al., 2003; Bonaguidi et al., 2005; Brozzi et al., 2009; Zamanian et al., 2012; Bellesi et al., 2015; Moet et al., 2016), which were also recently noted in a comparison of hESC-derived mHTT and normal glia (Osipovitch et al., 2019). Overwhelmingly, IPA terms associated with cell and process morphology were found to be enriched across both species and mHTT-variants. These terms included the organization of cytoplasm and cytoskeleton, cellular protrusion formation, and the branching of cells, functions for which ARC and DSCAM have been previously identified as major CNS contributors (Peebles et al., 2010; Maynard and Stein 2012). In addition, categories denoting regulation of morphology were found to be dysregulated in this common subset, including microtubule dynamics (MAP1B, MAP4, MAP6, KIF18A, and DYN2CH1) (Nguyen et al., 1997; Weaver et al., 2011; Bodaleo et al., 2016) and organization of the actin cytoskeleton (ARHGEF2, ARHGEF9, PAK3, CDC42EP4, and AUT2) (Boda et al., 2004; Hori et al., 2014; Ageta-Ishihara et al., 2015). These effector genes, identified at the intersection of these diverse cross-species and cross-gene length, mHTT-dependent gene sets, may comprise highly conserved targets by which to modulate or rescue glial phenotype in HD.

**Morphology of striatal astrocytes is altered in diseased R6/2 mice**

In light of the mHTT-dependent differential expression of both genes and gene ontologies associated with cell structure that was common to all four HD models, we asked whether the morphology of astrocytes was affected by mHTT expression. In particular, we compared the morphology of striatal astrocytes in R6/2 mice to that of WT controls. To that end, 1-, 5- or 11-week-old R6/2 mice were injected intrastriatally with astrocyte-specific lentivirus expressing an EGFP reporter. The lentivirus achieved astrocytic specificity by combining the human GFAP (ABC-D) promoter for astroglial expression with MIR124-target to prevent neuronal expression of the lentivirus (Figure 7A) (Lee et al., 2008; Taschenberger et al., 2017). The mice were injected 1 week prior to sacrifice, at 2, 6, or 12 weeks, and individual astrocytes imaged within 150-μm thick sections and their fiber arbors mapped in their entirety using 3D imaging via Neurolucida 360 imaging (MBF Biosciences). Complexity was then measured by Sholl analysis, by scoring the number of arbor intersections that occur at fixed 10-μm concentric circles originating from the soma (Sholl 1953) (Figures 7B and 7C).

This analysis revealed that the astrocytes were identical in their arbors (number of intersections as a function of radius) at 2 weeks. In contrast, by 6 weeks, the R6/2 astrocytes showed a significant reduction in their cellular arbors relative to WT controls, a difference that increased further by 12 weeks (Figures 7D–7F). Nonlinear regression analysis revealed that R6/2 striatal astrocytes differed gradually and significantly from those of the WT mice (R6/2 versus WT: 2 weeks: p = 0.1164, F[3,327] = 1.983; 6 weeks: p < 0.0004, F[3,327] = 21.79; 12 weeks: p = 0.0004, F[3,327] = 6.273). This decrease in cell complexity appeared in large part due to a significant reduction in the number of primary astrocytic processes in R6/2 mice (genotype effect: p < 0.0001, F[1,104] = 79.93 by 2-way ANOVA) (Figure 7G).

We next used fan-in radial analysis (Glaser and McMullen, 1984) to compare the relative fiber occupancy within astrocytic domains between R6/2 and control mice. We found that by 6 weeks of age, R6/2 astrocytes exhibited significantly less occupancy of their individual domains than did WT astrocytes (genotype effect: p = 0.0001, F[1,104] = 79.93 by 2-way ANOVA) (Figure 7G).

**Figure 4. Full-length and truncated mHTT drive distinct abnormal expression profiles in human astrocytes**

(A) Pathway enrichment (IPA) network analysis of human fetal astrocytes from Figure 3, with the addition of a previously published differentially expressed (DE) gene set of human ESC-derived astrocytes (HD versus control, RNA-seq) (Osipovitch et al., 2019). This network was constructed from differentially expressed genes (circles) for each comparison (squares) and their significantly dysregulated IPA terms (triangles). Unsupervised modularity determined four modules (M1–M4: M1, blue; M2, purple; M3, green; and M4, bronze) of closely related genes, annotations, and DE gene sets that were then clustered. Each module’s percent of total GO connectivity is listed. The sizes of the triangles (GO terms) and squares (DE genes) are proportional to the node degree.

(B) Heatmap of module-pertinent DE genes with their respective significance in each group.

(C) Significance of highly enriched GO terms within each module and group.
Figure 5. Integrated analysis of mouse and human HD models reveals shared astrocytic pathology

(A) Venn diagram of four HD astrocyte gene sets: Genea HD versus control; LV-HTTQ73 versus LV-EGFP; DEGs in R6/2 at either 6 or 12 weeks; and DEG in zQ175 at either 6 or 12 months. Three subsets were analyzed: common to full-length zQ175 and HD hESC-derived astrocytes (red); common only to truncated R6/2 and LV-HTTQ73 (blue); and common between a full-length and truncated model (green).

(B–D) Highly enriched IPA terms within each comparison.

(E) The heatmap highlights those genes significant in both a human and mouse model, as well as in both a full-length and a truncated mHTT astrocyte model.
Finally, the density of astrocytes was also significantly reduced. We found that the density of ALDHL1L-defined astrocytes was significantly lower in R6/2 mice as early as 2 weeks, a difference that became more prominent by 6 weeks. Interestingly, this difference abated by 12 weeks, likely reflecting the loss in neuropil and hence overall contraction of the striatal volume in R6/2 mice. This would have served to increase the intrastriatal packing density of these atrophic R6/2 astrocytes, consistent with the striatal involution with apparent astrogliosis that typifies the late-stage HD striatum (genotype effect: p < 0.0001, F[1,12] = 45.05; age effect: p = 0.5, F[2,12] = 0.7 by 2-way ANOVA) (Figure 7I). Together, these data show the progressive, age-linked structural involution of R6/2 astrocytes. This observation further implicates astrocytic pathology in the genesis and progression of HD, while providing a strong structural correlate to the conserved transcriptional defects noted in astrocytes across all four of our model systems.

**DISCUSSION**

Astrocytes generated from patients with HD and from transgenic mice exhibit cell-intrinsic pathology that has been causally associated to both neuronal dysfunction and neuropathology in HD. Nonetheless, the differences in mutant HTT gene structure and inclusion across species and models have been such that neither the commonalities of astrocyte pathology across models, which might be presumed critical to HD pathogenesis, nor those specific and limited to one model or another, which might be presumed artifactual, have hitherto been clearly identified. To address this issue, we compared the transcriptional profiles of...
Figure 7. Astrocytic domains differentially constrict with age in R6/2 mice

(A) Structure of the lentivirus LV-GFAP-EGFP-Mir124T used for astrocyte targeting. To achieve astrocyte specificity, EGFP and a MIR124 target were placed under the control of the human GFAP(ABC-D) promoter (Lee et al., 2008; Merienne et al., 2013).

(B) Sagittal section of WT mouse injected with LV-GFAP-EGFP-Mir124T. (B1) 2D projection image of an EGFP+ astrocyte; (B2) its corresponding 3D tracing by Neurolucida 360.

(C) Representative astrocytes imaged at 2, 6, and 12 weeks of age (n = 3 mice each).

(D–F) Lorentzian non-linear regression analysis (Motulsky and Brown 2006) shows a progressive reduction of the complexity of astrocytic arborization that becomes significant by 6 weeks (2 weeks: R6/2, n = 25 cells; WT, n = 15 cells; 6 weeks: R6/2 n = 35 cells; WT n = 28 cells; 12 weeks: R6/2, n = 15 cells; WT, n = 22 cells).

(G) R6/2 astrocytes have significantly fewer primary processes at all ages analyzed.

(H) Fan-in radial analysis, which estimates volume occupancy, shows that R6/2 astrocytes have significantly less volume coverage than diseased astrocytes at 6 weeks.

(I) Striatal astrocytic density, as defined by ALDH1L1+ cells, was reduced in presymptomatic R6/2 mice. Mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.0001 by 2-way ANOVA with Tukey’s post hoc tests (G–I); scale, 200 μm.
striatal astrocytes across four distinct human and mouse models of HD. The latter included R6/2 and zQ175 mice, which respectively include the exon1-only truncated form of mHTT and the full-length mHTT gene. In both transgenics, long CAG repeat expansions have been included to accelerate the disease course in these fundamentally short-lived mice. Similarly, our human models included astrocytes produced from the fetal human lateral ganglionic eminence and transduced with lentivirus expressing the truncated exon1-only mHTT and astrocytes derived from hESCs derived from HD embryos expressing the full-length mHTT gene. Thus, our design was intended to include both mouse and human astrocytes and examples of each expressing either exon 1-only or full-length mHTT.

Because of the dominance of striatal neuronal death in clinical HD, the glial contribution to HD pathology has been largely neglected; as a result, prior therapeutic trials of cell replacement in HD have focused on neuronal replacement—that has so far proven ineffective (Benraiss and Goldman, 2011). Nonetheless, it has become clear that glial cells are also impaired in HD, both fundamentally so and in a cell-intrinsic fashion (Clabough et al., 2013; Huang et al., 2015; Benraiss et al., 2016). Among other impairments, HD astrocytes exhibit deficient astrocytic glutamate and potassium uptake, which may underlie the neuronal hyperexcitability, excitotoxicity, and vulnerability characteristic of HD (Maragakis and Rothstein, 2001). Astrocytes display a reduced glutamate uptake that reflects the decreased expression of the glutamate transporter gene Glut1/Slc1a2 in both HD patient-derived cells and HD model mice (Arzberger et al., 1997; Liévens et al., 2001). Similarly, targeted expression of mHTT in astrocytes resulted in motor dysfunction, weight loss, and premature death, all of which were correlated with huntingtin aggregates and reduced glutamate transporter expression (Bradford et al., 2009). Accordingly, the transplantation of normal WT glia into R6/2 mice reduced interstitial potassium, improved motor coordination, and extended the lifespans of the treated mice (Benraiss et al., 2016). Similarly, the clearance of HTT from resident astrocytes improved both the neuropathological and electrophysiological phenotypes of BACHD mice (Wood et al., 2019).

Several studies have used whole genome expression profiling to investigate the molecular pathology of the neurodegenerative diseases, including that of HD (Luthi-Carter et al., 2000; Thomas, 2006; Runne et al., 2008; Bayram-Weston et al., 2015; Chandrasekaran and Bonchev, 2016; Moily et al., 2017; van Hagen et al., 2017; Switońska et al., 2019; Merienne et al., 2019; Ooi et al., 2019). However, whole tissue from anatomically defined regions is often used for RNA extraction, which makes it difficult to identify cell-specific contribution to the pathology. To overcome these limitations, we used Git1 as a cell-specific surface marker to label astrocytes for FACs, and CD11b as a sort marker for microglia, to allow specific enrichment of these glial populations (Lovatt et al., 2007; Auvergne et al., 2013). Both sort markers proved robust for their respective targeted populations. In particular, Git1—despite its relative downregulation in HD (Petr et al., 2013)—was still expressed at levels that permitted high-efficiency astrocytic enrichment by FACs.

Gene expression analysis of sorted mouse astrocytes, as well as of sorted mouse microglia, showed progressively more aberrant gene expression in both R6/2 and zQ175 mice over time. Transcriptional changes were far more prominent in R6/2 than in zQ175 mice, pari passu, with the greater severity of disease in the former. Interestingly, network analysis uncovered model-specific patterns of transcriptional dysregulation in R6/2 astrocytes. The most prominent of these R6/2-selective groups of enriched terms were those centered on cholesterol biosynthesis and metabolism. Yet, although R6/2 astrocytes exhibited significant downregulation of cholesterol pathway genes, such cholesterol pathway dysregulation was not noted in astrocytes derived from zQ175 mice, expressing full-length mHTT. This pattern of mHTT truncate-associated cholesterol pathway dysregulation was noted in human astrocytes as well, in that fetal striatal astrocytes transduced with lentivirus expressing truncated mHTT manifested significant cholesterol pathway dysregulation, whereas HD hESC-derived astrocytes expressing full-length mHTT did not do so. Thus, in human as well as mouse astrocytes, IPA terms pertaining to cholesterol metabolism and biosynthesis were differentially prominent in cells expressing truncated mHTT, indicating the distinct molecular pathologies of astrocytes expressing full-length and truncated mHTT.

Indeed, we noted a marked downregulation of the cholesterol synthetic pathway in truncated mHTT astrocytes, as derived from both mice (R6/2) and human cells (mHTT-73CAG-transduced). In particular, astrocytes derived from diseased R6/2 mice displayed a systematic downregulation of cholesterol regulatory and biosynthetic genes; the expression of virtually every gene in the cholesterol biosynthetic pathway was significantly downregulated in R6/2 mice. This likely contributed to the severe phenotype of these mice; cholesterol is essential for synapse and myelin formation and is a crucial component of the cell membrane and CNS. Interestingly, the astrocytic cholesterol transporter ABCA1 was upregulated in R6/2 astrocytes. This may be a result of the downregulation of miR-33, which controls ABCA1 expression (Gerin et al., 2010). miR33 is located in the intron of the SREBF2 gene, which is itself downregulated in R6/2 astrocytes. The expression pattern of cholesterol-related genes in R6/2 astrocytes may thus suggest an aberrant response to ambient cholesterol levels in the cells, with downregulated biosynthetic and import genes (SREBF2, the synthetic pathway enzymes, and LDLR), accompanied by the concurrent upregulation of ABCA1-mediated cholesterol transport.

Yet, notwithstanding the prominent suppression of the cholesterol synthetic pathway in astrocytes bearing truncated mHTT, no such downregulation of cholesterol biosynthesis was evident in either mouse or human glia expressing full-length mHTT (zQ175 mouse and hESC-derived astrocytes, respectively). Whether this reflects modifiers that attenuate the level of cholesterol pathway dysregulation in astrocytes with full-length mHTT, or whether those cells expressing truncated mHTT are in effect especially prone to a disturbance of cholesterol biosynthesis that may not otherwise occur, is unclear. Regardless of its basis, these data suggest that astrocytic cholesterol pathway dysregulation is not a feature of HD models using full-length mHTT and hence is unlikely to be a significant contributor to astrocytic pathology in clinical HD.

The importance of this topic extends beyond HD. In the brain, cholesterol is almost exclusively made de novo, and its homeostasis has been implicated in the pathophysiology of Alzheimer’s...
Multiple models to define conserved transcriptional signatures of disease. Compensatory mechanisms can mask cell-intrinsic causal molecular pathology, just as model-specific pathologies may highlight noncausal or even artifactual pathology. By combining rigorous identification and isolation of astrocytes and microglia with a cross-model and cross-species analysis, we have identified a core set of dysregulated genes and cognate pathways causally linked to the glial pathology of Huntington disease.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.109308.

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AUTHOR CONTRIBUTIONS

A.B. and S.A.G. designed the study. D.C.-M. prepared and sorted the cells used for the experiments. J.N.M., M.O., and A.C. analyzed the genomic data. M.S.W. and C.B.V. performed the quantitative image analysis. A.B., J.N.M., and S.A.G. wrote the paper. All authors have approved the final manuscript.
DECLARATION OF INTERESTS

S.A.G. is a part-time employee and stock-holder of Sana Biotechnology, a cell therapy company, and his lab receives sponsored research support from Sana. All other authors declare no competing interests.

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REFERENCES


Huntington’s Disease. Trends Neurosci.


# STAR★METHODS

## KEY RESOURCES TABLE

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**Critical commercial assays**

- Ovation PicoSL WTA System V2 NuGEN Cat#3312
- Prelude Direct Lysis Modul NuGen Cat#1400-24

**Deposited data**

- R Analysis Script and Processed data https://github.com/CTNGoldmanLab/HD_Astrocytes_2021 N/A
- Processed data Genialis https://ctngoldmanlab.genialis.com/rochester

**Experimental models: cell lines**

- hESC line: Genea 02 (normal HTT) GeneaBiocells Genea 02
- hESC line: Genea 17 (42 CAG) GeneaBiocells Genea 17
- hESC line: Genea 18 (44 CAG) GeneaBiocells Genea 18
- hESC line: Genea 19 (normal HTT) GeneaBiocells Genea 19
- hESC line: Genea 20 (48 CAG) GeneaBiocells Genea 20

**Experimental models: organisms/strains**

- Mouse: B6CBA-Tg(HDexon1)62Gpb/1J Jackson Laboratory Cat#002810
- Mouse: B6.129S7-Rag1tm1Mom/J Jackson Laboratory Cat#002216
- Mouse: zQ175 Charles river N/A

**Oligonucleotides**

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- Primer: Gfap Reverse: ATCCACAGGAGCCAGGGTGG This paper N/A
- Primer: Slc1a3(Glast) Forward: CGGGATTCTCAGGGCCGGTGC This paper N/A
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- Primer: Aqp4 Forward: ACAGAGGCTCCGAGCCGAGGCG This paper N/A
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(Continued on next page)
### RESOURCE AVAILABILITY

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Steven A. Goldman (steven_goldman@urmc.rochester.edu).

**Materials availability**
Cells and reagents generated in this study will be made available on request. All requests for materials and reagents should be directed to the Lead Contact (steven_goldman@urmc.rochester.edu). For some cellular reagents and plasmids, a completed Material Transfer Agreement may also be required.

**Data and code availability**
All gene expression data have been submitted to GEO, accession number GSE154141. Expression data are also available and may be further interrogated at https://ctngoldmanlab.genialis.com/rochester. R Analysis Script and processed data may be accessed at https://github.com/CTNGoldmanLab/HD_Astrocytes_2021. Any further information and requests for data and code should be directed to and will be fulfilled by the Lead Contact (steven_goldman@urmc.rochester.edu).

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EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal models
All experiments were approved by the Institutional Animal Care and Use Committee of the University of Rochester. Wild-type females with ovary transplants from R6/2*(150 ± 5 CAG) mice were purchased from Jackson Laboratories (Bar Harbor, ME). zQ175(Q190 ± 10 CAG) were provided by the CHDI Foundation. In R6/2 mice, HD-related symptoms typically begin by 8 weeks and are fully established by 12 weeks (Mangiaroni et al., 1998). zQ175 knock-in mice display a milder phenotype (Peng et al., 2016). These mice are pre-symptomatic at 6 months and become symptomatic by 8 months. All mice were bred on C57BL6 to minimize any alteration of gene expression due to background strain, and all were genotyped following weaning. R6/2 transgenic positive were further analyzed to determine their CAG repeat number through PCR with primers encoding a product spanning the repeat region as previously described (Benaïss et al., 2013). Upon weaning mice were housed at 5 mice per cage in pathogen-free conditions with a 12-h light/dark cycle and provided with food and water ad libitum. R6/2 mice were sacrificed at either 6 or 12 weeks of age, and zQ175 mice at either 6 or 12 months. All mice within each group, with each group defined by the intersection of genotype and time point (n = 3-5 mice/group) were pooled together, with an approximate 1:1 distribution of pooled male and female mice/sample.

Cellular models
The human ESC lines used are as noted in the Key Resources Table. These included 5 different hESCs from Genea Biocells: GENE02 (normal HTT); GENE19 (normal HTT); GENE17 (42 CAG); GENE18 (44CAG); and GENE20 (48 CAG). Cells were differentiated into astrocytes and isolated using CD44-based FACS, and their RNA expression profiles assessed as earlier reported (Ospovich et al., 2019). Briefly, undifferentiated hESCs were differentiated into glial progenitor cells using sonic hedgehog- and retinoic acid-mediated ventralization and neuralization followed by the serial application of neural and glial growth factors, as previously detailed (Wang et al., 2013; Ospovich et al., 2019). Using this protocol, significant numbers of CD140a-defined hGPCs are produced by 140 DIV, typically comprising over half of the cultured cells by that point (Wang et al., 2013). Astrocytic differentiation was then triggered by the addition of 20 ng/ml BMP4 (Sim et al., 2006), and the resultant cultures were sorted 4 weeks later for the astrocytic hyaluronan receptor CD44 (Liu et al., 2004; Cai et al., 2012) for astrocytic selection protocol, see also Liu et al., [2019]).

Fetal human striatal glial cells transduced to express mutant or normal HTT were sourced and derived as noted below (see Mutant HTT overexpression in fetal human glia). Briefly, the lateral ganglionic eminence (LGE) was dissected from aborted fetuses of 14–18 weeks gestational age, with maternal consent and under protocols approved by the University of Rochester-Strong Memorial Hospital Research Subjects Review Board.

METHODS DETAILS

Mouse brain dissection
The mice were euthanized with carbon dioxide, transcardially perfused with sterile Hank’s Balanced Salt Solution (HBSS), and the brain removed. The brains were immersed in ice-cold sterile HBSS for about 5 minutes to facilitate the microdissection. Under a dissecting microscope, the subventricular zone was removed and discarded and the striata from each mouse was dissected and placed in sterile HBSS on ice. Four to six striata pooled together as per genotype to form a sample. The striatal tissues were transferred to a Petri dish containing sterile HBSS then transferred into small pieces using sterile disposable scalpels, transferred into a sterile tube and then incubated in a papain/DNase dissociation solution at 37° C for 50 minutes. Minimum Essential Media plus 0.5% BSA (MEM-BSA) containing 5% serum was then added to inactivate the papain. The tissue was triturated by repeated pipetting in order to achieve a single cell suspension. The cells were then pelleted, resuspended into MEM-BSA and overlaid first onto a 90% Percoll gradient followed by a second 30% Percoll gradient and the solution centrifuged. Myelin and debris were removed from the tube and the cells were resuspended into MEM-BSA plus donkey serum for flow cytometry.

Flow cytometry and FACS
Flow cytometry analysis for astrocyte surface markers was performed on freshly dissociated striatal cells from wild-type or R6/2 mice. Cells were stained with primary antibodies against the astrocyte marker, GLT1 and the microglial marker, CD11b-PE (BD Biosciences). The primary antibodies, directly conjugated antibodies or their corresponding isotype controls were added to the cells and then incubated on ice for 15 minutes. 5 mL of MEM-BSA wash buffer was added to the cells and the cells were spun down. For the non-conjugated antibodies, the pelleted cells were resuspended in MEM-BSA plus 5% donkey serum and the appropriate secondary antibody, APC conjugated donkey anti-rabbit IgG, was added at a 1:200 dilution (Jackson ImmunoResearch). The samples were incubated on ice for 15 minutes and then washed with 5 mL of MEM-BSA wash buffer for 10 minutes. All samples were resuspended in MEM-BSA containing 20U/ml DNase to a concentration of 1-1.5x10^6 cells/ml and then passed over a 35 μm tube top cell strainer prior to flow cytometry. DAPI was added at 1 μg/mL. Flow cytometry and FACS were performed on a BD FACS-Aria IIIU (Becton Dickinson, San Jose, CA). The cells were analyzed by forward and side scatter, for PE fluorescence through a 582 ± 15 nm band-pass filter, for APC fluorescence through a 660 ± 20 nm band-pass, and for DAPI fluorescence through a 450 ± 50 nm band-pass. Unstained cells were used to set the background fluorescence; a false positive rate of 0.5% was accepted. The isolated GLT1+/ CD11b+ and CD11b+ striatal cells were pelleted, frozen on dry ice and stored at −80°C until the time of RNA extraction.
Mutant HTT overexpression in fetal human glia
The lateral ganglionic eminence (LGE) from human fetal brain tissue from aborted fetuses (gestational age 14-18 week human brains), with maternal consent and under protocols approved by the University of Rochester-Strong Memorial Hospital Research Subjects Review Board. Briefly, LGE tissue was minced and dissociated using papain and DNase as previously described, always within 2 h of extraction. The dissociated cells were maintained in DMEM/F12/N2-based medium supplemented with 2% PD-FBS and 10 ng/ml FGF2 until ready for lentiviral transduction. To express mutant or control HTT in LGE cells, the cells were transduced with lentivirus at 0.5 multiplicities of infection expressing EGFP in tandem with the first exon of either wild-type HTT (LV-HTTQ23), mHTT (LV-HTTQ73), or control expressing reporter only (LV-EGFP) (see Benraiss et al., 2016) for viral constructs and amplification. Transduced cells were isolated by FACS for EGFP in combination with antigenic selection of PSA-NCAM to isolate neuronal and astrocytic populations. RNA was extracted from the FACS-isolated cells and then used for microarray profiling.

RNA Preparation, amplification and labeling
RNA was extracted from the pelleted/frozen cells using the QIAGEN RNeasy Mini kit. An on-column DNase treatment was performed and the RNA concentration was determined using a Nanodrop. A portion of the RNA was then used for bioanalysis to confirm the integrity of the RNA. Mouse RNA isolated from GLT1+, CD11b+ and the GLT1+/CD11b- cells was labeled and hybridized to Affymetrix U430 arrays. Human fetal RNA was hybridized to Affymetrix Human Gene 2.0 ST Array V1.

Quantitative RT-PCR
Sorted Glit1+ /CD11b- cells were collected as described above (n = 3 for R6/2 and Wild-Type) at 12 weeks old were processed for RNA was extracted using TRIzol (Invitrogen) and purified via the RNeasy Mini Kit (QIAGEN) according to the manufacturer’s instructions. cDNA synthesis was carried out for 1 h at 37°C in 50 μL reactions containing 2 μg total RNA, reverse transcriptase and random hexamer primers (TaqMan Reverse Transcription Reagents; Fisher). 5 ng of RNA input was used for each reaction; these were performed using FastStart Universal Sybgreen Mastermix (Roche Diagnostics, Germany), on a real-time PCR instrument (CFX Connect Real-Time System thermocycler; Bio-Rad, USA). Amplification protocol included an initial denaturation at 95°C for 10 min followed by 40 cycles each consisting of denaturation at 95°C for 15 s, annealing at 62°C for 10 s, and extension at 72°C for 10 s, respectively. Dissociation-curve analysis was performed after each PCR to confirm the specificity of the reaction. Fold change between R6/2 and wild-type was analyzed using the ΔΔCt method against mouse GAPDH. The primer sequences are as follows:

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Microarray analysis
Following the import of raw data into R with the Affy package (R Development Core Team, 2020; Gautier et al., 2004), robust multi-array analysis (RMA) was utilized for pre-processing and normalization (frizarry et al., 2003). Probe annotations were fetched using biomaRt (Durinck et al., 2009), and probes targeting multiple ensemble IDs were removed. Glit1 and CD11b sample intensities were individually subset and analyzed for between-time point differential expression using limma (Ritchie et al., 2015). An adjusted p value of < 0.05 was deemed significant. For Figure 5A, differentially-expressed genes were pooled from both time points in R62 and zQ175 comparisons. For heatmap visualization of genes with multiple differentially expressed probes, differentially expressed probes with the highest average intensity were selected. All raw microarray data are available via GEO accession number GSE154141

For human fetal astrocyte and neuron microarray samples, raw data was imported into R via the oligo package (Carvalho and Irizarry, 2010) and initially processed as above. For removal of unwanted variance, SVA determined 4 surrogate variables to be added to the design formula (Leek et al., 2012) prior to differential expression analysis in limma where an adjusted p value of < 0.1 was deemed significant.

Taqman Low Density Array (TLDA)
RT-qPCR validation was performed with probes for 96 selected genes on TLDA plates using a commercial cholesterol assay and run on an ABI 7900HT instrument. Four biological and two technical replicates were profiled for each of 12wk R6/2 Glit1+/CD11b-, 12wk R6/2 CD11b+, 12wk WT Glit1+/CD11b-, and 12wk WT CD11b+. GAPDH and 18 s were included as endogenous controls. ABI RQ Manager was used for determination of cycle threshold (Ct) values and quality checking; outlier wells were omitted. These results were imported into R for ΔΔCt analysis, assisted by functions from the HTqPCR package (Dvinge and Bertone, 2009). Wells without detectable amplification within 40 cycles were assigned “NA.” Statistical significance was determined for R6/2 versus WT astrocyte.
RNA-Seq analysis of hESC-derived astrocytes

We previously reported RNA-Sequencing data from CD44-sorted human astroglia that we produced from human embryonic stem cells (hESCs) derived from five distinct hESC lines (Osipovitch et al., 2019). These included astrocytes produced from two healthy control lines (GENEA02 and 19) and three derived from HD embryos (GENEA17,18 and 20, with 42, 44 and 48 CAG repeats, respectively). Our raw gene expression data from these cells were reprocessed for this study and analyzed to provide human, full-length mHTT comparison data. The RNA-Seq data were obtained from GEO accession GSE105041, and reads then aligned to human genome GRCh38.p10 via STAR 2.6.0c (Dobin et al., 2013). Mapping and estimation of gene abundance and expected counts were carried out in RSEM 1.2.28 (Li and Dewey, 2011). RSEM summarized gene data were imported into R via tximport (Soneson et al., 2015). Genes from the RNA-Seq data-set were filtered for presence of high-quality probes in comparison microarray datasets. Log2 transformed transcripts per million (TPM) with a pseudocount of 1 added, were used for heatmap construction. The pooled differentially expressed gene set (p < 0.05, no log2 fold change cutoff) of HD Astrocytes versus controls was obtained from the original publication as hosted on Genialis https://ctngoldmanlab.genialis.com/rochester/visual_analysis/gene_view?_s=dc851949-5a55-4999-b21b-f770f8e08338).

Ingenuity Pathway Analysis and Network Analysis

For functional analysis, differentially expressed gene lists were analyzed in Ingenuity Pathway Analysis (QIAGEN). Each analysis was exported and its canonical, functional, and upstream signaling terms were filtered for relevance and significance (p < 0.001). A pathway enrichment network was then constructed with IPA terms as nodes connecting to their contributing genes as well as the DE comparison that they possession were encoded for. Modularity within the networks was determined in Gephi (Bastian et al., 2009) and networks were visualized in Cytoscape (Shannon et al., 2003). Nodes were clustered within their respective modules and repositioned slightly for aesthetics. Gene edges were hidden for clarity. The percent of degree weight was calculated for each module as the sum of node degrees within a module as a percentage of the total node degrees.

Immunofluorescence labeling

For in vivo immunolabeling, 2, 6 or 12 week-old wild-type or R6/2 mice were sacrificed, transcardially perfused (first with Hank’s Balanced Salt Solution (HBSS) and then 4% paraformaldehyde in 0.1M phosphate-buffered saline at pH 7.4), and their brains were dissected. Brains were post-fixed in 4% paraformaldehyde 4 hours at 4°C, then serially immersed in 6% and 30% sucrose in phosphate buffer, embedded and frozen in O.C.T. Compound (Sakura Finetek, Torrance, CA), and stored at –80°C until sectioning. Brains were cryosectioned into 20μm sagittal sections and then stained ALDH1L1 1:400 (Abcam, Cambridge, MA). Alexa Fluor 568-conjugated (Invitrogen Carlsbad, CA) anti-rabbit secondary antibodies was used at 1:400. For in vitro immunocytochemistry, cell were plated onto poly-ornithine- 24-well plate, fixed with 4% paraformaldehyde for 15 min at room temperature then permeabilized with 0.1% saponin plus 1% of either goat serum for 15 minutes at room temperature. Cells were further blocked with 5% of either goat serum plus 0.05% saponin for 15 minutes at RT. After incubation with anti mutant HTT(1/400), anti-GFAP(1/800) or anti-GFP(1/400) at 4°C overnight, the cells were next incubated with corresponding secondary antibody for 30 minutes at RT. All antibodies are listed in the Key Resources Table.

Astrocytic targeting of EGFP expression in vivo

To visualize astrocytes in vivo, we used a lentivirus designed to drive astrocyte-specific expression. The viral construct LV-GFAP-EGFP-Mir124T (pTANK-GFAP(ABC1D)-EGFP-MIR124T-WPRE) carries, in the 5’ to 3’ direction: the cPPT element (Zennou et al., 2000); the astrocyte-specific minimal GFAP(ABC1D) promoter (Lee et al., 2008); EGFP; a MIR124 target to abolish neuronal expression (Merienne et al., 2013; Taschenberger et al., 2017); and the WPRE (Zufferey et al., 1999). Viral particles pseudotyped with vesicular stomatitis virus G glycoprotein were produced in 293HEK cells and concentrated by ultracentrifugation, then titrated using Lenti-X P24 Rapid Titer Kit (Fisher Scientific NC17208960). Viral suspension was injected stereotaxically into the striatum at the following coordinates relative to bregma: antero-posterior (AP) 0.4 mm, medio-lateral (ML) ± 1.8 mm; dorsoventral (DV) –2.8. The mice were injected when 1, 5 or 11 weeks-old and killed a week later. Mice were perfused transcardially with Hank’s Balanced Salt Solution (HBSS) followed by 4% phosphate-buffered saline at pH 7.4, and their brains removed and sectioned coronally as 150 μm floating sections.

Quantification and Statistical Analysis

Sholl analysis of Astrocytes

EGFP-expressing astrocytes were imaged at 1000x on a Leica TCS SP8 confocal system. Computer assisted 3-D tracings were made of the imaged cells using Neurolucida 360 (MBF Biosciences, Williston, VT). Individual morphologies were evaluated by Sholl (1953) and Fan-in analyses (MBF Biosciences) (Glaser and McMullen, 1984).
Statistical analysis
Data were analyzed using GraphPad Prism 8.0 (GraphPad, San Diego, CA). Unpaired t test is used to compare two groups while 2-Way ANOVA (followed by Tukey’s post hoc comparison tests) was used to compare four or more groups. Sholl analysis data was presented as frequency distribution plots of the number of intersections of the cell processes as a function of distance from the soma. Non-linear regression with Lorentzian distribution curve fitting model was used. Quantitative results are shown as mean ± SEM and statistical significance was accepted at p < 0.05.