Decreased Lin7b Expression in Layer 5 Pyramidal Neurons May Contribute to Impaired Corticostriatal Connectivity in Huntington Disease

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Abstract
Motor dysfunction, cognitive impairment, and regional cortical atrophy indicate cerebral cortical involvement in Huntington disease (HD). To address the hypothesis that abnormal corticostriatal connectivity arises from polyglutamine-related alterations in cortical gene expression, we isolated layer 5 cortical neurons by laser-capture microdissection and analyzed transcriptome-wide mRNA changes in them. Enrichment of transcription factor mRNAs including foxp2, tbr1, and neuroD6, and neurotransmitter- and plasticity-related RNAs including sema5A, pclo, cntn1, and Lin7b were observed. Layer 5 motor cortex neurons of transgenic R6/2 HD mice also demonstrated numerous transcriptomic changes, including decreased expression of mRNAs encoding the Lin7 homolog b ([Lin7b] also known as veli-2 and mals2). Decreases in LIN7B and CNTN1 RNAs were also detected in human HD layer 5 motor cortex neurons. Lin7 homolog b, a scaffold protein implicated in synaptic plasticity, neurite outgrowth, and cellular polarity, was decreased at the protein level in layer 5 cortical neurons in R6/2 mice and human HD brains. Decreases in Lin7b and Lin7a mRNAs were detected in R6/2 cortex as early as 6 weeks of age, suggesting that this is an early pathogenetic event. Thus, decreased cortical LIN7 expression may contribute to abnormal corticostriatal connectivity in HD.

Key Words: Corticostriatal projection, DNA microarray, Huntingtin, LIN7, Polyglutamine disease, Striatum.

INTRODUCTION
Huntington disease (HD) is an autosomal dominant polyglutamine disease clinically characterized by motor and cognitive dysfunction. The clinical features of HD have been attributed to neurodegeneration of subcortical basal ganglia circuits (1), but there is increasing evidence that the cerebral cortex may play a primary role in the pathobiology of HD. There are numerous structural and physiological abnormalities in various cortical subregions, even in asymptomatic gene carriers (2). Transcranial magnetic stimulation, somatosensory-evoked potentials, and long latency reflexes show changes that suggest abnormal information processing and decreased excitability in motor and sensory cortices (3, 4). Morphometric magnetic resonance imaging studies also show that regionally selective thinning of the cortex occurs early in HD and correlates with cognitive impairment (5, 6). At the cellular level, neuronal intranuclear inclusions (NIIs) indicate that mutant huntingtin protein (mt htt) accumulates in cortical projection neurons of layers 5 and 6 (7–9). We and others have shown that degeneration of pyramidal neurons in layers 3, 5, and 6 also occurs in HD (10, 11). Interestingly, regionally selective layer 5 pyramidal neuron degeneration correlates with clinical heterogeneity in HD symptom profiles (12).

R6/2 mice express the N-terminal portion of human htt with approximately 150 CAG repeats, driven by the human huntingtin promoter (13). They exhibit severe learning and motor deficits, have NII from 3.5 weeks of age, and have a limited lifespan of 12 to 17 weeks. Electrophysiological recordings of striatal medium spiny neurons from R6/2 animals reveal frequent large-amplitude synaptic events that indicate dysregulation of cortical inputs (14), which is consistent with the presumed corticostriatal dysfunction in HD. R6/2 mice also exhibit the striatal and cortical gene expression changes that occur in HD (15–17). Indeed, 12-week-old R6/2 mice bear greater striatal transcriptomic similarity to that of HD...
than any mt htt-expressing mice that have been studied to date (18).

We hypothesized that gene expression changes in corticostriatal projection neurons contribute to their dysfunction and that cortical mechanisms contribute to the selective neurodegeneration of striatal projection neurons caused by disturbed connectivity. Therefore, we isolated layer 5 neurons of motor cortex (M1 and M2) from R6/2 and wild-type (WT) mice by laser-capture microdissection (LCM) and compared their gene expression profiles using high-density microarrays. Layer 5 pyramidal cells were selected because most corticostriatal projections originate from this layer (19, 20) and because of the involvement of these cells in HD (10–12). To test the plausibility of this explanation for the selective striatal vulnerability characteristic of human HD, we subsequently assessed whether the changes observed in HD mice could also be detected in Brodmann areas 4 and 9 of human HD brain. To our knowledge, this is the first study to provide transcriptome-wide gene expression analyses of layer 5 neurons of the motor cortex and to assess the gene expression dysregulation in these cells in HD.

MATERIALS AND METHODS

Animals

Female R6/2 mice and WT littermates were purchased from Jackson Laboratories (Bar Harbor, ME) and killed at 12 weeks of age. Repeat length of the R6/2 mice used was approximately 141 to 152. Brains for LCM studies of R6/2 and WT littermates (4 per group) were snap frozen and stored at −80°C. Brains for homogenate studies were dissected from the skull, and cortices of both hemispheres were immediately removed and snap frozen.

RNA Samples and LCM

Eight-micrometer-thick sections from mouse brains and from human tissue blocks were cut on a cryostat (Shandon, Cheshire, UK) and thaw mounted on uncoated glass slides (Gold Seal RITE-ON microslides, Thermo Fisher Scientific Inc, Rockford, IL). Sections were stained with methylene blue (Sigma, Steinheim, Germany), as previously described (21). Deep-layer cortical neurons (mainly layer 5 but potentially including some layer 6) were selected from primary and

FIGURE 1. Visualization of murine and human deep-layer cortical neurons for laser capture microdissection (LCM). (A–D) Sections from cortex of 12-week-old R6/2 mice show the 6 cortical layers (A), neurons chosen for dissection (B), the section after LCM (C), and the harvested neurons attached to a thermoplastic film (D). (E–H) Cortex from grade 2 Huntington disease show a layer 5, 6 in a coronal section (E), layer 5 neurons selected for LCM (F), the section after LCM (G), and harvested cortical perikarya (H). All are stained with methylene blue. Scale bar = 250 μm.
secondary motor cortices (M1 and M2) of coronal mouse brain sections between +1.5 mm and +0.8 mm from bregma (22). These laminae were defined in scanned images of each stained section by direct comparison to a mouse cytoarchitectonic atlas (22). Human deep-layer cortical neurons were also selected by anatomical location in the lower third of the cortical ribbon in consultation with a neuropathologist Dr. M. P. Frosch (Massachusetts General Hospital, Boston, MA). Larger triangular-shaped cell bodies of pyramidal cells were easily distinguishable from glia and interneurons on the basis of size and morphological criteria (Fig. 1). Cells were selected using the PixCell II LCM instrument (Arcturus, Mountain View, CA), diameter 7.5 μm, onto CapSure HS caps covered with a thermoplastic film.

The harvested cells were solubilized from the film in extraction buffer provided in the Arcturus Pico Pure RNA isolation kit for 30 minutes at 42°C and stored at −80°C. The solubilized cells underwent RNA extraction according to the Pico Pure RNA isolation kit instructions (Arcturus), including a DNAse treatment step (Qiagen, Valencia, CA). Total murine cortical pyramidal neurons are larger than striatal neurons (diameter 9–12 μm vs 7.5–10 μm), we started with 4,000 cortical neurons per animal, which we estimated to be equivalent to 5 ng of total RNA (21).

For layer 5 neurons, 20 ng of total RNA from each homogenized sample and RNA from 4,000 laser-dissected neurons per sample were used to prepare biotinylated fragmented cRNA according to the GeneChip eukaryotic small sample target labeling protocol, with products from Affymetrix. Mouse genome 430 2.0 Affymetrix GeneChip arrays were hybridized for 16 hours, washed, stained, and scanned using an Affymetrix Fluidics Station and Scanner (Bio- polymers Facility, Harvard Medical School, Boston, MA).

**Microarray Analysis**

Selected array measures were reported from Affymetrix GCOS software; microarray data analyses were performed using R and Bioconductor packages (www.bioconductor.org). To compare gene expression in deep-layer cortical neurons from R6/2 versus WT animals, we normalized 8 microarrays together (4 WT LCM and 4 R6/2 LCM) and quantified gene expression using robust multiarray analysis implemented in the R package affy (23–25). We identified differentially expressed genes in R6/2 LCM versus WT LCM by computing empirical Bayes t statistics with the R package limma (26). Values of p were corrected for multiple testing using the False Discovery Rate method (27). We used the same approach to assess gene enrichment in LCM versus homogenate samples, normalizing and subsequently testing data from the 4 WT LCM versus 4 WT homogenate microarrays. Microarray annotation was performed using the R package annotationTools (28) and Affymetrix annotation data.

**Human Brain RNA Samples**

Frozen blocks of motor cortex (BA4) and prefrontal association cortex (BA9) were obtained from Dr J. P. Vonsattel (New York Brain Bank, Columbia University, New York, NY). Pathological grading had been performed according to the Vonsattel scale (Table 1) (29). RNA quality was assessed by capillary electrophoresis with a Bioanalyzer 2100 (Agilent) using 500 ng of total RNA. RNA samples without sharp rDNA peaks were excluded from further processing steps; sample processing was performed as for mouse tissues.

<p>| TABLE 1. Human Brains Used for Quantitative Polymerase Chain Reaction and Immunohistochemistry |
|-------------------------------------------------|-------------------------------------------------|</p>
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<th>Diagnosis</th>
<th>Average Age ± SEM</th>
<th>Age Range</th>
<th>Average CAG Repeat Length</th>
<th>CAG Repeat Length</th>
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<td>BA4</td>
<td>8</td>
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<td>61.5 ± 4.0</td>
<td>44–82</td>
<td>NA</td>
<td>NA</td>
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<td>8</td>
<td>HD2, 1 HD3</td>
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<td>54–80</td>
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<td>40–45</td>
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<td>67.1 ± 4.1</td>
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<td>Control</td>
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<td>63.0 ± 2.6</td>
<td>56–68</td>
<td>42</td>
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Brains for the mRNA studies were obtained from the New York Brain Bank, Columbia University. BA4 and BA9 were taken from the same brains, except for 1 Huntington disease (HD) case and 1 control. Brains for the immunohistochemical studies were provided by the New Zealand Brain Bank, University of Auckland. HD grade was determined according to Vonsattel (29).

NA, not assessed.
Quantitative PCR Assays
Reverse transcription of RNA from human laser-dissected cortical neurons in BA4 and BA9 was conducted with a SuperScript First Strand Synthesis System for reverse transcription-polymerase chain reaction ([PCR] Invitrogen, Carlsbad, CA), using random hexamer primers according to the manufacturer’s instructions. Quantitative real-time PCR (q-PCR) assays were performed with a Bio-Rad iCycler (Hercules, CA) using SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA) carrying out 50 PCR cycles as described (21). Each cDNA sample (equivalent to RNA from 50 laser-dissected neurons) was run in triplicate for the target and the normalizing gene in the same 96-well plate. Amplicon specificity was monitored by melt curve analysis at the end of the run, gel electrophoresis, and DNA sequencing. Human primer sequences were as follows: LIN7B/MALS2 (NM_022165) CGCCCATCTACATCTCCC, AGCT CCACCGCCTTCTC; 28s rRNA (M11167) (3 0 )A A A C T C

<table>
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<th>Gene Symbol</th>
<th>Log2 FC</th>
<th>Raw p Value</th>
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Differentially expressed probe sets are presented as fold change of laser capture microdissection (LCM) neurons versus whole cortex. Values of p were corrected for multiple testing using the False Discovery Rate (FDR) method (cutoff FDR p ≤ 0.05). RNAs with the largest magnitude increases in LCM neurons are shown (FC, fold change). Probe sets for expressed sequence tags and genes without annotation are not shown. In cases where multiple probe sets represent the same mRNA, the probe set reporting the largest magnitude of change is shown. Full analysis is in the supplementary data.
TABLE 3. Selected mRNA Decreases in Layer 5 Motor Cortex Neurons of R6/2 Versus Wild-Type Mice

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Gene Symbol</th>
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<td>−1.54</td>
<td>6.61E-12</td>
<td>2.97E-07</td>
<td>cAMP-regulated phosphoprotein 19</td>
</tr>
<tr>
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<td>Camkk2</td>
<td>−1.48</td>
<td>4.20E-10</td>
<td>9.45E-07</td>
<td>Calcium/calmodulin-dependent protein kinase kinase 2, β</td>
</tr>
<tr>
<td>1427683_at</td>
<td>Egfr2</td>
<td>−1.47</td>
<td>1.70E-08</td>
<td>9.36E-06</td>
<td>Early growth response 2</td>
</tr>
<tr>
<td>1449961_at</td>
<td>Rph3a</td>
<td>−1.46</td>
<td>9.75E-08</td>
<td>3.16E-05</td>
<td>Rabphilin 3A</td>
</tr>
<tr>
<td>1418744_s_at</td>
<td>tesc</td>
<td>−1.45</td>
<td>1.42E-10</td>
<td>6.41E-07</td>
<td>Tescalcin</td>
</tr>
<tr>
<td>1448830_at</td>
<td>Dusp1</td>
<td>−1.43</td>
<td>5.08E-07</td>
<td>9.53E-05</td>
<td>Dual-specific phosphatase 1</td>
</tr>
<tr>
<td>1459847_x_at</td>
<td>Gfra2</td>
<td>−1.42</td>
<td>6.37E-09</td>
<td>5.82E-06</td>
<td>Glial cell line-derived neurotrophic factor family receptor α 2 (Gfra2), mRNA</td>
</tr>
<tr>
<td>1422256_at</td>
<td>Satr2</td>
<td>−1.42</td>
<td>3.09E-10</td>
<td>9.29E-07</td>
<td>Somatostatin receptor 2</td>
</tr>
<tr>
<td>1436275_at</td>
<td>Kenp2</td>
<td>−1.39</td>
<td>1.14E-07</td>
<td>3.33E-05</td>
<td>KCHIP2A protein splice variant (KCHIP2 gene)</td>
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<tr>
<td>1423756_s_at</td>
<td>Igfbp4</td>
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<td>8.78E-06</td>
<td>Insulin-like growth factor binding protein 4</td>
</tr>
<tr>
<td>1419845_at</td>
<td>Dlx1</td>
<td>−1.34</td>
<td>1.05E-05</td>
<td>7.24E-04</td>
<td>Distal-less homeobox 1 (Dlx1), mRNA</td>
</tr>
<tr>
<td>1421477_at</td>
<td>Cpx2</td>
<td>−1.32</td>
<td>8.89E-07</td>
<td>1.34E-04</td>
<td>Complexin 2</td>
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<td>1448823_at</td>
<td>Cxcl12</td>
<td>−1.27</td>
<td>4.52E-08</td>
<td>1.92E-05</td>
<td>Chemokine (C-X-C motif) ligand 12</td>
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<tr>
<td>1438934_x_at</td>
<td>Sema4a</td>
<td>−1.18</td>
<td>2.83E-07</td>
<td>6.26E-05</td>
<td>Sema domain, immunoglobulin domain (Ig), transmembrane domain and short cytoplasmic domain, (seminophorin) 4A</td>
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<td>1449980_a_at</td>
<td>Gabrd</td>
<td>−1.13</td>
<td>1.46E-08</td>
<td>8.78E-06</td>
<td>γ-Aminobutyric acid (GABA-A) receptor, subunit delta</td>
</tr>
<tr>
<td>1416561_at</td>
<td>Gad1</td>
<td>−1.10</td>
<td>4.55E-09</td>
<td>4.66E-06</td>
<td>Glutamic acid decarboxylase 1</td>
</tr>
<tr>
<td>1436634_at</td>
<td>Robo3</td>
<td>−1.09</td>
<td>2.71E-07</td>
<td>6.16E-05</td>
<td>Roundabout homolog 3 (Drosophila)</td>
</tr>
<tr>
<td>1416953_at</td>
<td>Ctgf</td>
<td>−1.03</td>
<td>1.04E-08</td>
<td>7.77E-06</td>
<td>Connective tissue growth factor</td>
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<tr>
<td>1439239_at</td>
<td>Lin7b</td>
<td>−0.99</td>
<td>5.74E-06</td>
<td>4.89E-04</td>
<td>Lin 7 homolog b (C. elegans), mRNA</td>
</tr>
<tr>
<td>1455785_at</td>
<td>Kcnal</td>
<td>−0.99</td>
<td>3.43E-07</td>
<td>7.09E-05</td>
<td>Potassium voltage-gated channel, shaker-related subfamily, member 1 (Kcnal1), mRNA</td>
</tr>
<tr>
<td>1421863_at</td>
<td>Vamp1</td>
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<td>2.26E-07</td>
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<td>1417312_at</td>
<td>Dkk3</td>
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<td>3.18E-05</td>
<td>Dickkopf homolog 3 (Xenopus laevis)</td>
</tr>
<tr>
<td>1450350_a_at</td>
<td>Jdp2</td>
<td>−0.83</td>
<td>4.78E-07</td>
<td>9.09E-05</td>
<td>Jun dimerization protein 2</td>
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<tr>
<td>1416505_at</td>
<td>Nr4a1</td>
<td>−0.80</td>
<td>1.15E-04</td>
<td>3.70E-03</td>
<td>Nuclear receptor subfamily 4, Group A, member 1</td>
</tr>
</tbody>
</table>

P values were corrected for multiple testing using the False Discovery Rate (FDR) method. Data are presented for a subset of probe sets meeting criteria of log2 FC less than −0.75 and FDR p < 0.05. The full analysis is available in supplementary data.
CGAGCGCCTATGACATGTG); Lin7b (NM_011698) TAA GGCCACAGTGGCTGCTTT, CCCATGATGTTGAAGCCC AA; Lin7c (NM_011699) ATATCGCGGATAATTCCAGG TG, ATGGTG CTCCCCTCAACACTC.

**q-PCR Data Analysis**

Expression of the mRNA of interest in each sample was calculated for q-PCR by normalization of Ct values to the reference RNA (28S rRNA) using the equation:

\[
V = \frac{(1 + E_{reference})^{(Ct_{reference})}}{(1 + E_{target})^{(Ct_{target})}}
\]

to correct for potential differences in PCR amplification efficiencies (21, 31), where V is the relative value of target gene normalized to reference (28S rRNA), E is the PCR amplification efficiency, and Ct is the threshold crossing cycle number. Differences between genotypes for human layer 5 cortical neurons were assessed using an unpaired 2-tailed Student t-test.

**Immunoblotting**

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and transfer of separated proteins to polyvinylidene fluoride membranes were performed as previously described (32). For protein electrophoresis, 15% polyacrylamide gels were used. The concentration of Lin7 antibody (Sigma) used for immunoblotting was 1 μg/mL. For quantification of Lin7 protein, equal amounts (10 μg) of proteins from each group (6Rx/2 and 6WT littermates) were loaded into each lane of the gel. Fyn kinase antibody was used as a loading control.

**TABLE 4. Selected mRNA Increases in Layer 5 Motor Cortex Neurons of R6/2 Versus Wild-Type Mice**

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Gene Symbol</th>
<th>Log2 FC</th>
<th>Raw p Value</th>
<th>Adjusted p Value</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1417457_at</td>
<td>Cks2</td>
<td>2.13</td>
<td>4.01E-11</td>
<td>3.61E-07</td>
<td>CDC28 protein kinase regulatory subunit 2</td>
</tr>
<tr>
<td>1438130_at</td>
<td>Taf15</td>
<td>1.76</td>
<td>1.44E-07</td>
<td>4.02E-05</td>
<td>TAF15 RNA polymerase II, TATA box binding protein-associated factor</td>
</tr>
<tr>
<td>1430127_a_at</td>
<td>Ccnd2</td>
<td>1.57</td>
<td>4.68E-10</td>
<td>9.71E-07</td>
<td>Cyclin D2</td>
</tr>
<tr>
<td>1456039_a_at</td>
<td>Vim</td>
<td>1.56</td>
<td>1.33E-09</td>
<td>1.94E-06</td>
<td>Vimentin</td>
</tr>
<tr>
<td>1418733_at</td>
<td>Twist1</td>
<td>1.27</td>
<td>5.06E-06</td>
<td>4.73E-04</td>
<td>Twist gene homolog 1 (Drosophila)</td>
</tr>
<tr>
<td>1455039_a_at</td>
<td>Sin3b</td>
<td>1.21</td>
<td>2.00E-09</td>
<td>2.65E-06</td>
<td>Transcriptional regulator, SIN3B (yeast)</td>
</tr>
<tr>
<td>1447669_s_at</td>
<td>Gng4</td>
<td>1.16</td>
<td>6.25E-09</td>
<td>5.82E-06</td>
<td>Guanine nucleotide binding protein (G protein), γ 4 subunit</td>
</tr>
<tr>
<td>1452959_a_at</td>
<td>Capn10</td>
<td>1.15</td>
<td>3.04E-07</td>
<td>6.52E-06</td>
<td>Calpain 10</td>
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<tr>
<td>1416698_a_at</td>
<td>Cks1b</td>
<td>1.08</td>
<td>1.86E-06</td>
<td>2.20E-04</td>
<td>CDC28 protein kinase 1b</td>
</tr>
<tr>
<td>1434637_x_at</td>
<td>Sin3b</td>
<td>1.05</td>
<td>7.15E-09</td>
<td>6.31E-06</td>
<td>Transcriptional regulator, SIN3B (yeast)</td>
</tr>
<tr>
<td>1417056_at</td>
<td>Psme1</td>
<td>1.04</td>
<td>1.31E-07</td>
<td>3.72E-05</td>
<td>Proteasome (prosome, macropain) 28 subunit, α</td>
</tr>
<tr>
<td>1417185_at</td>
<td>Ly6a</td>
<td>1.03</td>
<td>6.11E-04</td>
<td>1.14E-02</td>
<td>Lymphocyte antigen 6 complex, locus A</td>
</tr>
<tr>
<td>1442263_at</td>
<td>Rgs13</td>
<td>1.03</td>
<td>1.69E-05</td>
<td>1.01E-03</td>
<td>Regulator of G-protein signaling 13</td>
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<tr>
<td>1424355_a_at</td>
<td>Sin3b</td>
<td>0.96</td>
<td>3.66E-08</td>
<td>1.67E-05</td>
<td>Transcriptional regulator, SIN3B (yeast)</td>
</tr>
<tr>
<td>1419879_s_at</td>
<td>Trim25</td>
<td>0.94</td>
<td>3.83E-05</td>
<td>1.72E-03</td>
<td>Tripartite motif protein 25</td>
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<tr>
<td>1448236_at</td>
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<td>0.91</td>
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<td>2.50E-05</td>
<td>Radixin</td>
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<tr>
<td>1428209_at</td>
<td>Bex4</td>
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<td>1.72E-06</td>
<td>2.12E-04</td>
<td>Brain expressed X-linked 4</td>
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<td>1444908_at</td>
<td>Hapb4</td>
<td>0.89</td>
<td>3.60E-05</td>
<td>1.66E-03</td>
<td>Hyaluronic acid binding protein 4</td>
</tr>
<tr>
<td>1422269_at</td>
<td>Cntn6</td>
<td>0.87</td>
<td>2.76E-05</td>
<td>1.38E-03</td>
<td>Contactin 6</td>
</tr>
<tr>
<td>1426412_at</td>
<td>Neurod1</td>
<td>0.87</td>
<td>5.43E-06</td>
<td>4.69E-04</td>
<td>Neurogenic differentiation 1</td>
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<tr>
<td>1422457_s_at</td>
<td>Sumo3</td>
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<td>2.44E-06</td>
<td>2.66E-04</td>
<td>SMT3 suppressor of mir 23 homolog 3 (yeast)</td>
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<tr>
<td>1421840_at</td>
<td>Abca1</td>
<td>0.87</td>
<td>1.27E-06</td>
<td>1.70E-04</td>
<td>ATP-binding cassette, subfamily A (ABC1), member 1</td>
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<td>1450783_at</td>
<td>Ifi1</td>
<td>0.87</td>
<td>1.86E-04</td>
<td>5.11E-03</td>
<td>Interferon-induced protein with tricistriopeptide repeats 1</td>
</tr>
<tr>
<td>1439078_at</td>
<td>Khih4</td>
<td>0.87</td>
<td>1.78E-05</td>
<td>1.05E-03</td>
<td>Kelch-like 4 (Drosophila)</td>
</tr>
<tr>
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<td>Wdr6</td>
<td>0.87</td>
<td>2.83E-07</td>
<td>6.26E-05</td>
<td>WD repeat domain 6</td>
</tr>
<tr>
<td>1417327_at</td>
<td>Cav2</td>
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<td>1.10E-03</td>
<td>Caveolin 2</td>
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<tr>
<td>1428210_s_at</td>
<td>Chuk</td>
<td>0.86</td>
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<td>2.86E-04</td>
<td>Conserved helix-loop-helix ubiquitous kinase</td>
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<tr>
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<td>Aldh9a1</td>
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<td>Aldehyde dehydrogenase 9, subfamily A1</td>
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<tr>
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<td>Nuclear transcription factor-Y γ</td>
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<tr>
<td>1435645_at</td>
<td>Mnd</td>
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<td>9.18E-08</td>
<td>3.06E-05</td>
<td>Monocytome to macrophage differentiation-associated</td>
</tr>
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<td>Ciss</td>
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<td>1.55E-05</td>
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<td>Cathepsin S</td>
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<td>9.20E-03</td>
<td>REST corepressor 3 (Rcor3), mRNA</td>
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<td>Nuclear receptor corepressor 1</td>
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</table>

P values were corrected for multiple testing using the False Discovery Rate (FDR) method. Data presented are a subset of probe sets meeting the criteria of FDR less than 0.05 and log2 FC greater than 0.8. The full analysis is available in the supplementary data.
### TABLE 5. Comparison of mRNA Changes in R6/2 Layer 5 Motor Cortex Neurons to Human Huntington Disease Motor Cortex

<table>
<thead>
<tr>
<th>Mouse Probe Set</th>
<th>Log2 FC</th>
<th>Gene Symbol</th>
<th>Human Probe Set</th>
<th>Log2 FC</th>
<th>Gene Symbol</th>
<th>Gene</th>
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</thead>
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<td>1416287_at</td>
<td>-0.93</td>
<td>Rgs4</td>
<td>204338_s_at</td>
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<td>RGS4</td>
<td>Regulator of G-protein signaling 4</td>
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<tr>
<td>1417663_a_at</td>
<td>-0.50</td>
<td>Ndeg3</td>
<td>221082_s_at</td>
<td>-0.95</td>
<td>NDRG3</td>
<td>NDRG family member 3</td>
</tr>
<tr>
<td>1424474_a_at</td>
<td>-1.48</td>
<td>Camkk2</td>
<td>210787_s_at</td>
<td>-0.94</td>
<td>CAMKK2</td>
<td>Calcium/calmodulin-dependent protein kinase 2, β</td>
</tr>
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<td>1416561_at</td>
<td>-1.10</td>
<td>Gad1</td>
<td>206670_s_at</td>
<td>-0.80</td>
<td>GAD1</td>
<td>Glutamate decarboxylase 1 (brain, 67 kd)</td>
</tr>
<tr>
<td>1421863_at</td>
<td>-0.90</td>
<td>Vamp1</td>
<td>207101_at</td>
<td>-0.78</td>
<td>VAMP1</td>
<td>Vesicle-associated membrane protein 1 (synaptobrevin 1)</td>
</tr>
<tr>
<td>1433888_at</td>
<td>-0.69</td>
<td>Atpb2b</td>
<td>211586_s_at</td>
<td>-0.77</td>
<td>ATP2H2</td>
<td>ATPase, Ca++ transporting, plasma membrane 2</td>
</tr>
<tr>
<td>1420955_at</td>
<td>-0.46</td>
<td>Vsnl1</td>
<td>203798_s_at</td>
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<td>Visinin-like 1</td>
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<td>Gabrg2</td>
<td>206849_at</td>
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<td>GABRG2</td>
<td>γ-Aminobutyric acid (GABA) A receptor, γ 2</td>
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<td>Sst</td>
<td>213921_at</td>
<td>-0.56</td>
<td>SST</td>
<td>Somatostatin</td>
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<tr>
<td>1423362_at</td>
<td>-0.57</td>
<td>Sort1</td>
<td>212797_at</td>
<td>-0.76</td>
<td>SORT1</td>
<td>Synaptogamin 1</td>
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<td>1431191_a_at</td>
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<td>Syt1</td>
<td>203998_s_at</td>
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<td>SYT1</td>
<td>Synaptotagmin 1</td>
</tr>
<tr>
<td>1449724_at</td>
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<td>Itpr1</td>
<td>211323_at</td>
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<td>Inositol 1,4,5-triphosphate receptor, type 1</td>
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<td>Junctophilin 3</td>
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<td>Pak1</td>
<td>209615_s_at</td>
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<td>PAK1</td>
<td>p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)</td>
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<tr>
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<td>Kcnkl</td>
<td>204678_s_at</td>
<td>-0.62</td>
<td>KCNK1</td>
<td>Potassium channel, subfamily K, member 1</td>
</tr>
<tr>
<td>1450646_at</td>
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<td>Cyp51</td>
<td>216607_s_at</td>
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<td>CYP51A1</td>
<td>Cytochrome P450, family 51, subfamily A, polypeptide 1</td>
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<td>1426499_at</td>
<td>-0.24</td>
<td>Sh3glb2</td>
<td>218813_s_at</td>
<td>-0.58</td>
<td>SH3GL2</td>
<td>SH3-domain GRB2-like endophilin B2</td>
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<td>1422609_at</td>
<td>-1.54</td>
<td>Appr19</td>
<td>214553_s_at</td>
<td>-0.58</td>
<td>ARP-19</td>
<td>Cyclic AMP phosphoprotein, 19 kd</td>
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<td>1434472_at</td>
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<td>Dusp3</td>
<td>201537_s_at</td>
<td>-0.57</td>
<td>DUSP3</td>
<td>Dual-specificity phosphatase 3 (vaccinia virus phosphatase VHI-related)</td>
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Comparison of mRNA changes in layer 5 samples from R6/2 versus human Huntington disease BA4 cortex homogenates. Gene expression changes common to both data sets were selected using a false discovery rate adjusted p < 0.05 and an absolute log2 FC cutoff of 0.4. Probe sets for mRNAs chosen from among those showing the highest magnitude changes; decreases are above the horizontal bar; increases are below the horizontal bar. The complete list of common mRNA changes is available in the supplementary data.
Bands were visualized on film by enhanced chemiluminescence, and their net intensities were quantified using computer-assisted densitometry (Kodak 1-D System, Rochester, NY). The net intensities of the bands were expressed as a percentage of that in control cortex and the resulting values were used to calculate group means. Differences between groups were analyzed using analysis of variance with post hoc tests (Scheffe test). Statistical significance was taken to be \( p < 0.05 \).

**Immunohistochemistry of Mouse Brain Sections**

Six-micrometer-thick paraffin sagittal mouse brain sections from 4 R/2 and 4 WT littermates were run through xylene (Merck, Darmstadt, Germany) and a series of graded alcohols to phosphate buffered saline (PBS). Sections were then incubated in 10 mmol/L sodium citrate pH 6.0 for 10 minutes, washed 3 \( \times \) 5 minutes in PBS, and incubated in 40\% methanol and 1\% \( \text{H}_2\text{O}_2 \) for 10 minutes to block endogenous peroxidase. After washing (3 \( \times \) 10 minutes) in PBS, the sections were incubated in blocking solution (5\% rabbit serum + 0.3 Triton X-100 in PBS) for 30 minutes at room temperature (RT), washed (3 \( \times \) 10 minutes) in PBS, and then incubated overnight with the primary antibody (1:300, goat anti-Lin7b; ab5967; Abcam, Cambridge, UK) on a shaker at 4\( ^\circ \text{C} \). The sections were then washed (3 \( \times \) 5 minutes) in PBS and incubated in a biotinylated anti-goat Ig (H + L) secondary antibody (1:200; Vector Laboratories Ltd, Peterborough, UK) for 30 minutes at RT. After additional washing (1 \( \times \) 5 minute, PBS), the sections were incubated in ABC reagent (metal-enhanced 3,3\(-\)diaminobenzidine substrate kit; Thermo Fisher Scientific) for 30 minutes at RT and then exposed to 3,3\(-\)diaminobenzidine

**FIGURE 2.** Quantitative polymerase chain reaction analysis of mRNA expression levels in layer 5 cortical neurons in Huntington disease (HD) (n = 9) versus control (n = 8) samples. Bar graphs show relative expression measures (i.e. number \( \times 10^{\text{--6}} \)) normalized to 28S rRNA. White bar: control cortex BA4; black bar: HD cortex BA4; diagonally striped bar: control cortex BA9; dark gray bar: HD cortex BA9. Each sample was run in triplicate. Error bars = SEM; *\( p < 0.05 \): NS, not significant. (A) Lin7 homolog b was decreased to 47.8\% in HD BA4 versus control BA4 (\( p < 0.01 \)). Lin7 homolog b did not differ between HD and control BA9 (NS). Contactin 1 mRNA in deep-layer cortical neurons of BA 4 was decreased to 44.4\% in HD versus control (\( p < 0.02 \)); there is no difference in BA9 (NS). (B) Messenger RNA expression that is not significantly changed in human HD cortical layer 5 neurons. SYNCRIP shows a trend to a decrease in HD cortex that is not significant in either BA4 (\( p > 0.05 \)) or BA9 (\( p > 0.5 \)). VAMP1 does not show an HD-related change in either region (\( p > 0.2 \); \( p > 0.5 \)). ATP2B2 also is not significantly different between HD and control (\( p > 0.1 \); \( p > 0.2 \)). Additional mRNAs were assayed in BA4 layer 5 neurons but did not show an appreciable difference in HD versus controls (not shown).
solution for 5 minutes. The sections were then washed in PBS, dried and dehydrated through a graded alcohol series to xylene, and coverslipped with DPX mountant (Sigma Aldrich/Fluka, Steinheim, Germany).

**Immunohistochemistry of Human Brain Sections**

Tissues were obtained from the Neurological Foundation of New Zealand Human Brain Bank in the Center for Brain Research, University of Auckland. Fifty-micrometer-thick perfused-fixed coronal sections of BA4 (motor cortex) were processed free-floating in 6-well tissue culture dishes. The sections were washed in PBS and 0.2% Triton-X (PBST), incubated for 20 minutes in 50% methanol and 1% H$_2$O$_2$, and washed (3 × 10 minutes) in PBST; they were then incubated with the same goat anti-Lin7b antibody as for mouse tissues (1:250) for 2 to 3 days on a shaker at 4°C. The sections were then washed (3 × 10 minutes, PBST) and incubated overnight in a biotinylated anti-goat Ig (H + L) secondary antibody (diluted 1:200; Vector Laboratories Ltd). After washing (3 × 10 minutes, PBST), the sections were incubated for 1 hour at RT in ABC mix, exposed to metal-enhanced 3,3'-diaminobenzidine (Thermo Fisher Scientific) for 5 minutes, and then washed in PBST, and mounted on gelatin-coated slides (Marienfeld Laboratory Glassware, Lauda-Koenigshofen, Germany; gelatin from Sigma), rinsed, dehydrated, and coverslipped as previously described.

**Image Analysis**

Images of immunohistochemistry-stained sections were collected and analyzed in a blinded fashion with respect to the sample genotype. For the human sections, a contour of the area of interest (layer V) in the primary motor cortex was first drawn at low power (4×) using Stereoinvestigator program (MicroBrightField, Williston, VT) using an Olympus BX60 microscope (Olympus Schweiz, Volketswil, Switzerland). Images were taken at 40× from the area within the defined boundary of each normal human and HD case. For mouse sections, images were taken at 20× from the striatum and the layer 5 of motor cortex. Images were analyzed using ImageJ (http://rsbweb.nih.gov/ij/) and Adobe Photoshop to measure the mean gray value. The difference between the normal and HD samples was assessed using a 2-tailed Student t-test.

**RESULTS**

**RNAs Enriched in Layer 5 Cortical Neurons**

To identify genes that exhibit enriched expression in layer 5 pyramidal neurons, we compared LCM and homogenate samples of mouse motor cortex (M1 and M2) in WT animals. A total of 2,943 (of 45,037) array probe sets indicated differential mRNA expression between deep-layer cortical neurons from R6/2 versus WT mice. Of the largest fold changes (1.75-fold or higher), 246 probe sets detected decreases and 81 probe sets detected increases in mRNA levels. Selected RNAs showing differential expression in...
layer 5 R6/2 cortex are presented in Tables 3 and 4. Complete lists are in Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A156.

Gene expression changes detected in the LCM array analysis included several mRNAs previously shown to be decreased in R6/2 cortex, including cplx2, dbp, sst, penk, arpp19, dusp1, and igfbp5 (16). The mRNA encoding igfbp4, which modulates the IGF/AKT prosurvival pathway (44), was also downregulated by more than 2-fold in R6/2 versus WT layer 5 neurons. The mRNAs encoding the GABA$_A$ receptor (GABRD) and Shaker-related member 1 of the voltage-gated potassium channel superfamily (kcnal) also showed 2-fold downregulation in R6/2 versus control pyramidal cells (Table 3).

Some unanticipated changes in gene expression were also observed, including decreases in corticotropin-releasing hormone and its binding protein, crhbp; this parallels a decrease in pituitary gland corticotropin-releasing hormone levels in R6/2 mice (45). Other interesting changes revealed by this analysis included a decrease in the pyramidal cell–enriched protease prss23 and several genes involved in immune system function (Tables 2–4).

Intriguingly, there was upregulation of several mRNAs encoding transcriptional regulatory proteins previously linked to the biology of huntingtin (Table 4; Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A156). These include the known huntingtin-interacting protein NCor1 (nuclear corepressor 1) (46) and the repressor element 1

![Figure 4](http://jnen.oxfordjournals.org/)

**FIGURE 4.** Decreased Lin7 protein in R6/2 mouse brain. (A) Graph showing the intensity of Lin7b staining in layer 5 pyramidal neurons of the motor cortex in R6/2 mice versus wild-type (WT) mice. There is an average reduction of 12% in the R6/2 mice. (B) Lin7 homolog b immunostaining in layer 5 motor cortex pyramidal neurons (arrows) in WT and R6/2 mice. (C) Graph showing the intensity of Lin7b staining in fiber tracts in striatum of WT and R6/2 mice, with an average reduction of 17% in the R6/2 mice. (D) Lin7 homolog b immunostaining in striata of WT and R6/2 mice. Asterisks indicate significant differences (p < 0.05) by Student t-test (A, C). Photomicrograph magnification: 20×. Scale bar = (B, D) 100 μm.
silencing transcription factor corepressor 3 (RCoR3), a coregulator of the NRSF/REST transcriptional silencing complex (47). The mRNA expression of the ubiquitous corepressor sin3b (which is known to be sequestered in polyglutamine aggregates) was also increased.

Pyramidal Cell–Enriched mRNAs Show Dysregulation in R6/2 Cortex

Previous studies indicate that a defect in neurotransmission could account for cognitive and motor deficits in HD. Accordingly, we determined whether mRNAs enriched in layer 5 pyramidal cells were dysregulated with polyglutamine disease in brains of R6/2 mice. Several layer 5–enriched synaptic proteins, including the SNARE complex synaptic vesicle protein vampl, were downregulated. Brain-derived neurotrophic factor, a cortically expressed striatal neuron survival factor, showed a significant downregulation in HD layer 5 neurons, but there was no significant enrichment in layer 5. Lin7 homolog b, a PDZ domain protein initially identified in Caenorhabditis elegans (48), was also decreased in corticostriatal neurons of R6/2 mice (p < 0.0005; Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A156).

Similarities and Differences Between Transcriptomic Effects in Layer 5 R6/2 Neurons and Human BA4 HD Cortex

To assess whether cortical pyramidal mRNA changes in R6/2 mice might recapitulate mRNA changes in human motor cortex at a transcriptome-wide level, we compared LCM microarray profiles from R6/2 layer 5 cells to previously established microarray profiles of human BA4 cortex homogenates (17). Overlapping changes included decreased expression of mRNAs encoding neurotransmitter signaling pathway components (e.g. gad1, rgs4, ipt1), potassium channels (kcncl, kcncl2), and other proteins involved in neurotransmission (e.g. vamp1, cplx2, arpp19, syt1) (Table 5; Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A157). Interestingly, these comparisons also detected overlapping upregulation of mRNAs encoding neuronal signaling and viability regulators that were not reported in previous studies; these included increases in notch2 and Sirt1 (Table 5; Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A157). Surprisingly, however, some of the previously described changes, including the apparent decreases in GABRD and plasma membrane ATPase Ca2+–transporting plasma membrane 2 (ATP2B2) RNAs, did not show a corresponding change in human HD layer 5 pyramidal cells by q-PCR (see later). This indicates that the decreases observed in HD BA4 cortex may occur in a different cell type such as pyramidal neurons of cortical layers 2 or 3 or GABAergic interneurons.

HD-Related Dysregulation of Gene Expression in Human Layer 5 Pyramidal Neurons

To determine to what extent the polyglutamine-induced mRNA changes in R6/2 pyramidal neurons might recapitulate the molecular changes that occur in the corresponding cell population in HD, we examined a subset of these changes in human HD cases and controls using q-PCR on layer 5 cortical neurons from both Brodmann area 4 (primary motor cortex) and Brodmann area 9 (prefrontal association cortex) for each HD case or control (Table 1). Because HD brain revealed a downregulation of β-actin, the ribosomal 28s RNA was used for normalization.

Because layer 5 neurons degenerate selectively in HD cortex, we assessed genes enriched in layer 5 pyramidal cells. A similar hypothesis has been proposed for the selective degeneration medium spiny neurons in the HD striatum (49, 50). However, in contrast to findings in striatum, only a very small proportion of the tested layer 5–enriched genes were significantly downregulated in human HD pyramidal cells. The mRNAs with normal expression levels in HD layer 5 neurons included VAMP1, SYNCRI, voltage-gated potassium channel subfamily members KCNA1 (control, 60.1 ± 8.6; HD, 42.5 ± 9.9; p > 0.1), KCNK3 (control, 3.9 ± 1.3; HD, 2.5 ± 1.3; p > 0.5), ATP2B2, and metabotropic glutamate receptor 2 (control, 2.8 ± 0.95; HD, 3.1 ± 1.1; p > 0.5). On the other hand, the RNA-encoding CNTN1 was significantly decreased in human HD BA4 pyramidal cells (Fig. 2).

We next determined whether differential expression in R6/2 layer 5 motor neurons predicted corresponding layer 5 changes in human HD cortex. Although RNAs encoding voltage-gated potassium channels or GABRD subunit were not significantly diminished in either BA4 (control, 0.004 ± 0.001; HD, 0.002 ± 0.0007; p > 0.2) or BA9 samples, the RNA encoding Lin7b was significantly decreased in BA4 and showed a nonsignificant trend toward a decrease in BA9 (Fig. 2). The selective decrease in the expression of this neuronal connectivity-related molecule in human HD cortex motivated us to verify a change in Lin7 at the protein level and consider possible functional consequences.

Lin7b Protein Levels

R6/2 cortical protein lysates were analyzed by Western blot with a pan-Lin7 antibody. As shown in Figure 3A, we observed 2 bands corresponding to apparent molecular weights of 21 to 27 kd representing Lin7 isoforms Lin7a, Lin7b, and Lin7c or mals1, mals2, and mals3, consistent with previous observations (51). Comparison of the abundance of Lin7-positive species in R6/2 cortex with control revealed significant decreases in both Lin7-positive bands (Figs. 3A, B). To investigate these changes, we also performed immunohistochemistry analyses of LIN7B protein using a Lin7b-specific
We found that LIN7B was significantly decreased in layer 5 pyramidal cortical neurons in both R6/2 mice and in human HD brains (Figs. 4A, B; 5). Thus, Lin7b RNA levels are paralleled by decreases in Lin7b protein in cortical projection cells.

To assess whether Lin7b expression would also be decreased in cortical axons in the striatum, we performed additional immunohistochemistry on R6/2 mice. Quantification of Lin7b immunoreactivity in axon fiber–containing striatal white matter tracts also showed decreased Lin7b expression (Figs. 4C, D). Although it is unlikely that all such fibers terminate in the striatum, these results indicate that a parallel diminution of expression occurs in axonal as well as cell body compartments of cortical projection neurons.

To determine whether decreased Lin7b expression may contribute to HD-related dysfunction early in the disease process, we measured cortical RNA levels in younger R6/2 mice by q-PCR. Lin7b RNA was significantly decreased by 6 weeks of age, coincident with the onset of early motor deficits in these mice (52). Lin7b RNA was also significantly decreased at 8 and 10 weeks of age. Consistent with the Western blots in 12-week-old animals (Fig. 3), Lin7a RNA was also decreased significantly at 6, 8, and 10 weeks of age (Fig. 6). Thus, the early and persistent decreases in Lin7a and Lin7b expression are consistent with a role in HD etiology. In contrast, Lin7c RNA showed only a transient increase at 8 weeks, which would be more consistent with a compensatory effect than an etiologic role in HD (Fig. 6).

**DISCUSSION**

Diminished expression of LIN7A and LIN7B mRNAs and proteins in HD cortex provides novel evidence for a role of LIN7 in the pathogenesis of HD. In view of its known role in neuronal connectivity, we conclude that an HD-associated decrease in LIN7 expression in cortical projection neurons is a plausible mechanism of corticostriatal dysfunction.

**Cortical Pyramidal Cells and HD**

Abnormal connectivity between the cortex and the basal ganglia may account for cognitive and/or motor manifestations of HD. Dysfunction of corticostriatal projections has been considered in previous studies of R6/2 mice as electrophysiological recordings reveal decreased excitatory

**FIGURE 6.** Time course of Lin7 RNA changes in R6/2 mice. Lin7 homolog b RNA levels are significantly decreased in R6/2 mice at 6, 8, and 10 weeks of age (top panel). Lin7 homolog a RNA levels in R6/2 mice show a trend toward a decrease at 4 weeks of age and are significantly decreased at 6, 8, and 10 weeks of age (middle panel). Lin7 homolog c RNA levels are maintained in R6/2 mice at 4, 6, and 10 weeks of age, with a transient increase at 8 weeks (bottom panel). Quantitative polymerase chain reaction expression measures are presented as quotients of R6/2 versus wild-type (WT) values × 100 (% control) ± SEM (same scale). Samples comprised cortical RNA extracts from 4 WT and 4 R6/2 mice at 4 weeks, 12 WT and 12 R6/2 mice at 6 weeks, 8 WT and 8 R6/2 mice at 8 weeks, and 7 WT and 7 R6/2 mice at 10 weeks. Asterisks represent statistical significance of p < 0.05 by 2-tailed Student t-test.
postsynaptic currents in striatal medium spiny neurons and large-amplitude synaptic events that indicate dysregulation of cortical glutamatergic inputs (14). N-methyl-D-aspartic acid receptor currents of cortical pyramidal neurons from R6/2 mice become smaller than those of WT mice beginning at 21 days of age (53). Here, we addressed the possibility that transcriptional alterations contribute to the HD-related dysfunction of corticostriatal projection neurons.

To avoid the confounding effects of cellular heterogeneity and the potential for changes in cell ratios during the neurodegenerative process, we performed a targeted cellular expression analysis using LCM. Because most of the projections to the striatum originate from layer 5 (19, 20), we collected medium- and large-sized pyramidal cells of this lamina. Pyramidal neurons within layer 5 form 2 subtypes: type 1 pyramidal neurons projecting to the superior colliculus, spinal cord, basal pons, and striatum; and type 2 pyramidal neurons projecting to the contralateral cortex and ipsilateral striatum (20, 54). Both project to the striatum, and we did not differentiate between these 2 subtypes; presumably, both were sampled in their endogenous proportions. Because layer 6 cortical neurons cannot easily be distinguished from layer 5 neurons, it is also possible that some layer 6 cells were included in our samples; this should be inconsequential to the interpretation of the data because layer 6 neurons also project to the striatum.

The decreased GABA<sub>A</sub> receptor delta subunit expression observed in R6/2 layer 5 neurons (Table 3) and in human HD cortex homogenates (17) suggests that neocortical GABA<sub>A</sub>ergic signaling might be altered in HD, which would comprise an interesting area of future investigation. However, based on the human LCM data, additional data are required to attribute this change to a specific neuronal population in the human HD brain.

Some RNA changes observed in R6/2 layer 5 cells did not show corresponding changes in human BA4 or BA9. Possible explanations for this are that these changes do not occur to the same extent in the HD brain, or that they occur in a limited subset of cells that escaped detection in the current study. These changes may alternatively indicate possible mt htt effects in other cortical neurons, such as layer 3 pyramidal cells, other layer 5 pyramidal cells, or GABA<sub>A</sub>ergic interneurons. Indeed, comparisons with our previous microarray analyses of human HD brain also infer that transcriptomic perturbations occur in cortical cells other than layer 5 pyramidal neurons. For example, whereas human BA4 tissue homogenates show significant decreases in GABRD and ATP2B2 mRNAs, the present experiments demonstrate that layer 5 pyramidal neurons in BA4 do not harbor these changes. Thus, additional anatomical and molecular studies to clarify transcriptional perturbations in other cortical cell types are warranted.

**HD-Related Transcriptional Dysregulation in Corticostriatal Neurons**

Changes in gene expression shared by R6/2 layer 5 pyramidal cells and human HD BA4 likely represent early relevant molecular events in HD pathogenesis. The distribution of mRNA changes (i.e. 3 times more decreases than increases) is in accordance with previous microarray results from other neurons of R6/2 mice and from LCM samples of human HD grade 1 brains (17, 49).

Using LCM, we were able to identify cortical mRNAs that show enriched expression in layer 5 pyramidal neurons (Table 2). As expected, the expression of most layer 5 enriched genes did not change in HD-affected neurons; however, CNTN1 was both enriched in layer 5 and downregulated in human corticostriatal cells (Fig. 2). Contactin 1, an axonal glycoprotein belonging to the immunoglobulin family of cell adhesion molecules, was enriched in layer 5 cortical neurons, which is in line with data from an in situ hybridization histological study that showed expression of this molecule in layers 2, 3, and 5. It is thought to play an important role in axonal pathfinding in development, and its plasma membrane signal transduction activity has been shown to govern the formation and maintenance of synapses (40, 57). Therefore, we postulate that downregulation of CNTN1 in human HD BA4 cortex (to 44.4% of control levels) could contribute to the destabilization of corticostriatal connections.

Although there are biochemical data to suggest that the general transcriptional machinery is affected by mutant huntingtin, the discrete subpopulations of neuronal mRNAs affected in model systems suggest a more specific mechanism or set of mechanisms. We observed aberrant expression of RNAs encoding known huntingtin-interacting transcriptional regulators. Specifically, NCoR1 and REST corepressor 3 are upregulated in R6/2 cortical neurons, as well as sin3b, another coregulator that can be present in either NCoR or REST complexes. Whereas increased transcriptional repression by these factors has been previously attributed to direct interactions with mt htt (58), our data raise the possibility that increased expression levels of these factors contribute to a change in their activities. However, the extent to which these expression changes have functional relevance to primary or secondary disease events remains to be determined.

**Regional Differences in Human HD Cortex**

To assess the potential impacts of gene expression changes on both motor and cognitive dysfunction, we examined both BA4 (primary motor cortex) and BA9 (prefrontal association cortex). As expected, cortical neurons from BA4 revealed transcriptomic dysregulation, whereas layer 5 neurons from BA9 showed no significant changes in gene expression, although there was a trend toward downregulation for some mRNAs (Fig. 2). This is in accordance with a parallel microarray analysis of HD brain in which BA4 but not BA9 exhibited significant changes in mRNA levels (17). It is also in agreement with the findings functional magnetic resonance imaging–based volumetric regional thinning in BA4 of HD gene carriers (5).

**Decreased Levels of Lin7b as a New Potential Mechanism of Corticostriatal Dysfunction in HD**

Neurons are highly polarized cells with intrinsic functional specifications of presynaptic axons and postsynaptic dendrites (59); specialized polarity proteins participate in axon formation, growth, and synaptogenesis, in part through signaling to the actin and microtubule cytoskeletons. The 3 members of the mammalian Lin7 family of proteins comprise...
logic events might account for decreased communication extent the loss of Lin7b, Lin7a, or contactin 1, or other etiological aspects of the study. The authors and Dr Matthew P. Frosch for consultation on neuroanatomical and neurocytologic aspects of the study. The authors also thank Dr Ippolita Cantutii Castelvetri, Dr Sandra Dieni, and Markus Kelm for valuable discussions and technical support.

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3. Berardelli A, Noth J, Thompson PD, et al. Hypometabolism of striatal medium spiny neurons together with altered dendritic spine density, consistent with decreased structural or signaling plasticity (63). Still other recent evidence for deficits of brain-derived neurotrophic factor synthesis and transport along the corticostriatal axons (64) provides an additional candidate mechanism underlying decreased corticostriatal connectivity. Thus, further investigation is warranted to determine to what extent the loss of Lin7b, Lin7a, or contactin 1, or other etiological events might account for decreased communication between the cortex and the basal ganglia in HD.

PDZ domain–containing scaffolding proteins that have also been previously shown to couple cell adhesion to intercellular signaling (51, 60). Mice harboring null mutations of all 3 Lin7 isoforms die perinatally with respiratory problems and deficient synaptic transmission (61). Our data reveal enrichment of Lin7b in layer V pyramidal neurons and show that it is downregulated in both R6/2 mice and in human HD layer V neurons. Based on the R6/2 mouse results, it also seems possible that Lin7a expression may be decreased in HD brain. Reduced levels of either Lin7b or Lin7a might disrupt normal neuronal connectivity and/or plasticity, either within the cerebral cortex or between the cortex and the basal ganglia. Therefore, we hypothesize that decreased Lin7 levels contribute to functional deficits in HD brain, an issue warranting further assessment in future studies.

The present data add to the growing body of molecular and functional evidence that corticostriatal communication is abnormal in the HD brain. A recent electrophysiological study also revealed progressive derangement of long-term depression and short-term plasticity of corticostriatal neurons at perirhinal synapses in HD mice (62). Likewise, Golgi stains of human HD brain sections reveal marked recurving of dendritic branches in striatal medium spiny neurons together with altered dendritic spine density, consistent with decreased structural or signaling plasticity (63). Still other recent evidence for deficits of brain-derived neurotrophic factor synthesis and transport along the corticostriatal axons (64) provides an additional candidate mechanism underlying decreased corticostriatal connectivity. Thus, further investigation is warranted to determine to what extent the loss of Lin7b, Lin7a, or contactin 1, or other etiological events might account for decreased communication between the cortex and the basal ganglia in HD.
60. Butz S, Okamoto M, Sudhof TC. A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. Cell 1998;94:773–82