Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington’s disease mouse models reveal context-independent effects

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Received March 12, 2002; Revised and Accepted June 7, 2002

Recent evidence indicates that transcriptional abnormalities may play an important role in the pathophysiology of polyglutamine diseases. In the present study, we have explored the extent to which polyglutamine-related changes in gene expression may be independent of protein context by comparing mouse models of dentatorubral–pallidoluysian atrophy (DRPLA) and Huntington’s disease (HD). Microarray gene expression profiling was conducted in mice of the same background strain in which the same promoter was employed to direct the expression of full-length atrophin-1 or partial huntingtin transproteins (At-65Q or N171-82Q mice). A large number of overlapping gene expression changes were observed in the cerebella of At-65Q and N171-82Q mice. Six of the gene expression changes common to both huntingtin and atrophin-1 transgenic mice were also observed in the cerebella of mouse models expressing full-length mutant ataxin-7 or the androgen receptor. These results demonstrate that some of the gene expression effects of expanded polyglutamine proteins occur independently of protein context.

INTRODUCTION

At least eight human neurologic diseases result from trinucleotide expansions causing the extension of a polyglutamine [poly(Q)] domain in the disease-causing protein (1–3). Poly(Q) diseases share many features, including a poly(Q) length-dependent neurodegeneration and inclusion body pathology. Clinical manifestations of the various diseases comprise both common and distinct signs. In fact, prior to the identification of their respective gene mutations, differential diagnosis of these disorders was often difficult.

The primary sites of neuropathology vary between the poly(Q) disorders, but include the cerebellum, striatum, cerebral cortex, brainstem, spinal cord and thalamus. Interestingly, two of the poly(Q) diseases showing the greatest degree of clinical similarity—Huntington’s disease (HD) and dentatorubral–pallidoluysian atrophy (DRPLA)—have different regional pathologies. Whereas the striatum is the most affected brain structure in HD, the primary site of degeneration in DRPLA is the dentate nucleus of the cerebellum.

We have generated transgenic mouse models of both HD and DRPLA using the same transgene promoters and mouse strains (4,5). Although these mice both utilize the murine prion promoter to drive transgenes with similar numbers of CAG repeats, the animals develop distinct pathologic and clinical phenotypes (6). The cerebellar dentate nucleus develops intranuclear inclusions and undergoes neurodegeneration in DRPLA (At-65Q) animals, but is spared in HD (N171-82Q) mice. The brains of these two mouse models appear largely similar, however. Both lines of mice show diffuse and punctate nuclear staining for the transprotein in the majority of neurons in most brain regions, and the HD mice additionally show cytoplasmic inclusions in some neurons. In contrast to the similar neuropathology in these animals, their phenotypes are
quite distinct. The HD mice are hypoactive whereas the DRPLA mice are hyperactive, and the DRPLA mice exhibit more severe cerebellar symptoms (e.g. tremor and uncoordinated gait).

Since expanded poly(Q) repeats cause both atrophin-1 and huntingtin to aberrantly sequester proteins that are critical for transcription (7–12), we hypothesized that transcriptional profiles would be altered in At-65Q and N171-82Q mouse brains compared with controls. Because these mice utilize the same promoter and similar-sized CAG repeats [encoding poly(Q)], they provide an opportunity to segregate transcriptional changes that are due primarily to poly(Q) expansion from those that are context-dependent. In this study, we identify some mRNA changes that distinguish At-65Q from N171-82Q mice and others that are caused by expanded poly(Q) regardless of the protein context. Several of the latter changes were also observed in cerebella from spinocerebellar ataxia type 7 (SCA7), spinobulbar muscular atrophy (SBMA) and other HD (R6/2) mouse models.

RESULTS

The primary goal of this study was to globally compare the gene expression changes in N171-82Q (HD) and At-65Q (DRPLA) mice. Because microarray data were available from the R6/2 and YAC72 HD mice (13,14), these data were compared with the N171-82Q and At65Q data when appropriate to provide additional insight into the mouse models. Figure 1 shows a schematic diagram of the mouse model transgenes (15–17, and C.J. O’Brien, E.S. Chevalier-Larsen and D.E. Merry, manuscript in preparation).

Striatal gene expression changes

The number of mRNAs that were differentially detected in N171-82Q (HD) versus wild-type striata (20 mRNAs at \( P < 0.001 \)) was similar to that in At-65Q (DRPLA) versus wild-type striata (36 mRNAs at \( P < 0.001 \)) and much lower than 12-week R6/2 versus wild-type (147 at \( P < 0.001 \)). Although the number...
of detected changes was low, there was evidence that mRNAs that were decreased in N171-82Q and At-65Q mice striata encode proteins involved in neuronal signal transduction, transcription and metabolism. A list of selected mRNAs that were altered in striata of these mice is presented in Figure 2 and the complete dataset is in the supplemental data (Supplementary Figs 1 and 2; www.neumetrix.info). Because the number of gene expression changes in the striata of these mice was insufficient to address the primary goal of distinguishing transcriptional changes primarily caused by poly(Q) expansion from those that are protein-context-dependent. Thus, we repeated the experiment using cerebellar tissue and an increased number of replicates.

Cerebellar gene expression changes unique to one model

We examined cerebellar gene expression changes in At-65Q (DRPLA) and N171-82Q (HD) mice at ages when both show neurologic deficits and cerebellar neuropathology. In comparing these lines, we assumed that the differences in tremor and coordination exhibited by these animals might be attributed to gene expression changes that were unique to each model. The number of gene expression changes in the At-65Q versus wild-type comparison (469 at \( P < 0.001 \) and 926 at \( P < 0.01 \)) was higher than in the N171-82Q versus wild-type comparison (165 at \( P < 0.001 \) and 354 at \( P < 0.01 \)). A Venn diagram (Fig. 3) shows the relationship between the gene expression changes in these models and the R6/2 model (data from 13). The At-65Q mice showed a higher percentage of unique gene expression changes (70%) relative to the N171-82Q (29%) and R6/2 mice (47%).

Since atrophin-1 and huntingtin transgenic animals that contain a non-expanded poly(Q) repeat (At-26Q and N171-18Q) are asymptomatic, gene expression changes shared between expanded and non-expanded transgenics were considered to be non-pathogenic. To determine the extent to which gene
expression changes in the atrophin-1 transgenics (At-26Q and At-65Q) might be reporting the presence of the atrophin-1 transprotein, we assessed mRNAs differentially expressed in At-26Q versus wild-type comparisons. Of 573 mRNAs that were significantly different in At-65Q versus wild-type, but not in the other two expanded repeat models, 108 of these were also different (at $P < 0.01$) in At-26Q versus wild-type comparisons (supplemental data Figs 3 and 4 at www.neumetrix.info). These changes appear to be unique to atrophin-1 mice, since no clear trend is observed for these mRNAs in N171-82Q or R6/2 (N-terminal huntingtin transgenic) mice. These mRNAs may represent transcriptional changes caused by overexpression of atrophin-1 regardless of poly(Q) length.

The gene expression changes in At-65Q versus wild-type that are not shared with other expanded or non-expanded poly(Q) mouse models are most likely to reflect mutant atrophin-1-specific cerebellar pathology that results in symptoms unique to these animals. A partial list of the mRNA changes specific to At-65Q cerebellum is presented in Figure 4.

Cerebellar gene expression changes shared across models

The most striking finding of our comparisons between N171-82Q and At-65Q mice was that most of the mRNAs that changed in response to the mutant huntingtin N171 transgene changed similarly in response to the mutant atrophin-1 transgene. Most (63% at $P < 0.01$) of mRNAs that were changed in N171-82Q mice were also statistically different in At-65Q mice. Figure 5 lists mRNAs that are differentially expressed in both of these animal models and also the R6/2 mouse model of HD. The similarity between these three models likely extends beyond that demonstrated by statistical thresholds. For example, Figure 6 shows mRNAs that are changed in N171-82Q and At-65Q mice, but are not significantly different from controls in R6/2 mice. The trend for most of these mRNAs was the same in R6/2 mice as in the other two models. Thus, we conclude that the poly(Q) effect on transcription seen in all three models is largely independent of the transprotein context. The lack of gene expression changes in mice that express non-expanded poly(Q) transproteins (Figs 5 and 6; columns labeled ‘DRPLA 26Q vs WT’ and ‘N171 18Q vs WT’) further indicates that gene expression changes seen in N171-82Q and At-65Q mice are unique consequences of poly(Q) expansion.

A subset of the cerebellar gene expression changes determined by array analysis to be common to all three disease models were confirmed using northern blot analysis (Fig. 7). In all cases, northern analyses were consistent with microarray results.

We then asked whether the changes in gene expression seen in N171-82Q, At-65Q and R6/2 mice would be predictive of cerebellar mRNA changes in other poly(Q) diseases. In order to test this hypothesis, we conducted northern analyses of cerebella from mouse models of SCA7 and SBMA (Fig. 1). Cerebella SCA7 and SBMA mouse show changes in enkephalin, insulin-like growth factor-binding protein 5 (IGFBP5), myristoylated, alanine-rich C-kinase substrate (MARCKS) and neuronal visinin-like protein 1 (NVP-1) that parallel changes seen in the brains of the mutant huntingtin and atrophin-1 transgenic mice (Fig. 8). In addition, changes in the expression of Snf1-related kinase (SNFK1) and neuronal acidic protein 22 (NAP22) were observed in four of the five lines (Fig. 8B). The consistency of the cerebellar gene expression changes between five poly(Q) disease models clearly demonstrates that poly(Q) expansion leads to common gene expression events even in the context of distinct proteins. An important exception was the absence of these gene expression changes in YAC72 mice, which express full-length huntingtin with 72 consecutive glutamines (Figs 5 and 6; YAC data from 14). This raised the possibility that full-length huntingtin protein interferes with the poly(Q) effects that are prominent in the context of a short huntingtin fragment. The influence of huntingtin protein context is further addressed in a companion paper (14).

**DISCUSSION**

Although the poly(Q) diseases differ with regard to which neurons degenerate first, ultimately cause patient death by damaging many neuronal populations. In this study, we demonstrated for the first time that atrophin-1 with expanded poly(Q) repeats causes gene expression changes in At-65Q mouse brain. Some of these changes were uniquely observed in
DRPLA mice, but many were seen also in two different HD mouse models: N171-82Q and R6/2. A subset of these changes were also seen in mouse models of SBMA and SCA7, suggesting that some mechanisms of transcriptional dysregulation are common to multiple poly(Q) disorders.

Among the gene expression changes that were shared by multiple animal models, many of the decreased mRNAs encode proteins involved in neuronal signal transduction, including neurotransmitter receptors (e.g. the GABA_A receptor δ subunit, the NMDA receptor 2C subunit and the cannabinoid receptor), neuropeptides (e.g. enkephalin) and other signal transduction proteins (e.g. protein kinase C, protein tyrosine phosphatase, G-protein-coupled receptor kinase and protein phosphatase 1c γ). Other mRNA changes observed in the cerebella of DRPLA and HD mouse models were decreases in growth factor-related transcripts [e.g. brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF)-1, the FGF receptor, and IGFBP5]. Similar to previous analyses of R6/2 mice, we detected increased levels of mRNAs associated with cellular stress or inflammation (e.g. the proteasome activator PA28 α subunit and multiple interferon-inducible genes) (18).

Figure 4. Selected mRNA changes unique to cerebella of At-65Q mice compared with wild-type (WT) controls. Listed are gene expression changes meeting a P < 0.001 criterion in At-65Q mice but remaining unchanged (P > 0.05) in R6/2 or N171-82Q mice. Green bars represent mRNAs that were decreased, and red bars represent mRNAs that were increased. Parameters are described in the Figure 2 legend. A complete list of mRNAs that were changed At-65Q mouse striatum is posted at supplemental data (www.neumetrix.info). At animals (and corresponding wild-type mice) were analyzed at 12 months of age; N171 animals (and corresponding wild-type mice) were analyzed at 4 months of age; R6/2 animals (and corresponding wild-type mice) were analyzed at 12 weeks of age (data from 13). n = 2 arrays with independent comparisons for each line.
important upstream mediator of some of the other poly(Q)-
induced gene expression changes. This suggests that histone
phosphorylation should be studied in addition to the recent
effort to understand poly(Q)-related impairments in histone
acetylation (20–22).

Together with the previous report, this study provides new
information about the N171-82Q, At-65Q and R6/2 mouse
models of poly(Q) disease. For example, it no longer seems
reasonable to use cerebellum as ‘control’ tissue for experiments
focusing on striatal, cortical or hippocampal pathology in HD
mouse models. In fact, the overall gene expression phenotype
was more severe in the cerebellum than in the striatum of both
the N171-82Q and At-65Q mice. Because cerebellar granule
cells outnumber other cerebellar neurons by 1/C24 : 200 : 1, it is
possible that gene expression changes are easier to distinguish in
tissue homogenates that are highly enriched for a single type
of cell. A more likely explanation, however, is that the murine prion
promoter directs higher expression of the transgene in the
cerebellum than the striatum in the N171-82Q and At-65Q mice.

Different levels of transprotein expression may also underlie the
higher number of overall gene expression changes in At-65Q
than N171-82Q mice. Transprotein expression is estimated to be
between one-half and the same level of endogenous atrophin-1
in At-65Q animals, whereas it comprises only one-tenth the level
of endogenous huntingtin in N171-82Q animals (G. Schilling,
C.A. Ross and D.R. Borchelt, unpublished observations). An
additional explanation for the greater changes in the R6/2
compared with the N171-82Q mice may be the considerably
longer poly(Q) repeat in the R6/2 mice.

The At-65Q cerebellum showed a higher number of unique
gene expression changes than did the N171-82Q or R6/2
cerebellum. This would be predicted based on the effects of
distinct context sequences of the full-length atrophin-1 trans-
protein versus the N-terminal huntingtin fragment. Further
evidence for unique effects of the atrophin-1 transprotein are
found in the gene expression profile of the At-26Q animals,
which show some gene expression changes in common with the
At-65Q mice. Still a third subset of changes appear to be unique

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**Figure 5.** RNAs that are changed in N171-82Q, At-65Q and R6/2 cerebella at \( P < 0.01 \). GenBank accession no. and gene description are on the left. \( n = 4 \) arrays per group with independent pairwise comparisons for N171 and At samples. The format is the same as in Figure 2. Data on 12-week-old R6/2 animals (\( n = 2 \)) are from (13). Data from YAC72 mice, which express a full-length huntingtin transgene with 72 repeats, are from (14). The following mRNAs represented only by ESTs are shown in this figure: AA099309, AA710049, W41032, AA174489, AA166452, AA144045, AA260960, AA254387, AA270913, W36829, AA275391 and Z31235 (decreased); AA199023, AA407204, AA137679, AA575675, W80816, AA546670, W83858 and Z31269 (increased).
Figure 6. mRNAs that are similarly changed in N171-82Q and At-65Q cerebella. The mRNAs on this list are statistically different between N171-82Q versus wild-type (WT) and At-65Q versus wild-type cerebella \( (P < 0.01) \), but fail to meet this criterion in R6/2 mice. \( n = 4 \) arrays per group with independent pairwise comparisons for N171 and At samples. Data on R6/2 animals \( (n = 2) \) are from (13). The format is the same as in Figure 2. The following mRNAs represented only by ESTs are not shown in the figure: AA009039, AA221260, W53565, W99005, W53565, AA408229, AA060862, C81506, AA266791, AA591007, C76985, AA034800, AA166452, AA596794, AA638759, C79612, AA692269, AA035912, AA035912, AA409750, AA614954, AA571856, AA267017, AA275391, Z31267, R74641 and AA087673 (decreased); AA0108016, AA174883, AA681993, AA268367, W83858, AA170779, AA230776, AA388781 and W83858 (increased). Data from YAC72 mice, presented for sake of comparison, are from (14).
Figure 7. Northern analysis of overlapping gene expression changes in At-65Q, N171-82Q and R6/2 mice. Two micrograms of cerebellar RNA was hybridized to $^{32}$P-labeled cDNA probes as described in Materials and Methods. N171 animals (and corresponding wild-type mice) were analyzed at 4 months of age; At animals (and corresponding wild-type mice) were analyzed at 12 months of age; R6/2 animals (and corresponding wild-type mice) were analyzed at 12 weeks of age. Two representative samples from groups of three or four animals are shown. Protein kinase C $\delta$ isoform (PKC delta) expression in the three lines was determined to be 28%±3% of control for R6/2, 48%±2% of control for N171-82Q and 34%±2% of control for At-65Q. Quantitative data for other mRNAs are presented in Figure 8.
to the expanded atrophin-1 transprotein, however, since only a minority of the changes specific to the At-65Q transgenic animals were observed in the At-26Q cohort (19%).

In this study, we determined that poly(Q) expansions in the contexts of several different poly(Q) disease proteins affect cerebellar gene expression similarly. These results suggest that the mutant-length poly(Q) expansion either causes the change in gene expression directly or activates a common pathway responsible for the change. One intriguing new candidate mechanism arising from the present study is that mutant-length poly(Q) sequences may lead to an early and pronounced disruption of histone phosphorylation.

**MATERIALS AND METHODS**

**Mice**

At-26Q, At-65Q, N171-18Q, N171-82Q and wild-type littermates were obtained from the authors’ (G.S., C.A.R. and D.R.B.) colonies. At-26Q and At-65Q mice were analyzed at 12 months of age and compared with age-matched wild-type controls. N171-18Q, N171-82Q and age-matched wild-type controls were assessed at 4 months of age. In both At-65Q and N171-82Q mice, these ages represent a late symptomatic but premorbid stage of disease (4–6).

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![Figure 8](image_url)

**Figure 8.** Gene expression changes in HD and DRPLA mice are similar in SCA7 and SBMA mice. (A) Northern autoradiograms of SCA7 and SBMA mice show changes in IGFBP5, enkephalin, MARCKS and NVP-1 mRNAs. Two representative samples from groups of three or four animals are shown. (B) Bar graphs showing gene expression changes in DRPLA, N171 HD, R6/2 HD, SCA7 and SBMA mice. Data (expressed as $\beta$-actin ratios for three or four animals per group) were analyzed by one-way ANOVA using Schefee's post hoc test with Bonferroni–Dunn correction. Single asterisks indicate $P < 0.05$, double asterisks indicate $P < 0.005$ and triple asterisks indicate $P < 0.0005$. N171 animals (and corresponding wild-type mice) were analyzed at 4 months of age; At animals (and corresponding wild-type mice) were analyzed at 12 months of age; R6/2 animals (and corresponding wild-type mice) were analyzed at 12 weeks of age; SCA7 mice (and corresponding wild-type mice) were analyzed at 18–22 weeks of age; SBMA animals (and corresponding wild-type mice) were analyzed at 11.5 weeks of age (continued).
Ataxin-7 transgenic mice (PrP-SCA7-c92Q) (16) and wild-type littermates (18–22 weeks of age) were obtained from the author’s (A.R.L.) colony. At this age, the ataxin-7 transgenics manifest retinal degeneration and blindness, and exhibit a neurological phenotype characterized by gait ataxia, tremulousness and inactivity. Male mice expressing a mutant androgen receptor (PrP-112Q-34, SBMA mice) and male littermates were created by the author (D.E.M.: C.J. O’Brien, E.S. Chevalier-Larsen and D.E. Merry, manuscript in preparation). These mice were studied at 11.5 weeks of age, at which stage they exhibit early signs of disease, including hindlimb clasping and modest but significant rotarod deficits. While cerebellar granule cells in PrP-112Q-34 mice eventually develop poly(Q) protein aggregates, they are devoid of such structures at the age used in this study (D.E. Merry, unpublished observations). Moreover, no cerebellar neuronal loss has been observed.

Figure 8 continued.
ACKNOWLEDGEMENTS

Select data from YAC72 animals are presented in Tables 3 and 4 courtesy of Edmond Chan, Blair Leavitt and Michael Hayden (see also 14). The PrP112Q-34 mice used for this study were courtesy of Edmond Chan, Blair Leavitt and Michael Hayden.

REFERENCES