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

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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) lengthdependent 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

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Figure 1. Schematic diagram of transgenes used in mouse models. MoPrP signifies the mouse prion promoter. ITI5/htt signifies the human huntingtin promoter. The number of amino acids (aa) encoded by the transgene is shown below each and the number of polyglutamines (Q) encoded by the transgene is shown above each. See (15) and Materials and Methods for detailed descriptions of the mice.

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

Fig	ure 2 Sele	cted mRN/	A changes in striatum of AT-65Q and N171-82Q	mice			
Δ	Mouse	Genhank	Description P val		Other Criteria	DRPLA	N171 820 vs wt
	Signal trans	duction	Description	i value	other ontena	Q00 13 WI	02Q V3 W(
Dec		\//79953	adenvlate cyclase, type V*	< 0.001		-2.5	0.1
Dec	AT-650	W55507	D2 donamine recentor*	< 0.001		-13.2	-0.7
Dec	AT-65Q	D42051	glutamate decarboxylase	< 0.001		-2.6	0.4
Dec	AT-65Q	M13227	enkephalin	< 0.01	ISHH**	0.0	-1.4
Dec	AT-65Q	X67141	parvalbumin	< 0.01		-1.6	-0.3
Dec	N171-82Q	AF026537	prodynorphin	< 0.001		0.9	-3.6
Dec	N171-82Q	X55674	D2A dopamine receptor*		ISHH	-1.3	-1.3
Dec	N171-82Q	M13227	enkephalin		ISHH	0.0	-1.4
Dec	N171-82Q	U23160	DARPP-32		ISHH	No data	No data
Inc	AT-65Q	Z11664	son of sevenless 2.	< 0.01		2.1	1.3
Inc	AT-65Q	AA049022	guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-3*	< 0.01		1.6	1.5
Inc	N171-82Q	D37793	synaptotagmin	< 0.001		-0.6	5.5
Inc	N171-82Q	D21165	neural visinin-like Ca2+-binding protein type 1	< 0.01		0.1	1.8
	Transcription	n Factors					
Dec	AT-65Q	AA071612	transcriptional activoator FE65*	< 0.001		-7.2	1.8
Dec	AT-65Q	U24674	max interacting protein 1	< 0.001		-1.7	0.4
Dec	AT-65Q	W11621	zinc finger protein ZFMSA12A	< 0.001		-16.2	0.4
Dec	N171-82Q	X16995	N10 nuclear hormonal receptor		ISHH	1.3	-1.5
Inc	N171-82Q	U49251	transcriptional regulator T-Brain-1	< 0.001		-3.2	2.9
Inc	N171-82Q	U29086	neuronal helix-loop-helix protein NEX-1 (nex-1)	< 0.001		-0.8	5.3
	Metabolism						
Dec	AT-65Q	W75072	creatine kinase, B chain*	< 0.001		-1.1	-1.1
Dec	AT-65Q	W41817	cytochrome c oxidase subunit VIII precursor (Cox8I)	< 0.001		-0.8	-1.3
Inc	AT-65Q	X01756	cytochrome c	< 0.001		2.8	-1.0
Inc	AT-65Q	W08392	ATP synthase B chain*	< 0.001		39.3	0.0

sequence homologous to named gene

** ISHH = in situ hybridization histochemistry

Figure 2. Selected mRNA changes in striata of At-65Q and N171-82Q mice compared with wild-type controls. N171 animals (and corresponding controls) were analyzed at 4 months of age; At animals (and corresponding controls) were analyzed at 12 months of age. For each mouse line, independent replicates are shown as columns of red (increased compared with control) and green (decreased compared with control). Brighter colors represent increased confidence (e.g. net probe pair changes >35% are represented by the brightest colors, then 25–35% dimmer, then >15–25% dimmer, then black for <15%). The rightmost column for each brain region is the mean fold change in the designated model compared with control as calculated by Affymetrix GeneChip software version 4.0. P-values are those calculated from the array data (n = 2 arrays per group with independent pairwise comparisons; see Materials and Methods and 23), unless otherwise noted. ISHH data without P-values are from (18). A complete list of mRNAs that were detected as changed in each line by microarray analysis is posted in the supplemental data (www.neumetrix.info).

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 the N171-82Q and At-65Q transgenic animals was low, these data were insufficient to address the primary goal of distinguishing transcriptional changes primarily caused by poly(Q) expansion from those that are proteincontext-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 wildtype 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

At-65Q (DRPLA)



R6/2 (HD) N171 (HD)

Figure 3. Venn diagram showing relationship of cerebellar gene expression changes in N171-82Q, At-65Q and R6/2 mice. The numbers of mRNAs that were differentially detected in each mouse model at P < 0.01 are shown (n = 4 arrays per group with independent pairwise comparisons between transgenic and wild-type animals for N171-82Q and At-65Q samples). 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. Based on the error model, the number of predicted false discoveries was subtracted, so that the diagram represents the predicted number of truepositive changes (1:100 mRNAs assayed/group). In contrast to Figures 5 and 6, expressed sequence tags (ESTs) are represented in this diagram. Data on 12-week-old R6/2 animals (n = 2) are from (13).

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 N171-82O and At-65O 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 of SCA7 and SBMA mice 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, all 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

Figure 4 Selected mRNA changes unique to DRPLA (At-65Q) mouse cerebellum (P < 0.001)											
GenBank ID	Description	R6/2		DRPLA	DRPLA	1	N171				
			WТ	Q26 vs WT	Q65 vs	WT	Q82 vs WT				
X53028	chromogranin B (secretogranin I)		-1.2	0.6		-1.8		-1.3			
U93309	disks-large 1 (Dlgh1)		0.0	-0.3		-2.4		0.0			
U93309	disks-large 1 (Dlgh1)		1.2	-0.6		-1.4		0.0			
AJ002366	general transcription factor TFIIH		0.0	-1.6		-1.5		0.9			
AA500440	Gi alpha subunit*		-1.4	-0.7		-1.9		-1.2			
Y12257	glutamic acid decarboxylase 67 kD (GAD67)		-1.4	0.6		-1.5		-1.2			
X95403	GTP-binding protein Rab2		1.3	-0.1		-1.7		-0.6			
X13171	histone subtype H1		1.1	-0.6		-1.5		-0.6			
U50734	maid - negative regulator of bHLH transcription factors		-1.3	-1.1		-1.6		-1.3			
M63903	max homolog B/HLH/Z protein (Myn)		-1.1	0.1		-1.3		-1.1			
L03279	murine leukemia virus core-binding factor		0.0	-0.1		-1.6		0.0			
U31908	potassium channel beta 2 subunit		-3.9	0.6		-3.7		-2.7			
M30440	potassium channel MK2		-1.3	-1.2		-2.8		-1.1			
M27073	protein phosphatase type 1 (dis2m2) catalytic subunit		0.1	-0.2		-1.6		0.5			
AA200702	RhoGEF*		-1.5	-1.2	. – .	-2.5		-0.7			
M28489	Ribosomal protein S6 kinase (rsk)		-1.4	-1.2		-2.0		-0.7			
W57339	sodium/potassium ATPase beta-1*		-1.3	-0.5		-4.3		-1.3			
AA002979	sodium/potassium ATPase beta-2*		-1.3	-0		-2.4		-1.3			
U59761	sodium/potassium ATPase beta-3*		-1.1	-0.1		-1.5		-1.1			
U66141	sox-10 SRY-box containing transcription factor Sox-M		1.3	-0.6		-1.4		-0.7			
AJ001598	vesicular GABA and glycine transporter		-1.3	-0.2		-2.0		0.4			
W30059	casein kinase I, delta isoform*		-1.3	0.8		2.1		-0.7			
AA245242	F52 mRNA / MARCKS-like protein (Mlp)		0.2	-0.7		2.6		0.1			
D10011	glutamate receptor channel subunit gamma 2 (GluR5)		1.6	0.1		2.2		0.0			
AA673176	high mobility group protein		-1.2	-0.7		1.9		1.2			
M37736	histone H2A.1 (replication dependent)		1.3	0.8		3.6		-0.1			
Z37988	leukocyte antigen related (LAR) tyrosine phosphatase		0.1	0.6		6.0		2.5			
Z30940	mRNA for histone H2A		1.0	2.2		9.6		-1.6			
W63974	protein phosphatase PP2A		0.0	0.4		0.9		0.0			
W15568	small nuclear ribonucleoprotein (snRNP)*		-1.5	0.2		2.4		-0.5			
AF009414	sox-11		0.2	-0.9		0.9		-0.6			
X70298	sox-4		1.8	-0.2		3.0		-0.9			
X64587	splicing factor U2AF (65 kD).		0.4	-0.7		1.2		0.7			
X87817	Ulip/dihydropyramidase-like/collapsin response mediator		1.1	-0.7		2.2		0.6			

sequence homologous to named gene

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 12 weeks of age (data from 13). n = 2 arrays with independent comparisons for each line.

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).

Notable among mRNAs that changed in multiple poly(Q) disease models was a decrease in SNFK1 mRNA, which encodes a protein implicated in chromatin phosphorylation. Expression of this gene was also decreased in the MN1 cell line that overexpresses a mutant form of the androgen receptor (19). If this mRNA decrease results in reduced protein levels, it is possible that a defect in chromatin phosphorylation is an

Figure 5 mRNAs that are similarly changed in R6/2, DRPLA, and N171 cerebella compared to wild type														
GenBank ID Description		R6/2 Q150 vs	wт	DRPLA Q26 vs W	т	DRPLA Q65 vs WT	DRPLA N171 Q65 vs Q26 Q18 vs WT		N171 Q82 vs WT	N171 Q82 vs Q18		YAC72 Q72 vs WT		
U29762	albumin gene D-Box binding protein		-4.4		-0.1	-2.7		-2.7	0.3	-1	9	-2.2		-1.4
X98014	alpha-2,8-sialyltransferase.		-4.2		1.35	-2.3		-3.0	13.2	6	0	-2.9		-0.3
M13444	alpha-tubulin isotype M-alpha-4 mRNA, comple		-1.8		-0.1	-1.8		-1.7	-0.7	-1	9	-1.7		0.2
AF026489	beta III spectrin (Spnb3)		-2.5		0.08	-0.9		-0.8	-1.2	-1	3	-1.2		0.5
L24755	bone morphogenetic protein 1 (BMP-1)		-2.5		-0	-1.9		-1.8	1.3	-2	0	-2.5		0.6
AA000813	bone morphogenetic protein 1 precursor (BMP-1)*		-2.4		-0.9	-3.0		-2.5	0.6	-3	3	-3.4		0.0
W65178	bone morphogenetic protein 1 precursor (BMP-1)*		-2.4		0.08	-1.5		-1.6	1.4	-2	5	-2.9		0.1
X55573	brain-derived neurotrophic factor		-3.2		-0.7	-3.3		-2.8	-1.3	-4	3	-3.5		1.9
U37091	carbonic anhydrase IV gene		-1.9		-0.6	-4.2		-3.9	-0.7	-3	6	-3.3		0.8
AF016697	chemokine receptor gene		-3.2		-0.5	-2.2	_	-2.1	0.0	-1	5	-1.6		0.7
X61448	D3 clone.		-1.5		0.63	-1.5		-1.8	0.6	-1	4	-1.5		-0.7
AA000227	diacylglycerol kinase (retinal degenration A protein)*		-2.4		-0.8	-2.1		-1.7	1.2	-1	4	-1.7		-0.2
AA059718	DNA polymerase beta		-1.9		-0.3	-2.4		-1.3	1.1	-1	<i>′</i>	-1.9		-1.1
M13227			-1.8		0.7	-2.9		-3.2	-0.5	-2	0	-1.9		-0.3
AA006655	extensin precursor (cell wall hydroxyproline)		-1.9		-0.7	-2.3		-1.0	-0.6	-2	7	-2.0		1.0
019860	growth arrest specific mRNA, clone 3544		-2.1		0.03	-1.9		-1.9	1.1	-1	7	-1.8		-0.2
L12447	Insuln-like growth factor binding protein 5 (IGFBP5)		-3.0		0.03	-0.0		-0.0	-0.1	-3	2	-3.9		0.2
115442	L6 antigen		-0.7		-2.1	-0.0		-3.0	-0.5	-0	3	-/.1		3.1
AE004874	latent TGE beta binding protein 2 mPNA		-1.7		-0.7	-2.0		-3.0	-2.2	-3	6	-0.7		-3.2
X00305	MBK1 mRNA for brain potassium channel protein-1		-2.0		0.53	-1.3		-1.8	0.0	-1	7	-1.7		0.0
AE006467	membrane-associated phosphatidylinositol transfer protein		-1.9		-0.6	-1.4		-1.3	-0.7	-1	6	-1.5		0.2
1181317	myelin-associated/oligodendrocyte basic protein (Mohn)		-1.3		-0.0	-1.4		-10	-0.7	-1	1	-0.9		0.0
D21165	neural visinin-like Ca2+-binding protein type 1 (NVP-1)		-1.8		-1	-3.1		-2.1	-0.7	-2	6	-2.3		0.7
AA111277	neural visinin-like protein 3*		-3.3		-0.1	-2.5		-2.4	0.5	-1	4	-1.4		11
W50848	neural visinin-like protein 3*		-1.9		0.03	-1.5		-1.6	0.0	-0	7	-0.7		-0.2
U22948	neuronal cannabinoid receptor (CB1)		-5.2		-2.2	-3.3		1.1	-0.1	-3	6	-2.6		0.2
U62021	neuronal pentraxin 1 (NPTX1)		-2.2		0.65	-2.6		-2.9	0.6	-1	6	-1.8		0.7
U47543	NGFI-A binding protein 2 (NAB2)		-9.3		0.63	-5.4		-6.4	-1.5	-5	4	-3.0		-0.1
X70398	P311		-1.3		0.08	-1.9		-2.0	-0.5	-1	5	-1.6		-0.7
X67141	parvalbumin.		-4.9		0.58	-2.0		-2.1	0.1	-1	6	-1.8		-0.9
AA105564	protein kinase (Kin1)*		-2.2		-0	-3.1		-3.6	0.0	-4	3	-4.3		0.7
M69042	protein kinase C delta		-2.3		-0.5	-3.0		-3.4	0.6	-2	0	-2.2		0.1
X60304	protein kinase C-delta		-2.8		1.33	-3.2		-4.5	0.7	-2	1	-2.5		-0.7
L28035	protein kinase C-gamma		-6.8		-0.4	-7.3		-6.7	1.2	-1	5	-1.8		0.5
M90388	protein tyrosine phosphatase (70zpep)		-3.4		0.1	-4.3		-5.4	1.0	-2	6	-4.0		-0.2
AA444931	SNF1-related kinase*		-2.0		0.35	-2.2		-1.9	-0.6	-1	7	-1.7		0.5
AA444931	SNF1-related kinase*		-2.3		0.7	-2.1		-2.8	0.1	-1	9	-2.0	_	0.7
AA117100	stathmin (phosphoprotein P19, prosolin)*		-1.8		-0.5	-2.1		-1.7	0.0	-1	1	-1.6		-0.7
U61751	vesicle-associated membrane protein VAMP-1		-3.8		-1.7	-3.8		-0.3	0.1	-8	9	-7.4		-1.5
L01695	calmodulin-dependent phosphodiesterase		1.5		0.53	1.3		1.2	0.6	1	3	1.2		-0.7
X07751	c-erbA alpha2 mRNA for thyroid hormone receptor		2.2		-3.3	1.7		6.2	0.0	1	6	1.6		0.8
U72519	Ena-VASP like protein (EvI)		1.8		0.58	1.6		1.5	0.1	1	7	1.5		-1.3
AA031158	extensin precursor*		7.7		-1.4	11.0	_	15.6	-0.4	6	8	7.4		-1.5
U42385	fibroblast growth factor inducible gene 16 (FIN16)		2.8		-0.8	1.6		1.9	0.8	2	2	1.9		-0.1
AA165782	interferon regulated I-5111 precursor*		2.4		0.02	2.6	_	2.5	0.4	1	8	1.9		-0.4
009421	K3 opiate receptor mRNA		8.3		-0.8	1.8		2.2	-1.1	7	0	8.3		-0.8
AA221937	LTO-B antigen"		14.0		0.03	10.7		9.1	1.2	17	2	16.4		-1.0
1000474	myristoyiated alanine-rich C-kinase substrate (MARCKS)		1.9		-1.3	2.3		2.8	0.1	2	3	2.2		-0.2
005252	DA28 alaba automit		1.6		0.05	2.2		1.9	0.1		/	1.6		0.3
1 22072	MZO alpha subunit		2.1		0.0	2.3		2.5	-0.6	1	6	1.9		0.2
L329/3	tnymidylate kinase nomologue		2.3		-0.3	1.7		1.5	0.0		5	1.6		-0.3

* sequence homologous to named gene

Figure 5. mRNAs 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 not shown in the figure: AA009039, AA271049, W41032, AA174489, AA166452, AA144045, AA269960, AA254387, AA270913, W36829, AA275391 and Z31235 (decreased); AA199023, AA407204, AA137679, AA575675, W08016, AA546670, W83858 and Z31269 (increased).

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 N171-82O and At-65O mice. Because cerebellar granule cells outnumber other cerebellar neurons by $\sim 200:1$, 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 transprotein 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

Figure 6 mRNAs that are similarly changed in N171-82Q and AT-65Q cerebella															
GenBank ID Description		R6/2 Q150 vs WT	DRPLA Q26 vs V	ντ	DRPLA Q65 vs WT	DF Q6	RPLA 65 vs Q26	N171 Q18 vs WT		N171 Q82 vs W	N171 T Q82 vs Q18		8	YAC72 Q72 vs WT	
AA475426	alpha 4*	-1.4		-0.6	-1	1.7	-1.6		2.7		1.6		-1.3		0.2
M13444	alpha-tubulin isotype M-alpha-4 mRNA, comple	-1.8		-0.1	-1	1.8	-1.7		-0.7		-1.9		-1.7		0.2
E161908	anti-DNA immunoglobulin neavy chain IgG antibody	0.0		-0.1		1.9	-1.3		-1.4		-1.3		0.6		-0.5
008372	Balb/c asialogiycoprotein receptor (MHL-1) mRNA BALB/c invesion inducing protein (Tiam-1) mRNA	-2.7		0.8	-4	+.Z	-4.9		-1.4		-3.7		-1.9		-0.5
V07836	basic-belix-loon-belix protein	-1.0		0.5	-2	2.0	-2.1		-0.0		-1.1		-1.0		-0.2
U41465	BCL-6 mRNA	-1.9		-0.6	-4	1.3	-4.5		-4.8		-6.9		-0.6		1.3
AA072611	beta adrenergic receptor kinase I*	-0.2		-0.8	-1	1.0	-0.2		-1.1		-1.3		-1.2		0.6
AA000813	bone morphogenetic protein 1 precursor (BMP-1)*	-2.4		-0.9	-3	3.0	-2.5		0.6		-3.3		-3.4		0.0
W65178	bone morphogenetic protein 1 precursor (BMP-1)*	-2.4		0.08	-1	1.5	-1.6		1.4		-2.5		-2.9		0.1
W70714	bone morphogenetic protein 3 (BMP-3)*	-1.6		-0.7	-5	5.0	-4.4		-1.3		-1.4		-0.1		-0.9
M20480	brain neurofilament-L mRNA	-1.2		-1	-2	2.5	-2.0		-0.2		-1.5		-0.9		-0.2
AA116977	brain protein H5"	1.2		-1.1	-2	2.4	-0.7		0.5		-1.5		-1.5		1.1
X59005	cadnerin-11-	-1.1		-1.0	-4	2.3	-1.4		1.0		4.2		4.6		0.1
X61434	cAMP-dependent protein kinase C beta subunit	-3.0		-0.7		1 4	0.5		1.0		1.4		-4.0		-0.2
W85144	cell division control protein 4*	-1.6		-0.8	-3	3.6	-2.0		0.6		-1.7		-1.7		-12
AA088962	cell survace glycoprotein MUC18*	1.2		-0.9	-2	2.5	-1.5		0.7	_	-1.7		-1.8		0.8
AF016697	chemokine receptor gene	-1.5		-0.2	-4	4.1	-3.1		0.1		-1.9		-2.1		0.3
X61448	D3 clone.	-1.5		0.63	-1	1.5	-1.8		0.6		-1.4		-1.5		-0.7
AA059718	DNA polymerase beta*	-1.9		-0.3	-2	2.4	-1.3		1.1		-1.7		-1.9		-1.1
M13227	enkephalin mRNA	-1.5		0.1	-3	3.6	-3.5		-1.1		-2.2		-1.9		-0.7
W13227 X74052	ETL-2 mRNA	-1.8		0.7	-4	2.9	-3.2		-0.5		-2.0		-1.9		-0.3
110426	ets-related protein 81 (ER81) mRNA	-2.0		0.5	-4	17	-1.0		0.0		-1.9		-1.6		-0.2
AA008655	extensin precursor (cell wall hydroxyproline)*	-1.9		-0.7	-2	2.3	-1.8		-0.6		-2.1		-2.0		1.0
M33760	FGF receptor mRNA	0.1		-0	-1	1.7	-1.7		-0.6		-1.7		-1.6		1.2
U67610	fibroblast growth factor 1 (FGF-1) mRNA	-0.1		-0.7	-2	2.1	-1.6		0.7		-1.4		-1.6		0.8
W36620	G protein-coupled receptro kinase (GRK5)*	-2.2		-1.5	-2	2.0	-1.7		1.5		-2.8		-3.1		0.5
M60596	GABA-A receptor delta-subunit gene, exon 9	-1.6		-0.1	-1	1.9	-1.8		-0.6		-1.8		-1.7		1.2
J04953	geisolin gene	2.3		2.13	-2	2.9	-4.3		-3.5		-3.1		0.7		-1.1
A01034 X53825	gene for osteopontin. heat stable antigen	-1 0		-0.2	-2	2.3	-2.5		-0./		-0.8		-0.0		-0.8
107264	heparin-binding EGE-like growth factor precursor	-1.0		0.65		4	-3.0		-1.0		-1.0		0.6		-0.3
AA015415	kinesin light chain*	-0.2		-1.6	-2	2.8	-1.9		0.6		-0.9		-1.5		0.7
L15443	L6 antigen	-5.7		-0.7	-2	2.6	-3.0		-2.2		-3.4		-0.7		-3.2
M15525	laminin B1 mRNA	-2.1		0.75	-3	3.0	-2.7		-1.5		-3.5		-2.2		1.2
AF004874	latent TGF-beta binding protein-2 mRNA	-1.7		0.5	-1	1.5	-1.5		0.5		-1.6		-1.7		0.3
L35029	N-methyl-D-aspartate receptor subunit NR2C (NMDA2C)	-1.3		0.5	-1	1.4	-1.5		-1.1		-1.5		-1.4		-1.1
M35131	neurofilament component (NF-H) mRNA, complet	-1.4		0.5	-2	2.1	-2.3		-1.1		-1.6		-1.5		-1.1
VV55224	neurofilament, heavy (200kD)*	-1.2		-4./	-5	9.0	-2.4		-3.4		-8.2		-5.0		-0.8
1128068	neurogenic differentiation factor (neuroD)	-0.1		-5.0	-0	1.5	-5.4		-0.6		-1.5		-1		-0.7
W61845	neuron specific enolase (gamma)*	-1.3		-0.9	-2	2.1	-1.6		-0.1		-1.7		1.5		1.2
W59713	neuron specific enolase (gamma)*	-1.4		-1.9	-1	1.9	-0.5		-1.1		-1.7		-1.6		0.8
W50385	neuron specific enolase (gamma)*	-1.1		-0.8	-1	1.5	-0.1		0.0		-1.4		-1.4		0.8
W48081	neuron specific enolase (gamma)*	-1.4		-0.6	-1	1.6	-1.4		-0.5		-1.3		-1.3		0.2
AA118297	neuronal protein NP25*	-1.3		-1.1	-3	3.1	-1.2		-1.1		-1.6		-1.4		1.0
Y07692	NfiC1A-protein, splice variant.	-4.9		-3.3	-4	4.4	-3.0		-2.2		-6.9		-5.0		1.3
X70208	oral tumor suppressor nomolog (Doc-1)	-1.3		-0.1		1.0	-1.5		-0.1		-1.5		-1.5		1.1
X70398	pentylenetetrazol-related PTZ-17	-1.5		-0.5		2.1	-2.0		-0.3		-1.7		1.8		-0.7
W41070	phosphatidylcholine transfer protein*	-1.8		1.5	-4	1.1	-6.0		-0.1		-2.8		-3.6		-0.5
M90365	plakoglobin	-2.0		-0.7	-9	9.8	-7.7		-0.6		-9.6		-8.3		0.9
M90365	plakoglobin mRNA	-0.1		-0.6	-1	1.9	-1.8		-0.7		-6.0		-2.1		0.9
U76832	plasma membrane protein syntaxin-4	0.0		-1.2	-2	2.3	-1.6		-0.8		-1.6		-1.3		0.7
AA155318	polypyrimidine tract-binding protein*	1.4		-1	-1	1.4	0.5		0.9		1.4		0.1	_	-0.9
053456	protein phosphatase 1cgamma (PP1cgamma)	0.1		1.13	-1	1.7	-1.9		0.6		-1.4		-1.6		0.3
105683	recentor-type tyrosine kinase (rse)	-1.9		-1.0	-2	1.0	-0.1		-0.0	-	-1.1		-3.7		1.0
U94828	retinally abundant regulator of G-protein signaling mRGS-r	-1.5		0.5	-1	1.4	-1.4		0.0		-1.4		1.4		-0.1
AA071743	serine/threonine protein phosphatase PP1-gamma*	1.1		-0.2	-1	1.7	-1.0		-0.6		-1.4		-1.3		0.4
AA105755	sodium/potassium ATPase alpha-1*	-1.2		-1.3	-1	1.6	-1.1		-0.6		-1.3		-0.7		0.2
AA063844	sodium/potassium ATPase alpha-3 chain*	-1.4		-0.6	-1	1.5	-1.7		0.1		-1.5		-1.6		-0.2
X95279	spot14	-2.3		-0.9	-2	2.6	-1.2		-0.7		-1.1		-1.6		-0.2
088327	suppressor of cytokine signalling-2	-2.3		0.35		0.4	-3.7		0.6		-2.4		-2.8		-0.3
W59791	tubulin alpha-5*	_1 1		-1.0	-4	1.4	-1.8		-0.9 -0.6		-3.5		-2.7		-0.0
W84068	tubulin beta-5 chain*	-1.1		-8.3	-8	3.5	0.3		-0.8		-1.9		1.1		2.1
AF006824	TWIK-related acid-sensitive K+ channel (TASK)	-3.0		0.78	-4	4.1	-4.2		-3.5		-3.6		-1.2		-0.3
U61751	vesicle-associated membrane protein VAMP-1	-3.8		-1.7	-3	3.8	-0.3		0.1		-8.9		-7.4		-1.5
W87254	apomucin*	5.1		-1.3	4	4.0	6.5		4.1		7.4		3.7		-0.7
Z72000	BTG3	3.8		1.7	3	3.4	1.8		0.6		2.6		2.5		0.8
ET63017	cadherin-8.	0.2		0.05	1	1.3	1.6		0.2		2.4		2.5		-0.7
X74134	COUP-TF1.	0.1		0.13		1.9	1.6		0.4		1.4		1.5		0.4
L12147	earry B-cell factor (EBF)	-1.3		0.68		0.9	0.6		-0.5		1.3		0.9		-1.2
0/2519	Ena-VASP like protein (EVI)	1.8		0.58		1.6	1.5		0.1		1.7		1.5		-1.3
W71881	GCN5 transcriptional activator*	2.8		-0.8		1.5	1.9		0.8		2.2		1.9		-0.1
U12473	helix-loop-helix ubiquitous kinase (CHUK)	1.0		1.68		0.8	-0.8		0.7		2.2		0.9		0.7
W08166	interferon gamma up-regulatedI-5111*	0.5		-0.8	2	2.0	2.9		-0.1		2.6		1.6		-1.6
AF022371	interferon-inducible protein 203 (Ifi203)	-1.3		-0.9	2	2.4	2.4		0.8		2.8		1.5		0.6
M60474	myristoylated alanine-rich C-kinase substrate (MARCKS)	1.7		-1.2	2	2.4	2.8		0.1		2.2		2.2		0.7
AA168918	NA+/K+ ATPASE gamma	1.7		0.53	1	1.9	1.8		-0.5		2.4		2.4		1.5
X15052	neural cell adhesion molecule NCAM	1.4		0.03		1.4	1.3		0.1		1.5		1.4		0.7
005252	nuclear matrix attachment UNA-binding protein SATB1	1.6		0.05	- 2	2.2	1.9		0.1		1.7		1.6		0.3
AA4/3525	ovarian granulosa cell 13 kD protoin*	1.1		0.05		1.2	1.7		0.1		1.6		1.0		-0.7
U17259	ovanan granulosa cell 13 ko protein	1.1		0.06		1.2	1./		0.7		2.0		1.2		-0.7
AA008245	polyadenylate binding protein*	1.1		0.45		0.7	0.2		1.3		1.2		-1.1		-0.8
U58887	SH3-containing protein SH3P13	1.1		0.08	1	1.7	1.6		0.2		1.7		1.1		-0.3
AA537404	thymosin beta-10*	1.4		-0	2	2.8	3.0		-0.7		1.6		1.9		-0.3
070940	7:-2			0.0		1.2	4.6		0.0		4.4		4.5		1 0 1

* sequence homologous to named gene

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, AA06862, C81506, AA266791, AA591007, C76985, AA034800, AA166452, AA596794, AA638759, C79612, AA269960, AA692269, AA035912, AA254387, AA035912, AA409750, AA614954, AA571856, AA267017, AA624011, AA275391, AA175557, Z31267, R74641 and AA087673 (decreased); AA0108016, AA174883, AA681993, AA268367, W83858, AA170779, AA575675, AA275266, AA178190, 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 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 δ 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).



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 β -actin ratios for three or four animals per group) were analyzed by one-way ANOVA using Scheffe's *post hoc* test with Bonferroni–Dunn correction. Single asterisks indicate P < 0.05, double asterisks indicate P < 0.005. 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 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).



Figure 8 continued.

Ataxin-7 transgenic mice (PrP-SCA7-c92Q) (16) and wildtype 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.

Arrays

Affymetrix microarray studies were conducted with Mu 11K GeneChips as described in (13). For all N171 and At studies, poly(A)⁺ RNA was prepared using Oligotex mRNA isolation kits (Qiagen) from 30–80 µg total RNA. RNA from one animal per array was used for cerebellar samples (n = 4). For striatal samples (n = 2), RNA from three animals was pooled prior to poly(A)⁺ RNA isolation. Analysis of array data was performed as in (23).

Tissue harvesting and RNA extraction

Whole cerebella and striata were dissected rapidly, quick-frozen on dry ice, and stored at -80° C until processed. Total RNA was extracted using Tri-Reagent (Sigma-Aldrich) according to the manufacturer's protocol, except that the isopropanol precipitation step was conducted overnight at -20° C

Northern blot analysis

Northern blot studies were conducted as reported previously (18). Scan intensities for mRNAs of interest were normalized to those of β -actin (sequence and use of probe are described in 13). The cDNA probe for NVP-1 comprised IMAGE clone no. 2780415 (GenBank accession nos AW742820 and AW742598), obtained from ResGen/Invitrogen.

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