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Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses

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Gene expression studies conducted with mouse models of Huntington's disease (HD) have revealed profound modifications in gene transcription. However, the complexity of *in vivo* tissue hampers definition of very early transcriptional modifications and does not allow discrimination between cell-autonomous changes and those resulting from intercellular activity processes. To identify early, cell-autonomous transcriptional changes, we compared gene expression profiles of clonal striata-derived cells expressing different N-terminal 548-amino-acid huntingtin fragments (with 26, 67, 105 or 118 glutamines) under the control of a doxycycline-regulated promoter. In these cells, mutant huntingtin did not form aggregates or cause cell death; therefore, the gene expression profiles report transcriptional changes reflecting early pathogenic events. We found that genes involved in cell signaling, transcription, lipid metabolism and vesicle trafficking were affected, in some cases, within 12 hours of mutant protein induction. Interestingly, this study revealed differential expression of a number of genes involved in cholesterol and fatty acid metabolism, suggesting that these metabolic pathways may play a role in HD pathogenesis.

INTRODUCTION

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by motor, cognitive and behavioral dysfunction. It is caused by an expansion of a polymorphic CAG trinucleotide repeat in the coding region of the gene for huntingtin, a 348 kDa protein in which the CAG repeats are translated into a stretch of glutamines [poly(Q)] (1). In the normal population, up to 35 CAG repeats are present in the *HD* gene, while expansions of 36 or more repeats induce HD pathology. Because huntingtin is expressed ubiquitously, a most striking feature of HD is a selective neurodegeneration of the striatum and, to a lesser extent, other brain regions (2). Pathogenic events in HD appear to depend upon both the gained toxic activity conferred by the expanded poly (Q) stretch to the mutant protein and the loss of normal huntingtin function (3–8).

Caspases have been shown to cleave huntingtin *in vitro* in a CAG length-dependent manner, and N-terminal fragments have been detected in striatal neuron nuclei of HD patient specimens (9–11). The N-terminal portion includes the poly(Q) repeat and a proline-rich region, which are characteristic of proteins involved in gene transcription (12). With respect to the wild-type protein, mutant huntingtin differentially interacts with various transcription factors, repressors and co-activators, perhaps leading to many of the gene expression changes reported in human HD brain and laboratory models of HD (13–21).

Mouse models provide an opportunity to identify temporal and regional events in the context of intact neuronal circuitry. Interpretation of data from these complex systems may be confounded however, by tissue heterogeneity, homeostatic mechanisms, and concurrent physiologic or pathologic changes. An inducible, clonally derived, cell line expressing mutant huntingtin offers a stable and controlled genetic and transcriptional background in which to perform gene expression studies. In such a system, biological and experimental variability can be greatly reduced. Transcriptional changes can be identified that may provide insight into events that occur during embryogenesis or in presymptomatic HD patients.

To identify early, cell autonomous transcriptional changes, we engineered striatal cells to express, in a tightly regulated manner, the first 548 amino acids of wild-type or mutant huntingtin (N548-huntingtin and N548-mutant huntingtin, respectively). DNA microarray analysis revealed N548-mutant huntingtin-modulated mRNAs that encode proteins involved in lipid metabolism, signaling, vesicle trafficking and RNA processing. The novel finding that multiple genes involved in cholesterol biosynthesis are altered by N548-mutant huntingtin may provide important clues to early HD pathogenesis.

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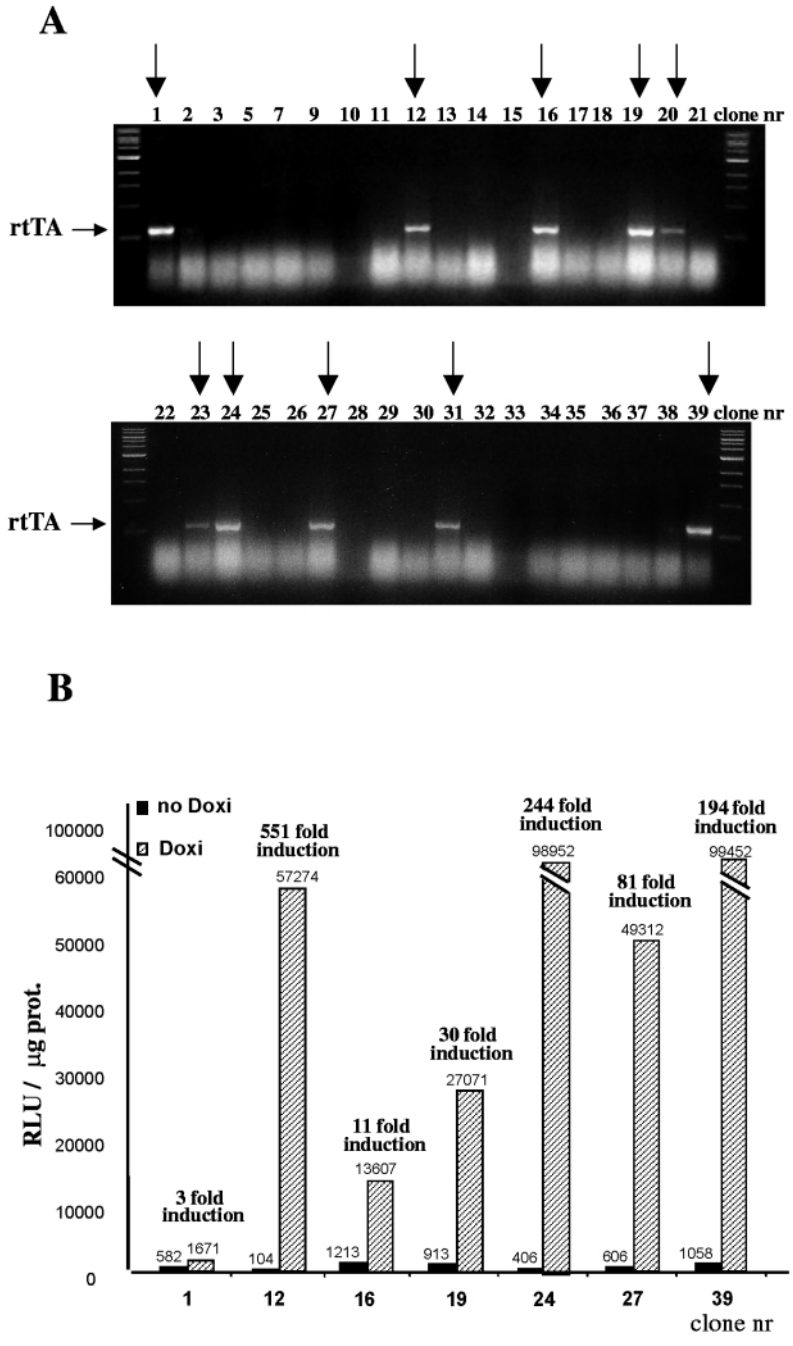

Figure 1. (**A**) RT–PCR screening for rtTA-expressing clones. Only a fraction of the clones screened are shown. Arrows indicate the product of specific amplification of the rtTA in positive clones. (**B**) rtTA-expressing clones were tested for their transactivation capability by transient transfection with a TRE-controlled luciferase reporter gene. Luciferase activity was measured after 48 h in the presence or absence of Doxy (1 µg/ml).

RESULTS

Generation of TetON huntingtin-inducible striatal cells

Striatal derived ST14A cells were used to generate an inducible cell system where expression of the N-terminal fragment of mutant huntingtin is driven by a tetracycline/doxycyline (Doxy)-responsive element (TRE) (22). Both reverse tetracycline-controlled transactivator (rtTA) and TRE–huntingtin constructs were delivered by retroviral infection to maximize single integrants and consistent performance of the transgenes in the descendant cells. To reduce the risk of insertional effects or silencing of the inserted transgene, we screened over 500 clones to identify those with no background expression and tight Doxy-dependent induction of N548-mutant huntingtin.

Figure 1A shows the results of the screening for rtTA-expression on pooled clones (each lane contains four or five pooled clones). Positive pools were further subcloned and

tested for rtTA ability to induce expression of a transiently transfected TRE–luciferase gene. Figure 1B shows that among the subclones, some had low luciferase activity in the absence of Doxy and high levels of induction (up to >500-fold increase) upon administration of the inducer. In the second step of the procedure, clone 12 was retrovirally transduced with a cDNA encoding the first 548 N-terminal amino acids of mutant huntingtin with 128 CAG repeats and under transcriptional control of a TRE sequence. Presumably as a result of retroviral processing, clones with different CAG repeat lengths were generated (Fig. 2A). Sequence analysis showed that three of the clones expressing exogenous N548-huntingtin contained 118, 105 and 104 CAG repeats [identified here as HD40(Q118), HD43(Q105) and HD27(Q104)], two clones expressed 67 CAG repeats [HD12(Q67) and HD14(Q67)], and CAG number was reduced to the human wild-type length in two clones [named HD19(Q26) and HD18(Q17)]. For each of these clones, a single sharp band was visible on agarose gel after RT–PCR, and its molecular weight was constant over high number of passages of the cells in culture.

Figure 2B and C show regulation of transgene expression in one of the clones used in this study. Typically, expression of N548-huntingtin was dependent on Doxy concentration, with a saturation effect evident at 0.5 μg/ml (Fig. 2B). Exogenous huntingtin was detectable 3–4 hours after Doxy treatment, and its expression was stable for at least 4 days following single administration of the antibiotic to the culture medium (Fig. 2C). All the other clones used in this study showed similar induction kinetics, protein levels and tight regulation of expression.

N548-mutant huntingtin in the inducible clones was detected as diffuse perinuclear staining, with no visible cytosolic or nuclear aggregates by 72 hours (Fig. 3). The mutant protein did not induce overt cellular toxicity or cell death. Nor did the presence of mutant huntingtin increased the susceptibility of the cells to proapoptotic stress driven by serum deprivation or 3-nitropropionic acid exposure (data not shown).

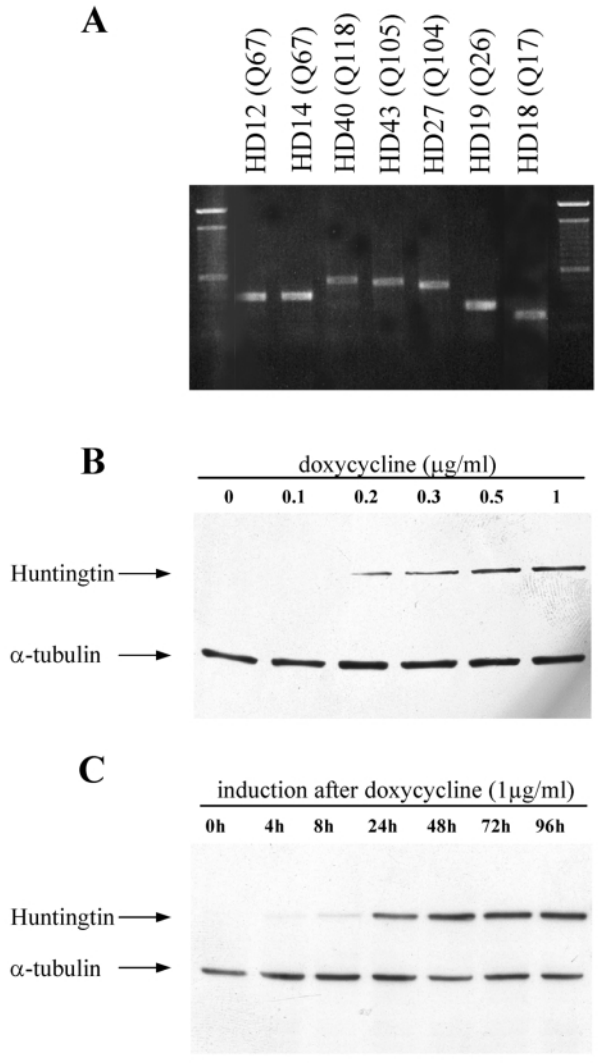

Figure 2. (**A**) RT–PCR, upon induction of mutant huntingtin in selected inducible clones, with primers flanking the CAG repeats. The CAG repeat length, shown in parentheses, has been obtained through sequence analysis. (**B**) Dose-dependent expression of exogenous N548-mutant huntingtin in clone HD43(Q105) upon administration of Doxy. Cells were grown in medium containing the indicated concentrations of Doxy for 48 h before harvesting. Maximal expression of the transgene was observed with 0.5 μg/ml doxycycline. No transgene expression was observed in the absence of the inducer. (**C**) Temporal pattern of induction of exogenous huntingtin in clone HD43(Q105).

Analysis of gene expression upon induction of mutant huntingtin in normal growth conditions

The lines described above were used to generate gene expression profiles prior to induction of huntingtin and at 12, 24, 48 and 72 hours post induction (for a description of the procedure used, see Materials and Methods).

Thresholds applied to identify changes in gene expression were rather conservative, especially considering the small amplitude of the changes observed. To best capture temporal changes in gene expression, regression analysis was performed on the same microarray datasets (23). This allowed the identification of a few more genes that were missed by the first approach for being just below the Affymetrix thresholds used. Significant genes identified with the two independent approaches were listed together.

Results from Q60 and Q100 lines were compared with lists of genes similarly generated for parental and HD19(Q26) cells. Genes modulated in parental cells as a result of Doxy administration itself were no longer considered for analysis. Figure 4 shows the list of mRNAs modulated upon induction of mutant huntingtin at 33°C, grouped by functional families. A small number of mRNAs were differentially detected in cells expressing either expanded or non-expanded huntingtin fragment (Fig. 4A), possibly as a consequence of huntingtin functions that are not affected by the CAG expansion. Most changes, however, were unique to expanded repeat clones (Fig. 4B). Among these were changes in mRNAs associated with lipid metabolism, vesicle trafficking and RNA processing (see Discussion). Expression levels of the genes involved in lipid metabolism are shown in Figure 5.

We further compared the Q105-118 lines with the Q67 lines (data not shown). This analysis revealed no more differences than observed between clones with the same number of CAG repeats (HD12(Q67) versus HD14(Q67) and HD40(Q105)

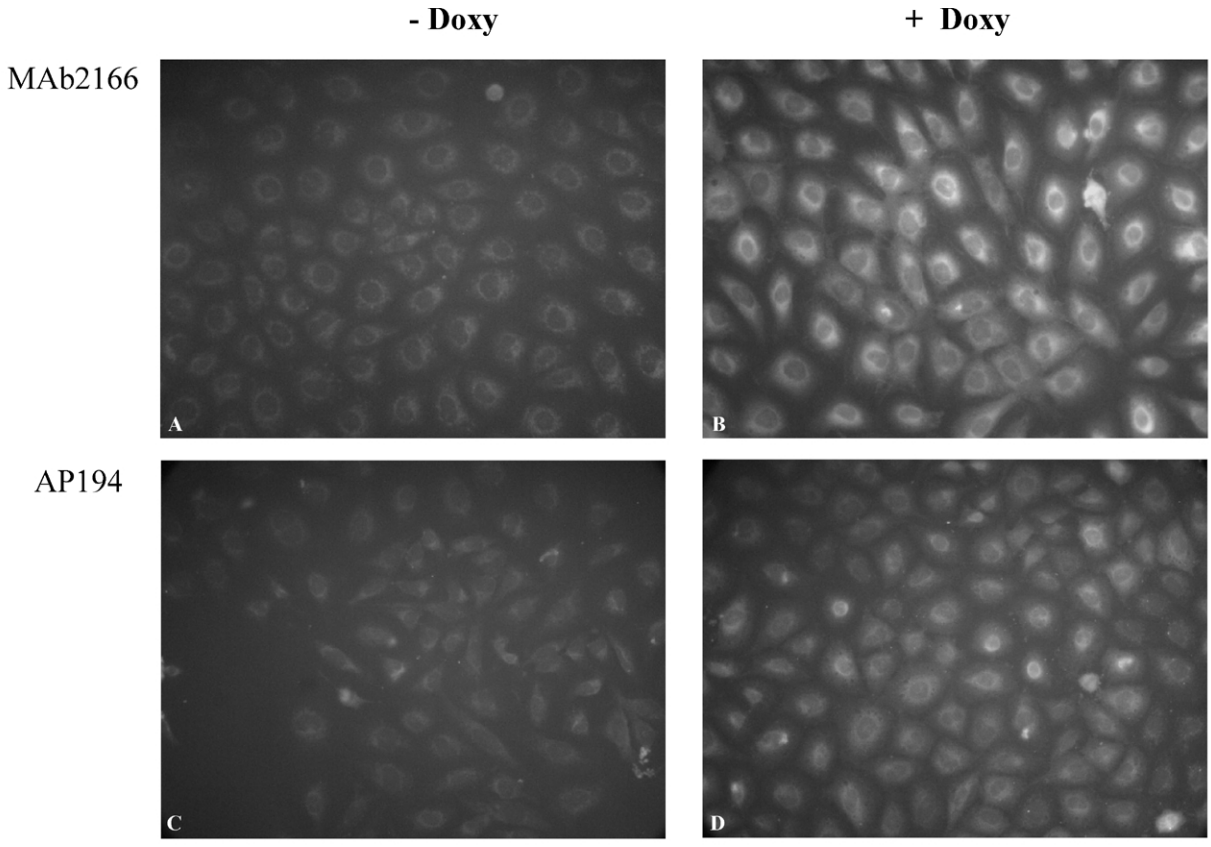

Figure 3. Immunocytochemistry performed on HD43(Q105) clone before (**A** and **C**) and 72 hours after induction of mutant huntingtin (**B** and **D**). The antibodies used were MAb 2166 (A and B) and AP194 (C and D). A diffuse cytoplasmic staining is present; no aggregates were detectable.

versus HD43(Q118)). From this, we conclude that differences caused by >100 poly(Q)s cannot be distinguished from those caused by 67 poly(Q)s.

Analysis of gene expression in post-mitotic conditions

At 33°C, ST14 cells are immortalized by a temperature-sensitive variant of largeTAg, which binds and sequesters p53 and the transcriptional regulator p300/CBP [cAMP response element-binding protein (CREB)-binding protein] (for a review, see 29). At the non-permissive temperature, 39°C, the largeTAg is degraded and cells stop proliferating. Because p53 binds to poly(Q) stretches (13,18) and CBP mediates poly(Q) toxicity (14,16), we wished to see how the presence of expanded repeat huntingtin at 39°C influenced gene expression as p53 and p300/CBP are released in response to disappearance of largeTAg.

Huntingtin induction and largeTAg temperature-sensitive repression were initiated simultaneously. As expected, the disappearance of largeTAg, with consequent release of p53 and p300/CBP in the cells, resulted in extensive transcriptional changes in all cell lines (see supplemental data at www.neumetrix.info).

Huntingtin fragments in Q105-118 cells markedly influenced gene expression dynamics of some p53 responsive genes. The levels of expression of these genes in the mutant, wild-type and parental subclones are shown in Figure 6.

In addition, we identified other mRNAs that responded to the presence of expanded poly(Q). Example mRNAs that preferentially increased following largeTAg depletion in the presence of Q105-118 HD fragments were notch, steroid hormone receptor, fatty acid translocase, lecithin–cholesterol acyltransferase, phospholipase Cβ, osteopontin, glucosidase-α, cellular retinol binding protein II and receptor-linked protein tyrosine phosphatase. mRNA encoding amyloid precursor protein increased 43-fold owing to largeTAg depletion in parental cells, and increased 11-fold in Q105-118 cells. Other mRNAs that increased more in parental cells than in Q105-118 cells included α1 type I collagen, nerve cell adhesion molecule (NCAM) and insulin-like growth factor I (IGF-I). The complete data set reporting differential response to largeTAg depletion in Q105-118 cells and controls is in supplemental data (www.neumetrix.info).

Confirmation of data by RT–PCR and northern blotting

We performed semiquantitative RT–PCR and northern blotting to confirm the differential expression in mutant clones for representative genes identified in our analysis. As shown in Figures 7 and 8, the results uniformly confirmed array data, showing early and progressive downregulation (or upregulation in the case of CAIN), of mRNAs affected by N-548 mutant huntingtin.

Table I - Genes modulated in cells expressing mutant huntingtin

A) - Genes modulated in cells expressing either mutant or wild-type huntingtin

GeneBank Acces.#		Gene and Function	HD19 (Q26)
Calcium-binding proteins			
AA800930		siminlar to junctin (NM_023066)	↑
Metabolism			
X02231	*	glyceraldehyde-3-phosphate-dehydrogenase	↓
AA892828		similar to pyruvate dehydrogenase beta-subunit	↑
Chromatin Remodeling			
D84418	*	chromosomal protein HMG2	↑
Others			
AA893667		EST	↓
AI169370		alpha-tubulin	↓
AA860030		similar to beta-tubulin (isotype Mbeta 5) (MMTUBBM5)	↓

B) - Genes modulated in mutant cells only.

GeneBank Acces.#		Gene and Function
Cell signaling		
D85435		protein kinase C delta-bindig protein
M83298		phosphatase 2A (PP2A)
D30040		RAC protein kinase alpha
AI105076		similar to RAC protein kinase beta - Murine thymoma viral (v-akt) oncogene homolog 2 (D30041)
U11681		rapamycin and FKBP12 target-1 protein (rRAFT1)
AA963674		similar to MAPKK related prot
AI236721	*	similar to 14-3-3 protein gamma (Ywhag) (NM_019376)
Ion channels and Receptors		
AF086758		Na-K-2Cl cotransporter (Nkcc1)
M58040		transferrin receptor
AF081366		ATP-regulated K+ channel ROMK2.1 isoform
Cell cycle and growth factors		
U23146		mitogenic regulation SSeCKS (322)
M65253		transformation-associated protein (34A)
X76454		similar to NM_017062.1| Reversion induced LIM gene (Ril)
M24604	*	proliferating cell nuclear antigen (PCNA/cyclin)
L05489		heparin-binding EGF-like growth factor
Apoptosis-related proteins		
X56729		calpastatin
AI070295	*	similar to GADD45 protein (U00937)
Chaperones and Heat Shock Proteins		
AI231547		similar to FK506 binding protein 4 (59 kDa) (BC003447)
AI136977		similar to FK506 binding protein 4 (BC003447)
S75280	*	pre-mtHSP70=70 kda heat shock protein precursor
AA859980		similar to t-complex protein (Tcp-1x) (M35797)
AI170685		similar to DnaJ (Hsp40) (NM_019794.1)
Lipid Metabolism		
M29249		3-hydroxy-3-methylglutaryl coenzyme A reductase gene
J02791		acyl coenzyme A dehydrogenase medium chain
D00729		delta3, delta2-enoyl-CoA isomerase
AF036761		stearoyl-CoA desaturase 2
AF036761		stearoyl-CoA desaturase 2
AA997614		similar to CyP51=Cyt. P450 lanosterol 14-alfa-demethylase (NM012941)
X13527	(a)	fragment for the acyl carrier protein domain of fatty acid synthetase
X52625	(a)	cytosolic 3-hydroxy 3-methylglutaryl coenzyme A synthase
M89945	(a)	farnesyl diphosphate synthase
U89905	(a)	alpha-methylacyl-CoA racemase
Vesicle trafficking		
D85183		SHPS-1
AF023621		sortilin
AI230406		similar to RAB10 (XM_002267)
D26154		RB109 (brain specific protein)
AA800743		similar to SH3P7r2 similar to drebrin (AB039818)
AF091834		N-ethylmaleimide sensitive factor NSF
S62096	(a)	Rab geranylgeranyl transferase component B alpha subunit
AI007824		similar to Mss4 (X70496)
Oxidative stress		
Y00497		manganese-containing superoxide dismutase (MnSoD)
Calcium-binding proteins		
AA893328	*	similar to calnexin (BC003552)
AI010725	*	similar to calnexin (BC003552)
M83107	(a)	SM22
RNA Processing and Ribosomal		
AF036335		NonO/p54nrb
X94351		CLK3 protein
AA799576		similar to nucleolar protein GU2 (AF334104)
X74565		TBFII mRNA for polypyrimidine tract binding protein
AI639474		similar to cleavage and polyadenylation specific factor 2 (BC013628)
D32209		leucine-rich acidic nuclear protein
AA799406		similar to 18S, 5.8S, and 28S ribosomal RNA (V01270)
X59051		ribosomal protein S29
Chromatin Remodeling,Transcription		
D37934		5E5 antigen
X62875		High Mobility Group Protein I (Y)
M24604		proliferating cell nuclear antigen (PCNA/cyclin)
M65251		angiotensinogen gene-inducible enhancer-binding protein
AB014722		rSALT-1(806)
AA900476		similar to transcription factor MRG1 (AF361476)
AB012230		NF1-B1
U20796		nuclear receptor Rev-ErbA-beta
AI177404		similar to acetyltransferase Tubedown-1 (AF237622)
AI009141		similar to M31 protein (X95399) or MOD1 (AJ132657)
Others		
AI009658		similar to MERANTES (X70675)
AA799803		similar to complement component 1r (BC004637)
M91652		glutamine synthetase (glnA)
M81639		stannin
AB013359		DPM2
AA858640		similar to cytochrome b (AF295545) or cytochrome b oxidase (Y173190)
K00750		cytochrome c
AA800179		similar to neiborough of Cox4 (AF034239)
L17127		proteasome RN3 subunit
AA685152		similar to Ubiquitin-like protein NEDD-8
AI010292		similar to beta-carotene 15, 15'-dioxygenase (Bcdo)(NM_053648.1)
X76489		cell surface glycoprotein
U82612		fibronectin
M28259		fibronectin
X05831		fibronectin
AA893857		EST
AA800693		EST
AA799804		EST
AA800908		EST
AA891209		EST
AA799740		EST
AA800711		EST
AA900769	*	vascular alpha-actin
U69272		interleukin-15
AA800814		similar to Pafaha/Lis1 exon 3 (U95119)
AI102814	*	similar to lysyl oxidase (Lox) (NM_017061)
AA893485		EST

Figure 4. Genes modulated at 33°C in cells expressing mutant huntingtin. Accession numbers in colored boxes indicate decreased (green) or increased (red) gene expression in the mutant clones. (**A**) Gene modulation in the wild-type HD19(Q26) is indicated by vertical arrows (arrow pointing up, increased expression; arrow pointing down, decreased expression). (**B**) Genes modulated in cells expressing mutant huntingtin only are grouped arbitrarily according to their main biological function. An asterisk indicates a potential p53 target (24–28). '(a)' indicates a gene found to be modulated using the regression analysis described in (23).

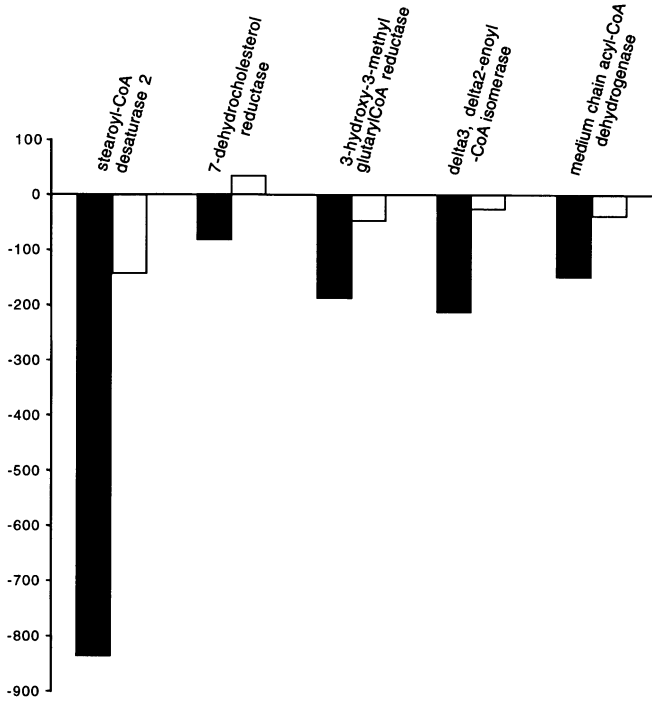

Figure 5. Changes in the expression levels of representative genes of lipid metabolism. The average difference change (Avg Diff Change) is an Affymetrix parameter that serves as an indicator of the difference of expression of a transcript between the sample and the baseline (no Doxy). Avg Diff Change values at 48 h upon induction of huntingtin were averaged for the mutant HD40(Q118) and HD43(Q105) clones (black columns) and wild-type cells (white columns).

We found that NF1 B1 (a silencer of gene expression), and Ssecks, were downregulated not only in the presence of N-548 mutant huntingtin, but also in parental cells exposed to 3-nitroproprionic acid (3NP) (Figure 8B), a mitochondrial toxin that induces a pattern of neurodegeneration and symptomatology closely resembling HD when administered to animals *in vivo* (30). A more extensive analysis of gene expression upon treatment of cells with 3NP is ongoing to assess whether there is a broader overlap with events induced by mutant huntingtin.

DISCUSSION

The primary goal of this study was to gain insight into early cellular and molecular dysfunctions caused by expanded poly(Q) huntingtin fragments. Striata-derived cells without large intracellular aggregates likely represent an early model of HD, where influence of mutant huntingtin on gene transcription can be studied in the absence of transcriptional protein recruitment into aggregates.

N-548 mutant huntingtin modulated several mRNAs involved in cholesterol biosynthesis, monounsaturated fatty acid synthesis and fatty acid β-oxidation, as reported in detail in Figure 5. Key enzymes of the cholesterol biosynthetic pathway, namely 3-hydroxy-3-methylglutaryl–CoA reductase (HMG–CoA Red) and 7-dehydroxycholesterol reductase, were found decreased at the mRNA level. Deficiency of 7-dehydroxycholesterol reductase is known to cause impaired brain development (31).

In the central nervous system, cholesterol is synthesized *de novo* rather than being imported from blood (32–34), and neurons depend on cholesterol provided by astroglia for normal synaptogenesis (35). Cholesterol enhances the production of presynaptic components and synaptic vesicles (36,37), and inhibitors of HMG–CoA reductase block dendrite outgrowth and axonal branching (38).

Consistent with the proposed role for cholesterol in synaptogenesis, genes involved in vesicle trafficking and synaptic vesicle formation were specifically downregulated upon induction of mutant huntingtin. Altered transcription of these genes is in agreement with a proposed role of huntingtin in vesicle trafficking (39) and with studies demonstrating endosome tubulation and authophagy (40), as well as impaired synaptic vesicle uptake (41) in cell and animal models of HD. Altered expression of genes involved in vesicle trafficking has been also shown in an animal model of HD (42).

mRNA encoding stearoyl–CoA desaturase 2, which influences membrane fluidity, was decreased in expanded poly(Q) ST14A cells and in an animal model of HD (43). These findings may further motivate the assessment of therapeutic use of essential fatty acids in HD mouse models (44). Beside downregulation of cholesterol and monounsaturated fatty acid biosynthetic enzymes, we also found reduced expression of genes involved in the rate-limiting steps of the β-oxidation pathway of fatty acids, namely medium-chain acyl coenzyme A dehydrogenase and Δ^3, Δ^2 enoyl–CoA isomerase. β-Oxidation is not the major metabolic pathway used by the brain to produce energy, and we speculate that the alterations observed in our striatal cell line may be more pronounced in tissues in which fatty acids are actively oxidized, such as liver and muscle.

Reduction of the β-oxidation pathway leads to esterification of long-chain fatty acids in triglycerides, which, accumulating in adipose tissue as well as in muscle and pancreatic β cells, become a risk factor for the development of type II diabetes (45). Interestingly, increased storage of fat has been observed in presymptomatic HD transgenic mice (46), and a higher incidence of diabetes has been reported in HD patients (47) and in HD animal models (46).

Another consequence of reduced utilization of fatty acids in β-oxidation is the increased utilization of glucose as a respiratory fuel to meet the demand for energy. Indeed, higher consumption of glucose in HD brains has been shown, including early stages of pathology (48). In line with these observations, enzymes involved in glycolysis and channeling of pyruvate into the Krebs cycle—GAPDH and pyruvate dehydrogenase, respectively—are upregulated in cells expressing mutant huntingtin.

Reduced β-oxidation could also explain, (at least in part), the wasting that characterizes later stages of the pathology (49,50)—a consequence of protein breakdown in HD muscle to produce energy when β-oxidation is reduced. In this perspective, administration of amino acids to HD patients might attenuate wasting.

We cannot assess here the mechanisms by which mutant huntingtin induces dysregulation of lipid metabolism. Enzymes involved in cholesterol synthesis and stearoyl–CoA desaturase are transcriptional targets of sterol regulatory element-binding proteins (SREBP-2 and SREBP-1c or -1a, respectively; for a review, see 51), which recruit p300/CBP to activate

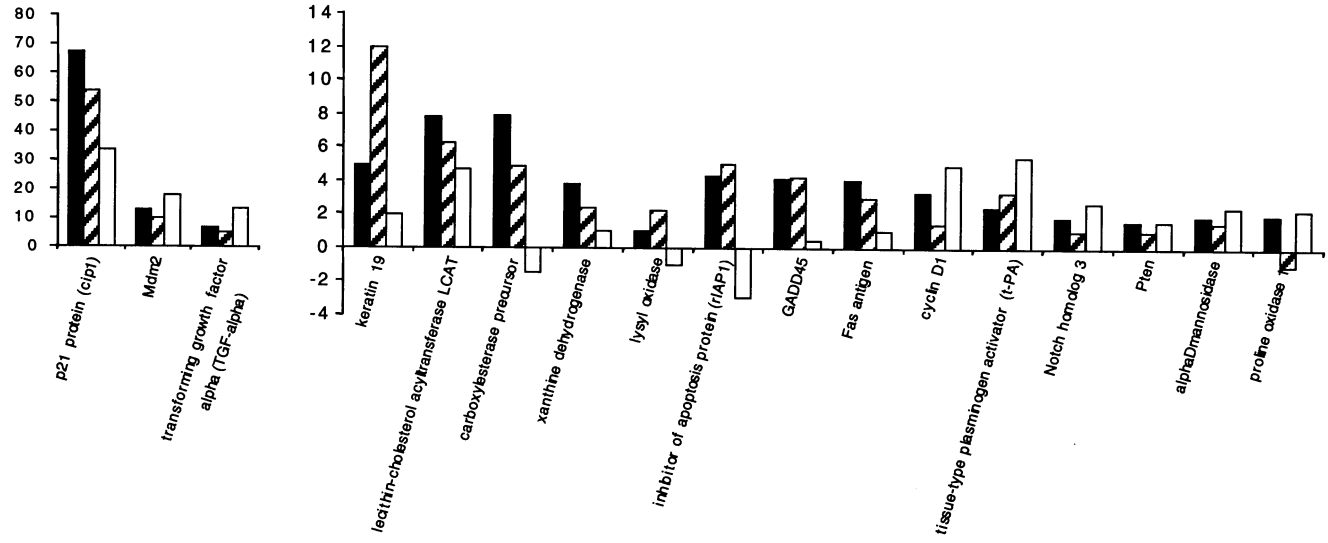

Figure 6. Effect of N548-mutant huntingtin on potential p53 target genes. Columns represent the average of the fold-change values obtained at 48 and 72 h after exposure to 39°C and administration of Doxy (1 μg/ml), for the mutant clones HD40(Q118) and HD43(Q105) (black columns), the wild-type HD19(Q26) (striped columns) and the parental cells (white columns).

transcription (51). It is possible that the inhibitory action of mutant huntingtin on p300/CBP (16,14) may, in turn, lead to reduced transcriptional activity of SREBP. However, other factors may play a role as well. SREBP 1c is known to be regulated by the liver X receptors (LXRs) (52), which are members of the nuclear hormone receptor superfamily whose obligate transcriptional partners are the retinoid X receptors (RXRs) (for a review, see 53). RXRS are also obligate partners of PPARα, and PPARα/RXR heterodimers activate the transcription of the β-oxidation enzymes (for a review, see 54). Factors that affect RXR activity consequently also affect both cholesterol and fatty acid metabolism. We can speculate that the effect of mutant huntingtin on lipid metabolism pathways may be mediated by reduced RXR activity. In support of this hypothesis, in previous studies on HD transgenic animals, it was shown that >20% of the genes that were found to be modulated by mutant huntingtin were retinoid targets (20). Finally, it has recently been reported that in HD transgenic mice and in presymptomatic and affected HD patients, the soluble form of mutant huntingtin interacts with Sp1, a transcription factor involved in the transcription of lipid metabolism genes (55,56), inhibiting its binding to DNA (57,58).

This study revealed that, in striata-derived cells, removal of largeTAg results in increased expression of many p53-regulated mRNAs and that many of these mRNAs were further modulated by mutant huntingtin (Fig. 6).

Not all of the known p53 target genes were found to be modulated by N548-mutant huntingtin. This is not surprising, however, because several studies have reported on heterogeneity of expression of p53 targets in different cell models (59,60). Further investigation is required to unravel the nature of mutant huntingtin involvement in the regulation of p53-mediated pathways and its importance in HD pathogenesis.

We developed a homogeneous, tightly regulatable inducible system that lacks visible intracellular inclusions. Through increased statistical power provided by multiple clones, replicates, and timepoints, the system revealed that, within 24–48 hours, N-548 mutant huntingtin enhances expression of potential p53 target genes and interferes with the transcription of mRNAs that encode critical enzymes involved in lipid metabolism.

MATERIALS AND METHODS

Cell culture

ST14A cells, previously derived from rat embryonic striatum (59), were used to generate inducible cell lines expressing the N-terminal fragment of huntingtin. ST14A cells and all subclones produced proliferate at 33°C but become postmitotic at 39°C. Cells were grown in DMEM supplemented as described in (61), unless otherwise specified. Tet-free fetal calf serum (FCS, Clontech) was used to supplement the medium for the inducible subclones (Tet-free medium).

Generation of inducible ST14A cell lines

Step 1: generation of rtTA-expressing subclones. Transduction of rtTA into ST14A cells was performed by infection with medium conditioned by RetroPack PT67 PackagingCell Line (Clontech), stably transfected with pRevTetON vector (Clontech).

Single colonies were tested for rtTA expression by RT–PCR using primers specific for rtTA (forward 5′-tgcttaatgaggtcg-gaatcgaa-3′; reverse 5′-acgcggacccactttcacat-3′) and the following cycling parameters: denaturation at 95°C for 7 min.; 95°C for 30 s, 52°C for 30 s, 72°C for 1 min (for 40 cycles); 72°C for 7 min. Subclones expressing the mRNA for rtTA were further analyzed for their transactivating capability and levels of background expression. Transient transfection with pRevTRE–Luc (Clontech) was performed using

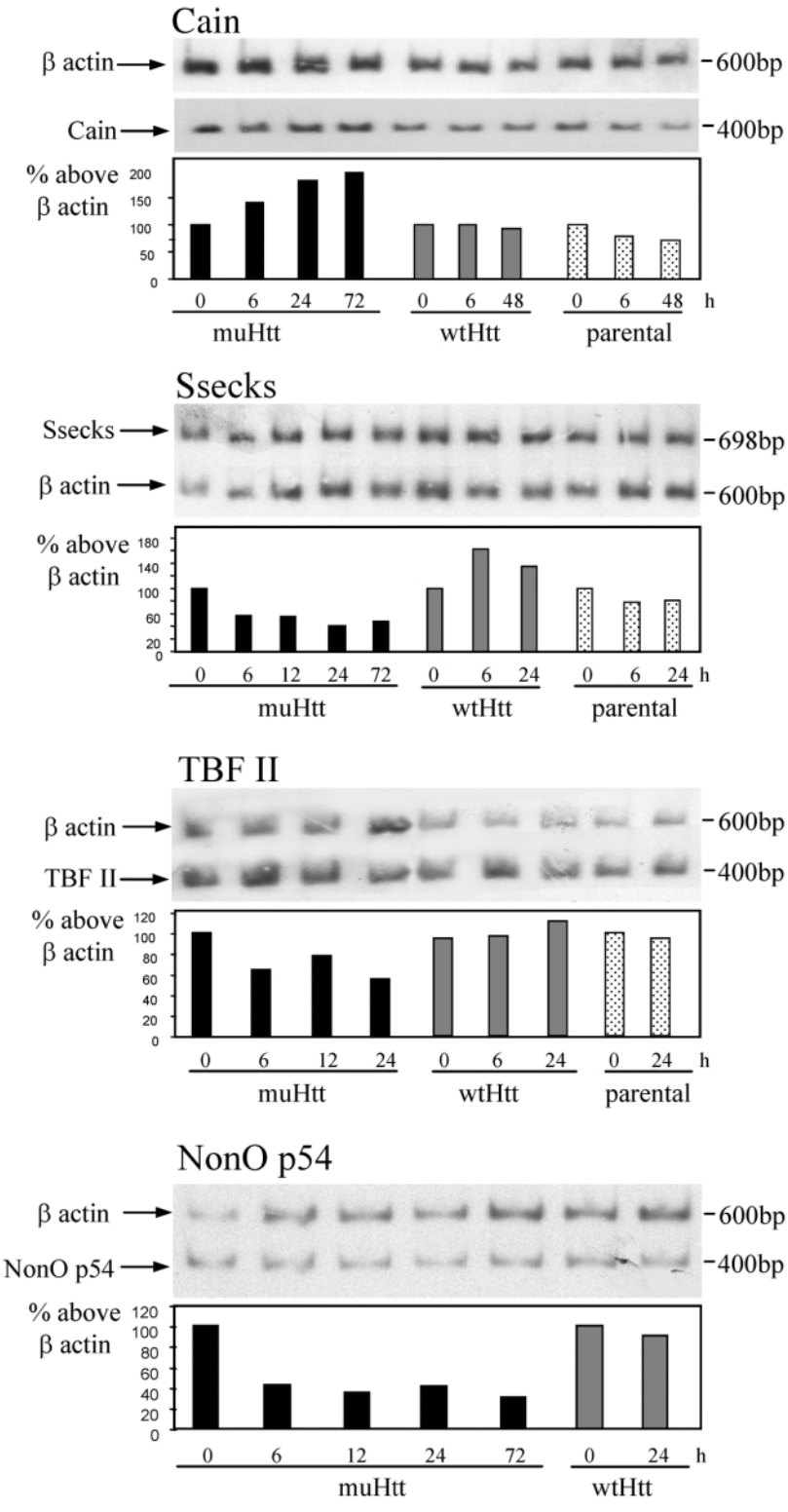

Figure 7. Semiquantitative radioactive RT–PCR for some representative genes modulated by N-548 amino acid mutant huntingtin fragment. HD43(Q105) clones were induced with 1 μg/ml Doxy for the indicated time before RNA extraction. Calcineurin inhibitor (Cain), Ssecks, TBFII and NonO p54, respectively, were co-amplified with β-actin. The intensity of the gene-specific radioactive band was normalized to the β-actin product. The ratio of gene intensity to actin intensity at timepoint 0 (no Doxy) is indicated as 100% in the graphs. All the other values are expressed as percentages with respect to timepoint 0 after normalization to β-actin.

Lipofectamine 2000 (Life Technologies). Transfected cells were grown for 48 h in the presence (1 μg/ml) or absence of the inducer, doxycycline (Doxy). For assessment of luciferase activity, cells were washed with PBS and incubated for 5 min in 25 mM Tris–phosphate, pH 7.8, 2 mM EDTA, 10% glycerol, 0.5% Triton X-100 and 2 mM DTT, at room temperature. Luciferase activity was measured in 20 μl of lysate by adding 100 μl of Luciferase Assay Reagent (20 mM Tricine, 0.1 mM EDTA, 1.07 mM $(MgCO_3)_4Mg(OH)_2{\cdot}5H_2O$ and 2.67 mM $MgSO_4$, pH 7.8, containing 33.3 mM DTT, 270 μM coenzyme A, 530 μM ATP and 470 μM luciferin) in a LUMAT LB 9501 Luminometer (Berthold). Luciferase activity was normalized to the protein content in each sample measured by a BCA-200 Protein Assay Kit (Pierce).

Step 2: generation of subclones expressing inducible N-548 huntingtin. The cDNA encoding for the first 548 amino acids of mutant huntingtin with 128 CAG (3,62) was blunt-end subcloned into the pRevTRE vector (Clontech) digested with *Hpa*I.

The recombinant pRevTRE vector was delivered to rtTA-expressing clones by infection.

Screening for huntingtin expression and inducibility in the presence or absence of 1 μg/ml Doxy was performed by western blot. Assessment of the number of CAG expressed was done by RT–PCR and sequence analysis. Total RNA was extracted using TRIAzol Reagent (Life Technologies), and RT–PCR was carried out with primers flanking the CAG stretch (forward 5′-cgaccctggaaaagctgatgaa-3′; reverse 5′-cacacggtctttcttggtagctga-3′) using Dynazyme Ext Polymerase (Fynnzyme) in the presence of 5% DMSO. Cycling parameters were 95°C for 30 s, 56°C for 30 s (for 35 cycles), 72°C for 7 min. Amplified products were purified with the Qiaquick Gel Extraction Kit (Qiagen) and sequenced by Primm (Milano, Italy).

Characterization of inducible huntingtin expression

For the dose–response analysis, cells were grown in the presence of various concentrations of Doxy for 48 h before harvesting. For the time course of huntingtin expression, cells were grown in 1 μg/ml Doxy and harvested at the indicated time points. Cell harvesting was performed by scraping in 100 μl of ice-cold RIPA buffer (0.15 M NaCl, pH 7.2, 1% Nonidet P-40, 1% sodiumdeoxcholate, 0.1% SDS and 2 mM EDTA) containing 1 × protease inhibitor cocktail (Sigma). Cell debris were pelleted at 10 000g for 15 min. Protein concentration was determined by the BCA-200 Protein Assay Kit (Pierce). Equal amounts of proteins (20–50 μg) of total cell lysate were used for SDS–PAGE and immunoblotting. Antibody MAb2166 (dilution 1 : 5000; Chemicon, Temecula, CA) was used to detect the N-terminal fragment of huntingtin. Monoclonal anti α-tubulin antibody (Sigma) was applied at 1 : 5000 dilution.

Immunocytochemistry

For immunocytochemistry, cells seeded onto polyornithine-coated glass coverslips were rinsed with PBS, fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 for 10 min and then incubated with MAb2166 antibody, recognizing amino acids 181–810 (Chemicon,Temecula, CA), or AP194, recognizing amino acids 1–194 (from A.H. Sharp, Baltimore, MD). Secondary fluorescinated antibody (Sigma) was used at 1 : 200 dilution. The cells were viewed with a Zeiss (Axioscop) microscope.

Analysis of gene expression

Time course of huntingtin induction. For analysis of gene expression at 33°C, we performed two independent time courses of induction of mutant huntingtin expression for two subclones bearing 67 CAG repeats and for two other clones bearing 118 or 105 CAG repeats. The day after plating in Tet-free medium, 1 μg/ml Doxy was added to the cells to be harvested after 72 h of induction and so on, backwards, for timepoints 48 h, 24 h and 12 h. Using this procedure, we were able to extract, on the same day and with a similar confluency, RNA from cells at different time points (12 h, 24 h, 48 h and 72 h) and from two baselines (defined as −6 and 0 h).

The parental clone expressing the transactivator rtTA but not exogenous huntingtin (Par) and one wild-type huntingtin-inducible subclone were included in each experimental set and treated with Doxy as described above. For each set of cells, two independent time courses were performed.

For analysis of gene expression at 39°C, cells were seeded at 80% confluence. The day after, cultures were shifted to 39°C for inactivation of largeTAg. At the same time, Doxy (1 μg/ml) was added to all plates except baseline controls. RNA was extracted at timepoints 48 and 72 h, when, as expected, largeTAg was no longer detectable in virtually all the cells in culture (46, 63; data not shown).

Microarray analysis. Preparation of labeled cRNA and Affymetrix Gene Chip hybridization were performed as previously described (46). Rat RGU34A oligonucleotide arrays were hybridized with 10 μg of cRNA. Data were analyzed using Affymetrix GeneChip software (version 4.0) and selected for presentation based on principles described in (23,64). Iterative comparisons of different datasets were done by spreadsheet analysis (Microsoft Excel). Datasets generated for each timepoint upon induction of mutant huntingtin were compared with the two independent baselines for the same experiment (−6 and 0 h). In addition, each timepoint was also compared to the baselines in the second experiment, giving a total of eight pairwise comparisons for each timepoint at 33°C or four comparisons for each timepoint at 39°C (in this last set of experiments, only one baseline at 0 h was produced). For example, timepoint 24 h experiment 1 was compared with each of the following: (i) baseline −6 h experiment 1, (ii) baseline 0 h experiment 1, (iii) baseline −6 h experiment 2, and (iv) baseline 0 h experiment 2. The same applied for timepoint 24 h experiment 2. For each comparison, we used the default parameters in the GeneChip4.0 software, to ‘call’ genes increased, decreased or not changed in the induced samples with respect to the baselines. Only genes that were called in at least half plus one of the comparisons (i.e. five out of eight for the experi-

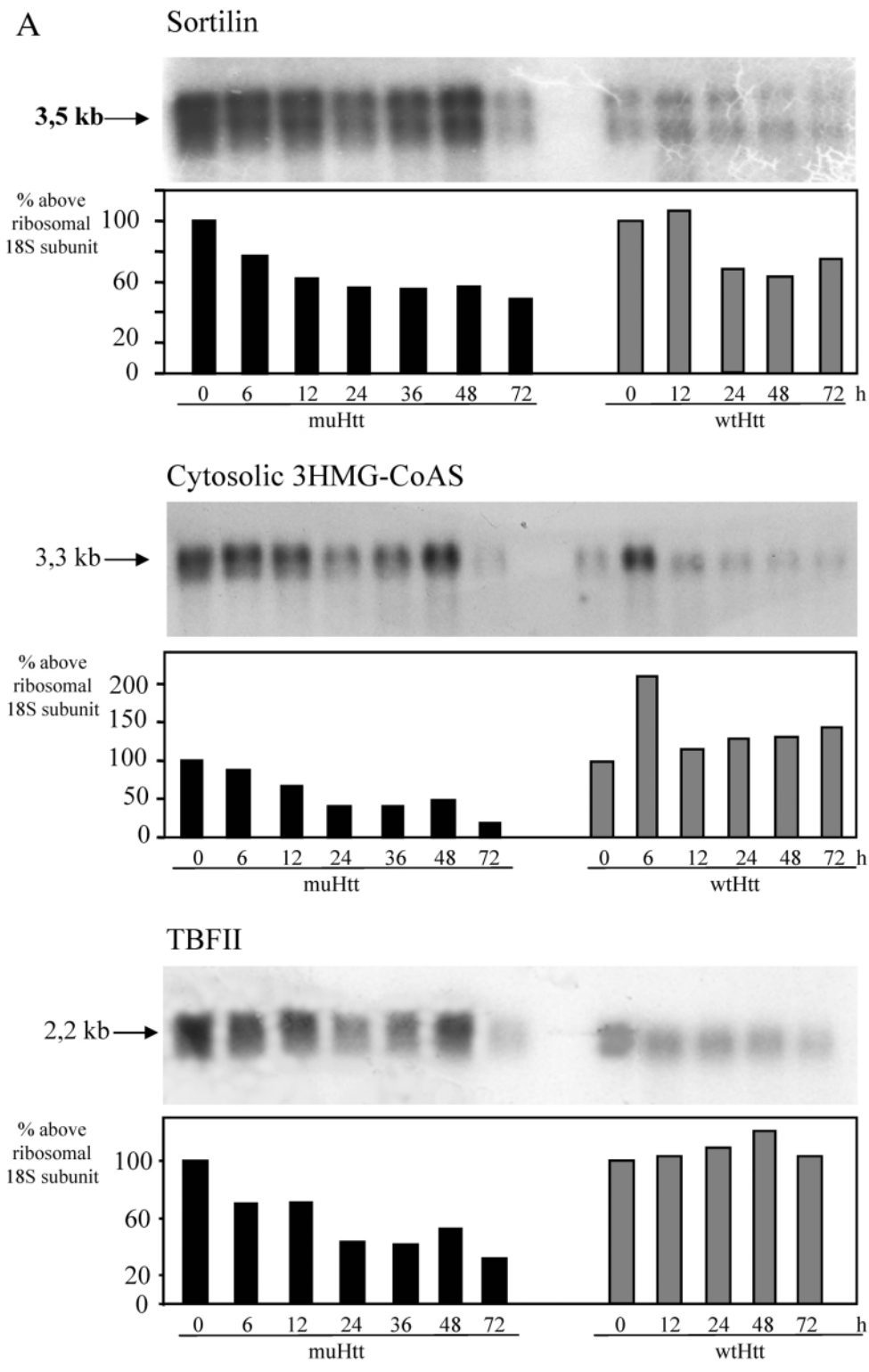

Figure 8. Northern blot analysis of representative genes modulated by mutant huntingtin. (**A**) HD40(118) and HD43(105) clones were induced by 1 μg/ml Doxy for the indicated time. Total RNA preparations from Q105-118 clones were pooled and used for northern blotting with the following probes: sortilin cDNA (nucleotides 1378–1777) cytosolic 3HMG–CoAS cDNA (nucleotides 1253–1652) and TBF II cDNA (nucleotides 1312–1711). The graphs show relative intensity of gene-specific bands normalized to the intensity of the corresponding UV-stained 18S rRNA. The ratio of gene intensity to actin intensity at timepoint 0 (no Doxy) is indicated as 100% in the graph. All the other values are expressed as percentages with respect to timepoint 0 after normalization to 18S rRNA. (**B**) Expression of NF1 B1 and Ssecks in the parental clone upon treatment with 3-nitroproprionic acid (3NP). Total RNA from parental cells grown for 24 h in the absence or presence of 3NP (1 mM) blotted and probed with NF1 B1 cDNA (nucleotides 241–644) or Ssecks cDNA (nucleotides 3726–4423). Normalization of expression levels was done as in (A).

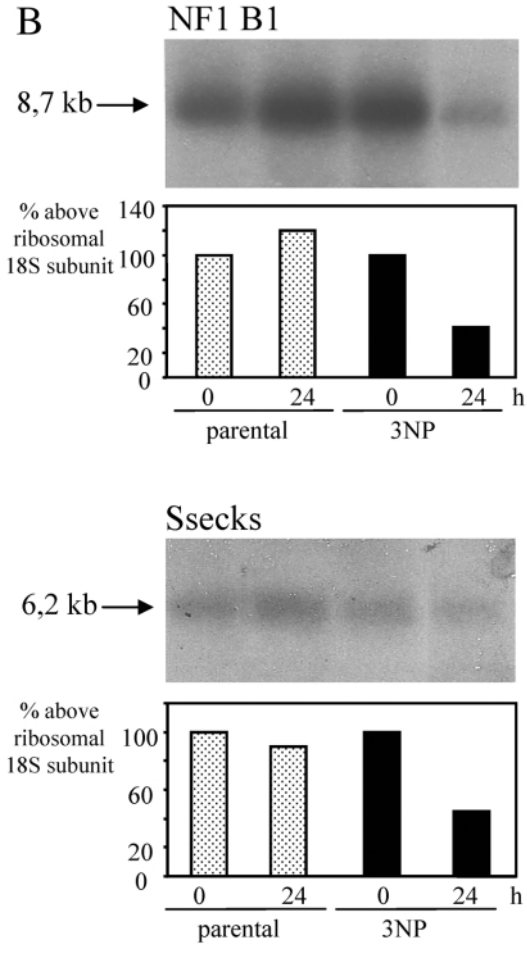

Figure 8 *continued.*

ments at 33°C and three out of five for the experiments at 39°C) were considered for further analysis. To the initial list of genes 'differently' called, we then applied a threshold of 30% to the number of probe pairs in a given probe set that had to show changes in the same direction in order for the gene to be 'called'. No additional fold-change thresholds were applied. Datasets were also analyzed with a randomization procedure described in (23). Significant genes identified by this method were included in the list previously described.

Semiquantitative RT–PCR. Equal amounts of total RNA were retrotranscribed. The obtained cDNAs were amplified with primers for the internal control (β-actin) and primers specifically designed for the gene of interest in the same reaction tube. PCR was performed in the presence of [α-^{32}P]dCTP for 25 cycles. The amplification products were run on an acrylamide gel and subsequently exposed to autoradiography. Normalization was done with respect to the β-actin band. Primers and reaction conditions were as follows: NF1 B1: forward 5′-tccccaagattgagcacttt-3′; reverse 5′-cctgaggctgtgtaggcttc-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; mitogenic regulation ssecks gene (Ssecks): forward 5′-ggtcaagagggtgaggttga-3′; reverse 5′-tctctctgggatgcgcttat-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; NonO p54: forward 5′-cctaccatgaacaccaagca-3′; reverse 5′-cttcccatcatggaaccact-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; cytosolic 3-hydroxy-3-methylglutaryl–CoA synthase (3HMG–CoAS): forward 5′-gtcacaaagatgccacacc-3′; reverse agtgctccccgttactgatg-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; sortilin (Sort): forward 5′-atcagcatctggggattcac-3′; reverse 5′-ggtacccatttgtggtcagg-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; polypyrimidine tract-binding protein (TBFII): forward 5′-aagctgcatgggaagtcagt-3′; reverse 5′-tggtggacttggaaaaggac-3′; cycling parameters: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; calcineurin inhibitor protein (Cain): forward 5′-tcatgatgcgctacctgaag-3′; reverse 5′-agccagtaaacacggaccac-3′; cycling parameters: 94°C for 30 s, 58°C for 30 s, 72°C for 30 s.

Northern blots. These were performed according to standard procedures (65). Transfer efficiency was evaluated by exposing the filter to ultraviolet light (254 nm). cDNA probes were obtained by PCR with primers specific for the genes of interest and labeled with [α-^{32}P]dCTP by random priming. Membrane hybridization was performed at 68°C in QuickHyb hybridization solution (Stratagene, La Jolla, CA). After washing, the blot was exposed to X-Omat AR film. Normalization of the signal intensity was done using the intensity of UV stained 18S ribosomal RNA band.

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REFERENCES

1. Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosome. *Cell*, **72**, 971–983.
2. Reiner, A., Albin, R.L., Anderson, K.D., D'Amato, C.J., Penney, J.B. and Young, A.B. (1988) Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl Acad. Sci. USA*, **85**, 5733–5737.
3. Rigamonti, D., Bauer, J.H., De-Fraja, C., Conti, L., Sipione, S., Sciorati, C., Clementi, E., Hackam, A., Hayden, M., Li, Y. *et al.* (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J. Neurosci.*, **20**, 3705–3713.

4. Cattaneo, E., Rigamonti, D., Zuccato, C., Goffredo, D., Squitieri, F. and Sipione, S. (2001) Loss of normal huntingtin function: new developments in Huntington's Disease research. *Trends Neurosci.*, **24**, 182–188.
5. Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B.R., Goffredo, D., Conti, L., MacDonald, M.E., Friedlander, R.M., Silani, V., Hayden, M.R. *et al.* (2001) Loss of Huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science*, **293**, 493–498.
6. Zoghbi, H.Y. and Orr, H.T. (1999) Polyglutamine diseases: protein cleavage and aggregation. *Curr. Opin. Neurobiol.*, **9**, 566–570.
7. Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L. and Bates, G.P. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell*, **90**, 537–548.
8. DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P. and Aronin, N., (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*, **277**, 1990–1993.
9. Wellington, C.L., Ellerby, L.M., Hackam, A.S., Margolis, R.L., Trifiro, M.A., Singaraja, R., McCutcheon, K., Salvesen, G.S. *et al.* (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.*, **273**, 9158–9167.
10. Wellington, C.L., Singaraja, R., Ellerby, L., Savill, J., Roy, S., Leavitt, B., Cattaneo, E., Hackam, A., Sharp, A., Thornberry, N. *et al.* (2000) Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J. Biol. Chem.*, **275**, 19831–19838.
11. Kim, Y.J., Yi, Y., Sapp, E., Wang, Y., Cuiffo, B., Kegel, K.B., Qin, Z.H., Aronin, N. and DiFiglia, M. (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. *Proc. Natl Acad. Sci. USA*, **98**, 12784–12789.
12. Gerber, H.P., Seipel, K., Georgiev, O., Hofferer, M., Hug, M., Rusconi, S. and Schaffner, W. (1994) Transcriptional activation modulated by homopolymeric glutamine and proline stretches. *Science*, **263**, 808–811.
13. Steffan, J.S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y.Z., Gohler, H., Wanker, E.E., Bates, G.P., Housman, D.E. and Thompson, L.M. (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA*, **97**, 6763–6768.
14. Steffan, J.S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B.L., Kazantsev, A., Schmidt, E., Zhu, Y.Z., Greenwald, M. *et al.* (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. *Nature*, **413**, 739–743.
15. Kegel, K.B., Meloni, A.R., Yi, Y., Kim, Y.J., Doyle, E., Cuiffo, B.G., Sapp, E., Wang, Y., Qin, Z.H., Chen, J.D. *et al.* (2001) Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. *J. Biol. Chem.*, **277**, 7466–7476.
16. Nucifora, F.C. Jr., Sasaki, M., Peters, M.F., Huang, H., Cooper, J.K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V.L. *et al.* (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science*, **291**, 2423–2428.
17. Holbert, S., Denghien, I., Kiechle, T., Rosenblatt, A., Wellington, C., Hayden, M.R., Margolis, R.L., Ross, C.A., Dausset, J., Ferrante, R.J., Neri, C. (2001) The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis. *Proc. Natl Acad. Sci. USA*, **98**, 1811–1816.
18. Wyttenbach, A., Swartz, J., Kita, H., Thykjaer, T., Carmichael, J., Bradley, J., Brown, R., Maxwell, M., Schapira, A., Orntoft, T.F. *et al.* (2001) Polyglutamine expansions cause decreased CRE-mediated transcription and early gene expression changes prior to cell death in an inducible cell model of Huntington's disease. *Hum. Mol. Genet.*, **10**, 1829–1845.
19. Cha, J.H. (2000) Transcriptional dysregulation in Huntington's disease *Trends Neurosci.*, **23**, 387–392.
20. Luthi-Carter, R., Strand, A., Peters, N.L., Solano, S.M., Hollingsworth, Z.R., Menon, A.S., Frey, A.S., Spektor, B.S., Penney, E.B., Schilling, G. *et al.* (2000) Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum. Mol. Genet.*, **9**, 1259–1271.
21. Hughes, R.E. and Olson, J.M. (2001) Therapeutic opportunities in polyglutamine disease. *Nat. Med.*, **7**, 419–423.
22. Gossen, M. and Bujard, H. (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl Acad. Sci. USA*, **89**, 5547–5551.
23. Kooperberg, C., Sipione, S., LeBlanc, M.L., Strandt A.D., Cattaneo, E. and Olson, J.M. (2002) Evaluating test-statistics to select interesting genes. *Hum. Mol. Genet.*, in press.
24. Kannan, K., Amariglio, N., Rechavi, G., Jakob-Hirsch, J., Kela, I., Kaminski, N., Getz, G., Domany, E. and Givol, D. (2001) DNA microarrays identification of primary and secondary target genes regulated by p53. *Oncogene*, **20**, 2225–2234.
25. Madden, S.L., Galella, E.A., Zhu, J., Bertelsen, A.H. and Beaudry, G.A. (1997) SAGE transcript profiles for p53-dependent growth regulation. *Oncogene*, **15**, 1079–1085.
26. Wang, L., Wu, Q., Qiu, P., Mirza, A., McGuirk, M., Kirschmeier, P., Greene, J.R., Wang, Y., Pickett, C.B. and Liu, S. (2001) Analyses of p53 target genes in the human genome by bioinformatic and microarray approaches. *J. Biol. Chem.*, **276**, 43604–43610.
27. Maxwell, S.A. and Davis, G.E. (2000) Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines. *Proc. Natl Acad. Sci. USA*, **97**, 13009–13014.
28. Zhao, R., Gish, K., Murphy, M., Yin, Y., Notterman, D., Hoffman, W.H., Tom, E., Mack, D.H. and Levine, A.J. (2000) Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.*, **14**, 981–993.
29. Ali, S.H. and DeCaprio, J.A. (2001) Cellular transformation by SV40 large T antigen: interaction with host proteins. *Semin. Cancer Biol.*, **11**, 15–23.
30. Palfi, S., Ferrante, R.J., Brouillet, E., Beal, M.F., Dolan, R., Guyot, M.C., Peschanski, M. and Hantraye, P. (1996) Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease. *J. Neurosci.*, **16**, 3019–3025.
31. Waterham, H.R. and Wanders, R.J. (2000) Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith–Lemli–Opitz syndrome. *Biochim. Biophys. Acta*, **1529**, 340–356.
32. Kabara, J.J. (1973) A critical review of brain cholesterol metabolism. *Prog. Brain Res.*, **40**, 363–382.
33. Danik, M., Champagne, D., Petit-Turcotte, C., Beffert, U. and Poirier, J. (1999) Brain lipoprotein metabolism and its relation to neurodegenerative disease. *Crit. Rev. Neurobiol.*, **13**, 357–407.
34. Dietschy, J.M. and Turley, S.D. (2001) Cholesterol metabolism in the brain. *Curr. Opin. Lipidol.*, **12**, 105–112.
35. Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A. and Pfrieger, F.W. (2001) CNS synaptogenesis promoted by glia-derived cholesterol. *Science*, **294**, 1354–1357.
36. Thiele, C., Hannah, M.J., Fahrenholz, F. and Huttner, W.B. (2000) Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat. Cell Biol.*, **2**, 42–49.
37. Lang, T., Bruns, D., Wenzel, D., Riedel, D., Holroyd, P., Thiele, C. and Jahn, R. (2001) SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. *EMBO J.*, **20**, 2202–2213.
38. Fan, Q.W., Yu, W., Gong, J.S., Zou, K., Sawamura, N., Senda, T., Yanagisawa, K. and Michikawa, M. (2002) Cholesterol-dependent modulation of dendrite outgrowth and microtubule stability in cultured neurons. *J. Neurochem.*, **80**, 178–190.
39. Velier, J., Kim, M., Schwarz, C., Kim, T.W., Sapp, E., Chase, K., Aronin, N. and DiFiglia, M. (1998) Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways. *Exp. Neurol.*, **152**, 34–40.
40. Kegel, K.B., Kim, M., Sapp, E., McIntyre, C., Castano, J.G., Aronin, N. and DiFiglia, M. (2000) Huntingtin expression stimulates endosomal–lysosomal activity, endosome tubulation, and autophagy. *J. Neurosci.*, **20**, 7268–7278.
41. He, Li, Shi-Hua, Li, Johnston, H., Shelbourne, P.F. and Xiao-Jiang Li (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity *Nat. Genet.*, **25**, 385–389.
42. Morton, A.J., Faull, R.L. and Edwardson, J.M. (2001) Abnormalities in the synaptic vesicle fusion machinery in Huntington's disease. *Brain Res. Bull.*, **56**, 111–117.
43. Luthi-Carter, R., Strand, A., Peters, N.L., Solano, S.M., Hollingsworth, Z.R., Menon, A.S., Frey, A.S., Spektor, B.S., Penney, E.B., Schilling, G. *et al.* (2000) Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum. Mol. Genet.*, **9**, 1259–1271.

44. Clifford, J.J., Drago, J., Natoli, A.L., Wong, J.Y., Kinsella, A., Waddington, J.L. and Vaddadi, K.S. (2002) Essential fatty acids given from conception prevent topographies of motor deficit in a transgenic model of Huntington's disease. *Neuroscience*, **109**, 81–88.
45. Bergman, R.N. and Ader, M. (2000) Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol. Metab.*, **11**, 351–356.
46. Fain, J.N., Del Mar, N.A., Meade, C.A., Reiner, A. and Goldowitz, D. (2001) Abnormalities in the functioning of adipocytes from R6/2 mice that are transgenic for the Huntington's disease mutation. *Hum. Mol. Genet.*, **10**, 145–152.
47. Farrer, L.A. (1985) Diabetes mellitus in Huntington disease. *Clin. Genet.*, **27**, 62–67.
48. Kuwert, T., Lange, H.W., Langen, K.J., Herzog, H., Aulich, A. and Feinendegen, L.E. (1990) Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. *Brain*, **113**, 1405–1423.
49. Sanberg, P.R. and Johnston, G.A. (1981) Body weight and dietary factors in Huntington's disease patients compared with matched controls. *Med. J. Aust.*, **1**, 407–409.
50. Pratley, R.E., Salbe, A.D., Ravussin, E. and Caviness, J.N. (2000) Higher sedentary energy expenditure in patients with Huntington's disease. *Ann. Neurol.*, **47**, 64–70.
51. Osborne, T.F. (2000) Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J. Biol. Chem.*, **275**, 32379–32382.
52. Repa, J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.M., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L. and Mangelsdorf, D.J. (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. *Genes Dev.*, **14**, 2819–2830.
53. Repa, J.J. and Mangelsdorf, D.J. (2000) The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. *Annu. Rev. Cell Dev. Biol.*, **16**, 459–481.
54. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.M., Heyman, R.A., Briggs, M., Deeb, S., Staels, B. and Auwerx, J. (1996) PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.*, **15**, 5336–5348.
55. Kim, J.H., Lee, J.N. and Paik, Y.K. (2001) Cholesterol biosynthesis from lanosterol. A concerted role for Sp1 and NF-Y-binding sites for sterol-mediated regulation of rat 7-dehydrocholesterol reductase gene expression. *J Biol Chem.*, **276**, 18153–18160.
56. Ikeda, Y., Yamamoto, J., Okamura, M., Fujino, T., Takahashi, S., Takeuchi, K., Osborne, T.F., Yamamoto, T.T., Ito, S. and Sakai, J. (2001) Transcriptional regulation of the murine acetyl-CoA synthetase 1 gene through multiple clustered binding sites for sterol regulatory element-binding proteins and a single neighboring site for Sp1. *J. Biol. Chem.,* **276**, 34259–34269.
57. Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N. and Krainc, D. (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science*, **296**, 2238–2243.
58. Li, S.H., Cheng, A.L., Zhou, H., Lam, S., Rao, M., Li, H. and Li, X.J. (2002) Interaction of Huntington disease protein with transcriptional activator Sp1. *Mol. Cell Biol.*, **22**, 1277–1287.
59. Kannan, K., Amariglio, N., Rechavi, G., Jakob-Hirsch, J., Kela, I., Kaminski, N., Getz, G., Domany, E. and Givol, D. (2001) DNA microarrays identification of primary and secondary target genes regulated by p53. *Oncogene*, **20**, 2225–2234.
60. Zhao, R., Gish, K., Murphy, M., Yin, Y., Notterman, D., Hoffman, W.H., Tom, E., Mack, D.H. and Levine, A.J. (2000) Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.*, **14**, 981–993.
61. Cattaneo, E. and Conti, L. (1998) Generation and characterization of embryonic striatal conditionally immortalized ST14A cells. *J. Neurosci. Res.*, **15**, 223–234.
62. Hackam, A.S. Singaraja, R., Wellington, C.L., Metzler, M., McCutcheon, K., Zhang, T., Kalchman, M. and Hayden, M.R. (1998) The influence of huntingtin protein size on nuclear localization and cellular toxicity. *J. Cell Biol.*, **141**, 1097–1105.
63. Cattaneo, E., Magrassi, L., Butti, G., Santi, L., Giavazzi, A. and Pezzotta, S. (1994) A short term analysis of the behaviour of conditionally immortalized neuronal progenitors and primary neuroepithelial cells implanted into the fetal rat brain. *Brain Res. Dev. Brain Res.*, **83**, 197–208.
64. Strand, A.D., Olson, J.M. and Kooperberg, C. (2002) Estimating confidence intervals for gene expression changes observed with oligonucleotide array. *Hum. Mol. Genet.*, in press.
65. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.