Huntingtin Modulates Transcription, Occupies Gene Promoters In Vivo, and Binds Directly to DNA in a Polyglutamine-Dependent Manner

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Huntingtin modulates transcription, occupies gene promoters *in vivo* and binds directly to DNA in a polyglutamine-dependent manner

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Abstract

Transcriptional dysregulation is a central pathogenic mechanism in Huntington's disease, a fatal neurodegenerative disorder associated with polyglutamine (polyQ) expansion in the huntingtin (Htt) protein. In this study, we show that mutant Htt alters the normal expression of specific mRNA species at least partly by disrupting the binding activities of many transcription factors which govern the expression of the dysregulated mRNA species. Chromatin immunoprecipitation (ChIP) demonstrates Htt occupation of gene promoters *in vivo* in a polyQ-dependent manner and furthermore, ChIP-on-chip and ChIP subcloning reveal that wild-type and mutant Htt exhibit differential genomic distributions. Exon 1 Htt binds DNA directly in the absence of other proteins and alters DNA conformation. PolyQ expansion increases Htt-DNA interactions, with binding to recognition elements of transcription factors whose function is altered in HD. Taken together; these findings suggest mutant Htt modulates gene expression through abnormal interactions with genomic DNA, altering DNA conformation and transcription factor binding.

Keywords

Transcription factor; chromatin immunoprecipitation; DNA microarrays; polyglutamine; gene expression; DNA conformation

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant, late-onset neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the *HD* gene which translates into a polyglutamine (polyQ) tract in the huntingtin (Htt) protein (HDCRG, 1993). Thus, HD belongs to a group of neurodegenerative disorders caused by polyQ expansion, which include spinal

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CONFLICT OF INTEREST STATEMENT
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and bulbar muscular atrophy (SBMA), dentatorubral pallidoluysian atrophy (DRPLA) and the spinocerebellar ataxias (SCA) types 1, 2, 3, 6, 7, and 17 (Bates et al., 2002). HD neuropathology is characterized by generalized brain atrophy, selective neuronal cell death, and the widespread occurrence of polyQ aggregates (DiFiglia et al., 1997).

Htt cleavage promotes nuclear localization and nucleocytoplasmic shuttling motifs have been identified within the Htt protein, suggesting that Htt can be transported in and out of the nucleus (Takano and Gusella, 2002; Xia et al., 2003). The first 17 amino acids of Htt can function as a cytosolic retention signal (Steffan et al., 2004; Cornett et al., 2005). While some wild-type Htt is normally localized in the nucleus, polyglutamine-expanded Htt exhibits more nuclear localization than non-expanded protein (Dorsman et al., 1999; Kegel et al., 2002). Nuclear-localized expanded polyglutamine protein is highly detrimental *in vitro* and *in vivo* (Klement et al., 1998; Saudou et al., 1998; Jackson et al., 2003; Schilling et al., 2004; Benn et al., 2005). In transgenic mice, exclusive nuclear localization of mutant exon 1 Htt in the nucleus is sufficient for the onset and progression of behavioral phenotypes, neurodegeneration and transcriptional dysregulation (Benn et al., 2005).

Transcriptional dysregulation is a central pathogenic mechanism in HD (Luthi-Carter and Cha, 2003). Human HD and mouse models of HD demonstrate down-regulation of the mRNA of specific genes (Luthi-Carter et al., 2000; Hodges et al., 2006). Indeed, comparison of gene expression studies has revealed remarkable concordance among mouse models and within human HD brain (Kuhn et al., 2007). Down-regulation is not due to post-transcriptional mRNA stability, but rather to decreased transcription from gene promoters (Hu et al., 2004; McCaw et al., 2004; Cui et al., 2006). The polyQ motif occurs in many transcription factors and can function as a transcriptional activation domain. Interestingly, polyQ repeat expansions in TATA binding protein (TBP) and androgen receptor (AR) cause the disorders SCA17 and SBMA, respectively.

While mutant Htt disrupts transcription in neurons, the underlying molecular mechanism is unknown. In this study, we used mRNA expression profiling to identify genes expressed exclusively in the presence of wild-type or mutant Htt. Additionally, many transcription factor activities were perturbed in response to mutant Htt. DNA immunoprecipitation using Htt specific antibodies demonstrated an increase in occupancy of mutant Htt at gene promoters *in vivo*. Furthermore, we have employed chromatin immunoprecipitation combined with DNA microarray analyses to identify the genomic binding sites of wild-type and mutant Htt. Finally, wild-type and mutant Htt bind directly to DNA *in vitro* in the absence of other proteins, and differentially alter DNA conformation. Thus, increased binding of mutant Htt to DNA modulates DNA conformation and alters transcription factor function, and ultimately results in transcriptional dysregulation.

MATERIALS AND METHODS

Transgenic R6/2 mouse striatum

R6/2 transgenic mice and wild-type littermate controls (Mangiarini et al., 1996) were sacrificed and brains rapidly removed, striata were dissected and flash-frozen in chilled isopentane and stored at −80°C until use. The guidelines for animal care and use were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (SRAC).

Immortalized striatal HD cell lines

Striatal cell lines established from wild-type (Q7/7) and homozygote mutant (Q111/111) Hdh knock-in embryonic mice (Trettel et al., 2000) were used in passages 5 to 16. ST*Hdh* cell lines

express full-length murine Htt with either 7 or 111 glutamines. Cells were kept at 33°C for propagation and were placed at 39°C for 48 h to stop proliferation.

Postmortem human brain tissue

Postmortem human brain tissue from HD patients (7 cases) and neurologically normal control patients (7 cases) were kindly provided by the Alzheimer's Disease Research Center at Massachusetts General Hospital under the auspices of Partners HealthCare Human Subjects Committee, as approved by the institutional review board. Postmortem intervals ranged from 13 to 24 h for the HD cases (corresponding to Vonsattel grade II (2 cases), grade III (2 cases), and grade IV (2 cases)) and from 10 to 21 h for the control cases. Unfixed frontal cortex was obtained from each case. Brain samples were stored at −80°C.

Nuclear extracts

Nuclear proteins were extracted from mouse brain tissue using a Sigma CellLytic™ NuCLEAR™ extraction kit according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO).

GST-fusion proteins

GST-Htt clones (HD20Q, HD32Q, and HD53Q) were a generous gift from Dr. Erich Wanker (Max-Planck Institute, Germany). GST-p53 was generously supplied by Dr. Joan Steffan (University of California, Irvine, CA), while the GST-CBP fusion proteins #1–5 (#1 = 1–450 amino acids, #2 = 419–1098 amino acids, #3 = 1069–1459 amino acids, #4 = 1459–1891 amino acids, #5 = 1892–2441 amino acids of CBP) were kind gifts from Riki Kurokawa in Christopher Glass' lab (University of California, San Diego, CA) (Kurokawa et al., 1998). GST fusion proteins were prepared as described (Yohrling et al., 2003). Verification of protein synthesis was performed with SDS-PAGE and Coomassie Blue staining (data not shown).

Gene expression profiling analysis

The gene expression data from the comparison study of ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines were analyzed as described (Sadri-Vakili et al., 2007) (Accession number GSE11358).

Transcription factor array

The TranSignal™ Protein/DNA array kit profiles binding activities of up to 345 transcription factors simultaneously and was utilized according to the manufacturers instructions (Panomics, Fremont, CA). Twenty ug of nuclear extract or 1 ug of GST protein were incubated with biotin-labeled DNA probe mix in binding buffer. Unbound probes were washed away, leaving protein-DNA complexes. Bound DNA probes were recovered from the complex and denatured prior to hybridizing to the array membrane. The biotin-labeled probe was detected with Streptavidin-HRP and chemiluminescently enhanced prior to imaging and densitometry with Alpha Innotech FluorChem Imager (Alpha Innotech, San Leandro, CA).

Correlating microarray gene expression profiling with transcription factor binding activity profiles

The gene expression profiling of the ST*Hdh* cell lines generated 4 lists of genes based on Presence/Absence and Up/Down criteria labeled as follows: a) Absent in ST*Hdh*$^{7/7}$, present in ST*Hdh*$^{111/111}$; b) Present in ST*Hdh*$^{7/7}$, absent in ST*Hdh*$^{111/111}$; c) Present in both lines, with relative up-regulation in ST*Hdh*$^{111/111}$; d) Present in both lines, with relative down-regulation in ST*Hdh*$^{111/111}$. In order to identify the relevant networks and pathways and related transcription factors for these sets of genes, we performed literature mining. Genomatix Bibliosphere was used to mine PubMed abstracts for gene-gene and gene-transcription factor

co-citations between each of the genes from the filtered lists. The criteria for the co-citation of the genes were: a) input genes + transcription factors, b) genes co-cited with at least 2 input genes, c) genes that are at least co-cited 2 times with 1 input gene, d) GFG level B3: restricted to sentences with order “gene - function word -gene”. We generated tables and interaction network maps with all the mined information. Transcription factors found involved in the network as a direct interaction with the input genes were compared with transcription factor binding activity profiles. Common transcription factors in both studies were combined.

Chromatin immunoprecipitation (ChIP) assay

The chromatin immunoprecipitation (ChIP) technique was performed as described (Chen-Plotkin et al., 2006). Antibodies used were S830 (raised against Htt exon 1 fusion protein) (kind gift of Gillian P. Bates, Kings College, London, UK), N18 (Santa Cruz Biotechnology Inc, Santa Cruz, CA), MAb1 (recognizes Htt amino-acids 1–17) (kind gift of Marian DiFiglia, Massachusetts General Hospital, Boston, MA), MAb2166 (raised against amino acids 181–810 of Htt), MAb2170 (raised against amino acids 1247–1646 of Htt) (Chemicon, Temecula, CA). Antibodies against RAP30 (sc-236) and RAP74 (sc-235) were obtained from Santa Cruz Biotechnology Inc. Positive control ChIP was performed with anti-acetylated histone H3 (Millipore, Billerica, MA) and negative controls included IgG (Jackson, West Grove, PA) and no antibody.

DNA quantitation

Immunoprecipitated DNA (from ChIP or DIP) was quantitated using the DNA Quantitation System which detects 10–500 pg/ul DNA (Promega, Madison, WI), according to manufacturer’s instructions. Briefly, a premix was made with a buffer solution containing sodium pyrophosphate, NDPK enzyme solution and T4 DNA polymerase, aliquoted and mixed with either known DNA quantities (for a standard curve) or experimental unknown DNA quantities. The reaction was incubated for 10 min at 37°C, chilled on ice for 10 min before mixing with ENLITEN™ luciferase reagent. The light output was immediately measured in a luminometer.

Real-time PCR

Input and IP samples were interrogated with gene promoter-specific primers in triplicate reactions (Supplementary Table 3) in real-time PCR analysis as previously described (Chen-Plotkin et al., 2006). Threshold amplification cycle numbers using iCycler software were used to calculate IP DNA quantities as percentages of corresponding inputs.

Ligation-mediated PCR amplification of ChIP products

We utilized a ligation-mediated PCR (LM-PCR) procedure in order to amplify the very small amounts of immunoprecipitated DNA recovered through ChIP, for subsequent ChIP-on-chip or ChIP-subcloning experiments. LM-PCR was performed as published (Lee et al., 2006).

ChIP-on-chip

ChIP-on-chip was performed as described (Boyer et al., 2006; Lee et al., 2006), in collaboration with Agilent Technologies (Santa Clara, CA). Briefly, LM-PCR-amplified DNA was labeled and purified using Invitrogen (Paisley, UK) Bioprime random primer labeling kits. Labeled DNA (immunoenriched DNA labeled with Cy5, input DNA labeled with Cy3) was combined (5 μg each of immunoenriched and input DNA) and co-hybridized to Mouse Proximal Promoter arrays (Whitehead format 13275 and 13276, with 4–5 60-mer oligonucleotide probes for 17,917 annotated transcription start sites) in Agilent hybridization chambers at 20rpm for 40 hr at 65°C. Arrays were washed and scanned on an Agilent scanner. The scanned image was analyzed using Feature Extraction Software. Background and noise were removed from the

data and the QC generated by feature extraction was monitored. Fluorescent intensities of Cy5/Cy3 for each individual feature on the array represent the binding events of the amplified ChIP product enabling identification of Htt binding sites.

ChIP-on-chip data normalization and analysis

Pre-processed data were normalized and analyzed using the ChIP Analytics Software 1.2. Replicates were analyzed together within the software (intra-array LOWESS intensity dependent normalization) and the results were averaged using the replicate support extended error model proposed by the software. Results of the combined analyses were used to generate reports of text files for the probes, segments and gene levels results, UCSC track files, Ensembl DAS files and quality control reports.

Identifying Htt-enriched regions at known gene locations

We selected probes with a weighted average Log_2 ratio (IP/input) >1 or < −1 with a p-value < 0.01 for the ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines, identified the genes with at least 1 probe significantly enriched and pulled down the results for all the probes for each of these genes. Results were mapped on the chromosomes using the UCSC Genome Browser as custom tracks. Binding ratios for both cell lines were compared at each probe and gene location. In order to determine the amplitude of difference between Htt binding in the 2 cell lines, we calculated the Log_2 ratio of the ST*Hdh*$^{111/111}$/ST*Hdh*$^{7/7}$ binding ratios.

Comparing Htt-promoter binding and gene expression data

We analyzed gene expression profiling data for genes identified through the ChIP-on-chip analysis. Probes which were absent (Affymetrix Absent/Presence call) in either ST*Hdh*$^{7/7}$ or ST*Hdh*$^{111/111}$ lines were removed as well as probes with no difference between the two lines (Log_2(ST*Hdh*$^{111/111}$/ST*Hdh*$^{7/7}$) <0.3 or > −0.3). Data from the remaining probes from the gene expression study and from the binding study were combined as a comparison of the Log_2 ratios (ST*Hdh*$^{111/111}$/ST*Hdh*$^{7/7}$) from the gene expression analysis compared to the binding ratios.

ChIP subcloning

In order to isolate transcription factor targets in an unbiased fashion, ChIP subcloning was performed using a modified method (Nelson et al., 2006). ChIP was performed as described with MAb2170 except that two sequential immunoprecipitations were performed. Annealed, phosphorylated oligonucleotides were ligated to the blunt-ended purified Htt ChIP product and amplified by PCR for 20 cycles of amplification as described in (Nelson et al., 2006). DNA from the ligation-mediated PCR amplification of the Htt-ChIP product was cloned into pBluescript, transformed into XL1 Blue (XRF', Stratagene, La Jolla, CA) *E. coli*, and positive clones were identified through blue/white colony selection. Initial analysis of bacterial clones was performed by picking each individual white bacterial colony into 200 ul of ddH_2O and vigorously vortexing. This preparation was used as a template in a PCR reaction with Promega buffer, 2.5 mM $MgCl_2$, 200 μM dNTPs, 10 ng/μl M13 forward and reverse primers and 0.5 U/μl Taq polymerase (Promega). Cycling conditions were 90 sec at 94°C, 35 × (30 sec at 94°C, 30 sec at 55°C, 90 sec at 72°C), 10 min at 72°C. Clones with an insert larger than 180 bp were miniprepped (Qiagen, Valencia, CA) and submitted for sequencing at the MGH DNA core using the M13 forward primer.

DNA immunoprecipitation (DIP)

DNA immunoprecipitation is essentially a modified GST-pulldown technique. Equal amounts (5 ug) of each GST protein (on sepharose beads) were incubated overnight with agitation at 4° C with 5 ug of DNA template in freshly-made GST binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.3% IEGPAL, and 20% glycerol). DNA

templates include genomic DNA restriction digested to completion with Sau3AI or short double-stranded DNA molecules such as EMSA probes (made from annealed oligonucleotides). Samples were cross-linked under UV light for 3 min prior to washing 3 times in 1 ml of GST-binding buffer, and resuspended in 50 ul of PBS containing protease inhibitors.

Micrococcal nuclease assay coupled with Southern blotting

MNase digestion was performed as described in Nature Methods (*2(9)*, 719–720), originally adapted from (Carey and Swale, 2000), except approximately 10^6 cells were used. It was not possible to measure genomic DNA amounts prior to MNase digestion due to its high-molecular weight state. After MNase-digestion (0.5–50 U/ml), DNA was visualized via agarose gel electrophoresis. Southern blotting was performed as described (Mangiarini et al., 1996), except that probes were generated by PCR amplification and confirmed by sequencing of the relevant gene promoter from genomic DNA prior to radiolabeling (primer information in Supplementary Table 3).

Conformation-dependent fluorescent quenching of YOYO-1/DNA complexes

pMDR1-luc, kind gift from Eric Stanbridge, (University of California, Irvine CA) and pWWP1-luc, generously given by Bert Vogelstein (Johns Hopkins, Baltimore, MD), plasmid DNA were mixed at a ratio of 50 bp of double-stranded plasmid DNA to 1 dye molecule at a final concentration of 200 nM of YOYO-1 dye (Invitrogen). The DNA-YOYO-1 complex was incubated in a buffer containing a final concentration of 100 mM NaCl and 20 mM HEPES at pH 7.4 at room temperature for 5 h in the dark. As a control, poly-L-lysine (Sigma) was added to a final concentration of 0.0045%, which corresponds to an approximate ratio of two poly-L-lysine molecules to one phosphate on the DNA backbone. GST fusion proteins were added (0.5 μM) to the DNA-YOYO-1 complex and incubated for up to 120 min. Steady-state florescent measurements were performed on a Vallac Victor 1420 Multilabel Counter (Perkin Elmer, Waltham, MA). The excitation wavelength was 485 nm and the emission was collected at 535 nm.

RESULTS

Genes whose expression is dependent on the presence of wild-type or mutant Htt

Specific gene expression changes occur in the presence of mutant Htt in human HD patient brains, as well as in multiple cell and mouse models of HD (Luthi-Carter et al., 2000; Sipione et al., 2002; Sugars et al., 2004; Hodges et al., 2006). Htt can modulate reporter gene transcription in a polyglutamine-dependent manner (Steffan et al., 2000; Kegel et al., 2002; Yohrling et al., 2003; Obrietan and Hoyt, 2004). Furthermore, the presence of mutant Htt fragments restricted to the nucleus *in vivo* is sufficient to induce transcriptional dysregulation (Schilling et al., 2004; Benn et al., 2005). However, the extent to which exclusive expression of wild-type or mutant Htt can modulate gene expression is not clear. We therefore performed microarray gene expression profiling on immortalized striatal cell lines expressing only wild-type Htt (ST*Hdh*$^{7/7}$), or homozygote for mutant Htt (ST*Hdh*$^{111/111}$) (Trettel et al., 2000). The exclusive presence of wild-type or mutant Htt confers a transcriptional phenotype, with distinct effects on gene expression profiles (Figure 1). We hypothesized that the most profound effect on gene expression would be presence versus absence of a mRNA species in one line compared to the other, and therefore generated lists of genes whose expression is dependent on or inhibited by either wild-type and mutant Htt (Figure 1) (Sadri-Vakili et al., 2007). More genes were expressed exclusively in the presence of mutant Htt (2098 probes); compared to the number of genes expressed in the presence of only wild-type Htt (1382 probes). We also interrogated mRNA species that were present (14233 probes) or absent (23424 probes) in both ST*Hdh*$^{7/7}$and ST*Hdh*$^{111/111}$ cell lines. For those mRNA species present in both cell lines, we

determined whether the expression is up- or down-regulated by mutant Htt. Genes expressed in both cell lines were more likely to be down-regulated in the presence of mutant Htt (1105 probes, 85% of which had a p-value <0.01) compared to genes that are up-regulated in the presence of mutant Htt (964 probes, 79% of which had a p-value <0.01). Therefore, we have identified four clusters of mRNA expression changes in response to the exclusive presence of wild-type or mutant Htt: (1) absent with wild-type Htt and present with mutant Htt, (2) present with wild-type Htt and absent with mutant Htt, (3) present in both wild-type and mutant but up-regulated by mutant Htt and (4) present in both wild-type and mutant but down-regulated by mutant Htt. Of these, the two most prevalent are clusters 1 and 4.

PolyQ expansion of Htt modulates binding of many transcription factors

The control of gene expression depends on the function of transcription factors. Htt interacts directly with many transcription factors, and for some of these factors, the interaction is altered with polyglutamine expansion (Holbert et al., 2001; Dunah et al., 2002; Kegel et al., 2002; Li et al., 2002; Yohrling et al., 2003; Zuccato et al., 2003; Zhai et al., 2005). However, other than the few transcription factors studied in HD, we do not know the effect of mutant Htt on transcription factor binding activities. We therefore interrogated transcription factor binding activity using an unbiased transcription factor array in the full length knock-in ST*Hdh* cell lines. There was an overall increase in transcription factor binding activities in ST$Hdh^{111/111}$ compared to ST$Hdh^{7/7}$ cells (Figure 2A).

In order for this finding to be relevant for HD pathogenesis, it needs to be confirmed in a separate model. R6/2 transgenic mice express only exon 1 of the human *HD* gene (Mangiarini et al., 1996). We therefore interrogated transcription factor binding activities at 12 weeks of age in wild-type and transgenic R6/2 mouse striatum. We observed an overall increase in transcription factor binding activity in 12 week old R6/2 transgenic mice (Figure 2B). The increased binding activities are an early event in R6/2 pathogenesis, as profiling of transcription factor activity at 4 weeks of age revealed the same trend toward increased activities at this pre-symptomatic time point (Figure 2C). For some transcription factors, altered binding activity was further confirmed by electromobility shift assay (data not shown). Thus, mutant Htt increases the binding activities of many transcription factors, in multiple HD models.

We next sought to determine the relationship between increased transcription factor binding and gene expression abnormalities in the ST*Hdh* cell lines (Figure 2D, Table 1, Supplementary Figure 1). To a large extent, altered transcription factor activity accounted for the differences in mRNA expression profiles. We found that there were groups of transcription factors with distinct gene expression effects, with the largest group of transcription factors potentially influencing all four types of gene expression changes (present in one cell line but absent in the other, or up/down-regulated in the homozygote cell lines) (Table 1). Other groups of transcription factors correlated with 1, 2 or 3 types of gene expression changes. Thus, increased transcription factor binding *in vitro* does not have unitary effects on gene expression, such as exclusive down-regulation of specific genes.

Huntingtin occupies gene promoters *in vivo*

The transcriptional machinery is clearly perturbed in HD, with altered mRNA gene expression profiles, abnormal transcription factor function, and altered occupancies of transcription factors at specific gene promoters in the presence of mutant Htt (Supplementary Figure 2) (Kegel et al., 2002; Zuccato et al., 2003; Hu et al., 2004; Zhai et al., 2005; Chen-Plotkin et al., 2006; Cui et al., 2006). However, the mechanism by which mutant Htt alters the presence of transcription factors at specific gene promoters is not clear. A simple possibility is that mutant Htt directly occupies gene promoters, interfering with normal transcription factor access. In

order to determine whether Htt is present at gene promoters *in vivo* we used chromatin immunoprecipitation (ChIP) assays.

DNA quantitation confirmed that Htt antibodies immunoprecipitate significantly more DNA than IgG or no antibody mock (Figure 3A). The DNA pull-down was not due to an antibody artifact, as we consistently observed the same result with different antibodies to Htt (Figure 3A and data not shown). Either equivalent amounts or more DNA was pulled down in HD conditions compared to control conditions in cells and mouse models (Figure 3B). Anti-Htt antibodies consistently immunoprecipitated DNA from human brain samples, although there was no difference in overall amounts of DNA immunoprecipitated between HD and control cortex (data not shown). We used the ChIP-Htt immunoenriched DNA from wild-type and R6/2 striatum as a template in real-time PCR with gene-specific primers to determine the association of wild-type and mutant Htt with gene promoters. We surveyed genes whose expression is unaffected in HD (*Actb*, *Grin1*), genes that are down-regulated in HD (*Drd2*, *Cnr1*, *Penk1*) and a gene that is not expressed in striatal cells (*Bdnf*). Immunoprecipitation with the MAb2170 antibody, which immunostains the nucleus (Kegel et al., 2002), showed increased Htt-promoter occupancy in R6/2 transgenic mouse brains, irrespective of the gene that was interrogated (Figure 3C). The MAb2170 antibody does not recognize the exon 1 transprotein, suggesting an increased occupancy of the endogenous wild-type protein in the presence of the mutant transprotein. These data demonstrate that Htt is present at gene promoters *in vivo*. ChIP experiments with MAb2170 in STHdh cell lines and with S830 antibody in R6/2 mouse brains further confirmed an association of the ChIP product with gene promoters, but failed to reveal clear differences in Htt association between wild-type and transgenic conditions that correlated with gene expression changes (data not shown). The possibility that Htt is present in transcriptional complexes *in vivo* is bolstered by the finding that addition of Htt antibodies supershifts several protein/probe complexes in an electromobility shift assay, indicating that Htt can assemble into transcriptional complexes *in vitro* (Supplementary Figure 3).

Wild-type and mutant Htt have different genomic binding sites

Chromatin immunoprecipitation with antibodies against Htt shows that wild-type and mutant Htt differentially occupy gene promoters, with mutant Htt present to greater extents at all gene promoters tested. A limitation of interrogating ChIP-immunoenriched DNA with gene-specific primers is that only a small number of genes can be interrogated. A broader and more unbiased approach is to survey the genome for all Htt binding sites. In order to determine the genomic targets of wild-type and mutant Htt, we used a ChIP-on-chip approach, hybridizing ChIP products to high-resolution genomic DNA microarrays probing proximal promoter regions. We employed the full-length ST$Hdh^{7/7}$ and ST$Hdh^{111/111}$ cell lines, facilitating equivalent wild-type and mutant Htt pull-down in ChIP with MAb2170. Analysis of Htt ChIP product binding at the individual gene level revealed that the level and pattern of binding to each probe is similar between the ST$Hdh^{7/7}$ and ST$Hdh^{111/111}$ cell lines, with relatively few probes showing significantly different binding patterns (Figure 4A–C). Htt was significantly immunoenriched at 419 (2.3%) of the promoter regions for known protein-coding genes in the wild-type (ST$Hdh^{7/7}$) cell line (Figure 4A), and at 338 (1.9%) of promoter regions in the homozygote (ST$Hdh^{111/111}$) cell lines (Figure 4B). This percentage of promoters bound is in the same range of magnitude of transcripts with altered expression as assessed by microarray analysis, typically 2–4% of transcripts (Luthi-Carter et al., 2000). Of the genes that were bound, only 62 genes (0.3%) were bound by Htt in both wild-type and homozygote cell lines, suggesting that the majority of gene targets of wild-type and mutant Htt may be distinct (Figure 4D, E and Supplementary Table 1). We used sequence information from the probes most significantly bound by wild-type and mutant Htt using BEST (Binding-site Estimation Suite of Tools) (Che et al., 2005) in order to determine whether a sequence-specific preference

existed in this paradigm. BEST analyses failed to reveal any distinct sequence preferences by either wild-type or mutant Htt (data not shown).

While ChIP-on-chip analysis yields insight into the binding of Htt at gene proximal promoters, many transcription factor binding sites are not limited to the proximal promoter region of a gene (Nelson et al., 2006). In order to determine whether Htt binds genomic regions other than proximal promoters, we also employed a ChIP subcloning approach. We performed ChIP with Htt antibody MAb2170 on human HD patient brains and normal controls, subcloned and sequenced the Htt-immunoenriched DNA. Sixty percent of clones from the HD patient brains but only 40% of clones from control patient brains were successfully and significantly aligned with a genomic region. Of the clones that were aligned, the majority were localized to intronic regions, followed by intergenic regions (Figure 4F and Supplementary Table 2). More than a third of clones from both control and HD sequences contained repetitive elements such as microsatellites that aligned to multiple genomic loci. Other clones did not significantly align with defined genomic regions and were thus unidentifiable. These results suggest that Htt binding sites are not limited to proximal promoters, but also include intronic or intergenic regions. More clones from the control source material were unidentifiable, whereas clones derived from HD patient brains appeared to derive from an interaction with DNA (Figure 4F).

We identified genes whose expression is modulated differentially according to the polyglutamine repeat length in Htt and additionally have identified some genes bound by wild-type and mutant Htt. In order to determine whether wild-type and mutant Htt binding events correlated with expression of nearby genes, we interrogated ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ microarray expression profiles (Figure 1 and (Sadri-Vakili et al., 2007)) for genes with significant ChIP-on-chip binding events for wild-type and mutant Htt and filtered the genes on the basis of whether they were expressed in the cell lines. We found no correlation between Htt ChIP-on-chip binding events and mRNA expression profiles at either a gene-by-gene level, or at a global level (Figure 5).

Exon 1 huntingtin binds directly to DNA in the absence of other proteins

ChIP-based experiments demonstrate that Htt is present at genomic loci, but these experiments cannot discriminate between direct binding of Htt to DNA and indirect binding via an interacting protein. In order to determine whether Htt can bind DNA directly, we have established and validated a DNA immunoprecipitation (DIP) technique using positive controls (known DNA-binding proteins) and negative controls (proteins known not to interact with DNA) (Figure 6A). We found that GST-exon 1 Htt fusion proteins interact with genomic DNA at significant levels. We then asked the question of whether it was possible to assess sequence specificity for DNA binding using double-stranded DNA fragments or plasmids containing transcription factor consensus sequences (Figure 6B and data not shown). Interestingly, GST-exon 1 Htt fusion proteins were able to interact with the p53 consensus sequence by this technique (Figure 6B). Furthermore, when GST-exon 1 Htt fusion proteins and GST-p53 fusion proteins were incubated together, DNA binding was inhibited rather than potentiated, suggesting competition for binding sites (Figure 6C). Thus, exon 1 Htt can bind DNA directly *in vitro*, in a manner that potentially interferes with the activities of other transcription factors.

In an attempt to determine DNA binding sequences for Htt, we incubated GST-Htt with labeled probes consisting of known DNA consensus sequences and hybridized the bound probes to a transcription factor array (Figure 6D). As expected, GST-p53 bound to the p53 consensus sequence in addition to some ectopic binding possibly due to the excess of pure protein. GST-only bound to some sequences, which were excluded from further analysis. Both wild-type and mutant exon 1 Htt proteins bound to the same motifs as GST-p53 but binding was not limited to these motifs. Expanded polyQ repeat protein binds more DNA sequences than wild-type protein, including response elements for transcription factors whose activity is modulated

by Htt such as p53 and the thyroid hormone receptor (Steffan et al., 2000; Yohrling et al., 2003). Interestingly, the transcription factor recognition elements that are bound by mutant Htt are remarkably similar to the transcription factors with increased activity in HD models (Figure 6E and Table 2).

Htt alters DNA conformation

ChIP-based and direct-DNA interaction assays suggest there is no sequence-specific basis for the Htt-DNA interaction. Mutant exon 1 Htt can differentially modulate DNA structure *in vitro*, as suggested by DNase footprinting assays (data not shown). In order to assess further whether DNA structure is affected by mutant Htt *in vivo*, we performed micrococcal nuclease assays on extracts prepared from ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines (Figure 7A). Titrating the micrococcal nuclease enzyme on equivalent amounts of cell extract lead to differential patterns of digestion; with more digestion occurring in the mutant 111/111 cell lines compared to the 7/7 cell lines for a given amount of enzyme, suggesting a more open DNA structure that is more accessible to the digesting enzyme. In order to further confirm that DNA structure is altered around gene promoters, we performed Southern hybridization with various gene-specific probes. We observed radiolabeled probe hybridization to both high and low molecular weight species of DNA in the MNase-treated 7/7 cells, but only to low molecular weight DNA species in MNase-treated 111/111 cells, consistent with the notion that the mutant 111/111 cell lines comprise a more open DNA structure that is more accessible to digestion by MNase (Figure 7B and data not shown).

In order to test the hypothesis that wild-type and mutant Htt alter DNA conformation directly, we chose to employ a DNA condensation assay to measure the direct effect of Htt protein on DNA structure. YOYO-1, a dimeric oxazole yellow cyanine dye that asymmetrically bis-intercalates into the DNA strand, has been used to assess changes in DNA structure (Rye et al., 1992; Wong et al., 2001; Krishnamoorthy et al., 2002; Krishnamoorthy et al., 2003). Validation of this assay with a known DNA-condensing agent (Poly-L-Lysine; (Wong et al., 2001)) confirmed quenching of YOYO-1 fluorescent signal (Figure 7C). In contrast, addition of GST-fusion proteins increases the YOYO-1 fluorescent signal, which is suggestive of a more open DNA conformation. We observed more fluorescence upon addition of GST-p53 than GST-only as expected. (Figure 7C). Furthermore, addition of the mutant Htt protein GST-HD53Q induces more fluorescence than the wild-type GST-HD20Q (Figure 7C). This raises the possibility that direct Htt binding can modulate genomic DNA structure.

DISCUSSION

We set out to identify the molecular mechanism underlying huntingtin-mediated transcriptional dysregulation in HD. We have identified genes whose expression is modulated differentially according to the polyglutamine repeat length in Htt. Moreover, mutant Htt modulates the binding activities of many transcription factors, which in part induces the aberrant mRNA expression profiles. In addition, Htt-ChIP product analyses by gene-specific PCR shows Htt occupation of gene promoters *in vivo*. Htt ChIP analyses were extended to ChIP-on-chip and ChIP subcloning, thus enabling us to identify genomic regions bound by wild-type and mutant Htt in an unbiased fashion. We found distinct localization patterns of wild-type and mutant Htt at proximal promoter regions within the genome. Furthermore, proximal promoter Htt binding sites may represent a subset of total Htt binding sites, as the majority of Htt binding sites appear to be within intronic and intergenic regions. Additionally, exon 1 Htt binds directly to multiple DNA sequences, and binding is enhanced with longer polyQ repeat lengths. Interestingly, transcription factors whose recognition elements are bound directly by Htt have altered binding activity in HD conditions. Finally, exon 1 Htt binding directly alters DNA conformation *in vitro*. The data presented in this manuscript together with previous observations that nuclear

mutant Htt fragments are sufficient for transcriptional dysregulation *in vivo* (Benn et al., 2005) suggest a model whereby mutant Htt interacts directly with DNA, altering DNA conformation and transcription factor binding and thus ultimately leads to transcriptional dysregulation (Figure 8).

Microarray gene expression profiling reveals selective mRNA alterations in the ST*Hdh*$^{111/111}$ compared to ST*Hdh*$^{7/7}$ cell lines. The mRNA expression profile does not appear to match that of global transcriptional suppression (Hoshino et al., 2006), suggesting that the molecular basis of mutant-Htt mediated transcriptional dysregulation is more selective. While the pattern of mRNA alterations did not correlate with published gene expression profiles from mouse models and human HD patient brain, gene ontology analyses suggest that the differences are attributable to profiling cell lines as opposed to intact brain tissues (Sadri-Vakili et al., 2007).

Transcriptional regulation requires an extensive network of proteins, with many proteins functioning at several levels and the integration of multiple signaling pathways. Htt is implicated in a wide range of cellular functions including, synaptic transmission, signal transduction and normal development (Bates et al., 2002; Woda et al., 2005). Furthermore, Htt interacts with a number of transcription factors, including but not limited to NCoR, p53, CREB, TBP, CtBP, Sp1 and its coactivator TAFII130 as well as proteins identified through their interaction with Htt, such as the DNA-binding protein p231 HYPB, recently identified as a histone methyltransferase (Steffan et al., 2000; Holbert et al., 2001; Rega et al., 2001; Dunah et al., 2002; Kegel et al., 2002; Li et al., 2002; Yohrling et al., 2003; Sun et al., 2005; Zhai et al., 2005). We confirm that wild-type and mutant Htt affect transcription factor binding activity, with increased binding of many transcription factors in mutant conditions at an early time point. These *in vitro* data support the hypothesis that transcriptional dysregulation does not arise simply from depletion of transcriptional factors in Htt inclusions (Yu et al., 2002). Indeed, we have previously found that the presence of inclusions has no effect on the levels of specific mRNA molecules in neurons (Sadri-Vakili et al., 2006). Taken together, mutant Htt does not merely reduce transcription factor levels, implicating a more direct role for mutant Htt in transcriptional dysregulation.

Many transcription factors had increased binding activity in the ST*Hdh*$^{111/111}$ compared to the ST*Hdh*$^{7/7}$ cell lines; and the dysregulated genes are governed by the transcription factors with altered activities. The sphere of effects for transcription factors is larger than expected, with many transcription factors correlating with varying effects on gene expression. The recognition elements bound directly by mutant exon 1 Htt and those transcription factors with increased binding activities are similar, suggesting that mutant Htt may block the access of these transcription factors to gene promoters. In this context, the observation that mutant Htt may alter DNA conformation is significant, as transcription factor access to DNA is dependent on the structural properties of DNA (Roeder, 2005). Indeed, given the larger number of transcription factors with altered activity, altered transcription factor binding as a consequence of direct Htt binding to DNA is a more parsimonious explanation than the less likely scenario in which Htt interacts with each transcription factor in protein-protein interaction.

We observed that both wild-type and mutant Htt occupy gene promoters *in vivo*, irrespective of whether these genes have altered expression in HD. In addition, the ChIP data demonstrates the presence of Htt at genomic loci *in vivo*. Cui *et al.* showed increased occupancy of mutant Htt at two gene promoters in a knock-in mouse model of HD, but did not interrogate genes whose expression was not dysregulated (Cui et al., 2006). Surveying genome-wide Htt binding in an unbiased fashion unmasked Htt binding at proximal promoter regions within the mouse genome for wild-type and mutant knock-in Htt cell lines. Binding levels of wild-type or mutant Htt were remarkably similar at many genomic loci, with a few distinct regions for which only

wild-type or only mutant Htt were bound, thus suggesting a potential role for wild-type Htt in the nucleus. However, quality-control data (gene-specific amplication after LM-PCR amplification) indicate that there may be compression of gene enrichment by LM-PCR, which suggests that we did not observe a full representation of Htt-DNA binding events in the ChIP-on-chip experiments. Furthermore, ChIP-on-chip using current technology is biased toward surveying proximal promoter regions. Genomic Htt-DNA binding sites identified through ChIP subcloning were predominantly localized to intronic or intergenic regions, which would be consistent with a widely distributed localization of Htt within the genome. Indeed, given that the majority of Htt binding sites do not reside within proximal promoter regions, attempting to correlate the ChIP-on-chip data with expression profiling data may not reveal the full picture of Htt-induced transcriptional events. Furthermore, restricting ChIP analyses to proximal promoter regions is a partial representation of all Htt binding sites. Given that the presence of wild-type and mutant Htt directly perturbs gene expression as assayed by reporter construct assays (Steffan et al., 2000; Yohrling et al., 2003; Cui et al., 2006), more investigation is clearly required to understand the precise nature of Htt's role in transcriptional regulation.

Htt does not appear to function as a typical transcription factor, as we have been unable to identify a DNA consensus sequence, although the transcription factor arrays suggest that exon 1 Htt does not bind indiscriminately to every DNA consensus sequence. While exon 1 Htt appears to be required for DNA binding, it remains possible that other domains within Htt may confer specificity, as evidenced by the distinct binding patterns of wild-type and mutant Htt at proximal promoter regions. An alternative possibility is that specificity may be bestowed through protein-protein interactions, a mechanism suggested by recent studies on other polyglutamine repeat disorders (Friedman et al., 2007; Goold et al., 2007; Lim et al., 2008). Yet another possibility is that the apparent lack of DNA sequence specificity exhibited by exon 1 Htt may implicate a role at a structural, higher order level of chromatin organization. This would be consistent with data showing clusters of deregulated transcripts at a genomic level, supporting a hypothesis of a genome-wide mechanism of disrupted transcription (Anderson et al., 2008). Interestingly, Htt has been shown to interact tightly with the genomic matrix (Kegel et al., 2002).

The striking finding that exon 1 Htt binds directly to DNA, and alters DNA conformation in a polyQ-dependent manner in the absence of other proteins at least *in vitro* points to a novel and thus far unknown function of Htt. Increased DNA binding by mutant Htt in itself is intriguing, given that many transcription factors possess a polyQ repeat domain, and the transactivation properties are altered with polyQ repeat expansion or abolition (Callewaert et al., 2003; Chen et al., 2004; Miyashita et al., 2005). Htt may thus belong to a growing number of polyQ repeat disease proteins with a transcriptional function. Of particular interest is the TATA-binding protein, TBP, a general transcription factor and a core component of the transcriptional initiation complex. PolyQ expansion of TBP leads to SCA17, and while the underlying molecular mechanism is unknown, this disorder highlights the paradox of how mutation of a general and heavily-involved transcription factor can lead to a late-onset neurological disorder affecting only specific neurons (Riley and Orr, 2006).

Taken together, we propose a molecular mechanism whereby mutant Htt perturbs normal transcriptional regulation: enhanced genomic DNA binding by mutant Htt alters the DNA conformation and subsequent binding of transcription factors thus disrupting the normal control of mRNA expression at an early time point in HD pathogenesis (Figure 8). This mechanism suggests that pharmacological modulation of aberrant Htt-DNA binding could be a novel, relevant and potentially effective therapeutic target in HD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AR
androgen receptor

ChIP
chromatin immunoprecipitation

DIP
DNA immunoprecipitation

DRPLA
dentatorubral pallidoluysian atrophy

GST
Glutathione-S-transferase

HD
Huntington's disease

Htt
Huntingtin

IgG
Immunoglobulin

IP
Immunoprecipitation

LM-PCR
ligation-mediated polymerase chain reaction

MNase
micrococcal nuclease

polyQ
: Polyglutamine

SBMA
: spinal and bulbar muscular atrophy

SCA
: spinocerebellar ataxias

ST*Hdh*$^{7/7}$
: Immortalised striatal cell lines containing 2 alleles of wild-type (7) glutamine repeats

ST*Hdh*$^{111/111}$
: Immortalised striatal cell lines homozygote for 111 glutamine repeats

TBP
: TATA binding protein

References

Anderson AN, Roncaroli F, Hodges A, Deprez M, Turkheimer FE. Chromosomal profiles of gene expression in Huntington's disease. Brain 2008;131:381–388. [PubMed: 18156153]

Bates, GP.; Harper, PS.; Jones, AL. Huntington's Disease. 3. Bates, GP.; Harper, PS.; Jones, AL., editors. Oxford: Oxford University Press; 2002.

Benn CL, Landles C, Li H, Strand AD, Woodman B, Sathasivam K, Li SH, Ghazi-Noori S, Hockly E, Faruque SM, Cha JH, Sharpe PT, Olson JM, Li XJ, Bates GP. Contribution of nuclear and extranuclear polyQ to neurological phenotypes in mouse models of Huntington's disease. Hum Mol Genet 2005;14:3065–3078. [PubMed: 16183657]Epub 2005 Sep 3023

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006;441:349–353. [PubMed: 16625203]Epub 2006 Apr 2019

Callewaert L, Christiaens V, Haelens A, Verrijdt G, Verhoeven G, Claessens F. Implications of a polyglutamine tract in the function of the human androgen receptor. Biochem Biophys Res Commun 2003;306:46–52. [PubMed: 12788064]

Carey, M.; Swale, S. Electromobility shift assays. In: M, C.; ST, S., editors. Transcriptional regulation in eukaryotes: concepts, strategies and techniques. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2000. p. 338-346.

Che D, Jensen S, Cai L, Liu JS. BEST: Binding-site Estimation Suite of Tools. Bioinformatics 2005;21:2909–2911. [PubMed: 15814553]

Chen-Plotkin AS, Sadri-Vakili G, Yohrling GJ, Braveman MW, Benn CL, Glajch KE, DiRocco DP, Farrell LA, Krainc D, Gines S, MacDonald ME, Cha JH. Decreased association of the transcription factor Sp1 with genes downregulated in Huntington's disease. Neurobiol Dis 2006;22:233–241. [PubMed: 16442295]Epub 2006 Jan 2024

Chen S, Peng GH, Wang X, Smith AC, Grote SK, Sopher BL, La Spada AR. Interference of Crx-dependent transcription by ataxin-7 involves interaction between the glutamine regions and requires the ataxin-7 carboxy-terminal region for nuclear localization. Hum Mol Genet 2004;13:53–67. [PubMed: 14613968]Epub 2003 Nov 2012

Cornett J, Cao F, Wang CE, Ross CA, Bates GP, Li SH, Li XJ. Polyglutamine expansion of huntingtin impairs its nuclear export. Nat Genet 2005;37:198–204. [PubMed: 15654337]Epub 2005 Jan 2016

Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 2006;127:59–69. [PubMed: 17018277]

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997;277:1990–1993. [PubMed: 9302293]

Dorsman JC, Smoor MA, Maat-Schieman ML, Bout M, Siesling S, van Duinen SG, Verschuuren JJ, den Dunnen JT, Roos RA, van Ommen GJ. Analysis of the subcellular localization of huntingtin with a set of rabbit polyclonal antibodies in cultured mammalian cells of neuronal origin: comparison with the distribution of huntingtin in Huntington's disease autopsy brain. Philos Trans R Soc Lond B Biol Sci 1999;354:1061–1067. [PubMed: 10434306]

Dunah AW, Jeong H, Griffin A, Kim YM, Standaert DG, Hersch SM, Mouradian MM, Young AB, Tanese N, Krainc D. Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 2002;296:2238–2243. [PubMed: 11988536]Epub 2002 May 2232

Friedman MJ, Shah AG, Fang ZH, Ward EG, Warren ST, Li S, Li XJ. Polyglutamine domain modulates the TBP-TFIIB interaction: implications for its normal function and neurodegeneration. Nat Neurosci 2007;10:1519–1528. [PubMed: 17994014]

Goold R, Hubank M, Hunt A, Holton J, Menon RP, Revesz T, Pandolfo M, Matilla-Duenas A. Down-regulation of the dopamine receptor D2 in mice lacking ataxin 1. Hum Mol Genet 2007;16:2122–2134. [PubMed: 17599952]

HDCRG. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell 1993;72:971–983. [PubMed: 8458085][see comments]

Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, Olson JM, Jones L, Luthi-Carter R. Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 2006;15:965–977. [PubMed: 16467349]Epub 2006 Feb 2008

Holbert S, Denghien I, Kiechle T, Rosenblatt A, Wellington C, Hayden MR, Margolis RL, Ross CA, Dausset J, Ferrante RJ, Neri C. 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 U S A 2001;98:1811–1816. [PubMed: 11172033]Epub 2001 Jan 1830

Hoshino M, Qi ML, Yoshimura N, Miyashita T, Tagawa K, Wada Y, Enokido Y, Marubuchi S, Harjes P, Arai N, Oyanagi K, Blandino G, Sudol M, Rich T, Kanazawa I, Wanker EE, Saitoe M, Okazawa H. Transcriptional repression induces a slowly progressive atypical neuronal death associated with changes of YAP isoforms and p73. J Cell Biol 2006;172:589–604. [PubMed: 16461361]Epub 2006 Feb 2006

Hu H, McCaw EA, Hebb AL, Gomez GT, Denovan-Wright EM. Mutant huntingtin affects the rate of transcription of striatum-specific isoforms of phosphodiesterase 10A. Eur J Neurosci 2004;20:3351–3363. [PubMed: 15610167]

Jackson WS, Tallaksen-Greene SJ, Albin RL, Detloff PJ. Nucleocytoplasmic transport signals affect the age at onset of abnormalities in knock-in mice expressing polyglutamine within an ectopic protein context. Hum Mol Genet 2003;12:1621–1629. [PubMed: 12812988]

Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, Sapp E, Wang Y, Qin ZH, Chen JD, Nevins JR, Aronin N, DiFiglia M. Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J Biol Chem 2002;277:7466–7476. [PubMed: 11739372]Epub 2001 Dec 7465

Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB, Zoghbi HY, Orr HT. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 1998;95:41–53. [PubMed: 9778246][see comments]

Krishnamoorthy G, Duportail G, Mely Y. Structure and dynamics of condensed DNA probed by 1,1′-(4,4,8,8-tetramethyl-4,8-diazaundecamethylene)bis[4-[[3-methylbenz-1,3-oxazol-2-yl] methylidine]-1,4–dihydroquinolinium] tetraiodide fluorescence. Biochemistry 2002;41:15277–15287. [PubMed: 12484766]

Krishnamoorthy G, Roques B, Darlix JL, Mely Y. DNA condensation by the nucleocapsid protein of HIV-1: a mechanism ensuring DNA protection. Nucleic Acids Res 2003;31:5425–5432. [PubMed: 12954779]

Kuhn A, Goldstein DR, Hodges A, Strand AD, Sengstag T, Kooperberg C, Becanovic K, Pouladi MA, Sathasivam K, Cha JH, Hannan AJ, Hayden MR, Leavitt BR, Dunnett SB, Ferrante RJ, Albin R, Shelbourne P, Delorenzi M, Augood SJ, Faull RL, Olson JM, Bates GP, Jones L, Luthi-Carter R. Mutant huntingtin's effects on striatal gene expression in mice recapitulate changes observed in human Huntington's disease brain and do not differ with mutant huntingtin length or wild-type huntingtin dosage. Hum Mol Genet. 2007

Kurokawa R, Kalafus D, Ogliastro MH, Kioussi C, Xu L, Torchia J, Rosenfeld MG, Glass CK. Differential use of CREB binding protein-coactivator complexes. Science 1998;279:700–703. [PubMed: 9445474]

Lee TI, Johnstone SE, Young RA. Chromatin immunoprecipitation and microarray-based analysis of protein location. Nature Protocols 2006;1:729–748.

Li SH, Cheng AL, Zhou H, Lam S, Rao M, Li H, Li XJ. Interaction of Huntington disease protein with transcriptional activator Sp1. Mol Cell Biol 2002;22:1277–1287. [PubMed: 11839795]

Lim J, Crespo-Barreto J, Jafar-Nejad P, Bowman AB, Richman R, Hill DE, Orr HT, Zoghbi HY. Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 2008;452:713–718. [PubMed: 18337722]

Luthi-Carter R, Cha JH. Mechanisms of transcriptional dysregulation in Huntington's disease. Clin Neurosci Res 2003;3:165–177.

Luthi-Carter R, Strand A, Peters NL, Solano SM, Hollingsworth ZR, Menon AS, Frey AS, Spektor BS, Penney EB, Schilling G, Ross CA, Borchelt DR, Tapscott SJ, Young AB, Cha JH, Olson JM. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. Hum Mol Genet 2000;9:1259–1271. [PubMed: 10814708]

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 1996;87:493–506. [PubMed: 8898202]

McCaw EA, Hu H, Gomez GT, Hebb AL, Kelly ME, Denovan-Wright EM. Structure, expression and regulation of the cannabinoid receptor gene (CB1) in Huntington's disease transgenic mice. Eur J Biochem 2004;271:4909–4920. [PubMed: 15606779]

Miyashita T, Tabuchi A, Fukuchi M, Hara D, Kisukeda T, Shimohata T, Tsuji S, Tsuda M. Interference with activity-dependent transcriptional activation of BDNF gene depending upon the expanded polyglutamines in neurons. Biochem Biophys Res Commun 2005;333:1241–1248. [PubMed: 15979572]

Nelson EA, Walker SR, Li W, Liu XS, Frank DA. Identification of human STAT5-dependent gene regulatory elements based on interspecies homology. J Biol Chem 2006;281:26216–26224. [PubMed: 16840779]Epub 22006 Jul 26213

Obrietan K, Hoyt KR. CRE-mediated transcription is increased in Huntington's disease transgenic mice. J Neurosci 2004;24:791–796. [PubMed: 14749423]

Rega S, Stiewe T, Chang DI, Pollmeier B, Esche H, Bardenheuer W, Marquitan G, Putzer BM. Identification of the full-length huntingtin- interacting protein p231HBP/HYPB as a DNA-binding factor. Mol Cell Neurosci 2001;18:68–79. [PubMed: 11461154]

Riley BE, Orr HT. Polyglutamine neurodegenerative diseases and regulation of transcription: assembling the puzzle. Genes Dev 2006;20:2183–2192. [PubMed: 16912271]

Roeder RG. Transcriptional regulation and the role of diverse coactivators in animal cells. FEBS Lett 2005;579:909–915. [PubMed: 15680973]

Rye HS, Yue S, Wemmer DE, Quesada MA, Haugland RP, Mathies RA, Glazer AN. Stable fluorescent complexes of double-stranded DNA with bis-intercalating asymmetric cyanine dyes: properties and applications. Nucleic Acids Res 1992;20:2803–2812. [PubMed: 1614866]

Sadri-Vakili G, Menon AS, Farrell LA, Keller-McGandy CE, Cantuti-Castelvetri I, Standaert DG, Augood SJ, Yohrling GJ, Cha JH. Huntingtin inclusions do not down-regulate specific genes in the R6/2 Huntington's disease mouse. Eur J Neurosci 2006;23:3171–3175. [PubMed: 16820007]

Sadri-Vakili G, Benn C, Kim M-O, Chawla P, Overland R, Glajch K, Xia E, Qiu Z, Hersch S, Clark T, Yohrling G, Bouzou B, J-HJ C. Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. Human Molecular Genetics. 2007

Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998;95:55–66. [PubMed: 9778247]

Schilling G, Savonenko AV, Klevytska A, Morton JL, Tucker SM, Poirier M, Gale A, Chan N, Gonzales V, Slunt HH, Coonfield ML, Jenkins NA, Copeland NG, Ross CA, Borchelt DR. Nuclear-targeting of mutant huntingtin fragments produces Huntington's disease-like phenotypes in transgenic mice. Hum Mol Genet 2004;13:1599–1610. [PubMed: 15190011]Epub 2004 Jun 1599

Sipione S, Rigamonti D, Valenza M, Zuccato C, Conti L, Pritchard J, Kooperberg C, Olson JM, Cattaneo E. Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Hum Mol Genet 2002;11:1953–1965. [PubMed: 12165557]

Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A 2000;97:6763–6768. [PubMed: 10823891]

Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, Slepko N, Illes K, Lukacsovich T, Zhu YZ, Cattaneo E, Pandolfi PP, Thompson LM, Marsh JL. SUMO modification of Huntingtin and Huntington's disease pathology. Science 2004;304:100–104. [PubMed: 15064418]

Sugars KL, Brown R, Cook LJ, Swartz J, Rubinsztein DC. Decreased cAMP response element-mediated transcription: an early event in exon 1 and full-length cell models of Huntington's disease that contributes to polyglutamine pathogenesis. J Biol Chem 2004;279:4988–4999. [PubMed: 14627700] Epub 2003 Nov 4918

Sun XJ, Wei J, Wu XY, Hu M, Wang L, Wang HH, Zhang QH, Chen SJ, Huang QH, Chen Z. Identification and characterization of a novel human histone H3 lysine 36-specific methyltransferase. J Biol Chem 2005;280:35261–35271. [PubMed: 16118227]Epub 32005 Aug 35222

Takano H, Gusella JF. The predominantly HEAT-like motif structure of huntingtin and its association and coincident nuclear entry with dorsal, an NF-kB/Rel/dorsal family transcription factor. BMC Neurosci 2002;3:15. [PubMed: 12379151]

Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, Cattaneo E, MacDonald ME. Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Hum Mol Genet 2000;9:2799–2809. [PubMed: 11092756]

Woda JM, Calzonetti T, Hilditch-Maguire P, Duyao MP, Conlon RA, MacDonald ME. Inactivation of the Huntington's disease gene (Hdh) impairs anterior streak formation and early patterning of the mouse embryo. BMC Dev Biol 2005;5:17. [PubMed: 16109169]

Wong M, Kong S, Dragowska WH, Bally MB. Oxazole yellow homodimer YOYO-1-labeled DNA: a fluorescent complex that can be used to assess structural changes in DNA following formation and cellular delivery of cationic lipid DNA complexes. Biochim Biophys Acta 2001;1527:61–72. [PubMed: 11420144]

Xia J, Lee DH, Taylor J, Vandelft M, Truant R. Huntingtin contains a highly conserved nuclear export signal. Hum Mol Genet 2003;12:1393–1403. [PubMed: 12783847]

Yohrling GJ, Farrell LA, Hollenberg AN, Cha JH. Mutant huntingtin increases nuclear corepressor function and enhances ligand-dependent nuclear hormone receptor activation. Mol Cell Neurosci 2003;23:28–38. [PubMed: 12799135]

Yu ZX, Li SH, Nguyen HP, Li XJ. Huntingtin inclusions do not deplete polyglutamine-containing transcription factors in HD mice. Hum Mol Genet 2002;11:905–914. [PubMed: 11971872]

Zhai W, Jeong H, Cui L, Krainc D, Tjian R. In vitro analysis of huntingtin-mediated transcriptional repression reveals multiple transcription factor targets. Cell 2005;123:1241–1253. [PubMed: 16377565]

Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D, Cattaneo E. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 2003;35:76–83. [PubMed: 12881722]

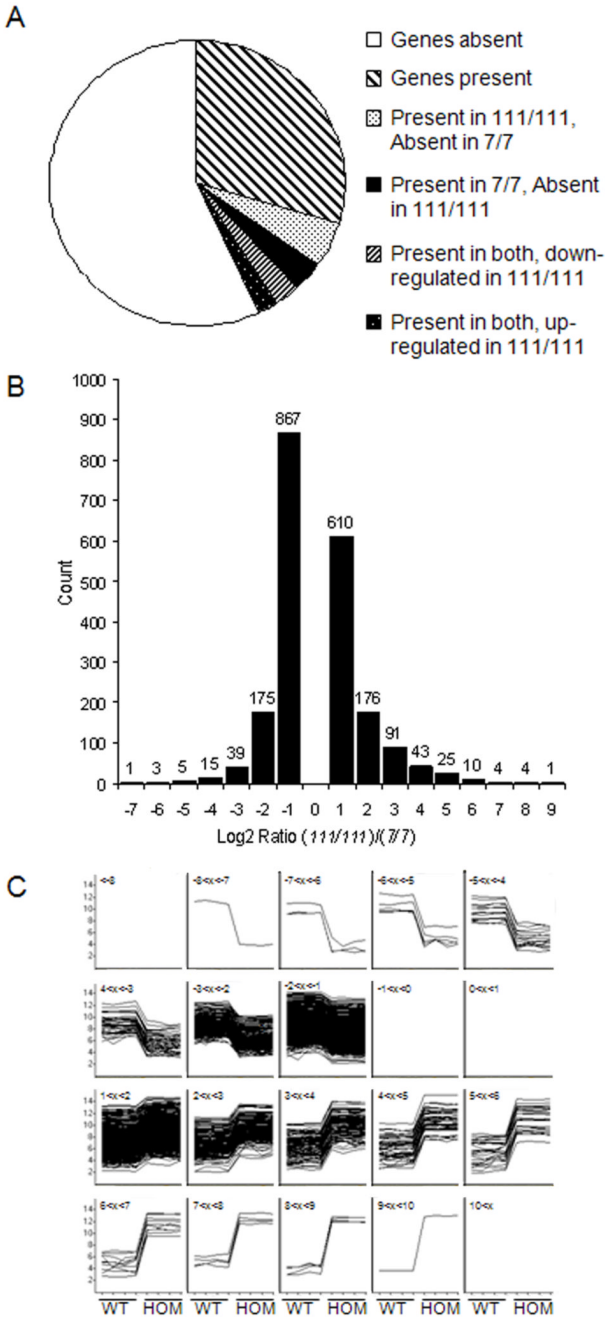

Figure 1. mRNA expression profiling reveals gene expression responses to wild-type or mutant Htt

Comparison of mRNA expression profiles yields numbers of genes present and absent in wild-type ST*Hdh*$^{7/7}$ compared to mutant ST*Hdh*$^{111/111}$ cell lines. **(A)** Pie chart showing the distribution of the Present and Absent probes. Probes called Present were present or marginal in at least 3 out of the 4 replicates, probes called Absent were absent in at least 3 out of 4 replicates, and probes were called Marginal if present and absent in 2 of the 4 replicates. More probes are Present in ST*Hdh*$^{111/111}$ and Absent/Marginal in ST*Hdh*$^{7/7}$. **(B)** Distribution of the binned Log_2(ST*Hdh*$^{111/111}$/ST*Hdh*$^{7/7}$) from the probes called present in both cell lines, with a Log_2 ratio > 1 or < −1 and a p-value < 0.05. More probes are down-regulated in the ST*Hdh*$^{111/111}$ versus the ST*Hdh*$^{7/7}$, but the amplitude of change (i.e. the difference in

expression) is larger for the probes up-regulated in the ST*Hdh*$^{111/111}$ versus the ST*Hdh*$^{7/7}$ lines. **(C)** A representation of the Log_2 normalized intensities for each replicate clustered by the Log_2 ratio bins determined from the probes called present in both cell lines, with a Log_2 ratio > 1 or < -1 and a p-value < 0.05, demonstrating the consistency of the results between the 4 replicates of each group and the amplitude of the differences between the 2 groups. The title of each box identifies which of the binned homozygote/wild-type binned data is shown −7 (at top left) to +9 (bottom right). The y-axis shows the magnitude of change for each sample, whereas the x-axis labels identify the wild-type (first four, left) and homozygote (last four, right) samples for each box. Thus, within each bin, the traces represent the fold change in wild-type cells to homozygote cells.

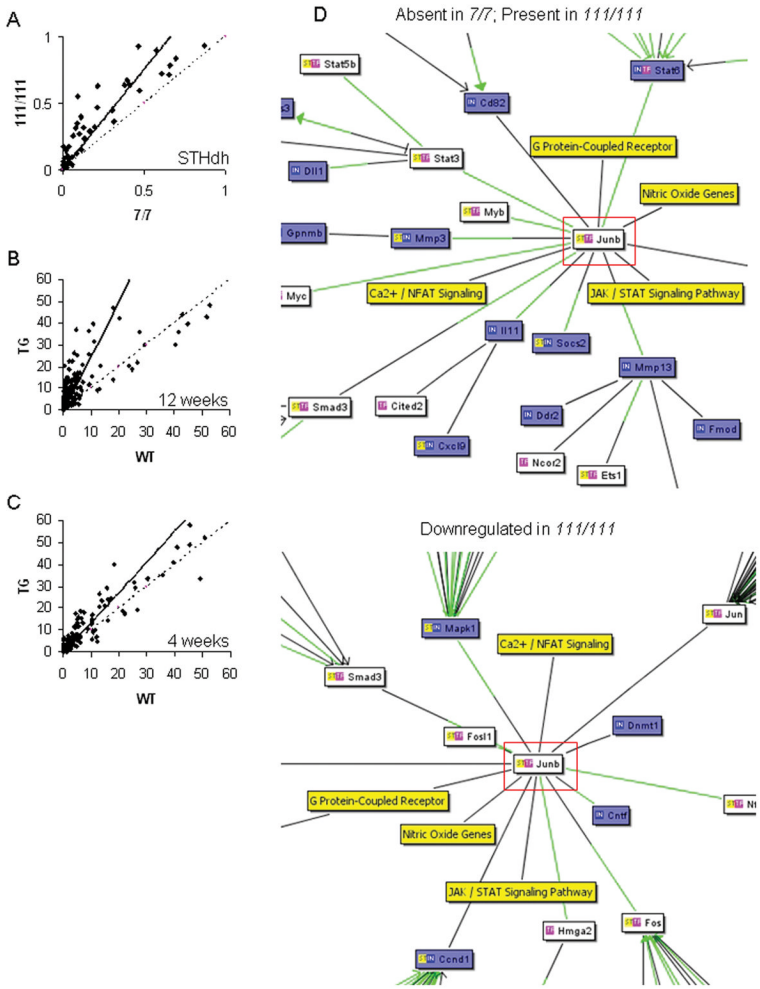

Figure 2. Mutant Htt alters the binding of many transcription factors

Densitometric analyses of transcription factor binding activity are scatter plotted, with each point representing the correlation of the binding activity of each transcription factor in two different conditions. Dashed line indicates the predicted correlation if there is no difference in binding activities between the two conditions, whereas the solid line indicates the actual correlation. **(A)** Transcription factor binding activities are increased in the homozygote ST*Hdh*$^{111/111}$ compared to ST*Hdh*$^{7/7}$ cells (measured by film densitometry). The progressive increase in binding of many transcription factors at **(B)** 12 weeks in the R6/2 transgenic mouse striatum is evident at **(C)** the presymptomatic age of 4 weeks. **(D)** Increased transcription factor activity does not have straightforward effects on gene expression profiles (Table 1), but correlates well with gene expression changes. Interaction networks from co-citations literature mining generated by the Bibliosphere Pathway View (Genomatix) display the relationship of transcription factors (white box), mRNA expression levels (blue box) and expression pathways (yellow box). The green lines represent a binding site for the relevant transcription factor on the gene promoter. Shown are part of the interaction networks for JunB (highlighted in red), demonstrating different interactions in ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines. JunB exerts two types of gene expression effects (Top: mRNA absent in ST*Hdh*$^{7/7}$ and present in ST*Hdh*$^{111/111}$ cells; Bottom section shows part of an interaction network for mRNA present in both cell lines but down-regulated in ST*Hdh*$^{111/111}$ homozygotes). Full interaction networks

generated by the Biblosphere Pathway View (Genomatix) for each of the 4 lists of genes are shown in Supplementary Figure 1.

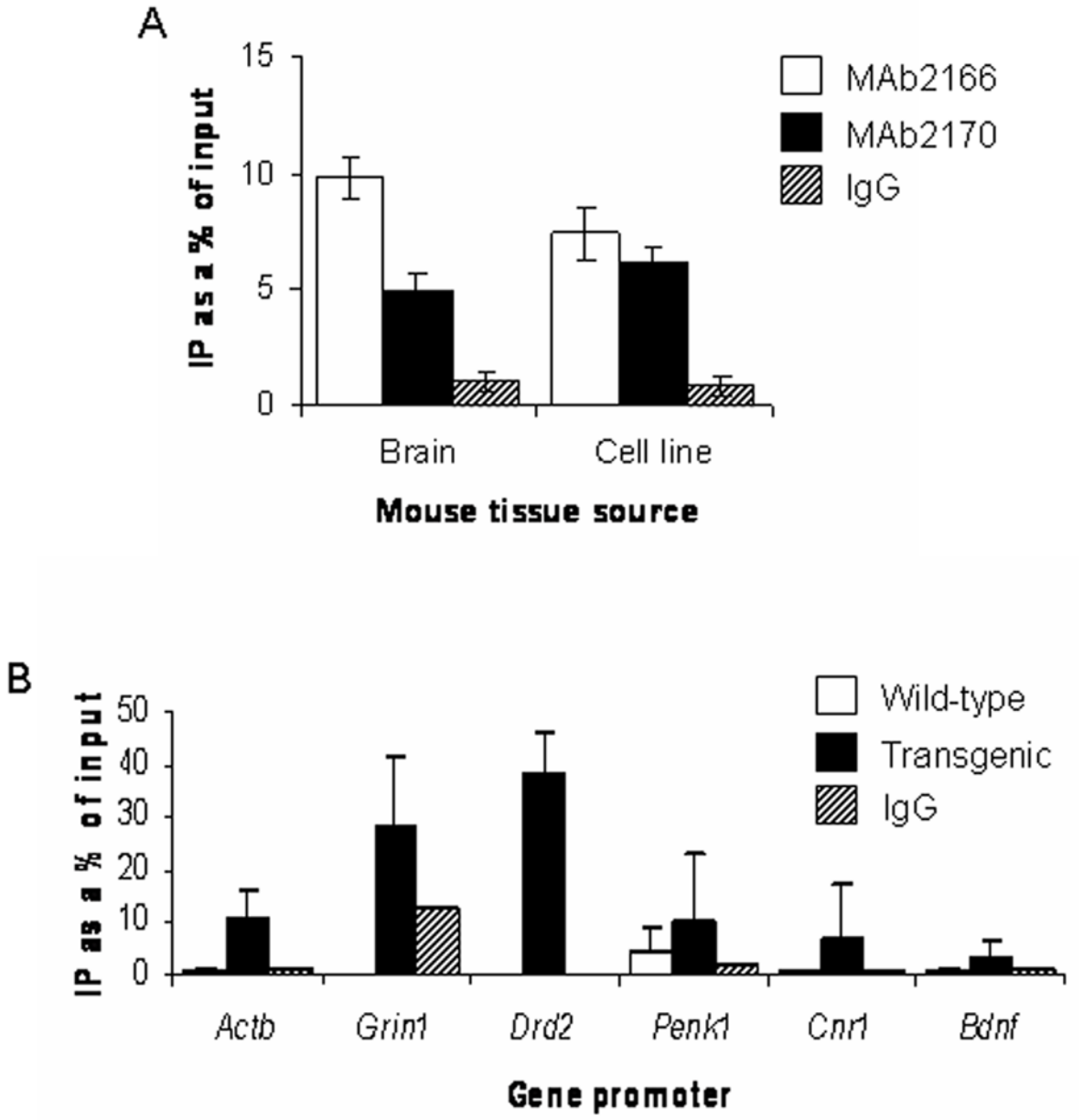

Figure 3. Htt-gene promoter occupancy is modulated by polyQ repeat length

(A) DNA Quantitation of ChIP using different Htt antibodies in wild-type brain and cell lines. Shown are MAb2166 (open bars) and MAb2170 (filled bars) compared to IgG or no antibody (hatched bars) shows significant amounts of DNA are pulled down. Error bars are S.E.M. (n=3–4). **(B)** ChIP with the MAb2170 Htt antibody in R6/2 mouse brain reveals non-selective increases in Htt-promoter occupancy in transgenic mice (filled bars) compared to wild-type mice (open bars), including those for genes whose expression is unchanged (*Actb* = β-actin, *Grin1* = NMDA receptor NR1 subunit), and those for genes down-regulated in HD (*Drd2* = dopamine D2 receptor, *Penk1* = preproenkephalin, *Cnr1* = cannabinoid receptor 1, *Bdnf* = brain derived neurotrophic factor, which is not expressed in striatal neurons). Hatched bars represent negative control conditions. Error bars are S.E.M. (n=2–4).

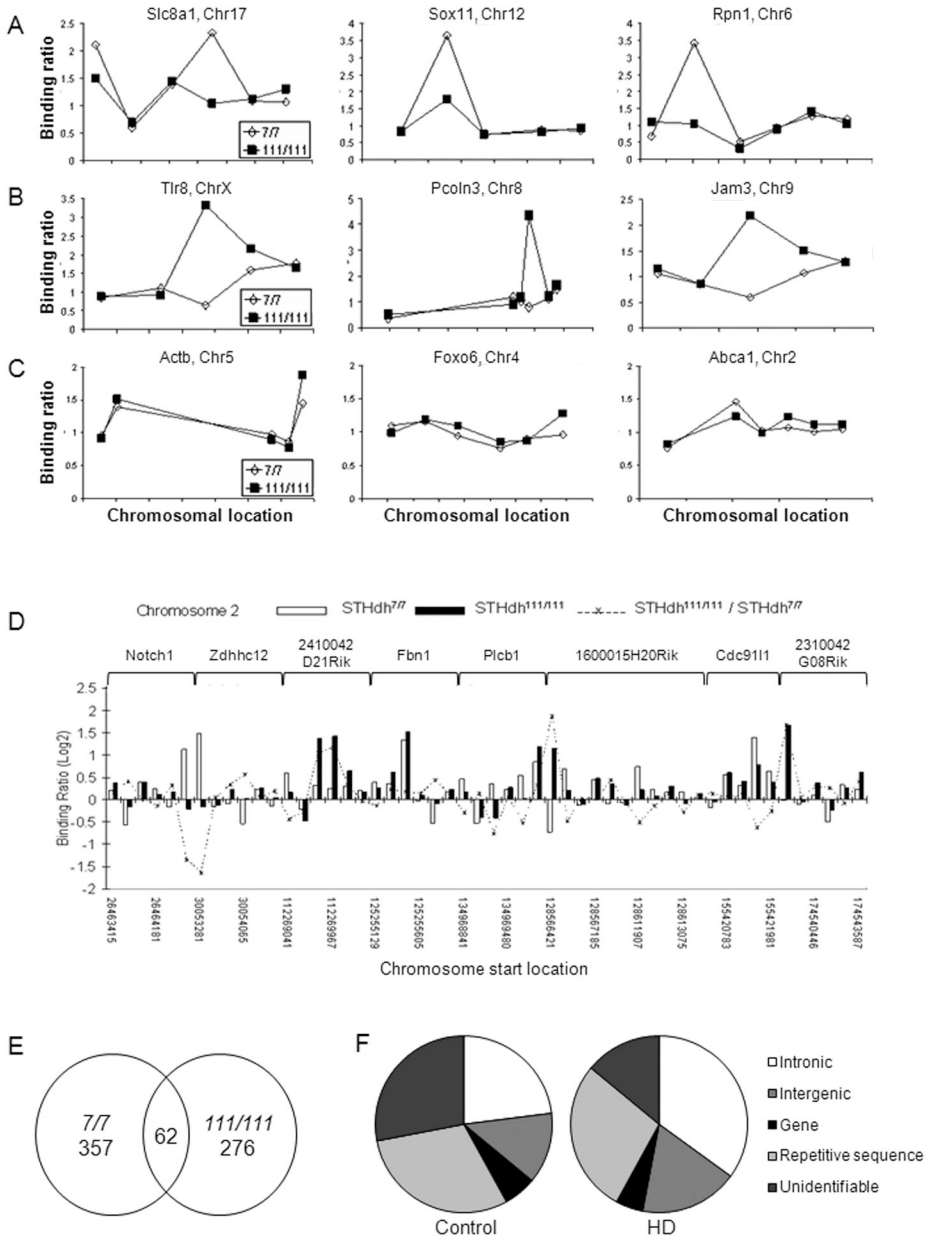

Figure 4. Genomic binding sites of wild-type and mutant Htt

Representative examples of binding events for **(A)** more wild-type Htt (open circles) bound: *Slc8a1* (solute carrier family 8, member 1), *Sox11* (SRY-box containing gene 11), *Rpn1* (ribophorin I); **(B)** more mutant Htt (filled squares) bound: *Tlr8* (Toll-like receptor 8), *Pcoln3* (procollagen (type III) N-endopeptidase), *Jam3* (junction adhesion molecule 3); and **(C)** genes showing no difference between wild-type and mutant Htt: *Actb* (cytoplasmic β-actin), *Foxo6* (forkhead box O6), *Abca2* (ATP-binding cassette, subfamily A (ABC1), member 2). For (**A–C**), binding ratios (y-axis) are plotted against chromosomal location (x-axis) for ST*Hdh*$^{7/7}$ (open circles) and ST*Hdh*$^{111/111}$ (filled squares), although the precise chromosomal location on the x-axis is omitted for simplicity. **(D)** Genes on chromosome 2 for which significant binding events have been identified include those bound more by wild-type Htt (open bars), and those with increased binding by mutant Htt (filled bars). Shown are relative

binding levels for each probe and the ratio of mutant/wild-type Htt binding (y-axis), plotted as a function of chromosomal probe localization (x-axis), with the genes indicated (top). **(E)** Genes bound in ST*Hdh*$^{7/7}$ (419) and ST*Hdh*$^{111/111}$ (338) cell lines show little overlap (62), suggesting that wild-type and mutant Htt may have distinct targets. **(F)** ChIP subcloning analysis using control and HD patient brain as a source material shows the majority of clones bind within intronic or intergenic regions as opposed to proximal promoters.

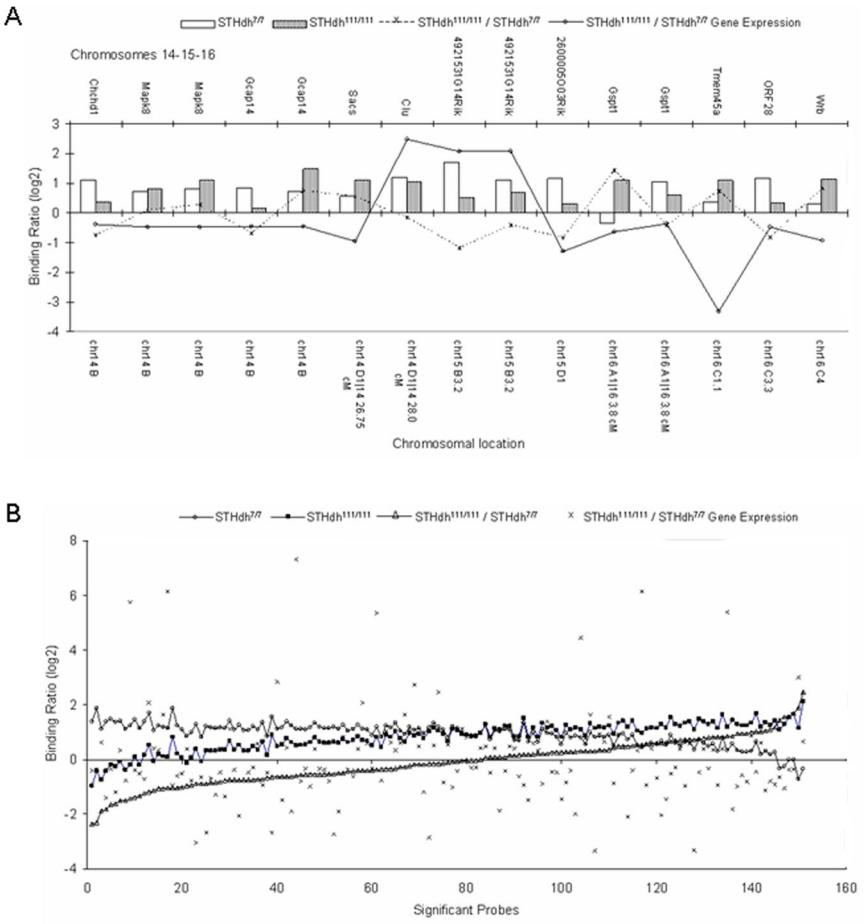

Figure 5. No correlation between ChIP-on-chip data and mRNA expression profiling

(A) ChIP-on-chip binding ratios (dashed line) for ST*Hdh*$^{7/7}$ (open bars) and ST*Hdh*$^{111/111}$ (filled bars) do not show straightforward correlation with mRNA expression level changes (solid line) at a gene-by-gene basis: shown is representative combined data from ChIP-on-chip and gene expression studies for significant genes on chromosomes 14, 15 and 16. **(B)** ChIP-on-chip binding ratios (open triangles, ranked in order) and gene expression profiles (crosses) show no correlation at a global level. Also plotted are ST*Hdh*$^{7/7}$ (open circles) and ST*Hdh*$^{111/111}$ (filled squares) binding ratios.

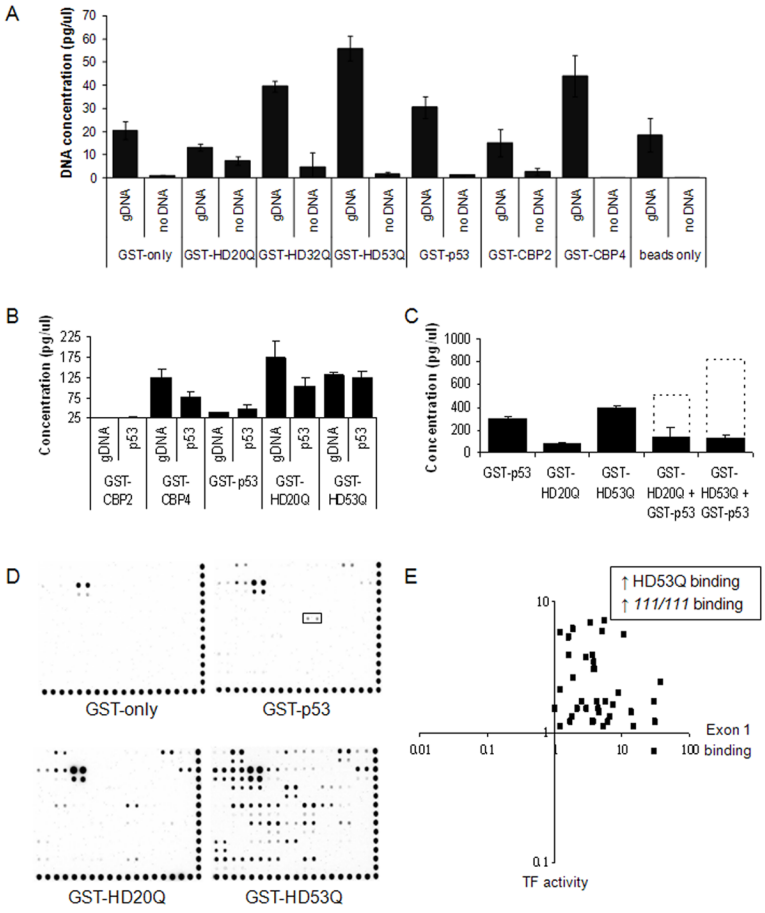

Figure 6. Exon 1 Htt binds non-specifically to DNA directly in the absence of other proteins in a polyQ dependent manner

(A) Validation of DIP technique with known DNA interactors (DNA binding domains of CBP (GST-CBP4, 1), GST-p53) and proteins known not to interact with DNA (GST-only, non-DNA binding domains of CBP (GST-CBP2, 5). Exon 1 Htt proteins bind significant amounts of genomic DNA, with expanded repeats (GST-HD32Q and GST-HD53Q) typically binding equivalent or more DNA than wild-type (HD20Q). Error bars are S.E.M. (n=4). **(B)** Exon 1 Htt proteins bind a p53 consensus sequence as does the non-specific DNA binding domain of GST-CBP4. **(C)** Exon 1 Htt proteins interfere with GST-p53 DNA binding. Dashed line indicates predicted amount of genomic DNA binding (estimated by adding level of binding of GST-p53 and the exon 1 Htt protein species). **(D)** DNA binding by GST-fusion proteins on a transcription factor array shows appropriate binding by GST-p53 to its recognition element (highlighted by box) in addition to some ectopic binding, some of which is explained through GST-only. The GST-HD20Q binding pattern resembles that GST-p53; however the polyQ repeat expansion in GST-HD53Q potentiates DNA binding. Binding levels were not normalized, as we assayed presence/absence of binding only. **(E)** Comparing GST-protein binding ratios (HD53Q/GST-HD20Q) with transcription factor activity in cell lines (ST*Hdh*$^{111/111}$/ST*Hdh*$^{7/7}$) shows that transcription factor recognition elements with more binding in HD conditions are those bound by pure mutant exon 1 Htt *in vitro* (Table 2).

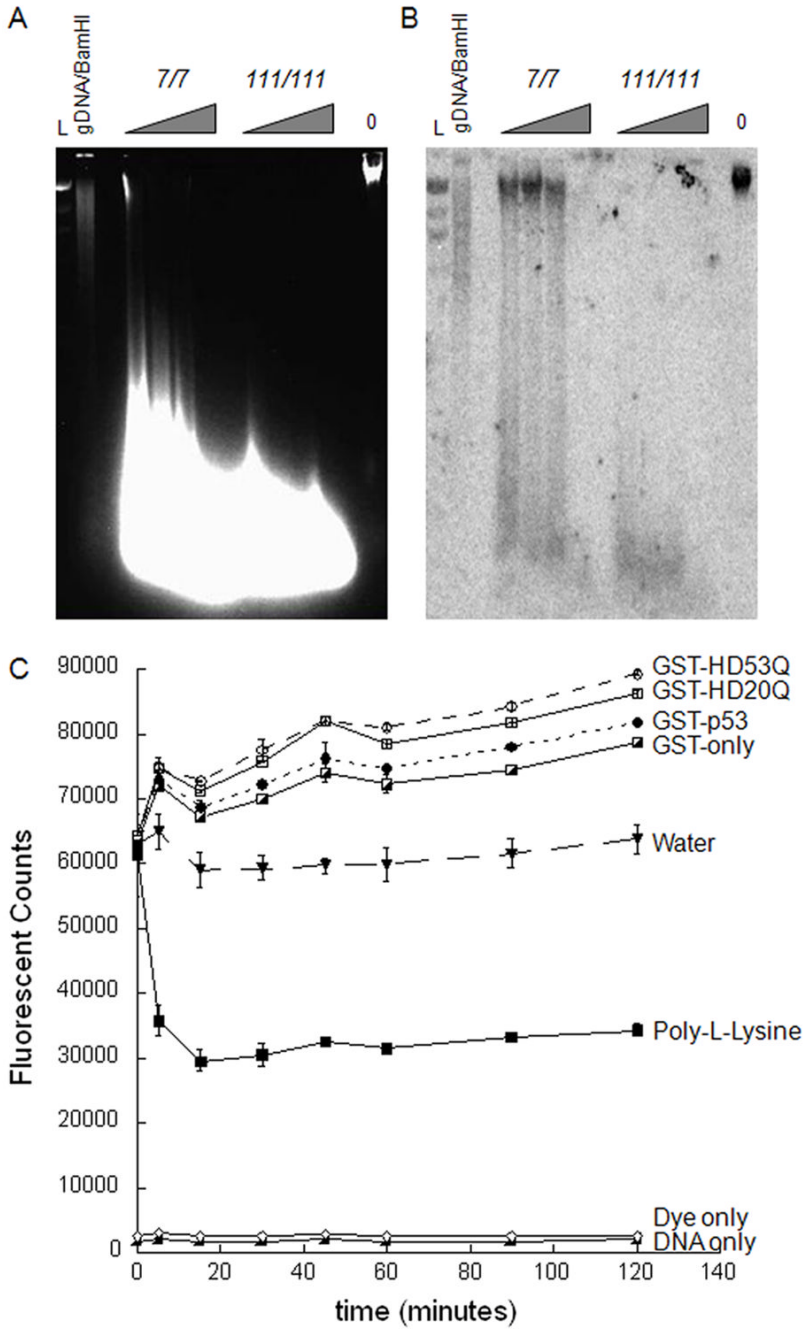

Figure 7. Mutant Htt may contribute to a more open DNA conformation

(A) Micrococcal nuclease assay on ST*Hdh*$^{7/7}$ and ST*Hdh*$^{111/111}$ cell lines show more digestion in the mutant Htt cell lines than the wild-type cell lines which suggests a more open conformation of DNA, facilitating access of the enzyme. Triangles represent amounts of titrated MNase enzyme (from 2.5 units to 12.5 units). Also shown is control genomic DNA from ST*Hdh*$^{111/111}$ cell lines with 0 units of MNase enzyme. L = λHindIII DNA ladder. **(B)** Southern hybridization of a Cathepsin H (*Ctsh*) specific probe to the MNase-digested chromosomal DNA revealed the presence of high-molecular weight fragments in the ST*Hdh*$^{7/7}$ cell lines only, with levels of radiolabeled probe binding to high molecular weight fragment much higher than levels of high molecular weight DNA in the native gel. In contrast,

the *Ctsh* probe hybridized only to low molecular weight fragments in the homozygote ST*Hdh*$^{111/111}$ cell lines, suggesting a more open chromatin conformation accessible to digestion by MNase. Probes specific for other genes have demonstrated a similar pattern (data not shown). **(C)** Exon 1 Htt exerts distinct polyQ-dependent effects on DNA conformation, as assayed by DNA-YOYO-1 fluorescence. Addition of 0.5 μM GST fusion proteins increases the YOYO-1 fluorescent signal, which corresponds to a more open DNA conformation. We observed more fluorescence upon addition of GST-p53 than GST-only as expected. Furthermore, addition of the mutant Htt protein GST-HD53Q induces more fluorescence than the wild-type GST-HD20Q. Quenching of the fluorescence by poly-L-lysine, a DNA condensing agent, confirms the assay. Data shown in C is a representative experiment from four independent trials.

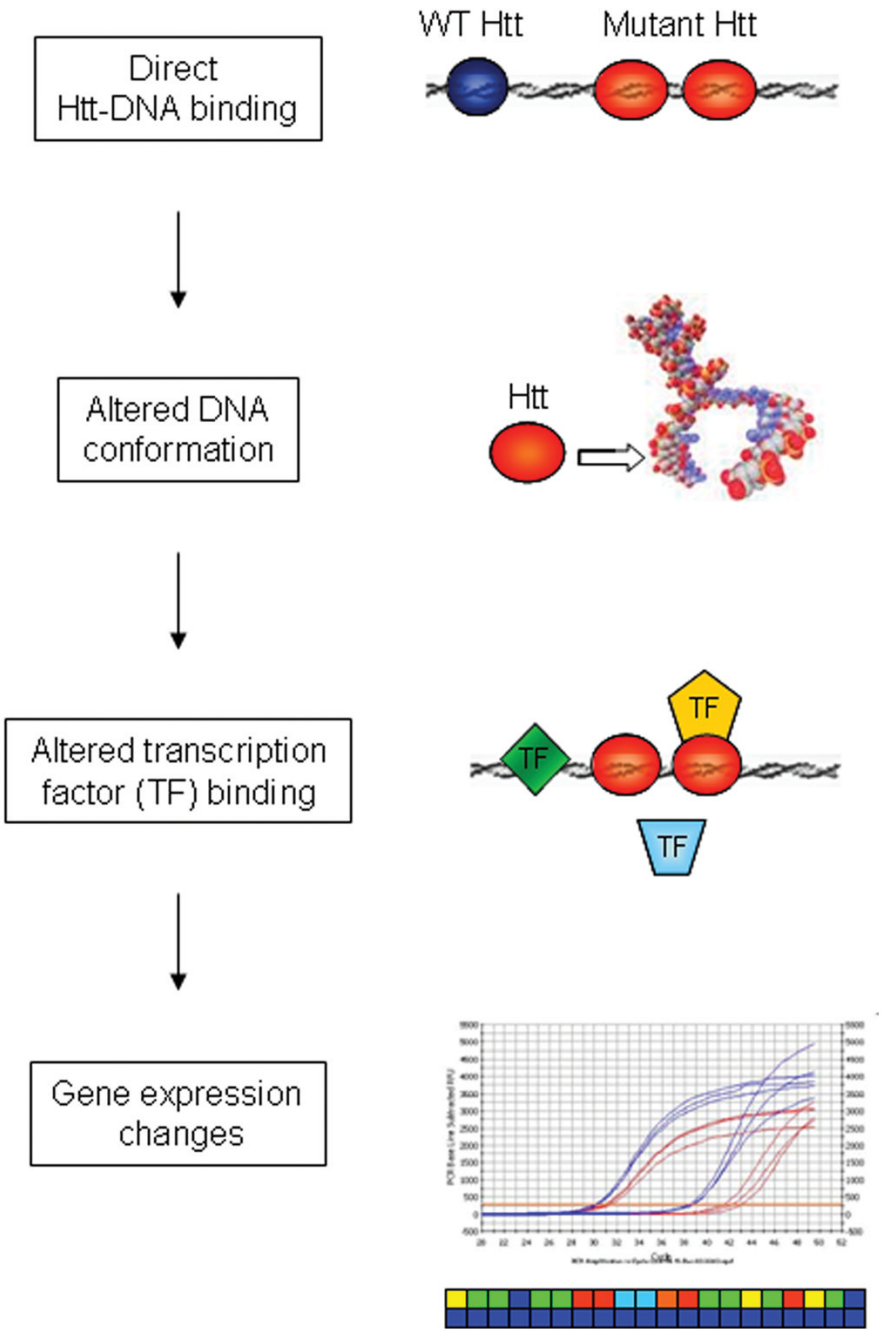

Figure 8. Schematic of wild-type and mutant Htt mediated effects on genomic DNA

Nuclear-localized mutant Htt facilitates direct Htt-DNA binding which in turn leads to altered DNA conformation and altered transcription factor binding, ultimately resulting in gene expression dysregulation. Altered DNA conformation could be reflected in chromatin structure alterations, as suggested by histone modification profiles (Sadri-Vakili et al., 2007).

Table 1

Increased transcription factor activities correlates with gene expression profiles

The Bibliosphere application was used to co-analyze the microarray and transcription factor array data to yield information about gene-transcription factor relations and hence gene expression regulators. Using this information, we have grouped transcription factors (TF) (column 2) according to the type of expression effect they have (column 1) (+ = type of gene expression effect present, shown along each row defining the group). Shown is the transcription factor binding activity in ST*Hdh* cell lines in the Panomics array (calculated as a ratio of binding activity, or spot intensity in ST$Hdh^{111/111}$ versus ST$Hdh^{7/7}$ cell lines) (column 3), and the number of gene changes in each of four groups (columns 4–7). As an example, group 2 contains the transcription factors involved in regulating two groups of genes: the group of genes that are not expressed (absent) in wild-type ST$Hdh^{7/7}$ cell lines, but expressed (present) in the homozygote ST$Hdh^{111/111}$ cell lines, and the group of genes that are down-regulated in ST$Hdh^{111/111}$ cell lines (compared to levels in ST$Hdh^{7/7}$ cell lines). Transcription factors in this group do not have any other type of gene expression effects, though some of the genes are regulated by other transcription factors. So, within group 2, Jun B regulates 6 genes that are absent in ST$Hdh^{7/7}$ and present in ST$Hdh^{111/111}$, and 5 genes which are down-regulated in ST$Hdh^{111/111}$. Similarly, genes that are regulated by JunB can be regulated by other transcription factors, both in the same group and those in other groups. Specifically, one of the downregulated genes in ST$Hdh^{111/111}$ cell lines, *Tgfb1* is regulated by JunB, but also regulated by the transcription factors Creb1 (Group 1), Stat6 (Group 2) and Pgr (Group 8), among others. However, within gene expression patterns, each gene is uniquely assigned to a type of gene expression (ie if it is down-regulated in ST$Hdh^{111/111}$, then it cannot belong to the group of genes that are upregulated in ST$Hdh^{111/111}$), thus each factor that is known to regulate a gene is correlated with the same type of expression effect.

Group	TF	Binding activity	Number of genes			
			Absent ST$Hdh^{7/7}$, Present ST$Hdh^{111/111}$	Present ST$Hdh^{7/7}$, Absent ST$Hdh^{111/111}$	Down-regulated in ST$Hdh^{111/111}$	Up-regulated in ST$Hdh^{111/111}$
1			+	+	+	+
	Creb1	*4.2*	10	7	9	7
	Egr1	*1.2*	9	4	12	13
	Esr1	*12.2*	9	6	8	5
	Ets1	*11.4*	6	4	7	4
	Fos	*2.35*	15	10	13	14
	Jun	*2.41*	28	13	24	29
	Nfkb1	*1.5*	37	11	19	35
	Myb	*1.3*	5	2	4	4
	Myc	*2.8*	12	15	15	14
	Pparg	*29.3*	17	8	13	7
	Rara	*2.8*	7	3	3	7
	Smad3	*1.3*	4	7	8	9
	Smad4	*1.3*	4	2	7	5
	Sp1	*2.8*	14	12	17	9
	Stat3	*2.9*	10	5	11	8
2			+		+	
	Junb	*2.41*	6		5	
	Hnf4a	*7.8*	3		4	
	Nfatc1	*19.5*	1		3	
	Ppara	*29.3*	7		11	
	Stat6	*3.7*	5		4	

Group	TF	Binding activity	Number of genes			
			Absent ST*Hdh*$^{7/7}$, Present ST*Hdh*$^{111/111}$	Present ST*Hdh*$^{7/7}$, Absent ST*Hdh*$^{111/111}$	Down-regulated in ST*Hdh*$^{111/111}$	Up-regulated in ST*Hdh*$^{111/111}$
3			+		+	+
	Crebbp	*4.2*	7		7	5
	Stat1	*1.7*	15		8	12
	Irf1	*3.9*	12		4	6
	Stat5a	*3.4*	6		6	6
4					+	
	Vdr	*1.4*			5	
	Esr2	*12.2*			4	
	Pou2f1	*2.9*			6	
	Rarb	*2.8*			5	
5				+	+	+
	E2f1	*4.8*		7	7	5
	Mycn	*2.8*		4	10	3
6			+			+
	Stat5b	*3.4*	3			2
	Gata2	*44.2*	1			3
7						+
	Hsf1	*3.6*				3
	Gata1	*44.2*				4
8	**Pgr**	*3.1*	5	4	4	
	Srf	*2.96*		3	5	
	Tbp	*4.2*			3	5
	Gata4	*44.2*		3		4
	Gata3	*44.2*	4			

Table 2

GST-fusion protein binding to transcription factor recognition sequences

Shown is the level of binding to a given transcription factor recognition sequence for each GST protein. The ratio of binding for GST-HD53Q/GST-HD20Q was calculated in order to compare with the ratio of binding of these transcription factors in ST*Hdh* cell lines. Key: + = some binding, ++ binding, +++ some binding at 1:10 dilution, ++++ binding at 1:10 dilution.

Transcription Factor	GST-only	GST-p53	GST-HD20Q	GST-HD53Q	53Q/20Q ratio	111/111/7/7 ratio
AP-1(1)				+	5.1	5.9
AP-2(1)		++	++	++++	1.9	2.6
Brn-3				+	8.9	2.0
C/EBP				++	21.1	
CBF		+	+	++	1.9	6.2
CDP						21.6
c-Myb		++	+++	+++	1.2	5.8
AP-1(2)			+	++	1.6	5.3
CREB(1)	+	+	++	++++	3.7	3.9
E2F1	+	++	+++	++++	1.6	3.9
EGR(1)	++++	++++	++++	++++	1.0	1.5
ERE				+++	10.7	5.5
Ets				++	5.5	7.1
Ets/PEA3				++	7.5	1.6
GAS/ISRE						2.9
AP-2(2)		++	++	+++	1.2	1.1
GATA					0.9	2.9
GRE				++	36.9	2.4
HNF-4		+	+	++	30.2	0.7
IRF-1						2.7
MEF-1			+	++++	4.0	3.0
MEF-2						3.8
Myc-Max			+	++	3.9	3.4
NF-1				+	4.5	1.5
NFATc						4.6
NF-E1			++	+++	2.5	1.7
NF-E2			+	++++	13.7	1.4
NFkB				++++	30.1	1.7
Oct--1				+	3.8	3.0
p53		++	++	++++	1.7	1.2
Pax-5			+	++	15.0	1.1
Pbx1				+	4.6	1.4
Pit1						8.6
PPAR						0.5
PRE				++	31.3	1.2
RAR(DR5)			+	+++	6.3	1.2
RXR(DR1)				+	5.3	1.1
SIE			+	+++	4.5	1.5
Smad SBE						1.8
Smad 3/4			+	++	3.7	1.2
Sp1		++	++	++++	3.0	3.7
Stat1				++	5.7	1.7
Stat3				++	6.5	1.3
Stat4			+	++	3.8	1.2
Stat5						0.9
Stat6						0.6
TFIID				+	1.2	2.1
TR		+	++	+++	3.5	6.8
TR(DR-4)				+++	4.3	1.7
USF-1		++	+	++	3.0	1.5
VDR(DR3)			+	++	2.2	1.5

Transcription Factor	GST-only	GST-p53	GST-HD20Q	GST-HD53Q	53Q/20Q ratio	111/111/7/7 ratio
HSE			++	+++	1.8	4.2
MRE						1.3