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ATF3 plays a protective role against toxicity by *N*-terminal fragment of mutant huntingtin in stable PC12 cell line

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Abstract

Huntington's disease is a progressive neurodegenerative disorder caused by a polyglutamine expansion near the N-terminus of huntingtin. The mechanisms of polyglutamine neurotoxicity, and cellular responses are not fully understood. We have studied gene expression profiles by cDNA array using an inducible PC12 cell model expressing an N-terminal huntingtin fragment with expanded polyglutamine (Htt-N63-148Q). Mutant huntingtin Htt-N63 induced cell death and increased the mRNA and protein levels of activating transcription factor 3 (ATF3). Mutant Htt-N63 also significantly enhanced ATF3 transcriptional activity by a promoter-based reporter assay. Overexpression of ATF3 protects against mutant Htt-N63 toxicity and knocking down ATF3 expression reduced Htt-N63 toxicity in a stable PC12 cell line. These results indicated that ATF3 plays a critical role in toxicity induced by mutant Htt-N63 and may lead to a useful therapeutic target.

Keywords

Activating transcription factor 3; Mutant huntingtin; Polyglutamine; Huntington's disease; Cell death

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1. Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by movement disorders and cognitive dysfunction resulting from an abnormal polyglutamine (poly Q) expansion in huntingtin (Htt). HD affects both sexes with the same frequency and is inherited in an autosomal dominant manner. (Borrell-Pages et al., 2006; Gatchel and Zoghbi, 2005; Ross, 2002). The molecular mechanisms underlying expanded poly Q-induced neuronal cell death remain poorly understood. Increasing evidence suggests that neuronal dysfunction may result from altered gene expression in an HD brain, with a potential role for a number of transcription factors (Cui et al., 2006; Ross and Thompson, 2006). The effects on transcription can occur through interaction of the mutant Htt with transcriptional activators and repressors (Cui et al., 2006; Jiang et al., 2003; Ross and Thompson, 2006). In addition, Htt may function as a transcriptional co-repressor by interacting with complexes that contain nuclear co-repressor proteins (Cornett et al., 2006; Gatchel and Zoghbi, 2005; Jiang et al., 2003; Nucifora et al., 2001; Steffan et al., 2000; Sugars and Rubinsztein, 2003). The full transcriptional gene expression profile however, is not completely understood.

Previously, we generated a stable inducible PC12 cell model in which expression of the N-terminal fragment of human Htt with an expanded polyglutamine region (Htt-N63-148Q) caused direct cell toxicity (Igarashi et al., 2003). To study the transcriptional gene expression profile, we performed a cDNA array analysis. We found that mutant Htt-N63 induced a change in gene expression profiles. Interestingly, we found that Htt-N63-148Q significantly up-regulated activating transcription factor 3 (ATF3) expression.

ATF3 is a member of the mammalian activation transcription factor/cAMP responsive element-binding (CREB) protein family of transcription factors and has a potential role in the stress response (Hai and Hartman, 2001). Previous studies indicate that the mRNA level of ATF3 is greatly increased when the cells are exposed to stress signals. In animal experiments, the signals include ischemia, ischemia coupled with reperfusion, wounding, axotomy, toxicity, and seizures; in cultured cells, the signals include serum factors, cytokines, genotoxic agents, cell death-inducing agents, and the adenoviral protein E1A (Allen-Jennings et al., 2001; Cai et al., 2000; Seijffers et al., 2007; Yoshida et al., 2008). The consequences of inducing ATF3 during stress responses are not clear. Here we found that expression of Htt-N63-148Q as a toxic protein increased ATF3 expression at mRNA and protein levels and enhanced ATF3 transcriptional activity. Overexpression of ATF3 protected against Htt-N63-148Q toxicity, and knock down of ATF3 increased Htt-N63-148Q toxicity. These results suggest a protective role for ATF3 in mutant Htt-N63-induced toxicity in a stable PC12 cell model.

2. Results

2.1 Induction of mutant Htt-N63 expression induces a transcriptional factor change in gene expression profiles

Gene expression profiling was carried out with a stable inducible PC12 cell line which previously described in Igarashi (Igarashi et al., 2003), designed to express an N-terminal Htt fragment with an expanded length polyQ region (Htt N63-148Q-myc) fused to a C-terminal myc tag (Cooper et al., 1998). To design microarray experiments in PC12 cells, it was important to establish the time period when measurable cell death occurred following the induction of expanded Htt expression. Therefore, we examined the time course of Htt N63-148Q expression and evaluated cell viability by using LDH assay (Fig. 1 A, B). To induce Htt expression the PC12 cells were differentiated and incubated in medium lacking doxycycline over a 6-day period. As shown in Fig 1A, expression of expanded Htt-N63-148Q-myc was observed on day 1, increased by day 2, and reached a peak on day 4. In contrast, in the non-inducing condition with doxycycline, cells did not express Htt. We further found that expression of Htt-N63-148Q

significantly induced cell death after 4 and 6 day induction, consistent with a previous report (Igarashi et al., 2003).

In order to study Htt-N63-148Q-induced early changes in gene expression of transcription regulators, we conducted cDNA arrays using total RNA from the cells with or without expressing mutant Htt at two time points: day 3 and day 4 post induction. We use the uninduced cells as controls. A total of 2588 genes showed more than 1.2-fold changes in PC12 cells expressing polyQ-expanded Htt on day 4, compared to uninduced “dox+” cells. Among these, 1396 genes were up-regulated and 1190 genes were down-regulated. The mRNA levels of 20 transcription-related genes were increased up to 1.5-fold of that of control cells on day 4 after induction of mutant Htt expression. We found that ATF3 increased significantly (24.5-fold, day 4, Table 1) at the top rank among the other genes. Even after a 3 day induction, ATF3 increased up to 5-fold over that in non-inducing conditions.

2.2 Mutant Htt increases ATF3 expression and enhances ATF3 transcriptional activity in a stable inducible PC12 cell model

To verify the cDNA array result that mutant Htt increased ATF3 expression, we used RT-qPCR and Western blot analysis. We found that there was an early increase in ATF3 mRNA beginning as soon as day 1, with a peak on day 4 after induction of mutant Htt expression (Fig 2A). ATF3 mRNA in cells expressing mutant Htt-148Q at day 4 was increased 14-fold over that of induction in control cells.

To determine the effect of mutant Htt-N63 expression on endogenous protein levels of ATF3, total cell lysates from Htt-N63-148Q cells were collected over a 6-day period and analyzed by Western blotting. Endogenous levels of ATF3 remained at a steady state in the absence of Htt-N63-148Q expression. Following induction of Htt-N63-148Q-myc expression, ATF3 protein noticeably increased by day 2, and reached a peak on day 4 (Fig 2B, C). No significant change in ATF3 protein was observed for cells expressing Htt-N63-23Q-myc (data not shown).

Next, endogenous ATF3 levels were assessed by immunofluorescence microscopy. Confocal images from a representative experiment on day 4 after induction of Htt expression demonstrated faint ATF3 staining in the absence of mutant N63-148Q-myc expression (Dox (+), Fig 2D). In contrast, strong ATF3 staining was observed in response to mutant Htt N63-148Q-myc expression (Dox (-), Fig 2D). Using the transient transfection method, we transfected wild type htt (htt-N63-23Q) or mutant htt (htt-N63-148Q) into PC12 cells. Mutant htt, but not wild type htt, increased endogenous ATF3 expression (Fig. 6). These results are consistent with the data we show in the stable cell line.

To determine whether mutant Htt N63 expression results in an increase in ATF3 transcriptional activity, we performed a luciferase reporter assay. The reporter construct comprised an ATF3 promoter region, including putative consensus elements for Ap-1, SRF, and ATF/CRE binding (Cai et al 2000; Liang et al., 1996), a TATA sequence, and the luciferase reporter gene (Fig 3A). ATF3 promoter activity was increased in response to Htt-N63-148Q-myc expression (Fig 3B). A negative control lacking the ATF3 promoter region (Delta ATF) showed no increase in luciferase activity with expression of mutant Htt. Transfection of a plasmid containing two point mutations known to block ATF/CRE binding (ATFm) resulted in a decrease in luciferase activity. These observations demonstrate that mutant Htt-induced ATF3 promoter activity required an intact ATF/CRE binding region. In contrast, no change in ATF3 promoter activity was observed in cells expressing Htt N63 with a normal length polyQ region. Taken together, these results establish that expression of mutant, but not wild type, Htt-N63-148Q in this PC12 cell model led to an increase in ATF3 transcription via ATF3/CRE DNA binding.

2.3 Elevated ATF3 levels protect against mutant Htt-induced cell death in the stable inducible PC12 cell model

To study the ATF3 role in mutant Htt N63-induced cell death, we transfected ATF3 into PC12 cells stable expressing Htt- N63-23Q or Htt-N63-148Q (Fig. 4A). Cell viability was assessed using the LDH-based assay. We found that overexpression of ATF3 reduced mutant Htt-induced cell toxicity compared to that of cells untransfected with ATF3 (Fig. 4B). Overexpression of ATF3 did not alter the cells stable expressing Htt-23Q (data not shown). To further investigate a protective function of ATF3, we used specific siRNA to knock down expression of ATF3. siRNA specific for ATF3 significantly reduced the levels of cellular ATF3 protein 4 days after transfection compared to a nonspecific RNA control (Fig 5A). Knockdown of ATF3 in cells expressing Htt- N63-148Q resulted in increased cell death compared to that of cells transfected with control RNA (Fig 5B). These data indicate that knockdown of endogenous ATF3 protein can compromise cell viability in response to mutant Htt: overexpression of ATF3 decreased mutant Htt-induced cell toxicity, suggesting that ATF3 has a protective role in mutant Htt-induced cell death.

3. Discussion

In this study, we found that inducible expression of mutant Htt-148Q caused a change in gene expression profiles of transcription regulators, increased ATF3 at both mRNA and protein levels, and enhanced ATF3 transcriptional activity via ATF3/CRE DNA binding. Knockdown of ATF3 expression resulted in an increase in mutant Htt-induced cell toxicity, whereas overexpressing ATF3 rescued cell death, indicating a protective role for ATF3.

Mutant htt-148Q significantly increased ATF 3 mRNA and protein expression level. This result is consistent with previous findings in other systems (Apostol et al., 2006; Luthi-Carter et al., 2002). Importantly, we found that mutant Htt-148Q increased ATF3 promoter activity through the ATF/CRE binding site, suggesting that mutant Htt can facilitate transcription via a CRE-dependent mechanism, as previously described (Obrietan and Hoyt, 2004).

ATF3 functions as a stress response gene and is induced on exposure to a variety of physiological and pathological stimuli (Liang et al., 1996). The consequences of ATF3 induction that follows stress signals are not completely understood. Depending on the promoter and cellular context, ATF3 can activate transcription of some target genes and repress transcription of others (Hai et al., 1999; Miyazaki et al., 2009; Yin et al., 2007). Consequently, the role of ATF3 in cell death and cell cycle progression remains uncertain. In our study, overexpression of ATF3 reduced Htt-148Q-induced toxicity, and knockdown of ATF3 enhanced Htt-148Q toxicity, suggesting that ATF3 has a protective effect against mutant Htt-induced cell death. In line with this idea, a previous report showed that ATF3 enhances neurite outgrowth in adult rat dorsal root ganglion neurons (Seijffers et al., 2007). Francis et al. reported increased ATF3 and resistance to cell death in rat hippocampal neurons following *in vivo* injection of kainic acid (Francis et al., 2004). Another study found that ATF3 overexpression in PC12 cells and in rat superior cervical ganglion neurons inhibited c-Jun N-terminal kinase-induced apoptosis, induced neurite elongation via Akt activation, and increased HSP 27 (Nakagomi et al., 2003). Taken together, these findings support a pro-survival function for ATF3 in response to mutant Htt. However, this pro-survival effect may not overcome the cell death events that occur as a result of consistent expression of mutant Htt for 4-6 days.

Previous studies suggest that the primary site of mutant Htt toxicity is the nucleus (Peters et al., 2002; Saudou et al., 1998), although other reports indicate that htt can induce cytotoxicity in the cytosol as well. (Gervais et al., 2002; Panov et al., 2002). Increasing evidence indicates that many transcription factors or cofactors are involved in huntingtin toxicity in both sites, especially in the nucleus. Our results showed that expression of mutant Htt induced a change

in the profile of transcription factors (Table 1) at the gene level. These changes included increases in several genes that control cell death [DNA-damage inducible transcript 3 (Cheng et al., 2008; Marciniak et al., 2004), v-jun (Shaulian et al., 2000), Egr-1 (de Belle et al., 1999; Huang et al., 1997)] and activator protein-1 family genes [Jun (c-jun, JunB and JunD) and Fos (c-Fos, Fos-related antigen-1fra-1 and fra2) (van Dam and Castellazzi, 2001)]. Forkhead box M1 gene (Foxm1), which has a dual DNA binding specificity (Major et al., 2004; Perez-Sanchez et al., 2000) and promotes cell proliferation (Kim et al., 2005), was significantly decreased in our cell line. These changes in genes that control cell death and regulate cell growth may converge on htt toxicity and overcome the ATF3 protective effect, thereby resulting in cell death.

In summary, we found that mutant Htt caused changes in the expression of genes that regulate transcription and increased ATF expression. These alterations may subsequently cause cells to have to fight for survival. These results provide insight into mutant Htt toxicity and may lead to a potential therapeutic target for ameliorating Htt-induced cell death at an early stage.

4. Experimental procedures

4.1 Constructs

Htt-N63-148Q-myc and Htt-N63-23Q-myc constructs were described by Cooper and colleagues. (Cooper et al., 1998). Htt-N63-23 (wild type) and Htt-N63-148Q (mutant Htt); ATF3 in pCG vector (Hai et al., 1999) plasmids are used for transient transfection experiment.

ATF3 promoter constructs prepared in pGL3 vector (Promega) were a gift from Dr. Kitajima, Tokyo Medical and Dental University, Tokyo, Japan (Cai et al., 2000), and are described as follows: ATF WT, ATF3 wild type promoter; Delta ATF, pGL3 vector lacking the ATF3 promoter region; ATFm, ATF3 mutant promoter with two point mutations in ATF/CRE binding site known to block ATF/CRE binding. The pTK-Renilla vector was obtained from Promega.

4.2 Cell culture and transfection

A stable inducible PC12 cell model designed to express a myc-tagged N-terminal Htt fragment with either an expanded or normal polyQ region was previously described (Igarashi et al., 2003). To initiate differentiation, cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 1% horse serum, 100 µg/ml G418, 200 µg/ml hygromycin, 200 ng/ml doxycycline, 100 units/ml penicillin, 100 units/ml streptomycin, and 50 ng/ml NGF (Roche). Mutant Htt expression was induced by washing the cells with PBS and eliminating doxycycline from the medium. Unless otherwise noted, all reagents were obtained from Invitrogen Life Sciences. PC12 tTA cells were used for transient transfection as described previously (Bae et al., 2005), Htt-N63-23Q or Htt-N63-148Q plasmids were transfected with lipofectamine 2000 (Invitrogen).

4.3 Cell viability studies

PC12 cells induced to express Htt-N63 were seeded on collagen-coated 24-well plates at a concentration of 10^4 cells/ml in either the presence or absence of doxycycline. Cell viability was assessed by lactate dehydrogenase (LDH) assay (Roche Applied Science) according to the manufacturer's instructions. For each experiment, values from three wells were averaged; data are expressed as mean $\pm$ SEM which collected from three separate experiment.

4.4 Western blot analysis

Protein extracts from differentiated Htt-N63 PC12 cells, grown for the indicated times, were prepared in M-Per lysis buffer (Pierce) supplemented with Complete Protease Inhibitor (Roche

Applied Science). Protein concentration was determined in each lysate by the BCA protein assay (Pierce). Proteins were transferred onto nitrocellulose (Schleicher and Schuell) at 30 V for 2 hr. Blots were treated with blocking buffer (PBS + 0.1% Tween-20 + 5% nonfat dry milk) for 1 hr at room temperature and probed with one of the following primary antibodies overnight at 4°C: mouse anti-myc (9E10) (Roche) at 1:500, rabbit anti-ATF3 (Santa Cruz) at 1:200, mouse antibody to expanded polyQ (1C2) (Chemicon) at 1:3000, mouse anti- α -tubulin (Santa Cruz) at 1:500, and mouse anti- β -actin (Sigma) at 1:5000. Peroxidase-labeled secondary antibody (Boehringer Mannheim) for 1 hr at room temperature. Immunoreactive bands were detected by chemiluminescence (Amersham, Buckinghamshire U.K.). For densitometry measurements, autoradiograms were scanned with an Epson scanner and immunoreactive bands were quantified by using Scion Image beta version 4.0.2 (Scion Corporation).

4.5 RNA isolation and real-time quantitative PCR (RT-qPCR)

Differentiated PC12 cells induced to express Htt-N63-148Q were rinsed twice with PBS and collected. Total RNA extraction was carried out using an RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. Cellular extracts were then treated with DNase (Qiagen) and total RNA was quantified on a Nanodrop spectrophotometer. Aliquots were prepared and either stored at -80 °C or used immediately. Two-step RT-PCR method were used (Ishigaki et al., 2002). First, after extraction of RNA, we immediately carried out reverse transcription to cDNA using a High Capacity Archive Kit (ABI) following the manufacturer's instructions; then we subjected the cDNA to PCR. Real-time quantitative polymerase chain reaction (qPCR) was carried out by the TaqMan Gene Expression Assay (ABI) according to manufacturer's instructions. In all experiments, actin (ID4352340E) was used as an endogenous control. PCR reactions were carried out in triplicate by using an ABI 7700 Prism Detection system (ABI) in a 50 μ l sample volume and 96-well plates under the following cycling conditions: 95°C for 10 min and 50°C for 2 min (1 cycle); 95°C for 15 sec and 60°C for 1 min (40 cycles). Each time for the q-PCR reaction, we used actin and ATF3 simultaneously. The data are expressed as the ratio of ATF3/actin, and the fold change of ATF3 was calculated as ATF3 of dox-/ATF3 of dox+. Negative control lacking the RT enzyme was routinely included.

4.6 Microarray data analysis

The mRNA samples were analyzed with Affymetrix GeneChip Rat genome 230 2.0 Arrays. Probe-level preprocessing was conducted with the statistical algorithm RMA (Robust Multi-array expression measure) (Irizarry et al., 2003). Using the bioconductor package *Affy* to produce probe set level signals. An empirical Bayes method with the lognormal-normal or Gamma-Gamma modeling, as implemented in the bioconductor package *EBarrays*, was then used to estimate the posterior probabilities of the differential expression of genes between the sample conditions (Kendzierski et al., 2003; Newton et al., 2001). The criterion of the posterior probability > 0.5, that is the posterior probability favors change, was used to produce the differentially expressed gene lists. All computation was performed under *R* environment (<http://www.r-project.org>) and all bioconductor packages are available at www.bioconductor.org.

4.7 Luciferase reporter assays

PC12 cells expressing either Htt N63-148Q-myc or Htt N63-23Q-myc were plated on 24-well plates at a density of 10⁴ cells per well and grown for 24 hr. Cells were pre-incubated for 4 hr with DMEM supplemented with 1% horse serum and then transfected with pTK-Renilla vector and one of the promoter constructs described above by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Following transfection, the medium was replaced with serum-free medium and doxycycline was added to Dox+ cells. After 24 hr, cells were harvested in luciferase lysis buffer (Promega) and luciferase activity was determined using a

dual luciferase assay kit (Promega). Data were collected in triplicate and normalized against pTK-renilla activity. The results shown represent the mean $\pm$ SEM of at least three separate experiments with statistical analysis performed by Standard Student's *t*-test.

4.8 ATF3 siRNA knockdown experiments

To silence the expression of endogenous ATF3, an siRNA specific for ATF3 was designed and prepared by Dharmacon (Dharmacon, Inc. Chicago, IL) (Rat ATF3, Cat. M-080-117-00). Stable Htt N63-148Q PC12 cells were transiently transfected with either ATF3 or control siRNA (non-targeting control pool, Cat. D-001206-13-20) 1 day after induction of mutant Htt expression (Lipofectamine 2000, Invitrogen). The conditioned medium was collected 4 days after transfection and analyzed by the LDH assay. Simultaneously, cells were collected, lysed, and analyzed by Western blotting to determine the extent of ATF3 knockdown.

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Fig 1.

Induction of mutant Htt expression in PC12 cells induces cell death. (A) Expression of mutant Htt-N63 in stable inducible PC12 cells detected by Western blot with use of an expanded polyQ-selective 1C2 antibody. The lower band in all samples represents α -tubulin, shown as a loading control. (B) Htt N63-148Q-myc induced cell death compared with non-inducing conditions measured by LDH toxicity assays; in contrast, Htt N63-23Q-myc did not. * $P < 0.001$ (by Student's *t* test), compare to non-inducing conditions.

Fig 2.

Mutant Htt-N63 increases ATF3 mRNA and protein levels. (A) RT-qPCR analysis was carried out in the presence or absence of mutant Htt-N63 expression. Data are reported fold as a-fold increase in ATF3 mRNA in Htt-expressing cells (Dox-) compared to the un-induced condition (Dox+) and show a peak in ATF3 mRNA 4 days after induction of mutant Htt expression. (B) Western blot analysis of endogenous ATF3 levels following induction of Htt N63-148Q-myc expression shows an increase in ATF3 over time, with a peak on day 4 after induction. Blots were probed with an antibody to ATF3. α -Tubulin is shown as a loading control. (C) Quantification of these data is shown in the lower panel. (D) Confocal images of Htt N63-148Q-myc stable inducible cells showing myc epitope (green), ATF3 (red), and Hoescht (blue) staining in the absence (Dox+, upper panels) or presence (Dox-, lower panels) of mutant Htt expression. Cells were fixed and immunostained 4 days after induction of mutant Htt expression. (Scale bar: 10 μ m)

Fig 3.

Mutant htt-N63 increases ATF3 transcriptional activity (A) Schematic diagram of ATF3 promoter plasmids with putative consensus elements for AP-1 and SRF, ATF/CRE and TATA binding sites, and the luciferase reporter gene. (B) Luciferase activity measured in Htt N63 PC12 cells transfected with ATF3 reporter plasmids in the presence (Dox+) or absence (Dox-) of doxycycline. Data were normalized against pTK-renilla activity. $P < 0.05$ by Student's *t*-test. Statistically significant differences between expressing wild type Htt (Htt-N63-23Q) and mutant Htt (Htt-N63-148Q) in stable PC12 cell line

Fig 4.

Overexpression of ATF3 reduced mutant Htt-N63 induced toxicity. (A) Htt N63-148Q stable cells were transfected with vector or ATF3 cDNA and incubated in inducing conditions for 2 days. Cells were harvested and subjected to Western blot analysis by using anti-ATF3 and anti-actin antibodies. (B) Overexpression of ATF3 reduced mutant Htt (Htt-N63-148Q) induced toxicity using LDH-based toxicity assay. $P = 0.027$ by Student's *t*-test.

Fig 5. Knockdown of ATF3 expression enhances mutant Htt-N63-induced cell toxicity. (A) Htt N63-148Q stable cells were transfected with siRNA targeting ATF3 or non-targeting control RNA, and incubated in inducing conditions for 4 days. Cells were harvested and subjected to Western blot analysis by using anti-ATF3 and anti-actin antibodies. (B) Knockdown of ATF3 enhanced mutant Htt-N63 induced toxicity by using LDH-based toxicity assay. $P < 0.05$ by Student's *t*-test.

Fig 6. An increase in endogenous ATF3 protein is associated with mutant Htt expression and aggregation in transiently transfected PC12 cells. Cells were fixed and immunostained 48 h after transfection with an antibody to myc epitope for Htt detection (green) and an antibody to ATF3 (red.) Nuclear staining with Hoescht is shown in blue. (A) A cell expressing mutant Htt-N63-148Q aggregates shows increased ATF3 expression. (B) A cell expressing normal Htt-N63-23Q with diffused staining for htt shows no increase in ATF3 protein. Quantification of these data is shown in C. * $P < 0.001$. 100 transfected cells were assessed in each individual experiment. Data are presented as mean $\pm$ SEM of three separate experiments. (Scale bar: 10 μ m.)

Table 1

Transcriptional factor changes in gene expression cause by expanded polyQ Htt in PC12 cells. The mRNA levels of 20 transcription-related genes were increased up to 1.5-fold of that of control cells on day 4 after induction of mutant Htt expression. Data shows fold change of mutant Htt induction condition compare to non-induction condition.

GenBank accession no.	Gene Product	Gene symbol	Fold change
NM_012912	Activating transcription factor 3	ATF3	24.559456
NM_012953	Fos-like antigen 1	Fosl1	3.69567
NM_021835	v-jun sarcoma virus 17 oncogene homolog (avian)	v-Jun	2.642766
NM_012551	Early growth response 1	Egr-1	2.353988
NM_024134	DNA-damage inducible transcript 3	Ddit3	2.06334
NM_012954	Fos-like antigen 2	Fosl2	1.91767
L38247	Pancreatic and duodenal homeobox gene 1		1.85513
NM_053727	Nuclear factor, interleukin 3, regulated	Nfil3	1.820948
NM_031642	Core promoter element binding protein	Klf6	1.75217
NM_022715	Major vault protein	Mvp	1.749057
BE113920	Signal transducer and activator of transcription 3		1.709783
BF565718	Core promoter element binding protein		1.666381
NM_031599	Eukaryotic translation initiation factor 2 alpha	Eif2ak3	1.655477
AF013965	Zinc finger protein 265		1.644508
BI289392	Eukaryotic elongation factor-2 kinase		1.590502
NM_053611	Nuclear protein 1	Nupr1	1.547344
BE329377	Jun D proto-oncogene		1.519918
AA957545	T-box 3 (ulnar mammary syndrome)		-1.500172
AI044110	Thymopoietin		-1.502719
NM_031633	Forkhead box M1	Foxm1	-1.851786