Truncated Peroxisome Proliferator-Activated Receptor- γ Coactivator 1 α Splice Variant Is Severely Altered in Huntington's Disease

Ashu Johri^a Anatoly A. Starkov^a Abhishek Chandra^a Thomas Hennessey^a
Abhijeet Sharma^a Sara Orobello^b Ferdinando Squitieri^b Lichuan Yang^a
M. Flint Beal^a

^aDepartment of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, N.Y., USA;

^bNeurogenetics and Rare Disease Centre, IRCCS Neuromed, Pozzilli, Italy

Key Words

Neurodegeneration • PGC1 α • Mitochondrial gene expression • Alternative splicing • Huntington's disease • Striatal neurons

Abstract

Background: Reduced peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC1 α) gene expression has been observed in striatal cell lines, transgenic mouse models of Huntington's disease (HD), and brain tissue from HD patients. As this protein is a key transcription regulator of the expression of many mitochondrial proteins, these observations strongly support the role of aberrant mitochondrial function in the pathogenesis of HD. The PGC1 α protein undergoes posttranslational modifications that affect its transcriptional activity. The N-truncated splice variant of PGC1 α (NT-PGC1 α) is produced in tissues, but the role of truncated splice variants of PGC1 α in HD and in the regulation of mitochondrial gene expression has not been elucidated. **Objective:** To examine the expression and modulation of expression of NT-PGC1 α levels in HD. **Methods and Results:** We found that the NT-PGC1 α protein, a splice variant of ~38 kDa, but not full-length PGC1 α is severely and consistently altered in human HD brain, human HD myoblasts, mouse HD models, and HD striatal cells. NT-PGC1 α levels were sig-

nificantly upregulated in HD cells and mouse brown fat by physiologically relevant stimuli that are known to upregulate PGC1 α gene expression. This resulted in an increase in mitochondrial gene expression and cytochrome c content. **Conclusion:** Our data suggest that NT-PGC1 α is an important component of the PGC1 α transcriptional network, which plays a significant role in the pathogenesis of HD.

Copyright © 2011 S. Karger AG, Basel

Introduction

Huntington's disease (HD) is a progressive neurological disorder caused by a mutation in the huntingtin gene resulting in an expanded polyglutamine repeat within exon 1 [1]. Aberrant transcriptional regulation, oxidative stress, and dysregulation of mitochondrial energy metabolism have been implicated in the pathogenesis of HD [2–4]. Recent findings [5, 6] suggest that mutant huntingtin may disrupt normal mitochondrial function by inhibiting the expression of peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC1 α), a transcriptional coactivator that has been shown to regulate the expression of nuclear-encoded mitochondrial proteins. cAMP signaling is a key activator of PGC1 α transcription in many tissues, promoting the binding of cAMP response ele-

ment (CRE)-binding protein (CREB) or the activating transcription factor-2 to a conserved DNA response element in the PGC1 α promoter. Mutant huntingtin occupies the PGC1 α promoter region and represses CRE-mediated transcription of PGC1 α , by interfering with the CREB/TAF4 transcriptional pathway, in the brains and cells of transgenic HD mice [5]. Reduced CRE-dependent transcription has been previously observed as an early abnormality in the pathogenesis of HD [7–9]. Moreover, it has been shown that cAMP levels are reduced in a striatal cell model of HD, i.e. STHdh^{Q111/Q111} cells [7]; treatment of these cells with 8-bromo-cAMP significantly up-regulated the activity of the PGC1 α reporter [5].

Several PGC1 α splice variants have been reported which might arise from differential internal splicing and/or alternative promoter usage [10–13]. The role of these variants in the regulation of transcription remains to be elucidated [14]. Zhang et al. [13] suggested that a truncated form of PGC1 α (NT-PGC1 α) may exhibit regulatory activity. This protein is produced by alternative 3' splicing between exons 6 and 7 that introduces an in-frame stop codon into PGC1 α mRNA. NT-PGC1 α retains the N-terminal transcriptional activation and nuclear-receptor interacting domains but lacks all domains within 268–797 amino acids of the full-length protein. NT-PGC1 α produces a cell context-specific subset of responses that complement, overlap, or prolong the actions of PGC1 α [13].

Here, we report that the NT-PGC1 α protein is the major variant of PGC1 α that is severely altered in human HD brain and in mouse and cell models of HD. Stimulation of the expression of NT-PGC1 α in HD cells results in upregulation of the expression of PGC1 α -controlled mitochondrial genes. NT-PGC1 α is responsive to cold stimuli (a well-established physiologic stimulus of PGC1 α) in brown adipose tissue (BAT). Thus, NT-PGC1 α appears to be an important component of the PGC1 α transcriptional network that may play a role in the pathogenesis of HD.

Animals and Methods

Transgenic Animals

The N171-18Q mice, N171-82Q mice, and R6/2 mice were from Jackson Labs. The N171 mice have the N-terminal fragment (171 amino acids) of human huntingtin and either 82 or 18 glutamines on a B6C3F1 background [15]. The R6/2 mice were created with a 1.9-kb human genomic fragment containing promoter sequences and exon 1 carrying expansions of approximately 130 CAG repeats on a CBA $\times$ C57BL/6 background [16]. The animals were

kept on a 12-hour light/dark cycle with food and water available ad libitum. All experiments were conducted in accordance with National Institutes of Health guidelines for animal research and were approved by the Weill Cornell Medical College Animal Care and Use Committee.

Striatal Cell Culture and Treatments

STHdh^{Q7/Q7} (Q7) and STHdh^{Q111/Q111} (Q111) cells, generated from striatal primordia of wild-type (WT) Hdh^{Q7/Q7} and homozygous mutant Hdh^{Q111/Q111} knock-in mouse embryos, respectively [17], were a gift from Dr. Marcy E. MacDonald. The cells were cultured in DMEM (33°C, 5% CO₂, 10% FBS, 1% antibiotic-antimycotic, and 400 μ g/ml G418) and treated with 10 μ M forskolin + 50 μ M 3-isobutyl-1-methylxanthine (IBMX) for 2 h.

Myoblast Cell Culture

Myoblasts were derived from muscle biopsies obtained from nondiseased subjects and HD patients (who gave their informed consent) and cultured as previously described [18]. Myoblasts were grown in HAM's F10 medium (GIBCO; Invitrogen, Carlsbad, Calif., USA) supplemented with 15% FBS (Invitrogen, San Diego, Calif., USA), 0.5 mg/ml bovine serum albumin, 4 ng/ml insulin, 10 ng/ml epidermal growth factor, 0.39 μ g/ml dexamethasone, 0.1 mg/ml streptomycin, and 100 U/ml penicillin.

Human Brain Samples

Human postmortem brain specimens from the putamen region (HD grade 2, n = 5; grade 3, n = 4, and grade 4, n = 2; mean age $\sim$ 63 years, range 53–75; age-matched nondiseased samples (n = 7), mean age $\sim$ 65 years, range 57–76) were generously provided by the New York Brain Bank at Columbia University, Taub Institute.

Cold Challenge

WT and R6/2 mice were exposed to 4°C for 4 h [19] and then sacrificed by cervical dislocation; BAT was harvested and frozen immediately.

Gene Expression Analysis by RT-PCR

Striatal cells were scraped and total RNA was isolated using Trizol reagent (Invitrogen). Genomic DNA was removed using RNase-free DNase (Ambion) in RNA pellets resuspended in DEPC-treated water (Ambion). Total RNA purity and integrity was confirmed by a ND-1000 NanoDrop (NanoDrop Technologies) and a 2100 Bioanalyzer (Agilent), respectively, with average 260/280 ratios for all study samples ranging from 1.9 to 2.1 and average RNA integrity numbers ranging from 7.5 to 9.0. Equal amounts of RNA were diluted in nuclease-free water (Ambion) to a final concentration of 10 ng/ μ l and reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA). Real-time RT-PCR was performed using the ABI prism 7900 HT sequence detection system (Applied Biosystems, Foster City, Calif., USA) to detect changes in mRNA expression using various primer pairs (table 1) at a final volume of 20 μ l. All qPCR plating was performed on ice. Expression of the gene cyclophilin B served as a control to normalize values. Thermal cycling conditions were 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative expression was calculated using the $\Delta\Delta$ Ct method.

Western Blot

Human brain tissues, striatum of WT and HD mice, myoblasts, and striatal cells were homogenized in cell extraction buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% SDS, 0.5% NP-40, and 0.5% deoxycholate supplemented with protease and phosphatase inhibitors (Sigma), and the protein concentration was determined using a BCA protein assay (Pierce, USA). Equal amounts of protein (40 µg) were loaded onto a 4–20% Tris-glycine gel (Invitrogen). The positive controls (cell lysates of COS1 cells transfected with the full-length PGC1α or NT-PGC1α) for full-length PGC1α and NT-PGC1α were obtained from Calbiochem. After transfer, membranes were blocked for 1 h at room temperature in Tris-buffered saline/Tween-20 (TBST) [50 mM Tris-HCl, 150 mM NaCl (pH 7.4), and 1% Tween-20] containing 5% nonfat dried milk. The membranes were incubated overnight at 4°C for PGC1α (1:750; Calbiochem), uncoupling protein-1 (UCP-1) (1:1,000; Calbiochem), and α-tubulin (1:10,000; Sigma). The membranes were then washed 3 times with TBST and incubated for 1 h with HRP-conjugated secondary antibody, and the immunoreactive proteins were detected using a chemiluminescent substrate (Pierce). Protein expression was quantified using Scion Image software (NIH, USA).

HPLC Assay for cAMP

The cultured cells in each well were washed twice with phosphate-buffered saline (PBS) and then lysed by adding 150 µl ice-embedded acetonitrile followed by 75 µl ice-cold sterile water. Lysates were centrifuged at 14,000 rpm and 4°C for 20 min. The supernatant was taken and diluted with an equal volume of water for HPLC assay. Fifty microliters of supernatant was applied to an HPLC system which comprises a Perkin Elmer M-250 binary LC pump, a Waters 717 plus autosampler, a Waters 2489 UV detector set at 260-nm wavelength, and an ESA 501 chromatography data process system. The gradient elution was built with 2 mobile phases at a rate of 1 ml/min through a 4.6 × 250 mm, 5-µm particle size TSK-GEL HPLC column. Mobile phase A contained 25 mM NaH₂PO₄ and 100 mg/l tetrabutylammonium (pH 5.5), and mobile phase B contained 10%(v/v) acetonitrile mixed in a buffer of 200 mM NaH₂PO₄ and 100 mg/l tetrabutylammonium (pH 4.0). Quantification was carried out using external standard calibration. The protein concentration was measured from the cell lysate.

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis was performed using GraphPad InStat software (GraphPad Software, Inc., San Diego, Calif., USA). The mean significant difference was determined using a 1-way ANOVA followed by a Tukey-Kramer post hoc multiple comparisons test or a 2-tailed Student's t test where appropriate. $p < 0.05$ was considered statistically significant.

Results

The NT-PGC1α Protein Is Altered in HD Human and Mouse Brain

Using an antibody that recognizes both the full-length ('PGC1α', ~113 kDa) form and the spliced form ('NT-

Table 1. Primer sequences used for real-time quantitative PCR

Gene name	Forward primer	Reverse primer
NT-PGC1α	tgccattgttaagaccga	ggctactggaagatatgg
Tfam	agccagggtccagctcactaa	aaaccaagaaagcatgtgg
NRF-1	tggtccagagagtgtctgtg	ttcctgggaaggagaagat
Cyclophilin B	ccatcgtgtcatcaaggactcat	cttgccatccagccaggaggtctt

PGC1α', ~38 kDa) of PGC1α, we analyzed several samples of human brain, human myoblasts, and mouse brain (fig. 1a–d shows representative Western blots) and Q111 HD cells (fig. 2a). The depletion of the full-length PGC1α protein was not consistent in human HD myoblasts and human and mouse HD brain samples (fig. 1a–d) or in Q111 HD cells (fig. 2a). However, the NT-PGC1α protein was prominently and consistently depleted in human HD myoblasts and mouse Q111 striatal cells (fig. 1b, 2a). In human brain, NT-PGC1α was depleted in grade 2 HD patients, while it was upregulated in grade 3 and grade 4 HD patients (fig. 1a). NT-PGC1α was severely depleted in young HD transgenic mice, R6/2 mice, and N171-82Q mice, but it was upregulated in older phenotypic HD mice (fig. 1c, d). It appears that the amount of NT-PGC1α rather than the amount of the full-length PGC1α variant is severely altered in HD human and mouse brain and in cultured HD myoblasts and striatal cells.

Elevated Intracellular cAMP Upregulates the Expression of Mitochondrial Genes and the NT-PGC1α Protein in HD Cells

PGC1α gene expression in HD cells can be significantly increased by cAMP [5]. We examined whether NT-PGC1α protein levels are affected by cAMP in Q111 HD cells. Consistent with earlier reports [7], cAMP was greatly reduced in the Q111 cells versus Q7 cells (fig. 2b). We treated Q7 and Q111 cells with forskolin + the phosphodiesterase inhibitor IBMX, which resulted in a 100-fold increase in cAMP levels in the Q7 and in a 710-fold increase in Q111 cells (fig. 2c). This treatment also resulted in a significant increase in NT-PGC1α protein (fig. 2a) and mRNA (fig. 2d) levels in Q111 striatal cells. We also observed about a 3-fold increase in the protein expression of NT-PGC1α in Q111 cells treated with a cAMP analog (8-bromo-cAMP; data not presented), which corroborates the finding that the expression of NT-PGC1α is modulated by cAMP levels in these cells (fig. 2a, d).

PGC1α acts upstream of nuclear respiratory factor NRF-1, which regulates the expression of nuclear-encoded

Fig. 1. Evidence of the altered protein levels of NT-PGC1α in the putamen of symptomatic HD patients (**a**), in human HD myoblasts (**b**), and in the striatum of 2 mouse models of HD, i.e. R6/2 (**c**), and N171-82Q (**d**). **a–d** Representative Western blots. Upper panels represent the full-length PGC1α (113 kDa) and NT-PGC1α (38 kDa) isoforms, and lower panels represent α-tubulin as a loading control. **a** Lanes 1–4 contain no disease control samples, lanes 5 and 6 are samples from grade 2 HD patients, lanes 7 and 8 contain samples from grade 3 HD patients, lanes 9 and 10 are samples from grade 4 HD patients, and lanes 11 and 12 contain the positive controls for NT-PGC1α and full-length PGC1α, respectively. Western blot analysis shows that the full-length isoform is downregulated in 1 out of 4 control samples and in all HD patients. The protein expression level of the short isoform is severely reduced in grade 2 HD patients and upregulated in grade 3 and grade 4 HD patients. **b** Lanes 1–3 contain no disease control (normal CAG repeat size) human myoblasts, and lanes 4–7 contain myoblasts from HD patients (CAG repeats: lane 4 = 40/46, lane 5 = 17/51, lane 6 = 18/48, and lane 7 = 10/42). In human myoblasts, there are

no consistent changes in the full-length isoform, while the short isoform is downregulated in all of the HD myoblasts. Lanes 8 and 9 contain the positive controls for NT-PGC1α and full-length PGC1α, respectively. **c** Representative Western blots (total number of animals used per group = 6). Lanes 1–4 contain striatum samples from 1-month-old WT (lanes 1 and 2) and R6/2 (lanes 3 and 4) mice. Lanes 5–8 contain striatum samples from 3-month-old WT (lanes 5 and 6) and R6/2 (lanes 7 and 8) mice. Lanes 9 and 10 contain the positive controls for NT-PGC1α and full-length PGC1α, respectively. **d** Representative Western blots (total number of animals used per group = 6). Lanes 1–4 contain striatum samples from 1.5-month-old N171-18Q (lanes 1 and 2) and N171-82Q (lanes 3 and 4) mice. Lanes 5–8 contain striatum samples from 4-month-old N171-18Q (lanes 5 and 6) and N171-82Q (lanes 7 and 8) mice. Lanes 9 and 10 contain the positive controls for NT-PGC1α and full-length PGC1α, respectively. In younger R6/2 and N171-82Q mice (**c**, **d**), NT-PGC1α is severely reduced compared to that in WT or N171-18Q mice, respectively, while it is upregulated in symptomatic R6/2 and N171-82Q mice.

ed mitochondrial genes such as mitochondrial transcription factor A (Tfam) and cytochrome c (Cyt c). We examined whether cAMP-mediated upregulation of NT-PGC1α in Q111 HD cells modulates the expression of its downstream targets. The mRNA expression levels of

NRF-1 and Tfam were significantly downregulated in Q111 cells versus Q7 cells (fig. 2e, f). Forskolin + IBMX produced similar changes in NRF-1 and Tfam mRNA expression (fig. 2e, f). Similarly, treatment with 8-Br-cAMP (data not presented) also resulted in an increase in NRF-

Fig. 2. Inducibility of NT-PGC1 α in striatal Q7 and Q111 cells upon chemical stimulation. **a** Representative Western blots (number of samples = 5 per group) showing protein expression levels for full-length PGC1 α and NT-PGC1 α (top panel) in untreated Q7 and Q111 cells (lanes 1 and 2, respectively) and in cells treated with a combination of forskolin + IBMX (FSK + IBMX) (lanes 3 and 4, respectively). Lanes 5, 6, and 7 contain, respectively, positive controls for NT-PGC1 α , full-length PGC1 α , and brain lysate from PGC1 α knock-out mice. Lower panel indicates α -tubulin as a loading control. NT-PGC1 α is severely suppressed in Q111 cells and responds to treatment with FSK + IBMX. **b**, **c** HPLC measurement of cAMP levels in Q7 and Q111 striatal cells. **b** cAMP levels in untreated Q7 and Q111 cells, expressed as nanomols per milligram of protein. cAMP levels are significantly lower in Q111 cells compared to Q7 cells at baseline. **c** FSK + IBMX produces a several-fold increase in the cAMP concentration in Q7 and Q111 cells. Data are expressed as means $\pm$ SEM from 3 experiments. * $p < 0.01$ compared to untreated Q7; $^{\S} p < 0.001$ compared to untreated Q7 or Q111, respectively. Quantitative real-time PCR

analysis (number of samples = 3 per group) of relative NT-PGC1 α (**d**), NRF-1 (**e**), and Tfam (**f**) mRNA expression normalized to cyclophilin B. The mRNA expression levels for NT-PGC1 α , NRF-1, and Tfam are significantly downregulated in untreated Q111 cells compared to untreated Q7 cells, and treatment with FSK + IBMX (10 μ M FSK and 50 μ M, 2 h) significantly increases the expression levels for NT-PGC1 α , NRF-1, and Tfam mRNA in Q111 cells. Data are presented as means $\pm$ SEM from 3 experiments. * $p < 0.05$ compared to untreated Q7; $^{\S} p < 0.05$ compared to untreated Q111 cells. **g** Changes in mitochondrial Cyt c in striatal neurons following treatment with FSK + IBMX detected by ELISA. The amount of Cyt c was calculated as nanograms per milligram of protein and expressed as a percent of that in untreated Q7 cells. Cyt c protein is significantly reduced in Q111 cells compared to Q7 cells. Treatment with FSK + IBMX significantly increases Cyt c levels in both Q7 and Q111 striatal cells. Data are expressed as means $\pm$ SEM from 3 experiments. * $p < 0.01$ compared to untreated Q7; $^{\S} p < 0.001$ compared to untreated Q7 or Q111 cells, respectively.

Fig. 3. Inducibility of NT-PGC1 α in BAT of R6/2 mice upon metabolic stimulation. Cold challenge produces an increase in the levels of NT-PGC1 α and UCP-1 protein in the BAT of WT and R6/2 mice. Representative Western blots (n = 6 animals per group) showing protein expression levels for full-length PGC1 α and NT-PGC1 α (top panel) and α -tubulin (lower panel). Lanes 1 and 2 represent untreated WT mice, lanes 3 and 4 represent untreated R6/2 mice, lanes 5 and 6 represent cold-exposed WT mice, lanes 7 and 8 represent cold-exposed R6/2 mice, lane 9 represents BAT from PGC1 α overexpressor mice, and lanes 10 and 11 contain positive controls for NT-PGC1 α and full-length PGC1 α .

1 and Tfam expression consistent with the expression of NT-PGC1 α . Thus, it appears that NT-PGC1 α is functionally important in HD cells.

To ensure that an increase in NT-PGC1 α protein results in an actual increase in mitochondrial proteins, we quantified cAMP-induced changes in mitochondrial Cyt c by ELISA. The amount of Cyt c protein in Q111 cells before treatment was ~50% of that in Q7 cells. Treatment of cells with forskolin + IBMX significantly increased Cyt c in HD (Q111) and Q7 cells (fig. 2g).

Cold Challenge Upregulated NT-PGC1 α in Mouse BAT

PGC1 α mRNA is strongly induced in BAT by cold exposure [19], resulting in enhanced mitochondrial heat generation during adaptive thermogenesis. We found that cold exposure of WT and R6/2 mice strongly increased NT-PGC1 α protein levels rather than full-length PGC1 α in BAT (fig. 3). Interestingly, the level of NT-PGC1 α protein is also significantly upregulated in mice overexpressing PGC1 α (no treatment or exposure to cold; fig. 3). It is known that PGC1 α activates the thermogenic

gene program of BAT through the control of UCP-1. We show here that UCP-1 protein levels are upregulated in concert with NT-PGC1 α levels (fig. 3).

Discussion

Reduced full-length PGC1 α expression has been observed in transgenic mouse models of HD, in postmortem brain tissue from HD patients [5, 6, 20], in muscle biopsies from HD patients, and in human HD myoblast cultures [21]. Our data are in line with this finding (fig. 1a–d). All the studies on the role of PGC1 α in HD to date have dealt with full-length PGC1 α . Although splice variants of PGC1 α have been reported earlier [10–13], their functional significance has not been elucidated. This is the first report to demonstrate that the amount of NT-PGC1 α protein is severely altered in human HD brain, mouse HD models, and HD striatal neurons. We found that NT-PGC1 α is significantly upregulated in HD cells by an increase in cAMP and by cold exposure in mouse BAT. An increase in NT-PGC1 α was associated with upregulation of gene expression and increased mitochondrial protein content.

PGC1 α has a complex structure with multiple domains. N-terminal leucine-rich domains (L2 and L3) mediate the interaction of PGC1 α with the ligand-binding domains of nuclear receptors, whereas central and C-terminal domains mediate the interactions with PPAR γ , NRF-1, and other transcription factors. PGC1 α also has a strong activation domain at the N-terminus, which interacts with a histone acetyltransferase complex, including steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP)/p300. Adjacent to the N-terminal domain is an inhibitory region that spans ~200 amino acids. The short variant of PGC1 α , NT-PGC1 α , lacks this inhibitory domain, which implies that NT-PGC1 α is likely a bona fide activator of gene expression; this is consistent with our data.

Regulation of PGC1 α expression includes a ‘positive feedback’ loop where an increase in expression causes a further increase in its expression [22]. The transcriptional activity of PGC1 α is also regulated by interaction with deacetylases such as SIRT1 [23, 24], which increases its transcriptional activity, and by changes in PGC1 α stability [23, 25].

Further studies are required to elucidate whether an increase in NT-PGC1 α protein content induced by cAMP in striatal cells or cold challenge in BAT was due to an increase in the stability of NT-PGC1 α or an increase in

expression of the PGC1 α gene. It is not clear why the NT-PGC1 α protein amount is strongly decreased in young animals that do not yet show a HD phenotype and at early stages of HD in humans but becomes strongly and consistently elevated in older HD animal models and in human brains with advanced HD stages. This may represent an adaptive increase in NT-PGC1 α as an attempt to compensate for the increasing severity of the pathology and loss of mitochondria which occurs with increasing pathologic grades [26]. It has been shown that the major ATP-buffering and redistributing system in cells, i.e. the creatine kinase/phosphocreatine system, is severely reduced in human HD [27]. In theory, this should put more demand on the ATP-producing systems, such as glycolysis and mitochondria. The grade-dependent increases in NT-PGC1 α levels in human postmortem brain could also be explained by the occurrence of inflammation and gliosis accompanying the death of neurons in later disease stages in which the glial population preferentially survives. The full-length PGC1 α protein is localized in the nucleus and has a fast turnover ($t_{1/2}$ <30 min), while NT-PGC1 α is predominantly cytoplasmic and relatively stable ($t_{1/2}$ >7 h). It has recently been shown that PGC1 α is primarily degraded in the nucleus via a ubiquitin proteasome pathway. It is evident that both isoforms are synthesized in HD tissues despite the presence of huntingtin on the PGC1 α promoter. The accumulation of NT-PGC1 α may therefore also be explained on the basis of its rela-

tively higher stability and slower degradation, although further studies are required to investigate this phenomenon.

Conclusions

We showed here that the NT-PGC1 α protein, a splice variant of ~38 kDa, but not full-length PGC1 α is severely and consistently altered in human HD brain, human HD myoblasts, mouse HD models, and HD striatal cells. The NT-PGC1 α levels were significantly upregulated in HD cells and mouse brown fat by physiologically relevant stimuli that are known to upregulate PGC1 α gene expression. This resulted in an increase in mitochondrial gene expression and Cyt c content. In summary, our data support the concept that NT-PGC1 α is the major variant of PGC1 α which is altered in HD brain and cells and that it is biologically active in controlling the expression of mitochondrial genes.

Acknowledgements

This study was supported by NIH grants NS39258 and P01AG14930 to M.F.B. and grant AG014930 to A.A.S. M.F.B. and A.J. are supported by the HDSA Coalition for the Cure. We would like to thank Dr. Bruce Spiegelman for providing knock-out samples.

References

- 1 Vonsattel JP, DiFiglia M: Huntington disease. *J Neuropathol Exp Neurol* 1998;57: 369–384.
- 2 Beal MF: Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol* 2005;58:495–505.
- 3 Browne SE, Beal MF: The energetics of Huntington's disease. *Neurochem Res* 2004;29: 531–546.
- 4 Browne SE, Beal MF: Oxidative damage in Huntington's disease pathogenesis. *Antioxid Redox Signal* 2006;8:2061–2073.
- 5 Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D: Transcriptional repression of PGC-1 α by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* 2006;127:59–69.
- 6 Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, Gilbert ML, Morton GJ, Bammler TK, Strand AD, Cui L, Beyer RP, Easley CN, Smith AC, Krainc D, Luquet S, Sweet IR, Schwartz MW, La Spada AR: Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1 α in Huntington's disease neurodegeneration. *Cell Metab* 2006;4:349–362.
- 7 Gines S, Seong IS, Fossale E, Ivanova E, Trettel F, Gusella JF, Wheeler VC, Persichetti F, MacDonald ME: Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. *Hum Mol Genet* 2003;12:497–508.
- 8 Sugars KL, Rubinsztein DC: Transcriptional abnormalities in Huntington disease. *Trends Genet* 2003;19:233–238.
- 9 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.
- 10 Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO: Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 2002;16:1879–1886.
- 11 Miura S, Kai Y, Kamei Y, Ezaki O: Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) mRNA in response to beta2-adrenergic receptor activation and exercise. *Endocrinology* 2008;149:4527–4533.
- 12 Yoshioka T, Inagaki K, Noguchi T, Sakai M, Ogawa W, Hosooka T, Iguchi H, Watanabe E, Matsuki Y, Hiramatsu R, Kasuga M: Identification and characterization of an alternative promoter of the human PGC-1 α gene. *Biochem Biophys Res Commun* 2009; 381:537–543.
- 13 Zhang Y, Huypens P, Adamson AW, Chang JS, Henagan TM, Boudreau A, Lenard NR, Burk D, Klein J, Perwitz N, Shin J, Fasshauer M, Kralli A, Gettys TW: Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1 α . *J Biol Chem* 2009;284:32813–32826.

- 14 Handschin C, Spiegelman BM: Peroxisome proliferator-activated receptor gamma co-activator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev* 2006;27:728–735.
- 15 Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuc JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA, Copeland NG, Price DL, Ross CA, Borchelt DR: Intracellular inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum Mol Genet* 1999;8:397–407.
- 16 Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Leach 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.
- 17 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.
- 18 Ciammola A, Sassone J, Alberti L, Meola G, Mancinelli E, Russo MA, Squitieri F, Silani V: Increased apoptosis, Huntingtin inclusions and altered differentiation in muscle cell cultures from Huntington's disease subjects. *Cell Death Differ* 2006;13:2068–2078.
- 19 Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM: Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* 2004;119:121–135.
- 20 McGill JK, Beal MF: PGC-1alpha, a new therapeutic target in Huntington's disease? *Cell* 2006;127:465–468.
- 21 Chaturvedi RK, Adihetty P, Shukla S, Hennessy T, Calingasan N, Yang L, Starkov A, Kiaei M, Cannella M, Sassone J, Ciammola A, Squitieri F, Beal MF: Impaired PGC-1alpha function in muscle in Huntington's disease. *Hum Mol Genet* 2009;18:3048–3065.
- 22 Amat R, Planavila A, Chen SL, Iglesias R, Giral M, Villarroja F: SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma Co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD. *J Biol Chem* 2009;284:21872–21880.
- 23 Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P: Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J* 2007;26:1913–1923.
- 24 Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P: Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 2005;434:113–118.
- 25 Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P: Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways. *FEBS Lett* 2008;582:46–53.
- 26 Kim J, Moody JP, Edgerly CK, Bardiuk OL, Cormier K, Smith K, Beal MF, Ferrante RJ: Mitochondrial loss, dysfunction and altered dynamics in Huntington's disease. *Hum Mol Genet* 2010;19:3919–3935.
- 27 Kim J, Amante DJ, Moody JP, Edgerly CK, Bardiuk OL, Smith K, Matson SA, Matson WR, Scherzer CR, Rosas HD, Hersch SM, Ferrante RJ: Reduced creatine kinase as a central and peripheral biomarker in Huntington's disease. *Biochim Biophys Acta* 2010;1802:673–681.