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mRNA expression levels of PGC-1α in a transgenic and a toxin model of Huntington's disease

Rita Török[a], Júlia Anna Kónya[a], Dénes Zádori[a], Gábor Veres[a], Levente Szalárdy[a], László Vécsei[a,b], Péter Klivényi[a]

[a] *Department of Neurology, Faculty of Medicine, University of Szeged, H-6725 Szeged, Semmelweis u. 6, Hungary*

[b] *MTA-SZTE Neuroscience Research Group, H-6725 Szeged, Semmelweis u. 6, Hungary*

Corresponding author:

Peter Klivenyi, MD, PhD, DSc

Department of Neurology, University of Szeged, H-6725 Szeged, Semmelweis u. 6, Hungary

Phone: +36 62 545348; Fax: +36 62 545597

E-mail: klivenyi.peter@med.u-szeged.hu

Abstract

Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ) Coactivator-1 Alpha (PGC-1α) is involved in the regulation of mitochondrial biogenesis, respiration and adaptive thermogenesis. The full-length PGC-1α (FL-PGC-1α) comprises multiple functional domains interacting with several transcriptional regulatory factors such as nuclear respiratory factors, estrogen-related receptors and PPARs; however, a number of PGC-1α splice variants have also been reported recently. In this study, we examined the expression levels of FL-PGC-1α and N-truncated PGC-1α (NT-PGC-1α), a shorter but functionally active splice variant of PGC-1α protein, in N171-82Q transgenic and 3-nitropropionic acid-induced murine model of Huntington's disease (HD). The expression levels were determined by RT-PCR in three brain areas (striatum, cortex and cerebellum) in three age groups (8, 12 and 16 weeks). Besides recapitulating prior findings that NT-PGC-1α is preferentially increased in 16 weeks of age in transgenic HD animals, we detected age-dependent alterations in both models, including a cerebellum-predominant upregulation of both PGC-1α variants in transgenic mice, and a striatum-predominant upregulation of both PGC-1α variants after acute 3-nitropropionic acid intoxication. The possible relevance of this expression pattern is discussed. Based on our results, we assume that increased expression of PGC-1α may serve as a compensatory mechanism in response to mitochondrial damage in transgenic and toxin models of HD, which may be of therapeutic relevance.

Keywords: PGC-1α, NT-PGC-1α, FL-PGC-1α, Huntington's disease, 3-nitropropionic acid

Introduction

Neurodegenerative diseases comprise a heterogeneous group of neurological disorders, characterized by a relatively selective damage of specific regions of the central nervous system. Mitochondrial dysfunction has been implicated in the pathogenesis of all of these disorders, including Huntington's disease . This is well underlined by the fact that there are several mitochondrial toxins, including the irreversible complex II inhibitor 3-nitropropionic acid (3-NP) for HD, which can be applied for the modelling of neuronal damage.

In respect of the major regulators of mitochondrial functioning, Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ) Coactivator-1 Alpha (PGC-1α) is assumed to be a key regulator of mitochondrial biogenesis, respiration, energy homeostasis and adaptive thermogenesis . **The human PGC-1α gene (reference gene) is located on chromosome 4p15.2 (chromosome 5 in mice) and encodes a full-length protein (FL-PGC-1α) containing 798 (human) or 797 amino acid (mouse).**

The PGC-1α protein has a complex structure with multiple domains, which enable the interaction with several transcriptional regulatory factors, such as nuclear respiratory factors (NRFs), estrogen-related receptors (ERRs) and PPARs . The N-terminal domain of the PGC-1α protein mediates interactions with nuclear receptors (NRs) and regulates the transcriptional activity, whereas the central and C-terminal domains mediate interactions with NRFs, PPARγ and FOXO1 . The activation of NRFs leads to the elevated expression of nuclear-encoded mitochondrial respiratory complex subunits and several mitochondrial factors, which control the mitochondrial DNA transcription; furthermore, the PGC-1α able to regulate the fatty acid oxidation via the induction of PPARγ .

Besides the FL-PGC-1α, several PGC-1α isoforms have been presented recently . Among isoforms, the N-truncated PGC-1α (NT-PGC-1α) is a major one, which is considerably shorter (267 amino acids) than FL-PGC-1α, but functionally active. NT-PGC-1α develops via an alternative 3' splicing between exons 6 and 7 that introduces an in-frame stop codon into PGC-1α mRNA. This protein retains the N-terminal transcriptional activation domains but lacks all domains within 268-797 amino acid of the FL-PGC-1α . **Beside these two major isoforms, novel tissue-specific PGC-1α isoforms have been described recently .**

The role of PGC-1α **system** in neurodegenerative disorders including HD has been reported both in human patients and in animal models . HD is an inherited neurodegenerative disease caused by the expansion of CAG trinucleotide repeat that encodes the polyglutamine region in the huntingtin protein. Transcriptional dysregulation, impaired energy metabolism and increased oxidative stress have all been implicated in HD pathogenesis . **In addition**, the interaction of mutant huntingtin with PGC-1α has been demonstrated in number of studies; in particular, mutant huntingtin is able to disrupt proper mitochondrial functioning via inhibiting the expression and/or influencing the activity of PGC-1α protein .

Accordingly, Chaturvedi et al. found significant reductions in the functioning of PGC-1α and its downstream genes in myoblasts from the skeletal muscle of HD transgenic mice and in muscle biopsy samples from HD patients . Previously, Cui et al. demonstrated that PGC-1α mRNA expression is significantly downregulated in HD striatal cells and tissues . Furthermore, the reduction in PGC-1α mRNA level was demonstrated in post-mortem HD brain tissues . All of these studies examined only the potential role of FL-PGC-1α expression in HD. Johri et al. were the first to demonstrate that the expression level of NT-PGC-1α protein is also altered in transgenic murine models of HD (including the N171-82Q transgenic mice), as well as in human HD brain tissues and in a striatal HD cell line. They found that the

NT-PGC-1α protein is depleted in human HD myoblasts and mouse Q111 striatal cells. Contrary, the level of this protein was decreased in human brains with early HD stages, but was strongly increased in advanced stages of HD. Furthermore, they demonstrated that the NT-PGC-1α protein was upregulated in the striatum of older N171-82Q and R6/2 HD mice compared to younger animals, but the NT-PGC-1α level was measured only in the striatum and not in other brain regions . Although the PGC-1α level was investigated in several transgenic model of HD, there is no data about its mRNA levels in 3-NP induced HD mouse model.

The aim of this study was to investigate the expression levels of FL-PGC-1α and NT-PGC-1α in the striatum, cortex and the cerebellum of N171-82Q transgenic mice and in the respective brain regions of the 3-NP-induced HD mice in three age groups.

Materials and Methods

Animals

C57Bl/6 mice (8, 12 and 16 weeks old; male) and N171-82Q mice (8, 12 and 16 weeks old, males and females equally distributed in each groups) were involved in this study. The latter animal strain was originally obtained from Jackson Labs (Jackson Laboratories, USA). **The symptoms of N171-82Q mice begin to develop at 8 weeks of age. The gait becomes abnormal, tremor, hypokinesia and reduced locomotor activity evolve. The body weight becomes progressively decreased, and the animals die at an average age of 110-120 days.** The animals were housed in cages under standard conditions with 12-12 h light-dark cycle and free access to food and water. The experiments were carried out in accordance with European Communities Council Directive (86/609/EEC) and were approved by the local animal care committee.

Treatment and sample handling

3-NP (Sigma Chemical, USA) was dissolved in phosphate-buffered saline (PBS; pH adjusted to 7.4) and was administered intraperitoneally (i.p.) to male C57Bl/6 animals (the reason for the exclusive application of male mice for 3-NP treatment was the fact that female mice are considerably resistant to toxin treatment with a relatively large variance). Animals of a particular age were randomly divided into four groups (n=6-7 in each group). The first group received a single i.p. injection of 100 mg/kg body weight 3-NP (acute treatment), the second one received 50 mg/kg body weight i.p. injection twice a day for 5 days (subacute treatment). The third and fourth groups served as the respective control (ctrl) groups, and were injected according to the above-detailed treatment regimen with the vehicle.

Ninety minutes after the last injection, the C57Bl/6 animals were deeply anesthetized with isoflurane (Forane; Abott Laboratories Hungary Ltd., Budapest, Hungary) and immediately perfused transcardially with artificial cerebrospinal fluid (composition in mM: 122 NaCl, 3 KCl, 1 Na_2SO_4, 1.25 KH_2PO_4, 10 D-glucose, 1 $MgCl*6H_2O$, 2 $CaCl_2*2H_2O$, 6 $NaHCO_3$) for 2 min with a flow rate of 10 ml/min by an automated peristaltic perfusor. The brains were rapidly removed on ice and immediately halved at the midline. The right hemisphere was dissected on ice, and the striatum, cortex and cerebellum were stored at -80 °C until the RT-PCR analysis. The left hemisphere was postfixed in 4 w/v% paraformaldehyde overnight, and then transmitted into glycerol (10 v/v%) until immunohistochemical sample processing in case of subacute treatment regimen.

In case of transgenic (tg; n=6-7 in each group) N171-82Q mice and the corresponding wild-type (wt; n=6-7 in each group) counterparts, both hemispheres were dissected into the proper brain regions and were stored at -80 °C until the RT-PCR analysis.

RT-PCR analysis

For RT-PCR analysis, total RNA was isolated from striatum, cortex and cerebellum with Trizol according to the manufacturer's protocol. RNA concentrations were determined using MaestroNano spectrophotometer, **and the integrity of RNA was confirmed by gel electrophoresis using 1% agarose gel.** cDNA was synthesized from 1 µg total RNA with random hexamer primers using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). cDNA were kept at -20 °C until further use.

Real-time PCR was performed on CFX 96 Real Time System (Bio-Rad, USA) to detect changes in mRNA expression, using various primer pairs at a final volume of 20 µl. **Thermal cycling conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s.** The relative mRNA level was calculated by the $2^{-\Delta\Delta Ct}$ method . A pre-optimized primer and probe assay for 18S rRNA was used as an endogenous control (Applied Biosystems, USA).

Immunohistochemistry

After postfixation in paraformaldehyde and cryoprotection in glycerol, 30-µm-thick serial sections were cut to obtain consecutive sections from the entire striatum. The sections were collected into 12 wells consecutively, i.e. the distance between the studied sections was 360 µm. The free-floating sections were rinsed in PBS and transferred in methanol, containing 0.3 w/v% H_2O_2 for 30 min. After washing in PBS containing 1 v/v% Triton-X 100 (PBS-T) and blocking for 1 h with PBS-T containing 2 v/v% normal horse serum, the sections were incubated for one night at room temperature in PBS-T containing the anti-NeuN primary antibody at 1:10,000 dilution (Millipore, USA). After rinsing in PBS-T, the sections were incubated in biotinylated anti-mouse IgG for 2 h, followed by the application of avidin-biotin-peroxidase complex in PBS-T for 2 h, and visualized with nickel ammonium sulphate-intensified 3,3'-diaminobenzidine (Sigma-Aldrich, USA). The sections were dried overnight

on glass slides, and coverslipped using DPX as mounting medium. The sections were analysed in a Zeiss Axio Imager M2 Upright Microscope (Carl Zeiss MicroImaging, Germany) supplied with an AxioCam MRC camera. Photographs were taken at 10x magnification. For the determination of NeuN density in the striatum, the MosaiX program feature of AxioVision program was used. Briefly, we measured the density of the whole striatum and the corresponding corpus callosum in the consecutive sections and substracted the values from each other to normalize the method for probable different background stain. After adjusting the differences for the parameters of the corresponding striatum, we calculated the average density/mm^2 values. Thereafter, we compared these derived values in ctrl and 3-NP-treated animals.

Statistics

All statistical analyses were performed with the SPSS Statistics 17.0 software (SPSS Inc., USA). The normality of data was checked by Shapiro-Wilk W test. As most of our data groups showed non-Gaussian distribution and/or displayed significant difference in the homogeneity of variance (checked with the Levene's test), we used non-parametric statistics (Mann-Whitney U test) for the comparison of the relative levels of expression between groups. Due to multiple comparisons, in cases of either FL-PGC-1α or NT-PGC-1α the necessary corrections were done on p values. After corrections, a $p < 0.05$ value was regarded as significant. We calculated the level of gene expression of all brain areas in all age groups relative to the level of FL-PGC-1α gene expression in 8-week-old wt/ctrl striatum. The data were plotted and expressed as median and interquartile range.

Since the data obtained from the quantitative analysis of immunohistochemical patterns showed Gaussian distribution, we used the independent t test for the comparison of ctrl and 3-NP treated groups in this respect. Data are presented as means ± SEM.

Results

HD transgenic mice

There were no detectable changes in the FL-PGC-1α or NT-PGC-1α levels of wt animals with aging. Furthermore, we could not detect differences in PGC-1α levels between male and female mice in tg/wt groups. The NT-PGC-1α expression level was approximately 30% of the mRNA level of FL-PGC-1α in all brain regions of wt animals. Our results demonstrated significantly reduced FL-PGC-1α expression in the striatum (0.59 (0.50–0.77); p=0.039) and cortex (0.83; (0.56–0.90); p=0.009) of 8-week-old tg mice compared to the values of wt animals (striatum: 0.96 (0.77–1.29); cortex: 1.49 (1.33–1.77)), but we could not detect significant changes in these brain regions of older mice (Fig. 1.A, C). The FL-PGC-1α mRNA showed a tendency to increase in the cerebellum of 8-week-old tg mice compared to wt animals, which changes became significant in 12-week-old (wt: 0.23 (0.20–0.30); tg: 0.82; (0.58–0.89); p=0.004) and 16-week-old tg mice (wt: 0.22 (0.15–0.27); tg: 0.74 (0.68–0.82); p=0.018, Fig. 1.E). NT-PGC-1α expression showed a mild tendency of decrease in the striatum and cortex of 8-week-old tg mice, but it was significantly upregulated in the striatum (wt: 0.25 (0.23–0.33); tg: 0.76 (0.53–0.86); p=0.018) and cortex of 16-week-old tg animals (wt: 0.45 (0.30–0.52); tg: 1.15 (0.89–1.33); p=0.018, Fig. 1.B, D). In the cerebellum, NT-PGC-1α expression was, however, significantly increased as early as 8 weeks of age in tg mice (0.18 (0.17–0.24); p=0.009) compared to wt animals (0.07 (0.07–0.13)). This difference also remained significant in 12-week-old (wt: 0.06 (0.05–0.09); tg: 0.37 (0.25–0.49); p=0.004) and 16-week-old tg mice (wt: 0.06 (0.04–0.07); tg: 0.47 (0.42–0.57); p=0.018) (Fig. 1.F).

Acute 3-NP treatment

After a single injection of 3-NP, the FL-PGC-1α expression had a tendency to be increased in the striatum and cortex of all three age groups of C57Bl/6 mice, which reached statistical significance in the striatum of 16-week-old animals (ctrl: 1.04 (0.89-1.09); 3-NP: 1.37 (1.20-1.76); p=0.036) and in the cortex of 12-week-old animals (ctrl: 1.10 (0.84-1.27); 3-NP: 1.67 (1.60-1.84); p=0.018) (Fig. 2.A, C), but not in other age groups. Contrarily, the alterations in the NT-PGC-1α expression were significant only in the striatum (Fig. 2.B), where its expression was significantly greater in 3-NP-treated mice at 12 weeks of age (ctrl: 0.32 (0.26-0.37); 3-NP: 0.62 (0.57-0.76); p=0.018) and 16 weeks of age (ctrl: 0.39 (0.36-0.41); 3-NP: 0.55 (0.51-0.65); p=0.036) compared to the respective ctrl animals.

We did not find differences in FL-PGC-1α and NT-PGC-1α levels in the cerebellum in either group (Fig. 2.E, F).

Subacute 3-NP treatment

The subacute 3-NP regimen resulted in a significant reduction (28%, ctrl: 3414.9 ± 157.4; 3-NP: 2443 ± 339.8; p=0.04) in the NeuN-related density values in the striatum of 3-NP-treated mice compared to ctrl animals, representing the toxin-induced neuronal damage **(Supplementary Fig. 1).** After subacute 3-NP treatment, however, no changes in the FL-PGC-1α or NT-PGC-1α levels were demonstrated between ctrl and 3-NP-treated animals in any brain area of any age group. (Fig. 3.A-F).

Discussion

Although it is known that an abnormal trinucleotide repeat expansion leads to the development of HD, the precise pathomechanism is still not fully understood.

The use of transgenic murine models of HD enables to get a better insight into the pathomechanism of the disease. The pathogenic process shows progression in transgenic murine models, including the N171-82Q strain. However, there are some age-dependent adaptive processes which may serve as protective tools to ameliorate the disease progression . Jarabek et al. observed that N171-82Q mice adapt to NMDA receptor-mediated excitotoxicity in an age-dependent manner, and the authors concluded that N171-82Q mice attempt to compensate for the progression of HD .

The involvement of mitochondrial dysfunction and the role of PGC-1α, which is a key regulator of mitochondrial biogenesis and antioxidant response, have been demonstrated in the pathogenesis of HD . **In particular, alterations in the expression of FL- and NT-PGC-1α protein in HD are the most well established . Notably, novel tissue-specific isoforms transcribed from an alternative upstream promoter have also been described recently in humans ; the altered expression of which, however, have not yet been described in neurodegenerative diseases.**

Supporting the role of PGC-1α gene in HD, it has also been implicated as a candidate modifier gene in HD; indeed, a number of variations demonstrated modifying effects on the age at onset (AO) . Among them, the rs7665116 polymorphism has been proven to have significant association with AO, i.e. its presence was related to a protective effect in three European HD cohorts . However, Ramus et al. could not confirm these results , which contradiction may be explained by the difference in the genetic backgrounds of the different populations examined. Furthermore, certain

haplotypes of a haplotype block where the promoter of novel PGC-1α isoforms is located have also shown association with AO in HD .

In this study, we aimed to demonstrate the age- and brain region-related changes in the **mRNA expression** of FL-PGC-1α and one of its main splice variants, NT-PGC-1α, in a transgenic and a toxin-induced murine model of HD. **Alterations of these two main isoforms have only been demonstrated in transgenic HD mice at the protein level, whereas the role of PGC-1α system in 3-NP model of HD has not yet been addressed.**

Recent studies reported male-specific roles of PGC-1α in neurodegenerative disorders . Notably, however, in our experiments, we could not detect any difference in PGC-1α levels between genders either in N171-82Q tg mice or their wt counterparts, therefore we were able to use pooled data in this case for further analyses.

In the N171-82Q transgenic mouse model, we measured reduced FL-PGC-1α mRNA levels in the striatum (the most affected region in HD) and the overlying cortex of 8-week-old tg mice, which difference became normalized in older HD mice. This could represent an age-dependent compensatory mechanism against the well-documented mitochondrial dysfunction in this transgenic mouse model of HD . However, we could not detect a difference in FL-PGC-1α mRNA expression between 8-week-old wt and tg mice in the cerebellum, which is known to be a relatively resistant region to the degenerative changes in HD . Interestingly, however, in older animals the expression level of FL-PGC-1α mRNA in the cerebellum showed a remarkable increase. Considering the possible neuroprotective roles of PGC-1α in HD , this cerebellum-predominant potentially compensatory phenomenon against mitochondrial dysfunction-related energy deficit might be an important factor underlying the relative resistance of cerebellar neurons against neurodegenerative processes in HD. This would be in line with the finding of reactive astrogliosis indicative of neuronal degeneration

in the cerebellar nuclei of FL-PGC-1α knockout mice, which is virtually absent in the striatum and the cortex of these animals .

Although the FL form of PGC-1α is the major gene product, the NT-PGC-1α splice variant could also have important functions in terms of neurodegeneration. The PGC-1α protein has a complex structure with multiple functional domains. The N-terminal domain of the PGC-1α protein mediates interactions with nuclear receptors, regulates a proportion of the PGC-1α-mediated transcriptional activity and contains a strong activation domain . Accordingly, the role of NT-PGC-1α has also been studied in HD pathogenesis . Johri et al. found that NT-PGC-1α protein was upregulated in the striatum of 4-month-old N171-82Q HD mice (and in 3-month-old R6/2 strain mice). They also studied the FL-PGC-1α level in the striatum and not in other brain regions. In addition to the detection of a rather similar elevation in NT-PGC-1α expression in the striatum of 16-week-old tg mice at the mRNA level as Johri et al. observed at the protein level, we also observed this type of change in the overlying cortex. Furthermore, we detected a considerable elevation of cerebellar mRNA expression of NT-PGC-1α as well, in a very similar pattern to that of FL-PGC-1α. The background of this preferential increased expression of the NT variant is currently unknown; however, it might represent an important shift in the transcriptional regulation of mitochondrial functions in HD, and implicate the potential role of the N-terminal domain-regulated nuclear receptors, especially the known neuroprotective PPARs , as endogenous compensatory mechanisms in HD.

Although the transgenic models have several undisputable advantages, the simplicity of the application of mitochondrial toxin models of HD provide a rapid assessment tool, e.g. for the screening of drug effects. Accordingly, the mitochondrial toxin 3-NP has been well characterized as an experimental model of HD . This toxin inhibits mitochondrial complex II, inducing a mitochondrial dysfunction which causes striatal lesion . **A number of studies**

demonstrated that female animals are considerably resistant to 3-NP toxin treatment . Since the mechanism of this differential effect of the toxin is not fully understood, it would be hard to distinguish if any difference between genders were due to CNS-specific mechanisms or merely due to secondary effects of differential pharmacokinetics of the toxin. Accordingly, since our aim was to examine the evoked effect of the toxin *per se* on expression patterns, we considered female mice inapplicable for animal modelling of HD in 3-NP experiments, and only male mice were involved. We measured the PGC-1α expression levels either after a single injection of 3-NP or after its application in a subacute dosing regimen.

After a single injection of 3-NP, the FL-PGC-1α expression was consistently increased in all three age groups in the striatum and cortex; however, it reached significance only in older animals after strict statistical correction. On the other hand, the NT-PGC-1α expression was increased significantly only in the striatum of older 3-NP-treated mice. We propose that this acute elevation after a single mitochondrial stress event might be an important compensatory response to acute energy deficit and/or oxidative stress due to 3-NP-induced mitochondrial dysfunction. In addition, the predominant elevation in aged animals may reflect the age-dependent vulnerability of the striatum to oxidative stress due to 3-NP treatment , as a more pronounced toxicity might evoke a more expressed compensatory response. Interestingly, however, we did not find any difference in the expression levels of PGC-1α in the cerebellum of either age group, which might be underlied by the fact that 3-NP is a rather selective striatal neurotoxin, and only limited cell death and energy deficit is observed in other brain regions .

Surprisingly, there were no differences in either the FL-PGC-1α or the NT-PGC-1α mRNA levels between toxin-treated and control animals in the striatum (nor in other brain regions) at any age group after subacute 3-NP treatment (a total of 10 injections within 5

days). We suggest that this may be the result of a reduced neuronal adaptive capability of the striatum due to the histologically verified neuronal damage.

Although 3-NP model is one of the most widely applied toxin model of HD, this model cannot mimic all alterations of genetically determined HD. Indeed, 3-NP is a mitochondrial toxin which inhibits complex II and causes preferential striatal cell death, whereas in the transgenic model other brain regions are affected as well. We believe that the findings that NT-PGC-1α expression is elevated only in the striatum but not in other brain regions in the acute 3-NP model may represent this preferential effect of 3-NP in comparison with the transgenic model.

Supported by the prior findings that PGC-1α-deficient neurons have increased sensitivity to mitochondrial toxicity , our results suggest that upregulation of PGC-1α system may represent and important compensatory mechanism against mitochondrial dysfunction in HD.

Conclusions

To our knowledge this is the first report on the alterations in the expression profile of the main PGC-1α splice variants at the mRNA level in multiple brain regions of different murine models of HD. Besides recapitulating the prior findings of Johri et al. that NT-PGC-1α at the protein level is increased in 16 weeks of age in transgenic HD animals, we detected age-dependent alterations in the PGC-1α expression pattern that may correspond with known predilections of neurodegeneration in HD. Based on our results, we suggest that elevated expression of PGC-1α could be an important tool for the compensation of mitochondrial damage in both transgenic and toxin models of HD. Although it is of question whether this mechanism alone would be enough to ameliorate the neurodegenerative process, it may surely serve as an important target for drug development in this currently incurable disease.

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Conflict of interest

The authors declare there is no conflict of interest.

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Figure 1.

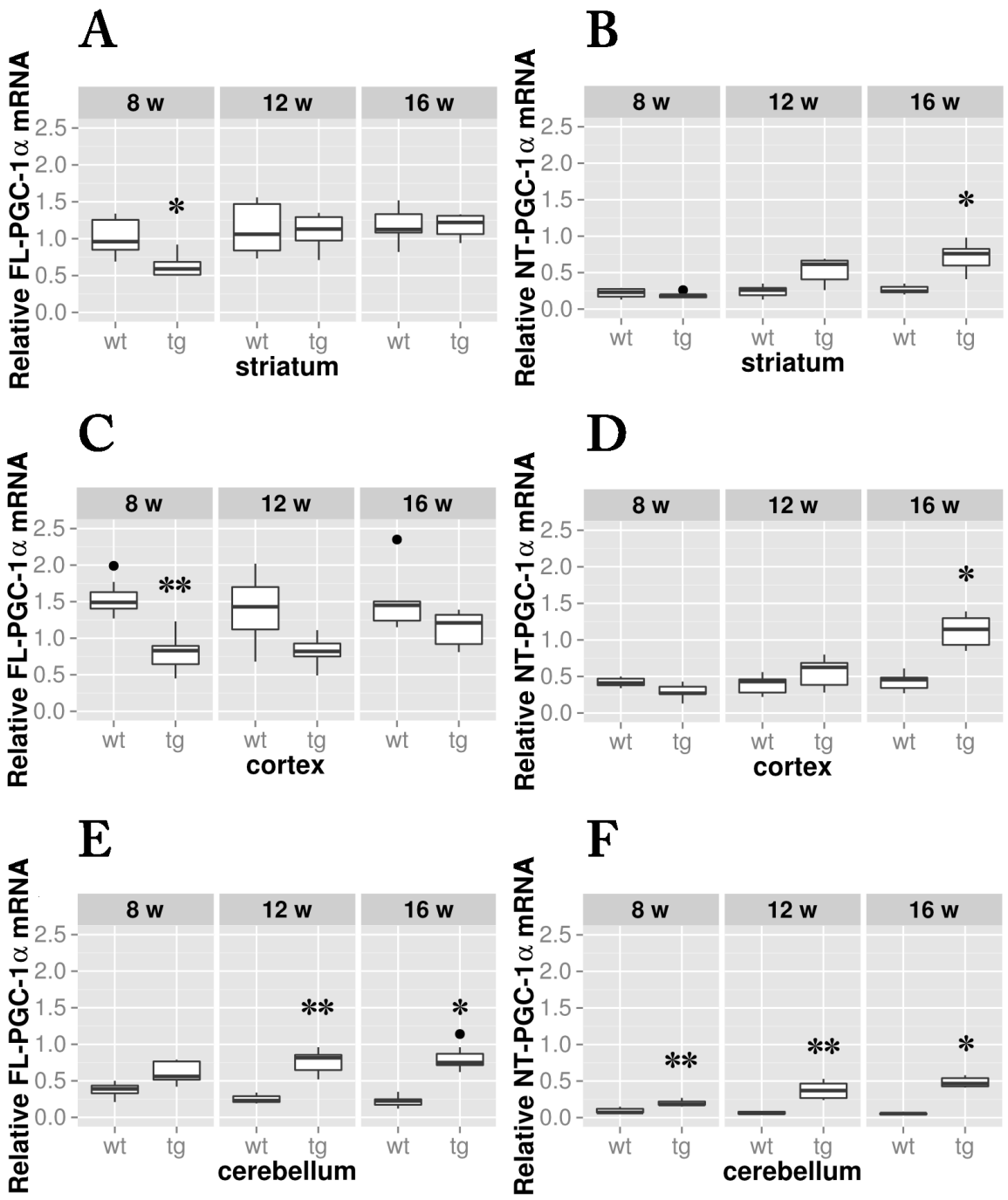
A
B
C
D
E
F
Relative FL-PGC-1α mRNA
Relative NT-PGC-1α mRNA
8 w
12 w
16 w
wt
tg
striatum
cortex
cerebellum
0.0
0.5
1.0
1.5
2.0
2.5
*
**

Figure 2.

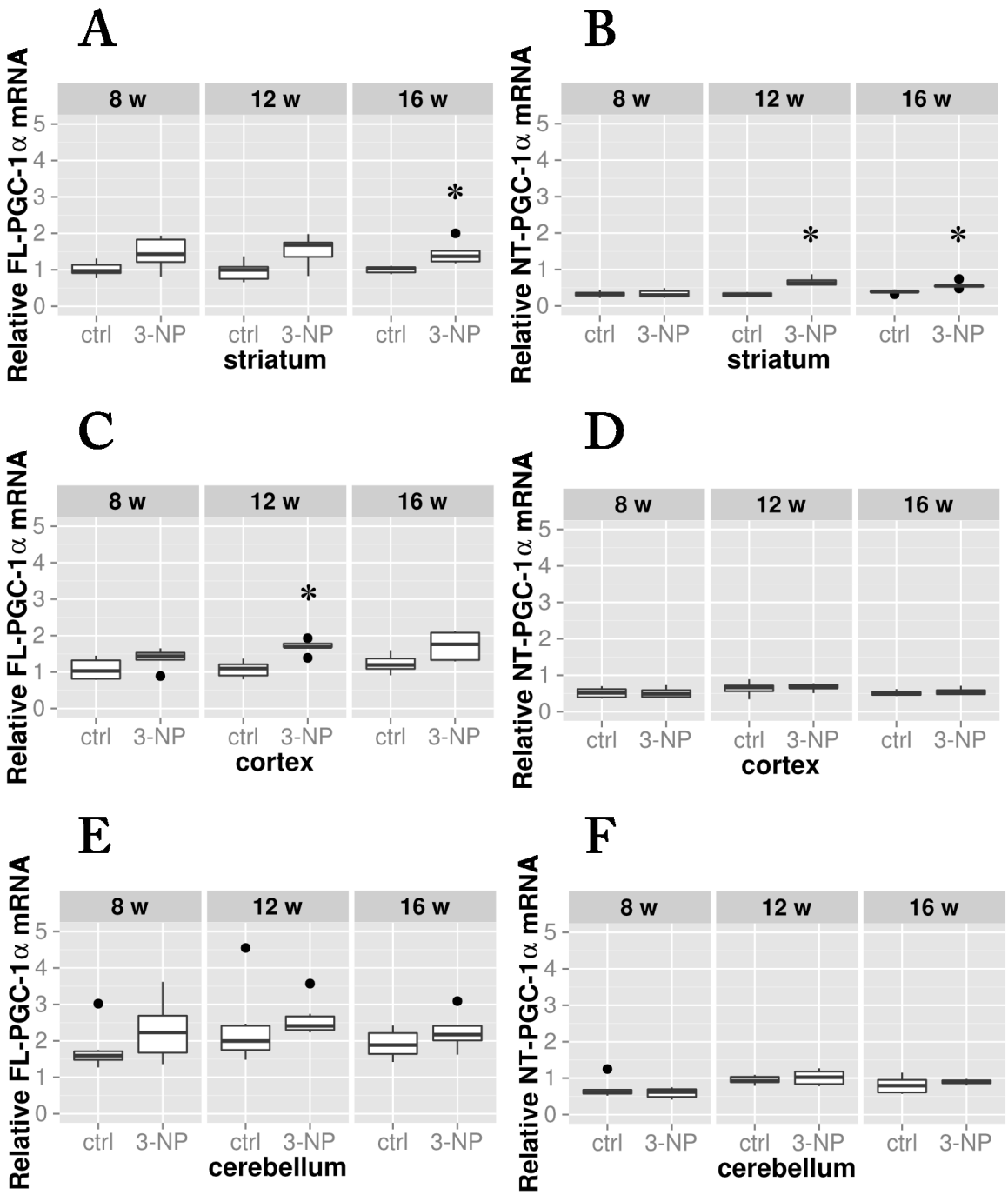
A
B
C
D
E
F
8 w
12 w
16 w
Relative FL-PGC-1α mRNA
Relative NT-PGC-1α mRNA
ctrl
3-NP
striatum
cortex
cerebellum
0
1
2
3
4
5
*

Figure 3.

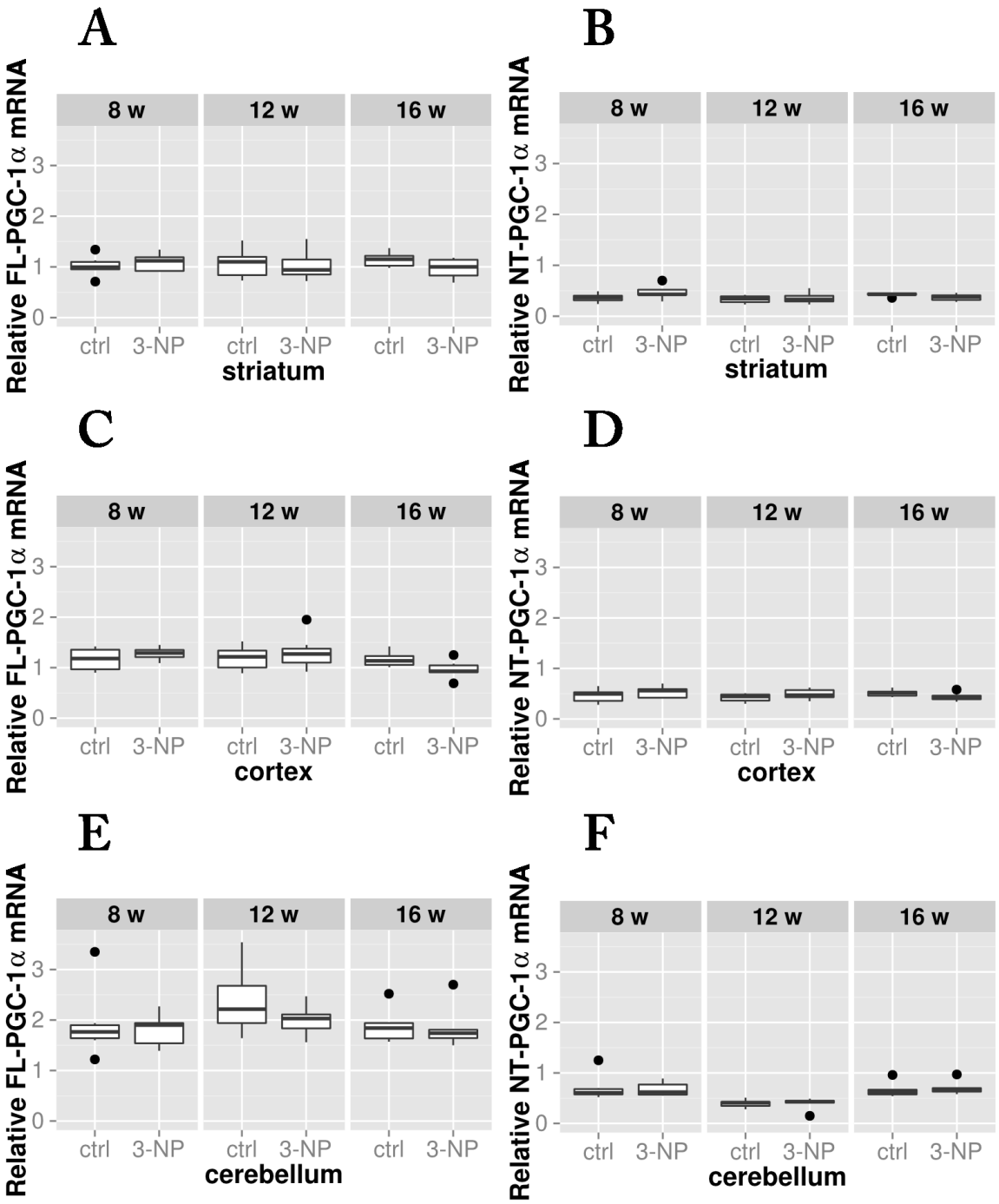
A
B
C
D
E
F
8 w
12 w
16 w
Relative FL-PGC-1α mRNA
Relative NT-PGC-1α mRNA
0
1
2
3
ctrl
3-NP
striatum
cortex
cerebellum

Fig. 1 Relative mRNA expression of FL-PGC-1α and NT-PGC-1α in the striatum, cortex and the cerebellum of N171-82Q transgenic and respective wild-type mice in three age groups. The FL-PGC-1α was significantly reduced in 8-week-old tg striatum and cortex (A, C respectively). In the cerebellum, FL-PGC-1α mRNA was significantly increased in 12-week-old and 16-week-old tg mice (E). The NT-PGC-1α was significantly upregulated in the striatum and cortex of 16-week-old animals (B, D respectively). The NT-PGC-1α expression was increased in all three age groups in the cerebellum (F). Values are plotted as medians and interquartile range; *p<0.05, **p<0.01; *tg* transgenic, *wt* wild-type, *w* weeks.

Fig. 2 FL-PGC-1α and NT-PGC-1α levels in the striatum, cortex and the cerebellum in three age groups of mice following acute 3-NP intoxication. The FL-PGC-1α expression was significantly increased in the striatum of 16-week-old 3-NP treated mice and in the cortex of 12-week-old 3-NP treated mice (A, C respectively). The FL-PGC-1α expression did not change in the cerebellum of 3-NP treated mice (E). The NT-PGC-1α mRNA was significantly upregulated in the 12-week-old and 16-week-old 3-NP treated mice striatum (B). In the cortex and cerebellum of 3-NP treated mice there were no differences in the NT-PGC-1α mRNA levels (D, F respectively). Values are plotted as medians and interquartile range; *p<0.05; *ctrl* control, *3-NP* 3-NP-treated, *w* weeks.

Fig. 3 Analysis of relative FL-PGC-1α and NT-PGC-1α mRNA expression of subacute 3-NP-treated mice in three age groups. We measured the FL-PGC-1α level in the striatum (A), cortex (C) and cerebellum (E) and the NT-PGC-1α levels in the striatum (B), cortex (D) and cerebellum (F), but we could not detect differences between ctrl and 3-NP-treated mice. Values are plotted as medians and interquartile range; *ctrl* control, *3-NP* 3-NP-treated, *w* weeks.