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Histone H3 Acetylation in the Postmortem Parkinson's Disease

Primary Motor Cortex

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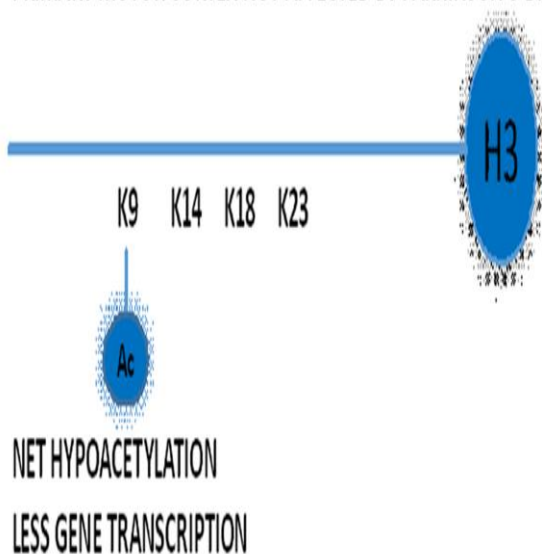
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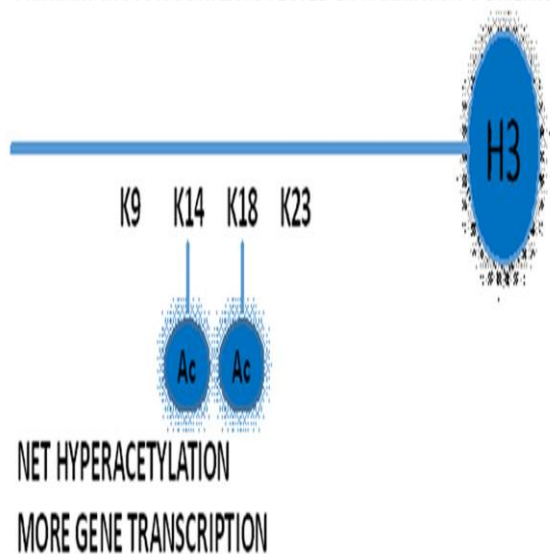
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Graphical abstract

PRIMARY MOTOR CORTEX NOT AFFECTED BY PARKINSON'S DISEASE



PRIMARY MOTOR CORTEX AFFECTED BY PARKINSON'S DISEASE



Highlights:

- Net histone H3 acetylation was increased in the PD primary motor cortex.
- H3K14 and H3K18 acetylation were increased in the PD primary motor cortex.
H3K9 acetylation was increased in the PD primary motor cortex.
- No between-groups difference in H3K23 acetylation was detected.
- The data suggest enhanced gene transcription in the PD primary motor cortex.

Abstract

Although the role of epigenetics in Parkinson's disease (PD) has not been extensively studied, α -synuclein, the main component of Lewy bodies, decreased histone H3 acetylation. Here, we determined if there were histone acetylation changes in the primary motor cortex which, according to the Braak model, is one of the last brain regions affected in PD. Net histone H3 acetylation, histone H3 lysine 9 (H3K9), histone H3 lysine 14 (H3K14), histone H3 lysine 18 (H3K18), and histone H3 lysine 23 (H3K23) acetylation was assessed in the primary motor cortex of those affected and unaffected by PD. There was net increase in histone H3 acetylation due to increased H3K14 and H3K18 acetylation. There was a decrease in H3K9 acetylation. No between-groups difference was detected in H3K23 acetylation. Relationships between Unified Lewy Body Staging scores and histone H3 acetylation and substantia nigra depigmentation scores and histone H3 acetylation were observed. No relationships were detected between postmortem interval and histone H3 acetylation and expired age and histone H3 acetylation. These correlational data support the notion that the histone H3 acetylation changes observed here are not due to the postmortem interval or aging.

Instead, they are due to PD and/or factors that covary with PD. The data suggest enhanced gene transcription in the primary motor cortex of the PD brain due to increase H3K14 and H3K18 acetylation. This effect is partially offset by a decreased H3K9 acetylation, which might repress gene transcription.

Abbreviations

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP; 6-hydroxydopamine, 6-OHDA; acetylated histone H3, AchH3; acetylated histone H3 lysine 9, AchH3K9; acetylated histone H3 lysine 14, AchH3K14; acetylated histone H3 lysine 18, AchH3K18; acetylated histone H3 lysine 23, AchH3K23; brain-derived neurotrophic factor, BDNF; α -synuclein, α -syn; dopaminergic, DAergic; glia cell line-derived neurotrophic factor, GDNF; histone deacetylase inhibitor, HDACI; histone deacetylase inhibitors, HDACIs; histone H3 lysine 9, H3K9; histone H3 lysine 14, H3K14; histone H3 lysine 18, H3K18; histone H3 lysine 23, H3K23; Parkinson's disease, PD; polyvinylidene difluoride, PVDF; postmortem interval, PMI; substantia nigra, SN; total histone H3, tH3.

Keywords: epigenetics, Parkinson's disease, histone acetylation, lysine residues, gene transcription, Unified Lewy Body Staging

1. Introduction

Parkinson's disease (PD) is a progressive neurological disorder affecting approximately 2% of the population 65 years old or older [4]. PD is diagnosed clinically by the combination of motor symptoms including tremor, bradykinesia, and rigidity [8]

and neuropathologically by a severe dopaminergic (DAergic) neuron loss in the substantia nigra (SN) and the presence of Lewy bodies [8]. Importantly, the SN damage in PD is always accompanied by extensive extranigral pathology. Indeed, according to the Braak model, the appearance of Lewy bodies in PD is observed in a stereotypic, temporal pattern that ascends caudorostrally from the lower brainstem through susceptible regions of the midbrain (i.e., the SN and the pedunculopontine tegmental nucleus), forebrain (e.g., amygdala), and the cerebral cortex. At Braak stage 6, the Lewy body pathology extends into first order sensory association cortices, premotor cortex, and primary sensory and motor cortices [5]. Although the cortical pathology that occurs in PD has not been extensively studied, a loss of pyramidal cells and GABAergic interneurons has been observed in the temporal cortex [28], a loss of pyramidal cells has been observed in the pre-supplementary motor cortex [21], and a loss of Betz cells has been observed in the primary motor cortex [29].

The majority of PD cases is thought to arise from the combination of environmental factors and susceptibility genes in ways that are not fully understood. The term “epigenetics” refers to heritable changes in gene expression that are not due to DNA sequence alterations [15]. Epigenetic changes are acquired throughout the lifespan and are affected by environmental factors such as lifestyle, diet, and toxin exposure. Importantly, histone modifications are a key epigenetic mechanism whereby environmental factors can alter gene expression. DNA is packed into the nucleus of eukaryotic cells through its chromatin organization. The nucleosome, the fundamental unit of chromatin structure, consists of 147 base pairs of DNA wrapped around two copies of each of the core histones H2A, H2B, H3, and H4 [18] and one copy of the

linker histone H1 [14,18]. N-terminal regions of these histone proteins, the histone tail domains, protrude from the nucleosome and are susceptible to interactions with other proteins. Post-translational modifications of histone tails domain residues include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation [18,27]. Of these modifications, histone acetylation has been the most studied and appreciated [13]. Acetylation of specific lysine residues in the amino tail domains of the core histones play a fundamental role in transcriptional regulation [17, 24]. Specifically, the acetylation of specific lysine residues found within histone tails is thought to generally activate gene transcription and deacetylation of these same lysine residues is thought to generally repress transcription [10]. Importantly, in histone H3 of humans, the focus of the present study, the main acetylation sites are lysines 9 (H3K9), 14 (H3K14), 18 (H3K18), and 23 (H3K23) [25].

Although the role of chromatin remodeling in PD has not been extensively studied, the protein α -synuclein (α -syn), the main component of Lewy bodies, decreased the level of acetylated histone H3 in cultured cells and produced cell death [16]. In addition, histone deacetylase inhibitor (HDACI) treatment had a neuroprotective effect in primary dopamine cell cultures exposed to the neurotoxin, 6-hydroxydopamine (6-OHDA), and in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD [12,31].

The aim of the present study was to determine if histone H3 acetylation changes would be observed in a brain area that had not yet been profoundly affected by PD. According to the Braak model, the primary motor cortex is one of the last brain regions affect by PD [5]. Thus, primary motor cortex, a brain area not profoundly affected by PD, was the focus of the present

study. We tested the hypothesis that there is decreased net histone H3 acetylation in the primary motor cortex of the human PD brain compared to the primary motor cortex of brains of those unaffected by PD. Since the net acetylation of histone H3 is due to acetylation at H3K9, H3K14, H3K18, and H3K23, we assessed the acetylation state of each of these lysine residues in the PD and non-PD primary motor cortex.

2. Materials and methods

2.1. Unified Staging System scores

Human primary motor cortex tissue samples were obtained from Banner Sun Health Research Institute Brain and Body Donation program [2]. The cases used here were stained immunohistochemically for α -syn according to a published method [3]. The density of α -syn-immunoreactive Lewy bodies was scored by an observer blind to the diagnosis to establish a Unified Staging System score [1]. Neurologically normal control samples were classified as stage 0. Those with Lewy bodies present only in the brainstem were classified as stage 1. Those with Lewy bodies present in the brainstem and limbic system were classified as stage 2. Those with Lewy bodies present in brainstem and limbic system and diffusely in the neocortex were classified as stage 3 [1].

2.2. Degree of SN depigmentation

The degree of SN depigmentation, a pathological feature of PD related to the loss of neuromelanin [11], was assessed histologically as previously described [1]. Histologic evaluation of SN pigmented neuron loss was graded as none, mild, moderate, or severe on hematoxylin and eosin-stained sections taken at or close to the level of the oculomotor nerve. These descriptive terms were converted to numerical scores ranging from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) for statistical purposes.

2.3. Western blots

Protein extracts were prepared from human primary motor cortex tissue samples (control, $n = 8$, PD, $n = 9$), provided by Banner Sun Health Research Institute Brain and Body Donation program [2]. The protein concentration of each sample was determined using bicinchoninic acid assay (BCA, Pierce Biotechnology, Rockford, IL) according to the manufacturer directions. Proteins (30 μ g per well) were separated on 4-20% Mini-PROTEAN TGX pre-cast gels (Bio-Rad Laboratories, Los Angeles, CA) and transferred to polyvinylidene difluoride (PVDF) membrane. Membranes were probed with rabbit anti-histone H3 (1:1,000, Cell Signaling, Danvers, MA), rabbit anti-acetyl-histone H3 (1:20,000, EMD Millipore), rabbit anti-acetyl-histone H3K9 (1:500, EMD Millipore), goat anti-rabbit acetyl-histone H3K14 (1:1,000, EMD Millipore), goat anti-rabbit acetyl-histone H3K18 (1:25,000, EMD Millipore), or goat anti-rabbit acetyl-histone H3K23 (1:200,000, EMD Millipore). The secondary antibody was goat anti-rabbit IR Dye 800CW (1:15,000, LI-COR Biosciences, Lincoln, NE). Immunoblots were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Bands of interest were analyzed and quantified using Scion Image. A researcher blind to the treatment groups conducted the Western blots, blot visualization, and quantification of bands of interest. All assays were performed in triplicate.

2.4. Data analysis

Relationships between optical density data and postmortem interval (PMI) and optical density data and expired age were analyzed via Pearson's r . Relationships between optical density data, expressed as an acetylated protein to total protein ratio, and Unified Lewy Body Staging scores and optical density data and SN depigmentation scores were analyzed via Spearman rank order correlation coefficient. The Unified Lewy Body Staging scores and SN

depigmentation scores were analyzed via Mann-Whitney U tests. Expired age, optical density, and PMI data were analyzed via independent-samples t -tests. The α level was set at 0.05. All data are represented as mean \pm *S.E.M.*

3. Results

3.1. Characteristics of the participants

We analyzed the data from the individuals who provided the primary motor cortex tissue samples (control, $n = 8$, PD, $n = 9$). No between-groups difference in expired age was detected (control group: 78.00 ± 1.85 years; PD group: 78.89 ± 1.33 years; $t(15) = -0.397$, $p = 0.697$, **Table 1**). No between-groups difference in PMI were detected (control group: 2.89 ± 0.15 hours; PD group: 2.85 ± 0.23 hours; $t(15) = 0.130$, $p = 0.898$, **Table 1**). Importantly, the Unified Lewy Body Staging scores and SN depigmentation scores were significantly greater for those with PD versus those without PD (Mann-Whitney U s = 0.000, $ps < 0.001$, **Table 1**). In fact, all of the members the PD group exhibited Lewy body pathology in the olfactory bulb, brain stem, and limbic regions; they all were classified as stage 3. In contrast, Lewy bodies were not detected in the brains of the members of the control group; they all were classified as stage 0. Eight of the 9 members the PD group exhibited severe SN depigmentation. One member of the PD group exhibited a moderate degree of SN depigmentation. SN depigmentation was not detected in the brains of those of the control group.

3.2. Total H3 and net acetylated H3

It was important to rule out the possibility of a between-groups difference in total histone H3 (tH3) immunoreactivity in the primary motor cortex. No between-groups

difference in tH3 was detected (control group: mean \pm S.E.M. = 4,262 \pm 325 arbitrary units; PD group: mean \pm S.E.M. = 4,139 \pm 273 arbitrary units; $t(15) = 0.291$, $p = 0.775$, data not shown). The acetylated histone H3 (AcH3) to tH3 ratio was used to index the level of AcH3 while accounting for individual variations in tH3. The AcH3: tH3 ratio was significantly greater in the primary motor cortex of those with PD versus those without PD ($t(15) = -2.354$, $p = 0.034$, **Fig. 1a**).

3.3. The acetylation state of specific histone H3 lysines

Since the net acetylation of histone H3 is due to acetylation at H3K9, H3K14, H3K18, and H3K23, we compared the acetylation state of each of these lysine residues in the primary motor cortex of the human PD brain to the acetylation state of these lysine residues in the primary motor cortex of brains of individuals not affected by PD. The acetylated H3K9 (AcH3K9) to tH3 ratio was used to index the level of AcH3K9 while accounting for individual variations in tH3. The AcH3K9: tH3 ratio was significantly greater in the primary motor cortex of those without PD versus those with PD ($t(15) = 2.239$, $p = 0.042$, **Fig. 1b**). The acetylated H3K14 (AcH3K14) to tH3 ratio was used to index the level of AcH3K14 while accounting for individual variations in tH3. The AcH3K14: tH3 ratio was significantly greater in the primary motor cortex of those with PD versus those without PD ($t(15) = -2.395$, $p = 0.030$, **Fig. 1c**). The acetylated H3K18 (AcH3K18) to tH3 ratio was used to index the level of AcH3K18 while accounting for individual variations in tH3. The AcH3K18: tH3 ratio was significantly greater in the primary motor cortex of those with PD versus those without PD ($t(15) = -2.286$, $p = 0.037$, **Fig. 1d**). Finally, the acetylated H3K23 (AcH3K23) to tH3 ratio was used to

index the level of AcH3K23 while accounting for individual variations in tH3. No between-groups difference was detected in the AcH3K23: tH3 ratio ($t(15) = -0.363$, $p = 0.797$, **Fig. 1e**).

3.4. Correlational analyses

Correlational analyses were performed to determine the relationship between the data collected from the individuals who participated in the present study and the acetylated protein to total protein ratios. Significant relationships were detected between Unified Lewy Body Stage score and acetylated protein to total protein ratio and SN pigmentation score and acetylated protein to total protein ratio for H3: tH3 (Unified Lewy Body Stage score: $r_s = 0.457$, $p = 0.032$; SN pigmentation stage score: $r_s = 0.458$, $p = 0.032$, **Fig. 2A, B**), H3K9: tH3 (Unified Lewy Body Stage score: $r_s = -0.409$, $p = 0.048$; SN pigmentation stage score: $r_s = -0.507$, $p = 0.019$, **Fig. 2C, D**), H3K14: tH3 (Unified Lewy Body Stage score: $r_s = 0.601$, $p = 0.005$; SN pigmentation stage score: $r_s = 0.520$, $p = 0.016$, **Fig. 2E, F**), H3K18: tH3 (Unified Lewy Body Stage score: $r_s = 0.529$, $p = 0.014$; SN pigmentation stage score: $r_s = 0.446$, $p = 0.037$, **Fig. 2G, H**), but not H3K23: tH3 (Unified Lewy Body Stage score: $r_s = -0.072$, $p = 0.392$; SN pigmentation stage score: $r_s = -0.072$, $p = 0.392$, **Fig. 2I, J**). Finally, no relationships were detected between PMI and the acetylated protein to total protein ratios ($ps > 0.05$) and expired age and the acetylated protein to total protein ratios ($ps > 0.05$).

4. Discussion

This is the first study to show a difference in histone H3 acetylation in the primary motor cortex of those affected by PD versus those unaffected by PD. Here we observed, in the PD brain, a net increase in histone H3 acetylation due to increased

H3K14 and H3K18 acetylation. In contrast, we observed a decrease in H3K9 acetylation in the PD brain. No between-groups difference was detected in H3K23 acetylation. The correlational data support the notion that the histone H3 acetylation changes observed here are not due to the PMI or aging. Instead, they are due to PD and/or factors that covary with PD. Since the acetylation of lysine residues on the H3 histone tail is thought to generally activate gene transcription [10], the data suggest enhanced gene transcription in the primary motor cortex of the PD brain due to increased H3K14 and H3K18 acetylation. Since the deacetylation of these same lysine residues is thought to generally repress transcription [10], the aforementioned effect is partially offset by a decreased H3K9 acetylation, which might repress gene transcription.

Although it is impossible to discern whether the pattern of results reported here characterize a cellular response to neurodegeneration or the phenotype of neurodegeneration-resistant cells, support for the latter notion comes from studies of histone deacetylation. Treatment with HDACIs, which produce a net increase in histone acetylation (including histone H3) and a more open and relaxed chromatin structure that favors transcriptional activation, have neuroprotective [6,7,19-21,26,30], neurotrophic [6,23,30], and anti-inflammatory effects [7,23]. For example, pretreatment with structurally dissimilar HDACIs protected dopamine cells *in vitro*, attenuated a pro-inflammatory response, and increased histone H3 acetylation [7]. HDACI treatment increased α -syn protein levels in neurons, hyperacetylated histone H3, and protected against glutamate excitotoxicity [20]. Moreover, treatment of midbrain neuron-glia cultures with HDACIs upregulated the expression of glia cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), increased histone H3

acetylation, and promoted dopamine neuron survival [30]. Finally, treatment with an HDACI attenuated motor deficits, reduced markers of oxidative stress and neuroinflammation, increased net histone H3 acetylation, and increased BDNF levels in 6-OHDA-treated rats [23].

Conclusions

Cells in the PD primary motor cortex exhibited a net increase in histone H3 acetylation, due to increased H3K14 and H3K18 acetylation, and a decrease in H3K9 acetylation. It is impossible to discern whether this pattern of results characterizes a cellular response to neurodegeneration or the phenotype of neurodegeneration-resistant cells. Future research is needed to determine if histone H3 acetylation changes occur in brain areas more profoundly affected by PD such as the SN and the striatum and if H3K14- or H2K16-specific HDAC inhibitors have the potential to restore and/or prevent loss-of-function in PD patients.

Conflict of interest

The authors have no conflict of interest to declare.

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Figure captions

Fig. 1. a, The ratio of acetylated histone H3 to total histone H3 is greater in the PD primary motor cortex. **b,** The ratio of acetylated histone H3 lysine 9 to total histone H3 is greater in the primary motor cortex of those not affected by PD. **c,** The ratio of acetylated histone H3 lysine 14 to total histone H3 is greater in the PD primary motor cortex. **d,** The ratio of acetylated histone H3 lysine 18 to total histone H3 is greater in the PD primary motor cortex. **e,** There was no difference between those affected and those unaffected by PD in the ratio of acetylated histone H3 lysine 23 to total histone H3. Parkinson's disease, PD; AcH3, acetylated histone H3; total histone H3, tH3; ratio of acetylated histone H3 to total histone, H3AcH3: tH3; acetylated histone H3 lysine 9, AcH3K9; ratio of acetylated histone H3 lysine 9 to total histone H3, AcH3K9: tH3; acetylated histone H3 lysine 14, AcH3K14; ratio of acetylated histone H3 lysine 14 to total histone H3, AcH3K14: tH3; acetylated histone H3 lysine 18, AcH3K18; ratio of acetylated histone H3 lysine 18 to total histone H3, AcH3K18: tH3; acetylated histone H3 lysine 23, AcH3K23; ratio of acetylated histone H3 lysine 23 to total histone H3, AcH3K23: tH3; * $p < 0.05$.

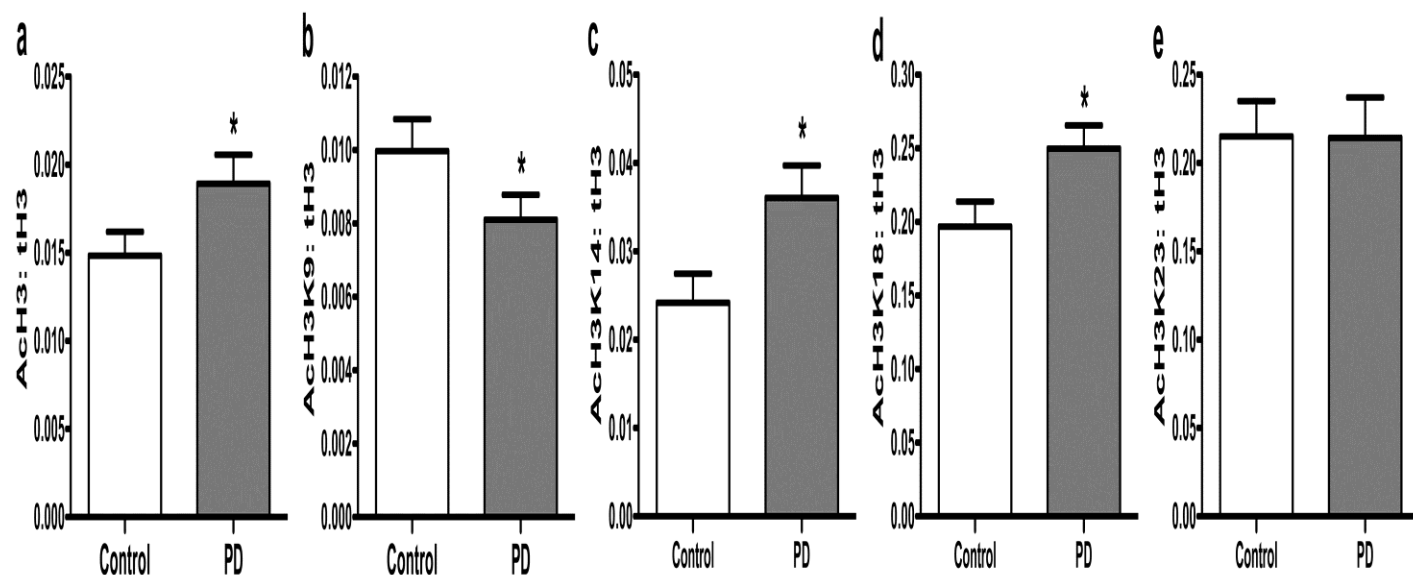


Fig. 2. A correlation matrix illustrating the relationships between Unified Lewy Body Stage score and acetylated protein to total protein ratio for H3: tH3 ($r_s = 0.457$, $p < 0.05$, **a**), H3K9: tH3 ($r_s = -0.409$, $p < 0.05$, **c**), H3K14: tH3 ($r_s = 0.601$, $p < 0.05$, **e**), H3K18: tH3 ($r_s = 0.529$, $p < 0.05$, **g**), and H3K23: tH3 ($r_s = -0.072$, $p > 0.05$, **i**) and the relationships between SN pigmentation score and acetylated protein to total protein ratio for H3: tH3 ($r_s = 0.458$, $p < 0.05$, **b**), H3K9: tH3 ($r_s = -0.507$, $p < 0.05$, **d**), H3K14: tH3 ($r_s = 0.520$, $p < 0.05$, **f**), H3K18: tH3 ($r_s = 0.446$, $p < 0.05$, **h**), and H3K23: tH3 ($r_s = -0.072$, $p > 0.05$, **j**).

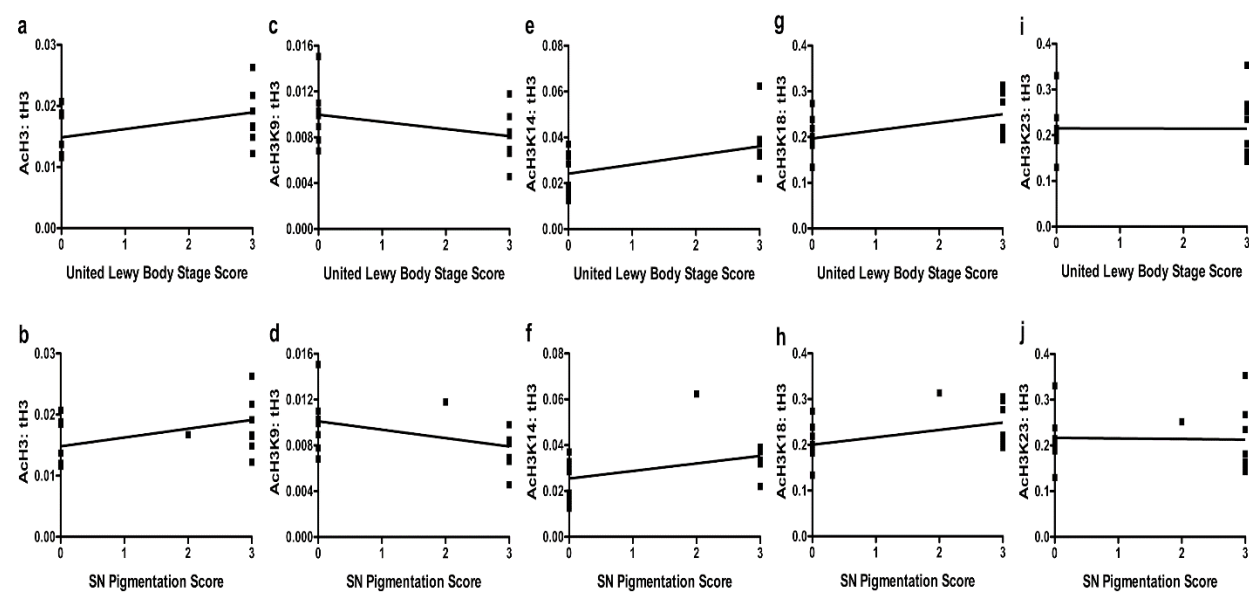


Table 1. Characteristics of the individuals who participated in the present study. Gender, 1 = male, 2 = female; expired age is the individual's age at death, data given as mean (*S.E.M.*); PMI, postmortem interval, the number of hours between death and brain removal, data given as mean (*S.E.M.*); Unified LB Stage, Unified Lewy Body Staging score, 0 = no Lewy bodies, 1 = Lewy bodies present in the brainstem only, 2 = Lewy bodies present in the brainstem and limbic system, 3 = Lewy bodies present in brainstem and limbic system and diffusely in the neocortex; SN Depigmentation, substantia nigra depigmentation, 0 = none, 1 = mild, 2 = moderate, 3 = severe.

Case ID	Condition	Gender	Expired Age	PMI	Unified LB Stage	SN Depigmentation
10-70	Control	1	74	3.25	0	0
10-63	Control	1	79	3.00	0	0
09-57	Control	1	80	3.50	0	0
08-90	Control	1	81	2.25	0	0
08-55	Control	1	71	3.00	0	0
08-40	Control	1	76	2.33	0	0
07-11	Control	2	75	2.75	0	0
01-37	Control	2	88	3.00	0	0
			78.00 (1.85)	2.88 (0.15)		
99-11	PD	1	80	2.33	3	3
99-28	PD	1	77	4.00	3	3
11-51	PD	2	87	1.92	3	3
10-45	PD	1	80	3.00	3	2
10-29	PD	1	73	2.50	3	3
10-28	PD	1	75	2.33	3	3
10-04	PD	1	81	3.55	3	3
06-44	PD	1	79	2.50	3	3
02-15	PD	2	78	3.50	3	3
			78.89 (1.33)	2.85 (0.23)		