NEUROPATHOLOGICAL CHARACTERIZATION OF FL-PGC-1α-DEFICIENT MICE – IMPLICATIONS FOR MITOCHONDRIAL ENCEPHALOPATHY

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TABLE OF CONTENT

I. INTRODUCTION

I.1. Mitochondrial respiration and adaptive biogenesis – the role of PGC-1α

Mitochondria are membrane-bound intracellular organelles evolutionary originating from the endosymbiosis of an ancient aerobic alpha-proteobacterium with an early eukaryotic host cell [1]. Harboring their own maternally inherited, double-stranded, circular genome (mtDNA), supplemented by the presence of several ancillary, structural and regulatory proteins encoded by the nuclear DNA (nDNA), mitochondria host a number of molecular processes essential for cellular life, development, aging, and death. These include the production of biologically utilizable energy through the joint function of respiratory complexes I–V within the electron transport chain (ETC) in processes involving terminal oxidation and oxidative phosphorylation (OXPHOS), the adaptive thermogenesis via the uncoupling of energy production, as well as the regulation of cellular calcium homeostasis, cell-cycle, and programmed cell death. Mitochondria are dynamic organelles capable of undergoing fission in a process in which mitochondria grow and divide in response to an increased energy demand. This process is often referred to as mitochondrial biogenesis. In addition, mitochondria are also prone to constant fusion with each other resulting in the mixture (and possibly recombination) of normal and potentially mutated genomes (‘heteroplasmy’), which, on the one hand, can protect mitochondria and their host cells from the potentially deleterious effects of mtDNA mutations, and on the other hand, somewhat counterbalances the limited possibility of genomic recombination resulting from the uniparental inheritance.

The role of PGC-1α in adaptive respiration and mitochondrial biogenesis

In multicellular organisms, the ability to adaptively regulate and activate mitochondrial biogenesis and functions in response to a variety of conditions is essential to maintain energetic homeostasis and cellular viability. Several lines of evidence obtained in the past decade suggest that peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator 1-alpha (PGC-1α), a nuclear-encoded coactivator of a wide range of transcriptional factors, plays a key role in the transcriptional cascade of such adaptive processes [2]. PGC-1α-mediated coactivation of genes such as nuclear respiratory factor 1 and 2 (NRF-1 and -2), PPARs, estrogen-related receptors (ERRs), and myocyte-specific enhancer factor 2C (MEF2C) leads to an increased

expression of a spectrum of proteins involved in mitochondrial transcription, replication, as well as the import and assembly of a number of nuclear-encoded respiratory complex subunits; furthermore, it boosts OXPHOS and thermoregulation in a tissue-dependent manner, enhances gluconeogenesis and fatty acid oxidation [3], and increases the defense against oxidative stress [4] (Figure 1). The inducing effect of physical exercise (mediated by calcineurin A-linked MEF2 activity, calcium/calmodulin-dependent protein kinase IV (CaMKIV)-linked cyclic AMP (cAMP) response element-binding protein (CREB) activity, and the p38 mitogen-activated protein kinase (MAPK)-linked activating transcription factor 2 (ATF-2) activity), as well as cold exposure and starvation (mediated by catecholamine- and glucagon-induced cAMP elevation and a subsequent phosphorylation and activation of CREB by protein kinase A (PKA)) on PGC-1α expression is well-documented [3]. Furthermore, energy deprivation through a high AMP/ATP ratio leads to an increased AMP-activated protein kinase (AMPK) activity and a subsequent phosphorylation of PGC-1α protein, priming PGC-1α for deacetylation and thereby activation by silent information regulator 2 homolog 1 (Sirt-1) [5, 6], the expression of which is also increased in conditions with energy shortage, *i.e.*, starvation or exercise, in response to a high NAD+/NADH ratio [7]. These posttranslational modifications on PGC-1α play pivotal roles in adaptive mitochondrial biogenesis. The roles of impaired mitochondrial function and more recently a decreased function of the PGC-1α cascade in the pathogenesis of degenerative central nervous system (CNS) disorders are of extensive research interest.

I.2. Mitochondrial dysfunction, free radicals, and cell death – regulation by PGC-1α

Under physiological conditions, the efficiency of reducing of oxygen during terminal oxidation is approximately 97–99%, while 1–3% undergo incomplete reduction to superoxide ($O_2^{-\bullet}$), a highly reactive free radical. $O_2^{-\bullet}$ can be transformed into hydrogen peroxide (H_2O_2) both spontaneously and through a reaction catalyzed by mitochondrial manganese superoxide dismutase (Mn-SOD) in the matrix. H_2O_2 normally undergoes degradation by glutathione peroxidase (GPX) and catalase (CAT) enzymes, yielding water.

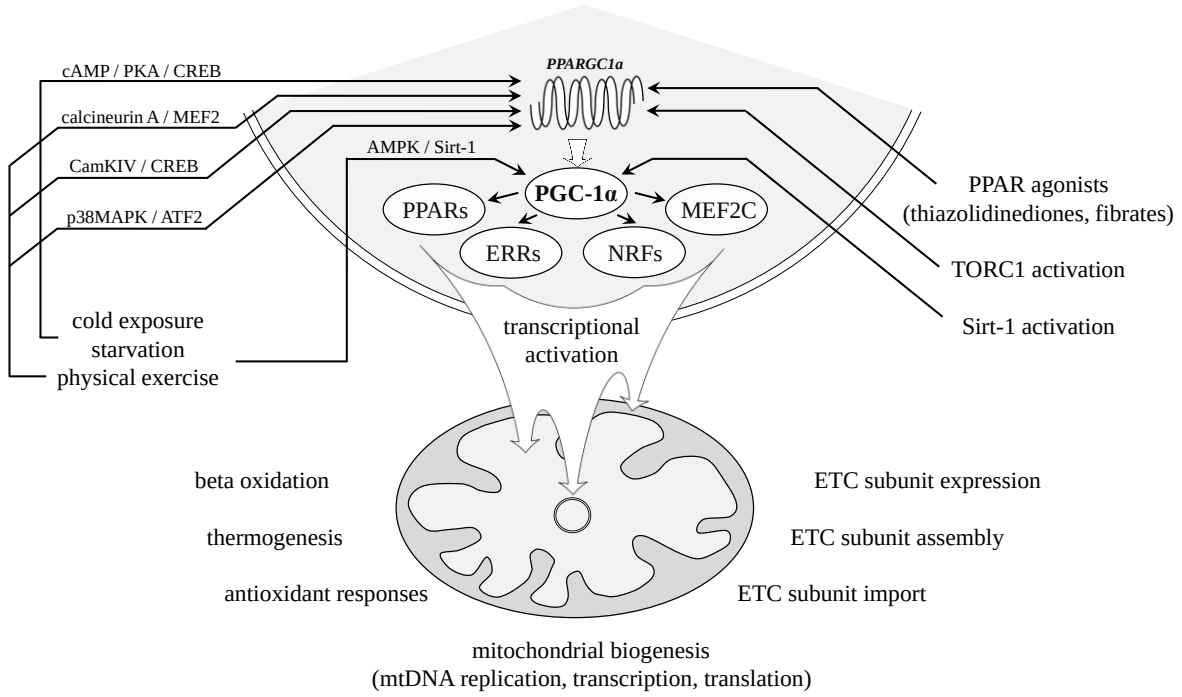

Figure 1. The PGC-1α cascade. Transcriptional upregulation or posttranslational activation of PGC-1α due to fasting, physical exercise, cold exposure, or pharmacological manipulations lead to the transcriptional activation several nuclear-encoded proteins involved in mitochondrial functioning at multiple levels, including mitochondrial biogenesis, adaptive metabolism, antioxidant responses**,** and proper ETC assembly/import. Adapted from Szalárdy et al., 2015 [2].

In case of an impaired function of the mitochondrial ETC, leakage of excess electrons from complex I and III leads to a higher amount of $O_2^{-\bullet}$ and subsequent H_2O_2 production, which when exceeding the degradative capacity of the mitochondria can be transformed into the extremely toxic hydroxyl radical ($HO^{\bullet}$), through reaction with transition metals (Fe^{2+} and Cu^{2+}; Fenton reaction). $HO^{\bullet}$ in turn can react with nucleic acids and phospholipids, yielding the formation of further toxic radicals and consequent severe functional impairments of the affected macromolecules. In addition, $O_2^{-\bullet}$ can also react with nitric oxide ($NO^{\bullet}$), yielding the highly toxic peroxynitrite anion ($ONOO^-$), which can evoke injury to proteins via nitration and nitrosylation. These toxic radicals are known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and their damaging effects on macromolecules are referred to as oxidative and nitrative/nitrosative stress, respectively.

With mitochondrial ETC being the main source of ROS and RNS production, macromolecular components of the mitochondria are extremely exposed to injury due to

oxidative/nitrative/nitrosative stress. The injury to mitochondrial respiratory complex subunits has two main consequences: 1) it leads to decreased energy production due to impaired OXPHOS; 2) it decreases the efficacy of terminal oxidation, which results in an increased production of ROS/RNS, generating a vicious circle.

The proximity to the main source of free radical production and the relatively high proportion of coding sequences render the mitochondrial genome particularly sensitive to ROS/RNS-mediated injury [8]. Indeed, the mutation rate of mtDNA relative to nDNA is approximately 10:1 [9]. Defensive processes of the mitochondria to counteract excessive free radical production involve low molecular weight antioxidants, an enzymatic redox apparatus to clear ROS/RNS (*e.g.*, SOD, CAT, GPX, and peroxiredoxin), as well as an nDNA-encoded repair machinery. Notably, the ability to cope with oxidative/nitrative/nitrosative stress declines with aging [10]; therefore, the rate of mtDNA mutations further increase in the elderly [11].

Of note, excessive ROS and RNS accumulation within the mitochondria can trigger the opening of mitochondrial permeability transition pores (mtPTP), which, on the one hand, decreases the mitochondrial membrane potential further aggravating the initial OXPHOS impairment, and, on the other hand, leads to the release of proapoptotic factors (including apoptosis-inducing factor (AIF), procaspase-9, and cytochrome *c*) from the intermembrane space to the cytosol. This is, in severe cases, followed by cellular death, which can be either apoptotic or necrotic, depending on the severity of the initial insult and subsequent energy deprivation [12]. Furthermore, mitochondrial energy deprivation leads to an overactivation of extrasynaptic *N*-methyl-D-aspartate-sensitive (NMDA) glutamate receptors, which by resulting in an excessive influx of calcium into the cytosol likewise leads to the opening of high-conductance mtPTPs, aggravating the vicious circle in a process referred to as excitotoxicity.

The role of PGC-1α in concerting antioxidant responses

A number of evidence link PGC-1α to the regulation and activation of mitochondrial antioxidant responses. In a comprehensive study [4], the expression of PGC-1α significantly increased after H_2O_2 challenge in 10T1/2 cells, which effect was recapitulated by others on C_2C_{12} muscle cells [13]. This is in correspondence with our recent findings of significantly increased PGC-1α mRNA expression in the CNS of mice intoxicated with the neurotoxin 3-nitropropionic acid (3-NP), an irreversible inhibitor of complex II [14], and with a similar

finding by others on SH-SY5Y cells using 1-methyl-4-phenylpyridinium (MPP+), an irreversible inhibitor of complex I [15]. Furthermore, RNAi against PGC-1α reduced the baseline expression of Mn-SOD (SOD2), Cu/Zn-SOD (SOD1), and GPX in 10T1/2 cells, whereas the expression of Mn-SOD, Cu/Zn-SOD, and peroxisomal CAT was found reduced in the heart and brain of PGC-1α-deficient mice [4]. Similarly, overexpression of PGC-1α in C_2C_{12} myotubes lead to increased expression of Mn-SOD and GPX, in association with decreased ROS production [16]. Moreover, PGC-1α-deficient fibroblasts exhibited blunted response to ROS challenge and an increased sensitivity to oxidative stress. This is in correspondence with an increased sensitivity of PGC-1α-deficient mice to intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a prodrug of MPP+, as well as to that with the excitotoxin, kainate [4], and to the overexpression of α-synuclein [17].

I.3. The central role of mitochondrial dysfunction in degenerative CNS diseases – the contribution and therapeutic potential of PGC-1α

A number of general observations and considerations explain the special susceptibility of the CNS to suffer injuries due to mitochondrial disturbances. Indeed, the CNS has an especially high energy demand as it represents merely 2% of the total body mass and accounts for some 20% of bodily oxygen consumption [18]. Besides, unlike astrocytes, neurons store low amounts of glycogen, and have a poor ability to enhance glycolysis under conditions when mitochondrial respiration is impaired [19]. Therefore, neurons depend on the constant availability of oxygen and glucose to maintain their functions. Furthermore, the CNS contains high amounts of polyunsaturated lipids, which are highly susceptible to oxidative injury by means of lipid peroxidation, and the antioxidant capacity of neurons is known to be relatively poor [20]. The high sensitivity of neurons as opposed to the relative resistance of astrocytes to oxygen or glucose deprivation is well known; however, recent studies suggest that oligodendrocytes are among the most sensitive cell types within the CNS to mitochondrial stress, exceeding the vulnerability of neurons [21, 22], a feature which may have implications for the pathogenesis of characteristic myelinopathies in chronic conditions with mitochondrial dysfunction, including aging and mitochondrial encephalopathies. The roles of mitochondrial dysfunction with particular focus on the involvement of PGC-1α in degenerative CNS disorders

is presented by a brief overview on the respective features of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and mitochondrial encephalopathies.

Alzheimer's disease

AD is a chronic, neurodegenerative disorder characterized by hippocampal, cortical, and basal forebrain cholinergic neurodegeneration and atrophy, associated with late-onset progressive dementia, which manifests in the loss of short-term and spatial memory and eventually most of the cortical functions. The process is accompanied by the formation of predominantly extracellular plaques of β-amyloid (Aβ) and intracellular deposits of neurofibrillary tangles constituted by hyperphosphorylated tau proteins (pTau).

A line of evidence links the dysfunction of mitochondria to the pathogenesis of AD, which include a significant decrease in mitochondrial complex IV activity [23, 24], the excessive amount of mtDNA mutations [25], and the dysregulation of mitochondrial dynamics in AD neurons [26]. Additionally to the indirect elevation of ROS production through disrupted respiration, Aβ fragments *per se* generate free radicals [27], and there is a co-localization of the dense core plaques with the fluorescent signaling of free radicals, demonstrating a direct connection between Aβ and ROS generation [28]. Importantly, impaired protein processing in AD, *i.e.*, the development of Aβ and Tau/pTau depositions, has been causatively linked to mitochondrial dysfunction, and in particular to oxidative stress [29] (Figure 2).

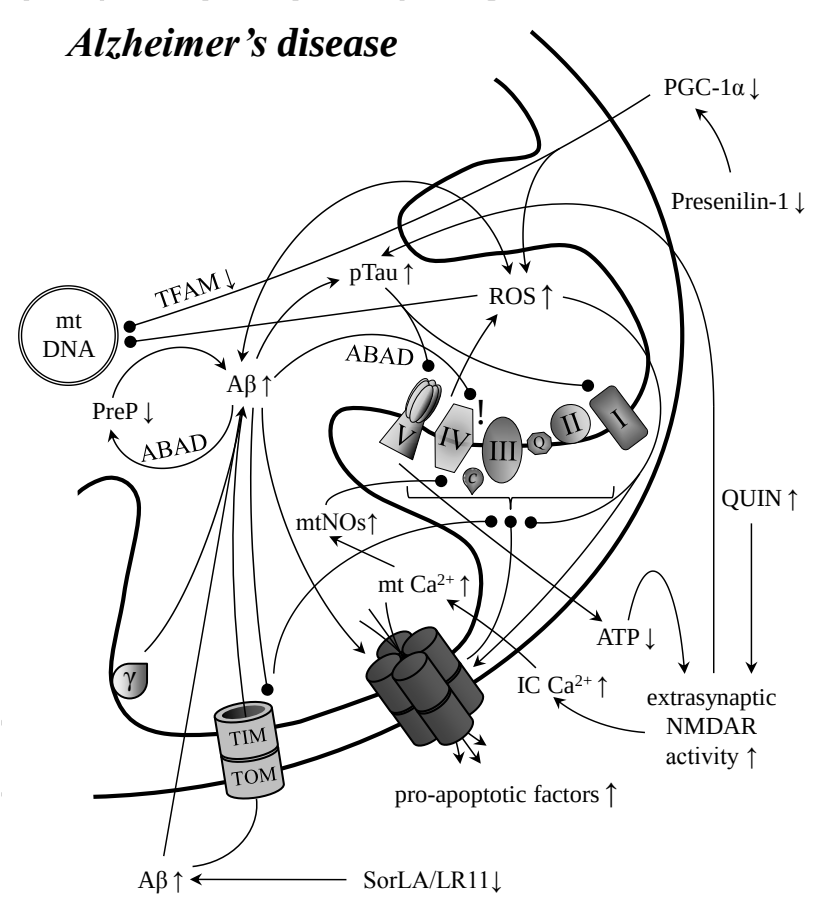

Figure 2. The involvement of mitochondrial dysfunction in Alzheimer's disease. Complex IV deficiency, the predominant ETC disorder in sporadic PD has long been linked to the deleterious effects of β-amyloid accumulation, a pathognomonic alteration in AD. Disturbed OXPHOS leads to a vicious circle eventually resulting in cell death. Novel findings link PGC-1*α* dysfunction to the pathogenesis of AD, the restoration of which may hold therapeutic value. (↑ = increased presence/expression/activity; ↓ = decreased presence/expression/activity; arrow = promotion; bulb-headed arrow = inhibition/deterioration.) Adapted from Szalárdy et al., 2012 [29].

With regard to a potential role of PGC-1α in AD, Qin et al. reported an inverse correlation of hippocampal PGC-1α protein content with the severity of cognitive decline, neuritic plaque pathology and hippocampal $A\beta_{x\text{-}42}$ burden in AD patients. This group also provided *in vitro* evidence that PGC-1α deficiency might contribute to the accumulation of AD-type Aβ in the brain via decreasing the non-amyloidogenic α-secretase activity [30]. On the other hand, impaired Presenilin-1 γ-secretase activity on amyloid precursor protein (APP) has recently been shown to downregulate PGC-1α expression in mouse embryonic fibroblasts and

in APP-deficient murine brains, contributing to decreased mitochondrial respiration and reduced levels of ATP *in vitro* [31]. Notably, exogenous upregulation of PGC-1α increased non-amyloidogenic APPα secretion and decreased amyloidogenic Aβ production via the deregulation of β-secretase in a PPARγ-dependent manner *in vitro*, a feature that may hold therapeutic relevance in AD, whereas downregulation of PGC-1α increased Aβ production [32].

Parkinson's disease

PD is a progressive, chronic neurodegenerative disorder, the pathognomonic alterations of which include loss of dopaminergic neurons and the presence of α-synuclein-rich deposits of Lewy bodies in the substantia nigra pars compacta (SNpc), with a subsequent decrease in striatal dopamine levels [33]. The leading clinical symptoms include bradykinesia, rigidity, resting tremor, and postural instability, evolving into severe akinesia, dementia, and eventually death.

The development of sporadic PD is linked to a complex interplay of genetic and environmental factors, which have multiple implications for mitochondrial involvement (Figure 3). The first implication for the role of mitochondrial dysfunction in PD came from serial cases of intoxication by the side-product of a synthetic illicit drug, MPTP, which evokes parkinsonian symptoms and recapitulates the majority of PD-related pathologies [34]. Its active metabolite MPP^+ selectively and irreversibly impairs the function of mitochondrial complex I in dopaminergic neurons [35, 36]. Similar effects can be achieved by known environmental chemicals including the herbicide paraquat and the insecticide rotenone [37]. Corresponding with the ability of complex I inhibitors to evoke parkinsonism, a decreased activity and/or expression of respiratory complex I has been detected in the SNpc [38, 39], striatum [40] frontal cortex [41], platelets [42, 43], and skeletal muscle [44, 45] of sporadic PD patients, suggesting a systemic impairment of mitochondrial functions in this disease. In addition to the apparent involvement of mitochondrial dysfunction in sporadic PD, the majority of monogenic familial forms of PD are linked to a set of genes having direct implications in mitochondrial dysfunction (*i.e.*, parkin, PINK1, DJ-1, LRRK2, and SNCA (encoding α-synuclein) [29].

Alpha-synuclein, the main constituent of Lewy-bodies, may have central roles in PD, as its mitochondrial accumulation results in complex I inhibition [46, 47], a pathognomonic alteration in PD. The protein appears to play pivotal roles in modulating oxidative stress, as its transgenic overexpression leads to enhanced sensitivity against intoxication with paraquat and MPTP [48], whereas α-synuclein-deficiency leads to resistance against intoxication with MPTP and other mitochondrial toxins in mice [49]. Notably, there seems to be a bidirectional relationship between mitochondrial dysfunction and impaired protein handling in PD, as mitochondrial dysfunction itself can lead to the formation of α-synuclein inclusion bodies [50].

An increasing body of evidence suggests that PGC-1α may add important contributions to the pathogenesis of PD. Indeed, a comprehensive genome-wide meta-analysis found a set of 425 PGC-1α-responsive nuclear-encoded mitochondrial genes underexpressed in sporadic PD, representing pinpoint defects in glucose metabolism and mitochondrial ETC [51]. Furthermore, associations of single nucleotide polymorphisms (SNPs) of PGC-1α has been reported with the risk of PD, the age of onset, and the longevity [52]. These appear to be in correspondence with the decreased expression of PGC-1α and its target gene NRF-1 in the SN and striatum of PD patients as well as in the midbrain of conditional parkin knockout mice [53]. In line with a decreased ATP production and impairments in mitochondrial OXPHOS [54] and antioxidant responses [4], mice deficient in PGC-1α have enhanced susceptibility to MPTP- [4] and α-synuclein-induced toxicity [17], with alterations in mitochondrial dynamics preferentially affecting nigral dopaminergic neurons [17]. Corresponding to the observations that mitochondrial dysfunction can promote the aggregation of α-synuclein [50], the reduced expression of PGC-1α *in vitro* lead to enhanced α-synuclein oligomerization [55].

Calling for a potential therapeutic relevance in PD, the overexpression of PGC-1α demonstrated neuroprotection against α-synuclein-induced toxicity *in vitro* [51, 56] and in PGC-1α-deficient mice *in vivo* [17], against MPP+ [15] and rotenone toxicity *in vitro* [51], and in a parkin interacting substrate (PARIS) overexpression model of PD *in vivo* [53]. In line with these, transgenic overexpression and resveratrol-induced activation of PGC-1α (via deacetylation by Sirt-1) both rendered neuroprotection against MPTP toxicity in mice [57]. Similarly, the PPARγ agonist pioglitazone, capable of enhancing the activity and expression of PGC-1α [58], was also protective in MPTP studies [59, 60]. However, contrasting preclinical results have also been published [61, 62], and, disappointingly, a phase II safety and futility

clinical trial with pioglitazone on patients with early PD has recently demonstrated no clear benefit compared to placebo with the doses applied (NCT01280123) [63].

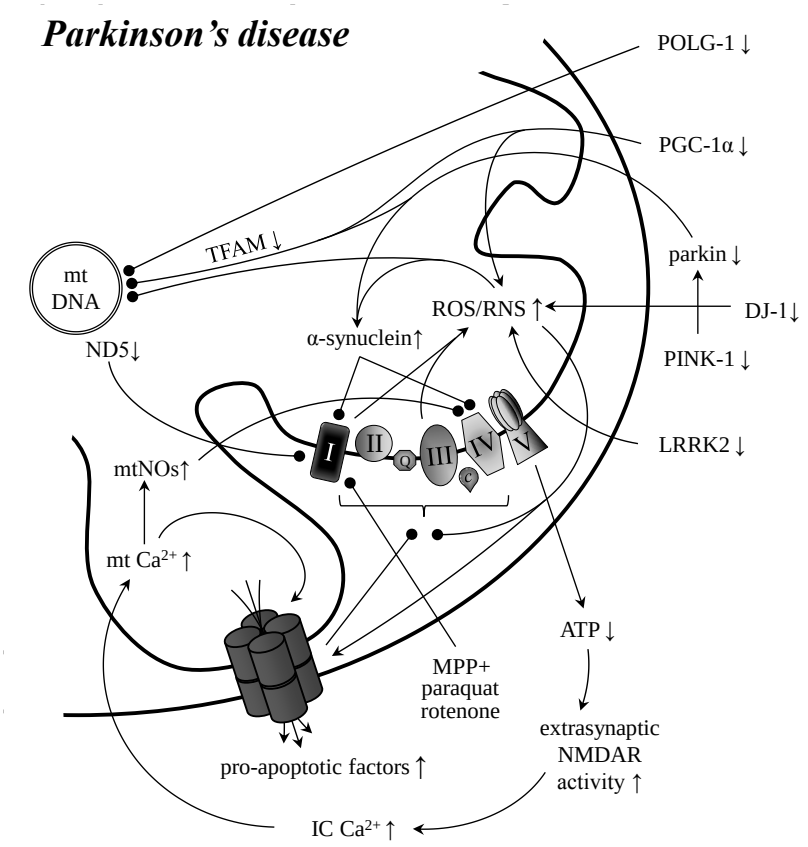

Figure 3. The involvement of mitochondrial dysfunction in Parkinson's disease. Complex I deficiency, the predominant ETC disorder in sporadic PD is linked to the deleterious effects of α-synuclein aggregation, a pathognomonic alteration in PD, and inhibitors of complex I (such as MPTP, rotenone, and paraquat) are used in experimental modeling of PD. Most of the genes associated with familial PD have direct implications in mitochondrial dysfunction. Disturbed OXPHOS leads to a vicious circle eventually resulting in cell death. Novel findings link PGC-1α dysfunction to the pathogenesis of sporadic PD, the restoration of which may hold therapeutic value. (↑ = increased presence/expression/activity; ↓ = decreased presence/expression/activity; arrow = promotion; bulb-headed arrow = inhibition/deterioration.) Adapted from Szalárdy et al., 2012 [29].

Huntington's disease

Huntington's disease (HD) is a monogenic, progressive neurodegenerative disease of autosomal dominant inheritance. The genetic alteration is the expansion of CAG trinucleotide repeat sequence on the interesting transcript 15 (IT15) gene on chromosome 4 encoding huntingtin, with increasing number of repeat associating with earlier onset and more rapid progression [64]. The disease onset is usually between 40-50 years of age, presenting with behavioral alterations and hyperkinetic movement disorders (*i.e.*, chorea, ballism) in the early stages, subsequently associating with pyramidal symptoms, dystonia, dementia, and psychosis. The pathognomonic alteration is the preferential loss of the striatal γ-aminobutyric acid (GABA)-ergic medium-sized spiny projection neurons (MSNs), and the presence of intracytoplasmic and intranuclear protein inclusions of mutant huntingtin, widely distributed in neuronal as well as extraneuronal tissues.

The characteristic decreased activity of respiratory complex II, especially in the striatum, has early linked HD to mitochondrial dysfunction [65], an alteration that appears to be crucial in HD, as its overexpression demonstrated marked restorative effect in animal models [66-68]. In line with these, irreversible or reversible inhibition of complex II by 3-NP [69, 70] and malonate [71], respectively, effectively recapitulates most of the clinical and histopathological characteristics of HD. Supportive data for a primary role of mitochondrial dysfunction in mediating the effects of mutant huntingtin include an increased presence of oxidative stress [72, 73], an increased amount of mtDNA mutations [74], disturbances in mitochondrial trafficking [75], a gradually decreasing mitochondrial number [76], and an impairment of mitochondrial calcium handling [77] observed in HD patients, with an enhanced sensitivity to calcium-induced opening of mtPTP and cytochrome *c*-mediated cell death [78, 79] (Figure 4).

A PGC-1α-dependent thermoregulatory defect in transgenic HD mice was the first to suggest the contribution of PGC-1α- dysfunction in the pathogenesis of HD [80]. Indeed the expression of PGC-1α has been found downregulated in the striatum of HD patients [76, 80-82], in transgenic HD animals [14, 80, 81, 83-85], and in *in vitro* HD models [81, 82, 85, 86]. Correspondingly, the decreased expression of several PGC-1α target genes have been identified in the striatum of HD patients [76, 80] and transgenic HD mice [80, 83, 84]. A possible

mechanism through which mutant huntingtin can lead to the downregulation of PGC-1α can be secondary to its effect to enhance the expression [87] and activity of NR2B subunit-containing NMDA receptors [88], features characteristic of transgenic HD mice [89], which in turn results in a decreased striatal CREB signaling [89], and a subsequent downregulation of PGC-1α [90].

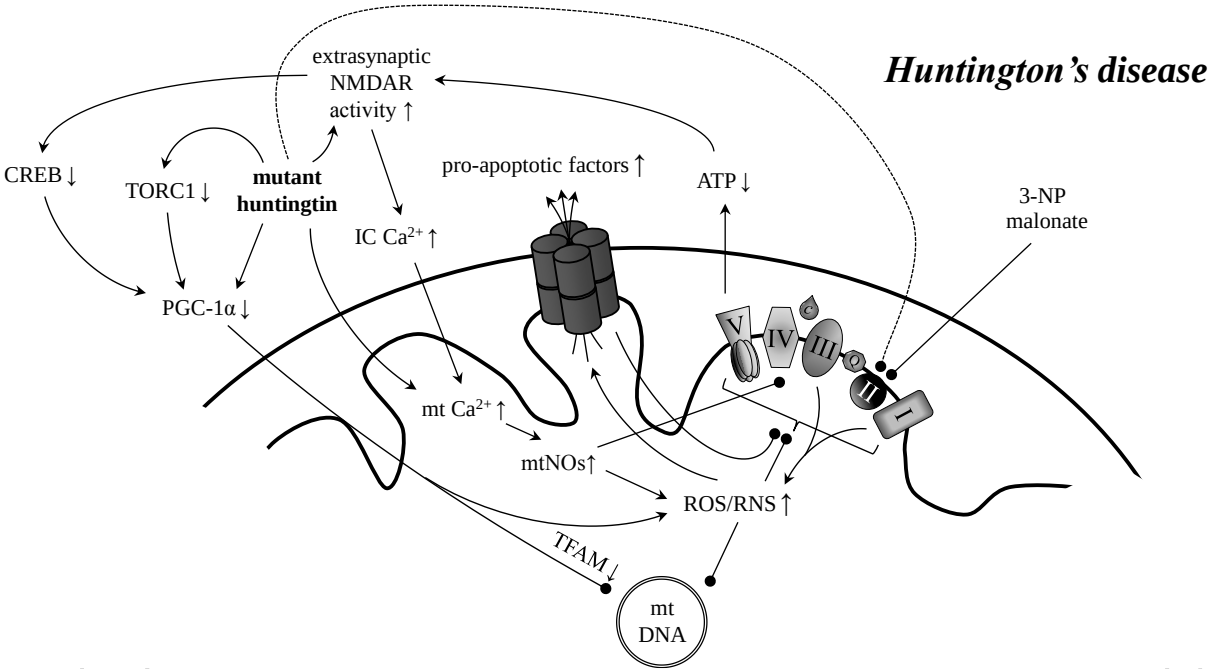

Figure 4. The involvement of mitochondrial dysfunction in Huntington's disease. Complex II deficiency, the predominant ETC disorder in HD has long been linked to the deleterious effects of mutant huntingtin aggregation, a pathognomonic alteration in HD, and inhibitors of complex II (such as 3-NP and malonate) are used in experimental modeling of the disease. Disturbed OXPHOS leads to the development of a vicious circle, eventually resulting in cell death. Novel findings link PGC-1*α* dysfunction to the pathogenesis of HD at multiple levels, the restoration of which may hold therapeutic value. (↑ = increased presence/expression/activity; ↓ = decreased presence/expression/activity; arrow = promotion; bulb-headed arrow = inhibition/deterioration.) Adapted from Szalárdy et al., 2012 [29].

A possible mechanism through which mutant huntingtin can lead to the downregulation of PGC-1α can be secondary to its effect to enhance the expression [87] and activity of NR2B subunit-containing NMDA receptors [88], features characteristic of transgenic HD mice [89], which in turn results in a decreased striatal CREB signaling [89] and a subsequent downregulation of PGC-1α [90]. In addition, a decreased expression of transducer of regulated CREB-binding protein 1 (TORC1), an activator of CREB-mediated PGC-1α expression, has been found in *post mortem* HD striatum, in transgenic HD mice and in an *in vitro* HD model, which may contribute to the downregulation of PGC-1α expression in HD [86], whereas others suggest that PGC-1α repression may be secondary to the downregulation of PPARγ in HD [85]. Corresponding with the concept of an important contribution, polymorphisms in the PPARGC1a gene encoding PGC-1α have been found to modify the age at onset of HD [91, 92].

Supporting a potential therapeutic relevance, a line of evidence suggests that mechanisms associated with the upregulation of PGC-1α can exert neuroprotection in experimental models of HD. Indeed, PPARγ agonists thiazolidinediones (such as rosiglitazone and pioglitazone) were proven to be protective in transgenic [84, 85, 93], quinolinic acid-induced [94] and 3-NP-induced rodent [95], and in *in vitro* models of HD [85, 93, 96]. Similarly, the pan-PPAR agonist, bezafibrate, exerted protection in transgenic HD mice [97]. Furthermore, TORC1 activation displayed protective and restorative effects on viability and mitochondrial functions in a striatal HD cell line model exposed to 3-NP [86]. Likewise, transgenic overexpression or Sirt-1 overexpression-induced upregulation of PGC-1α restored the diminished mitochondrial number in 120Q huntingtin-overexpressing cortical neurons [56]. The protective effect of resveratrol, a polyphenol with potent Sirt-1/PGC-1α-activating properties, has also been demonstrated in transgenic murine and nematode models [98], in a 3-NP-induced murine model [99], and in an *in vitro* model of HD [98]. A phase III clinical trial with resveratrol in HD is currently recruiting its participants (NCT02336633).

Mitochondrial spongiform encephalopathies

Mitochondrial diseases are a group of multisystemic disorders where the characteristic pathologies affecting organs with high energy demand (*i.e.*, brain, liver, heart, skeletal muscle, and kidney) are due to mitochondrial dysfunction as a consequence of a sporadic or inherited genetic alteration either in the mtDNA or in the nDNA. The deleterious loss of functions may affect several components of proper mitochondrial functioning, including genes encoding respiratory complex subunits, proteins responsible for mtDNA transcription/translation, mitochondrial tRNAs and rRNAs, as well as nuclear-encoded ancillary proteins of mitochondrial function [100]. The diseases are distributed to characteristic syndromes based on the clinical manifestation and the observed neuropathological alterations, including Leigh syndrome (LS), Kearns-Sayre syndrome (KSS), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neuropathy, ataxia, retinitis pigmentosa (NARP), and mitochondrial neurogastrointestinal encephalopathy (MNGIE) [101-104]. In these diseases, impaired ATP production with various defects in respiratory complexes and excess ROS production in the affected tissues have widely been documented and have been excessively reviewed [105]. Though in somewhat different patterns, mitochondrial encephalopathies are collectively characterized by various degrees of tissue vacuolation in the white (WM) and grey matter (GM) of the CNS, accompanied by region-selective reactive astrocytosis with-or without neurodegeneration (the latter case is also referred to as ‘pseudonecrosis’).

Though no mutations of PGC-1α have been directly associated with a phenotype of mitochondrial disease, considering the spectrum of roles of PGC-1α in regulating and promoting mitochondrial functions and the fact that a number of genes involved in disease-causing mutations and/or that involved in modeling mitochondrial disease have direct or indirect interactions with PGC-1α (*e.g.*, adenine nucleotide translocator-1 (ANT-1), mtDNA polymerase γ 1 (POLG1), Tfam, NRF-1, NRF-2, PPARs, ERRs, Mn-SOD, and CREB), the rationale for PGC-1α induction to provide symptomatic benefit in these currently intractable groups of diseases can be accepted [106]. Indeed, transgenic or bezafibrate-induced overexpression of PGC-1α delayed the onset of symptoms in a cytochrome *c* oxidase-deficient murine model of mitochondrial mypopathy [107]. Similarly, transgenic overexpression of PGC-1α ameliorated the phenotype and increased the activity of mitochondrial respiratory

complexes in POLG1 mutant 'Mutator' mice [108]. Furthermore, adenoviral overexpression of PGC-1α partially restored respiratory deficits in fibroblasts obtained from patients with mitochondrial disease of various origin (though to different efficacy) and in MELAS cybrids [109]. These together suggest a potential therapeutic relevance of boosting mitochondrial biogenesis via PGC-1α-mediated approaches in diseases with a genetic mitochondrial disorder.

I.4. PGC-1α-deficient animals – intriguing contradictions

PGC-1α has recently been described to undergo an alternative 3' splicing between exons 6 and 7, which produces an in-frame stop codon at amino acid 268, resulting in a shorter but functionally active splice variant of PGC-1α called N-truncated or N-terminal fragment of PGC-1α (NT-PGC-1α; comprising 267 amino acids) [110] (Figure 5). In the past years, two independently developed whole-body knockout murine strains of PGC-1α have been generated. Despite its well-established contributions in mitochondrial functions, the absence of PGC-1α is compatible with life. Correspondingly, the developed knockout mice are viable and fertile [111, 112], suggesting the presence of functional compensatory pathways. Importantly, the first whole-body knockout strain generated by Lin et al. represents a complete knockout of PGC-1α [111], displaying no residual expression of any fragments of the protein (PGC-1α -/-). Meanwhile, the parallel-developed whole-body knockout strain described by Leone et al. [112], however, lacks the expression of the full-length protein (FL-PGC-1α -/-) but readily expresses a slightly shorter form of the splice variant NT-PGC-1α, denoted as NT-PGC-1α^{254}, which has recently been shown to be functionally identical with the complete NT splice variant [113] (Figure 5). Notably, both PGC-1α whole-body knockout strains were reported to display astrogliosis and tissue vacuolation in the brain, predominantly in the striatum, which led to the conclusion that PGC-1α deficiency may model neurodegeneration, more particularly HD. These appeared to be in correspondence with early findings of reduced striatal expression of PGC-1α and its target genes in the striatum of HD patients [80, 81] as well as in its transgenic *in vivo* [14, 80, 81] and *in vitro* models [80, 81], as discussed above. It must be noted, however, that no reports have yet provided direct evidence of striatal neuronal loss in PGC-1α-deficient mice. Notably, a number of interesting differences were observed between the two strains, including an intact hepatic gluconeogenesis and the adult onset obesity seen in FL-PGC-1α -/- mice. More intriguingly, in the first pioneering publication, the complete PGC-1α -/- mice were

reported to exert hyperactivity, a behavioral feature which was interpreted as HD-like hyperkinetic phenotype [111]. Contrastingly, the second pioneering publication on FL-PGC-1α -/- mice reported hypomotility and weakness [112].

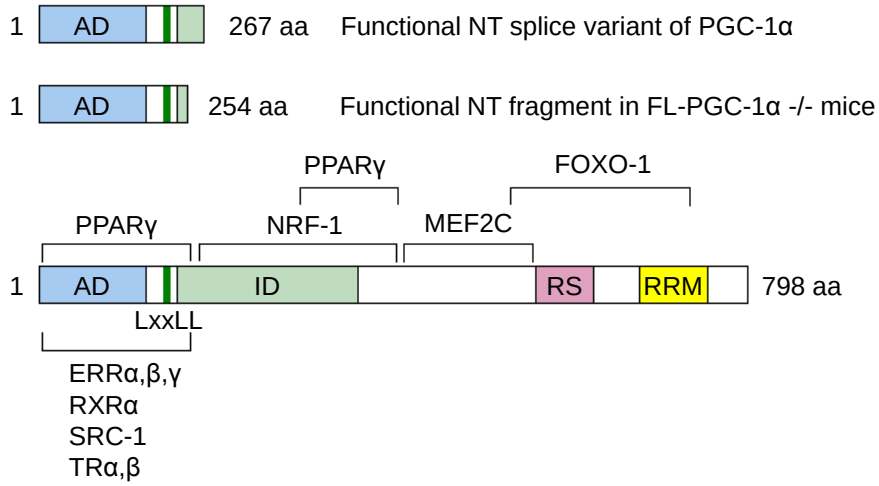

Figure 5. Full-length (FL) and N-truncated (NT) isoforms of PGC-1α protein, and the residual fragment expressed in FL-PGC-1α -/- mice. While a number of coactivator domains are functional in the NT isoform, coactivator domains of target proteins essential in mitochondrial biogenesis and respiration are apparently lacking. Adapted and modified from Knutti and Kralli, 2001 [114].

II. OBJECTIVES

In light of the above-reviewed literature about the contribution of mitochondrial and in particular PGC-1α-related dysfunction in the different aspects of the pathogenesis of degenerative CNS diseases, our objectives were to further explore and characterize the effects of PGC-1α deficiency *in vivo* along two pillars.

1) Based on the concordant data linking mitochondrial dysfunction and PGC-1α deficiency to the dysregulation of neuronal protein processing and handling in neurodegenerative diseases (*i.e.*, demonstrated enhancement of accumulation of α-synuclein [55] and β-amyloid [30] due to PGC-1α deficiency in *in vitro* models of PD and AD, respectively), we performed a systematic neuropathological characterization of adult mice lacking the expression of FL-PGC-

1α, with special focus on the immunohistochemical pattern of neurodegeneration-related proteins.

2) PGC-1α-deficient animals were reported to exhibit vacuolation and reactive gliosis in the striatum [111, 112] accompanied by hyperkinetic behavior [111], which were interpreted as features typical of HD. As neither brain vacuolation (which was, however, not isolated to the striatum [111]) nor hyperkinetic behavior is typical feature of transgenic or systemic toxin-induced model mice of HD, our aim was to create a comprehensive neuropathological lesion profile of FL-PGC-1α -/- animals, characterizing neuronal, axonal, astroglial, oligodendroglial, and myelinic pathologies. As the initial results linked the neuropathological phenotype to mitochondrial disease, the animals were systematically compared with human brain tissue samples from a case with definite mitochondrial encephalopathy.

III. MATERIALS AND METHODS

III.1. Animals

FL-PGC-1α -/- mice developed on C57Bl/6J background were generated in Kelly Lab (Sanford-Burnham Institute for Medical Research at Lake Nona, Orlando, FL, USA) [112] and bred further in the Department of Neurology, University of Szeged. The animals were housed in cages (maximum 4 per cages) in standard conditions with 12-12 h light-dark cycle and *ad libitum* access to standard pellet food and water. The experiments were performed in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the local animal care committee.

III.2. Histopathology, immunohistochemistry, and electron microscopy

For the first set of neuropathological work-up, 15 FL-PGC-1α -/- (8 male, 7 female) and 16 (8 male, 8 female) age-matched 30-week-old wild-type C57Bl/6J mice were involved. Brains were removed on ice and halved at the midline immediately following decapitation. Half brains were fixed in 4% paraformaldehyde overnight and kept in 10% glycerol in 4°C until embedding in paraffin. The other half of the brains were used for frozen sections (*i.e.*, for Oil Red O staining). 4-μm-thick sagittal sections of paraffin-embedded murine brain halves were

evaluated. We performed the stainings at two levels, one in the level containing the septal nuclei, the medial thalamus, and the retroflex fascicle, and one in the level of the caudate-putamen, containing also the substantia nigra. In addition to Hematoxylin and Eosin (H&E), Klüver-Barrera (Luxol and Fast red), and Bielschowsky silver stainings, the following monoclonal antibodies (cross-reacting with mouse) were used for immunohistochemistry: anti-pTau AT8 (pS202/pT205, 1:200, Pierce Biotechnology, Rockford, IL, USA), anti-α-synuclein (1:10.000, clone 4D6, Signet, Dedham, MA, USA), anti-Aβ (1:50, clone 6F/3D, Dako, Glostrup, Denmark), anti-APP (1:500, Millipore, Billerica, MA, USA), and anti-prion protein (PrP; 6H4, 1:1000, Prionics, Schlieren, Switzerland; epitope 144-152). Furthermore, the following polyclonal antibodies were used: anti-ubiquitin (1:1000, Dako), anti-glial fibrillary acidic protein (GFAP, 1:3000, Dako) anti-TDP-43 (1:100, ProteinTech Group, Chicago, IL, USA), anti-tau (1:100, Dako; cross-reacts with the tau-equivalent protein in mouse), and anti-FUS (1:1000, Sigma-Aldrich, St. Louis, MO, USA) antibodies. The DAKO EnVision detection kit, peroxidase/DAB, rabbit/mouse (Dako) was used for visualization of antibody reactions. When applying mouse antibodies, we used the M.O.M. kit (Vector Laboratories, Burlingame, CA, USA) to prevent the aspecific background staining of endogenous mouse immunoglobulins. As positive controls for the immunostaining, we used tissue sections from human AD (pTau and Aβ), PD (α-synuclein) and frontotemporal lobar degeneration (FTLD) with TDP-43 or FUS inclusions (TDP-43 and FUS) as well as tissue sections from scrapie-infected mice (PrP, RML strain, Hamilton, MT, USA).

During neuropathological profiling, alterations (vacuolation and astrogliosis) were semiquantitatively evaluated in several anatomical regions (Table 1). Respecting the fact that vacuole-like alterations may also develop due to histological preparatory processes, vacuoles were scored 0–4 as follows (Figure 6A): 0) No vacuoles or scattered vacuole-like alterations but not more than 1 per visual field at 40x magnification; 1) Scattered vacuole-like alterations but not more than 3 per visual field at 40x magnification; 2) Mildly vacuolated lesion(s) with less than 10 vacuoles per visual field at 40x magnification; 3) Moderately vacuolated lesion(s) with less than 20 vacuoles per visual field at 40x magnification; 4) Severely vacuolated lesion(s) with more than 20 vacuoles per visual field at 40x magnification. Astrogliosis were scored 0-3 as follows (Figure 6B): 0) No astrocytes or scattered resting asctrocytes; 1) Cloudy positivity with no signs of reactivation; 2) Moderate astrogliosis with multiple reactive astrocytes in a

patchy distribution; 3) Severe astrogliosis with abundant reactive astrocytes in a confluent pattern.

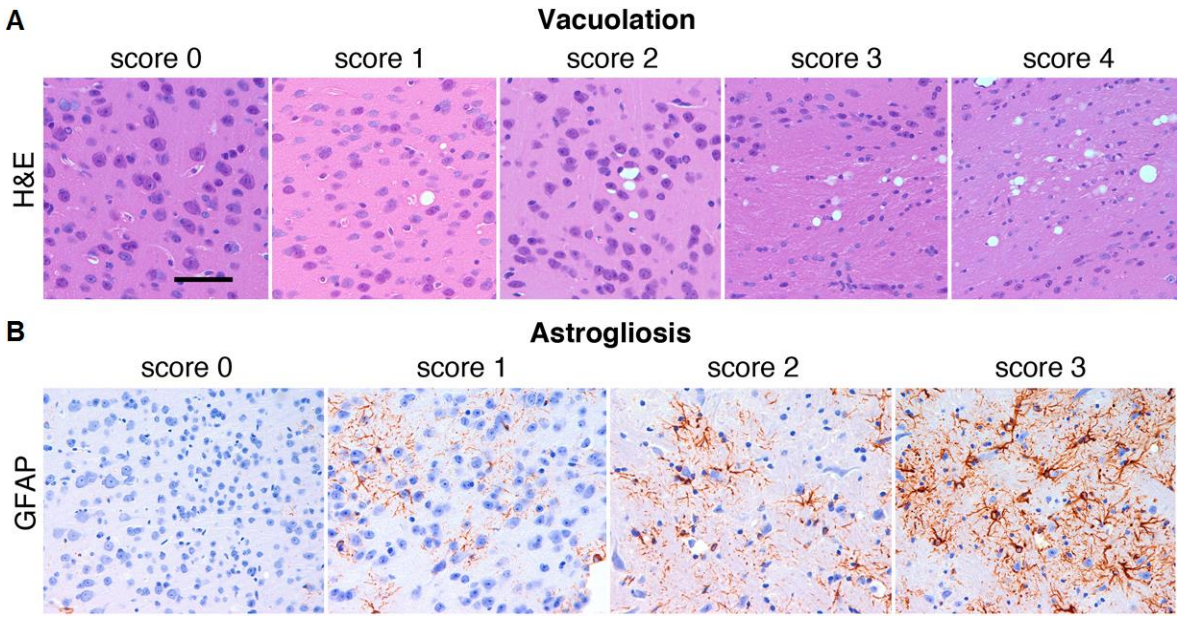

Figure 6. Representative images corresponding to the semiquantitative scores used to evaluate vacuolation (**A**) and astrogliosis (**B**) in FL-PGC-1α -/-. Bar in a represents 20 μm. Original publication in: [115].

For the second set of neuropathological work-up, 8-8 remarkably older 70-75-week-old FL-PGC-1α -/- and age-matched wild-type C57Bl/6J male mice were sacrificed and evaluated with half brains prepared and fixed as above. A total of 6 (four 30-35-week-old, one 70-week-old and one 100-week-old) FL-PGC-1α -/- animals and 3 wild-type (one from each age group) used for electron microscopy; animals were anesthetized with isoflurane and were perfused transcardially with modified Hamori fixative (1.5% glutaraldehyde and 1% formaldehyde in phosphate buffer) for 18 min with a 10 ml/min flow, prior to decapitation, subsequent postfixation, and sample preparation.

Based on the results of the first set of neuropathological examinations [115], the alterations were systematically compared with corresponding samples from human brain tissue

of a male patient with a mitochondrial disease died at 22 years of age, who carried a 4.9 kbp common deletion in mtDNA with a heteroplasmy ratio of 50%, characteristic of KSS. The mother gave informed consent prior to the neuropathological work-up, and the study was adhered to the tenets of the most recent revision of the Declaration of Helsinki.

Formalin-fixed paraffin-embedded blocks of different regions of the KSS brain, including several neocortical areas, basal ganglia, thalamus, hippocampus, amygdala, brainstem, and cerebellum, as well as a double hemispheric block at the levels of WM lesions were examined. For electron microscopy, human KSS samples from the basal ganglia (internal capsule) and WM lesions were immersion-fixed in 4% glutaraldehyde for 3 days. Human and murine brain sections for conventional histology were stained with Klüver-Barrera and Oil Red O stainings. The murine brains were subjected to staining with anti-pTau AT8, anti-α-synuclein, anti-Aβ monoclonal, and anti-ubiquitin, anti-tau, anti-TDP-43, and anti-FUS polyclonal antibodies for immunohistochemical assessment of neurodegeneration-related proteins, as described above. In addition, monoclonal antibodies (cross-reacting with mouse) against APP, phosphorylated and non-phosphorylated neurofilaments (clones SMI-31 and SMI-32, markers of axons and neuronal cell bodies; 1:5000 and 1:200, respectively, Covance, Berkeley, CA, USA), TPPP/p25 (1:2000; a marker of mature oligodendrocytes [116]) and microtubule-associated protein-2 (MAP-2, marker of neuronal cell bodies and dendrites; 1:500, Millipore), as well as polyclonal antibodies against GFAP, myelin basic protein (MBP; 1:400, Dako) and anti-Iba1 (1:1000, Wako Chemicals, Osaka, Japan) were used. The DAKO EnVision detection kit was also used for visualization of antibody reactions. We used the M.O.M. kit as above where appropriate.

For electron microscopic evaluation, the obtained samples from both human and murine brains were postfixed in 1% osmium tetroxide for 1-2 h, dehydrated through a series of graded ethanols and propylene oxide, and then embedded in Embed 812 resin (Electron Microscopy Sciences, EMS 14120). Semi-thin sections were stained with toluidine blue, blocks trimmed, and ultrathin sections stained with lead citrate and uranyl acetate. Specimens were examined using a JEM-100C transmission electron microscope.

III.3. Statistical analysis

The statistical evaluation of the data was carried out by SPSS Statistics 17.0 software®. For evaluation of scored neuropathological alterations, cross-tabulation analyses of the discrete variables were performed using the Fisher's exact test. In structures large enough to be present in both the paramedian and the lateral sections, we performed a statistical analysis for the estimation of difference within the FL-PGC-1α -/- animals. A p value < 0.05 was regarded as significant. Structures with statistically significant medio-lateral difference were dealt separately; otherwise, pooled data were used for the comparative analysis of FL-PGC-1α -/- and wild-type brain structures.

IV. RESULTS

IV.1. Immunostaining for neurodegeneration-related proteins

Immunostaining for PrP revealed moderate staining of the neuropil, and for α-synuclein we observed immunopositivity in presynaptic structures. Anti-APP and anti-phospho-independent tau antibodies showed cytoplasmic immunoreactivity, while for TDP-43, FUS, and ubiquitin, nuclear staining pattern was observed. All of these were the same in wild-type and FL-PGC-1α -/- animals in any age group examined. Moreover, there was a complete lack of tangle-like structures, pretangles, glial or astrocytic tau pathology, extracellular plaques (Aβ or PrP) and TDP-43, FUS, or ubiquitin-immunopositive inclusion bodies (nuclear or cytoplasmic) in all animals and brain regions (Figure 7). This picture was virtually identical when examining 70-75-week-old FL-PGC-1α -/- mice in a subsequent experimental setting (data not shown).

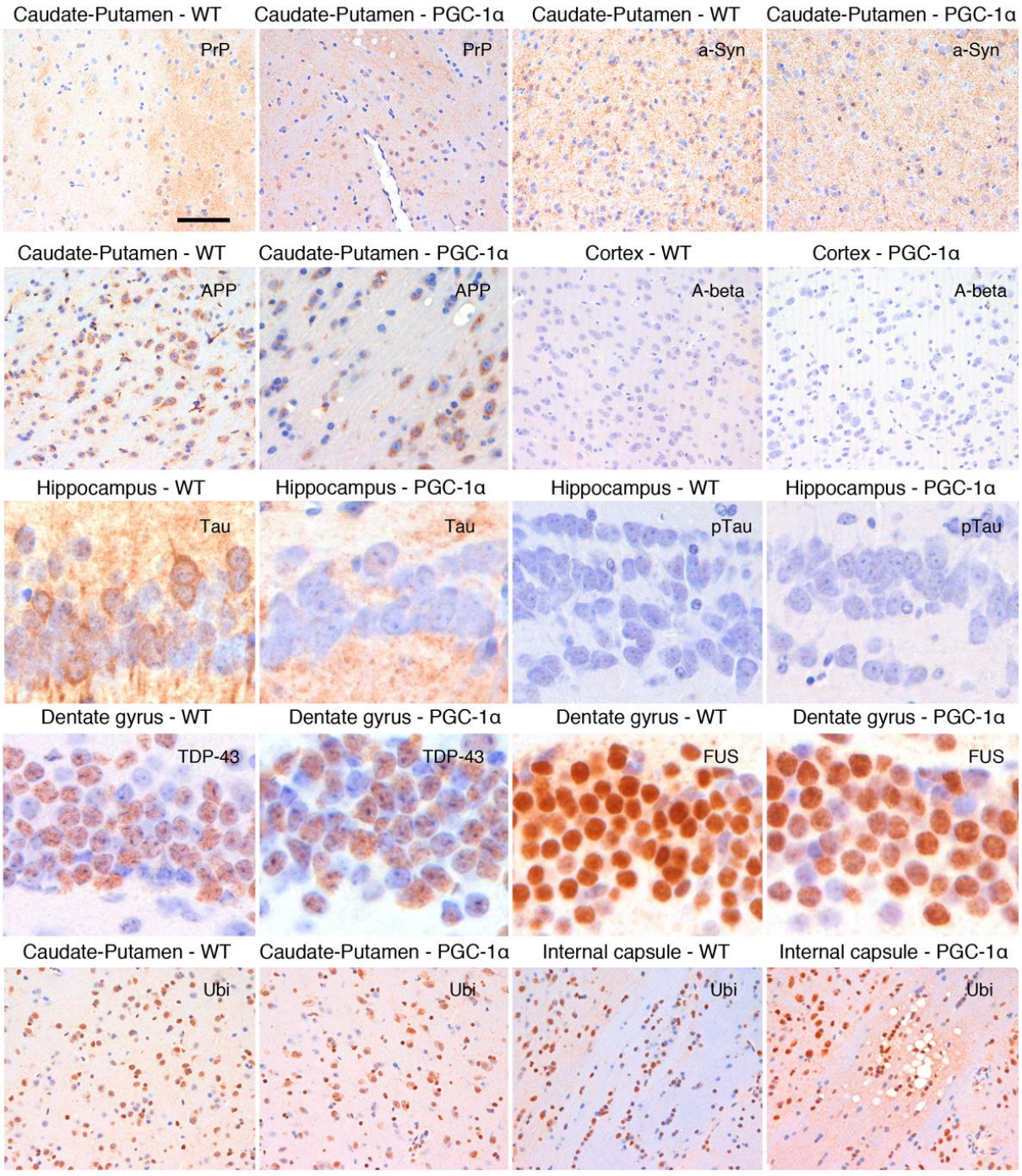

Figure 7. The absence of FL-PGC-1α expression is not associated with the accumulation of neurodegeneration-related proteins in 30-week old adult mice. Immunostaining for prion protein (PrP), α-synuclein (a-Syn), amyloid precursor protein (APP), amyloid-beta (A-beta), Tau, pTau, TDP-43, FUS, and ubiquitin (Ubi) in various anatomical regions in wild-type (WT) and FL-PGC-1α -/- (PGC-1α) animals. The representative images demonstrate the lack of pathological protein aggregation and inclusion body formation with physiological staining patterns throughout the brain of mature FL-PGC-1α -/- mice. Original publication in: [115].

IV.2. Lesion profile analysis of adult FL-PGC-1α -/- mice

At 30 weeks of age, neuropathological changes included vacuolation, predominantly in the WM, and reactive astrogliosis in certain brain regions.

Spongiform alterations were present throughout the brain in FL-PGC-1α -/- mice. The caudate-putamen was the most severely affected GM structure (Figure 8A), followed by the antero-lateral nuclei of the thalamus (reticular and ventral posterolateral area), but consistent mild-to-moderate alterations could be observed in the pontomedullary brainstem, tegmental midbrain, nucleus accumbens, globus pallidus, medial thalamus, mammillary body, substantia nigra, the cerebellar nuclei, as well as throughout the neocortex (Table 1). However, vacuolation was lacking in the cerebellar cortex, tectal midbrain, and septal nuclei. Vacuoles within the neocortex predominated in the deep cortical layers; furthermore, the analysis of cerebral cortices in the lateral sections revealed a preference towards the posterior (visual and sensory) versus the anterior (motor, insular, and piriform) cortices (Fisher's exact value = 19.184; $p < 0.001$). Spongiform change in the pontomedullary brainstem was more severe in the paramedian than in the lateral sections (Fisher's exact value = 9.282; $p = 0.018$).

In WM structures, robust vacuolation was observed in the internal capsule (Figure 8A) and the retroflex fascicle, mild-to-moderate alterations were noted in the cerebellar WM, the fimbria hippocampi, the stria terminalis, the anterior commissure, and the olfactory tract, whereas the optic tract remained consistently unaffected. Vacuolation of the myelin, which was arranged in chains in the severely affected WM regions, was not associated with apparent axonal injury, as indicated by the lack of argyrophilic axonal swellings (Figure 8B), excluding robust morphological evidence of a disturbance in axonal transport. Notably, in the hippocampus, consistent vacuolation was mainly observed in the WM bundles of the lacunosum molecular layer of the cornu Ammonis (CA)1 (Figure 8C), whereas its GM structures, the CA1-3 and the dentate gyrus, were relatively preserved. Similarly, the Klüver-Barrera staining of the caudate-putamen revealed that the vacuoles within the caudate-putamen were the most abundant in the streaming fibers of the anterior internal capsule (pencil fibers) (Figure 8C). All these raised the possibility that at least a proportion of vacuoles previously considered to be localized within the neuropil might be situated also within WM structures.

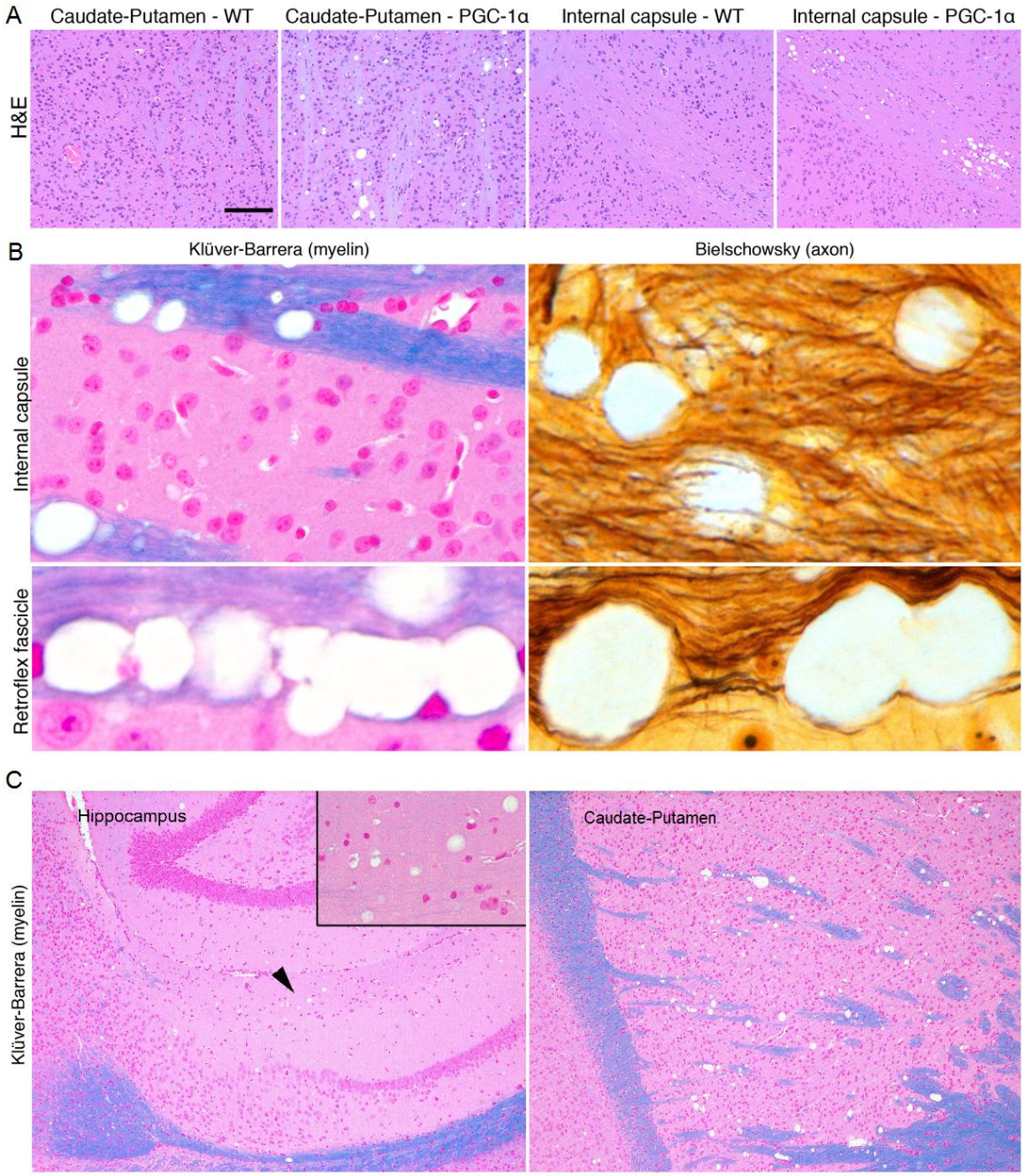

Figure 8. Vacuolation in the brains of adult FL-PGC-1α -/- mice is reminiscent of human mitochondrial leukoencephalopathies. (**A**) H&E staining in different anatomical regions in wild-type (WT) and FL-PGC-1α -/- (PGC-1α) animals demonstrates prominent vacuolation in the caudate-putamen and the internal capsule in FL-PGC-1α -/- animals. (**B**) Vacuolation in the WM (as shown by Klüver-Barrera staining on the middle left) often exhibits chain-like appearance and is associated with seemingly well-preserved axons, pushed towards the edge of the vacuoles (as shown by Bielschowsky silver staining on the middle right). (**C**) Vacuoles in the neuropil appear to associate with WM structures as revealed by Klüver-Barrera staining of the hippocampus (lacunosum molecular layer) and the caudate-putamen (pencil fibers). Original publication in: [115].

Indicative of chronic neuronal degeneration, prominent reactive gliosis was present in the brainstem and within the cerebellar nuclei of FL-PGC-1α -/- mice. This was the most striking in the paramedian sections of the pontomedullary brainstem, whereas moderate astrogliosis was noted in the midbrain, the cerebellar nuclei, and the lateral pontomedullary brainstem (Figure 9). The median predominance of the involvement of the pontomedullary brainstem was statistically significant (Fisher's exact value = 10.758; p = 0.004). No other regions of the brain displayed any signs of astrocytic reactivation. Moreover, we did not observe pathological degree of vascular proliferation in any examined region (data not shown).

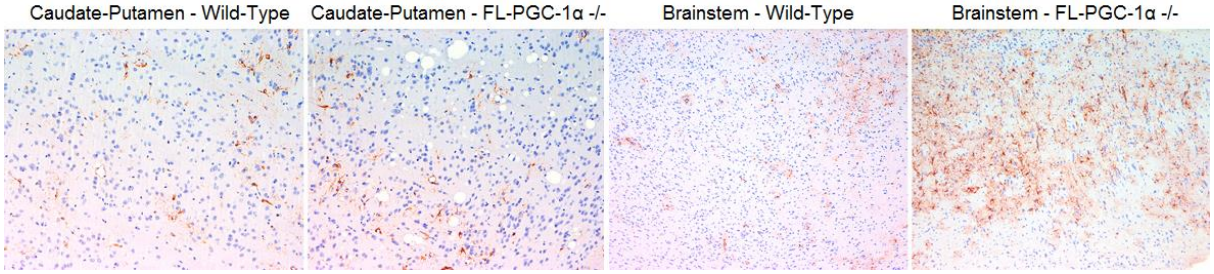

Figure 9. Immunostaining for GFAP showed reactive astrogliosis indicative of chronic neuronal degeneration in the pontomedullary brainstem and certain deep cerebellar nuclei but not in the caudate-putamen of adult FL-PGC-1α -/- animals. Original publication in: [115].

Concluding these initial results, the observed lesion profile did not recapitulate features characteristic of neurodegenerative diseases (including HD, AD, and PD) either in terms of impaired protein processing or the localization of signs of neuronal degeneration. However, with wide-spread vacuolation predominantly affecting the WM of the basal ganglia, thalamus, brainstem, and cerebellum, as well as reactive astrogliosis in the brainstem and deep cerebellar nuclei, the observed picture was remarkably reminiscent of that seen in mitochondrial spongiform leukoencephalopathies, in particular the KSS (Table 2) [115].

This prompted us to further explore and characterize the observed lesion profile in a systematic comparison of aged FL-PGC-1α -/- animals and a human brain with KSS by the use of further immunohistochemical and electron microscopic techniques, with a special focus on the origin of vacuoles [117].

Typical degree of vacuolation	Region	Mean	*p*-value
Mild	cerebellar WM	*1.833*	*< 0.001*
	pontomedullary brainstem [l] *	*1.923*	*0.005*
	substantia nigra	*1.375*	*0.004*
	hippocampus [m]	*1.133*	*0.006*
	hippocampus [l]	*1.933*	*< 0.001*
	fimbria hippocampi	*1.182*	*0.007*
Moderate	cerebellar nuclei *	*2.150*	*< 0.001*
	pontomedullary brainstem [m] **	*2.933*	*< 0.001*
	midbrain *	*2.571*	*< 0.001*
	nucleus accumbens	*2.600*	*< 0.001*
	globus pallidus	*2.692*	*< 0.001*
	mammillary body	*2.143*	*< 0.001*
	cerebral cortex	*2.305*	*< 0.001*
	thalamus [m]	*2.067*	*< 0.001*
	anterior commissure	*2.533*	*< 0.001*
	stria terminalis	*2.786*	*< 0.001*
	olfactory tract	*2.071*	*< 0.001*
Severe	caudate-putamen	*4.000*	*< 0.001*
	thalamus [l]	*3.533*	*< 0.001*
	internal capsule	*3.615*	*< 0.001*
	retroflex fascicle	*4.000*	*< 0.001*

[l] lateral section; [m] paramedian section; * accompanied by moderate astrogliotic reaction; ** accompanied by severe astrogliotic reaction.

Table 1. Lesion profile in the brains of FL-PGC-1α -/- mice. Comprehensive mapping of lesion profile in FL-PGC-1α -/- animals, demonstrating the typical degree of involvement of an anatomical region in the spongiform vacuolation and reactive astrogliosis. The differences between FL-PGC-1α -/- and wild-type animals are represented by the means and the corresponding *p* values in the respective anatomical regions. Original publication in: [115].

IV.3. Comparison of lesion profiles between human KSS and aged FL-PGC-1α -/- mice

Vacuolation

Klüver-Barrera staining revealed widespread spongiform change in both the KSS brain and 70-75-week old aged FL-PGC-1α -/- mice. In both, the vacuolation predominated in the WM; however, vacuoles in the GM neuropil were also observed with apparently lower frequency (Figure 10A-F). The vacuolation commonly affected the internal capsule, striatal pencil fibers, cerebellar WM, thalamic fascicules, pyramidal tracts, and, less intensively, the corpus callosum. Vacuoles were commonly present in the neuropil, most intensively in the brainstem, but also consistently present in the basal ganglia, thalamic nuclei, and, less intensively, in the neocortex, where they showed a predilection towards the deep cortical layers. The severity of vacuolation was more prominent in the KSS brain than in experimental animals; in some regions with coarse cystic-necrotic lesions and myelin pallor, whereas in some others with demyelinated foci (*e.g.*, postcentral region, cerebellar WM, and some of the pencil fibers) (Figure 10C and F). Demyelination and cystic-necrotic lesions were undetected in FL-PGC-1α -/- mice. Notably, the examined aged wild-type brains presented vacuoles showing similar appearance and distribution as their FL-PGC-1α-deficient counterparts; however, their frequency was remarkably lower in all examined regions (Figure 10A-B and D-E).

Astrogliosis

GFAP staining revealed moderate-to-severe reactive astrogliosis in the brainstem and cerebellum of both the KSS and the FL-PGC-1α-deficient brains (Figure 10G-I). In the KSS brain, astroglial reaction was observed in the tectal midbrain, the area of the inferior olive in the medulla oblongata, the dentate nucleus of the cerebellum, and the Purkinje cell layer (*i.e.*, Bergmann gliosis). In the FL-PGC-1α-deficient mice, severe reaction was present in the medulla oblongata and the pons often in a confluent pattern involving large areas without being limited to certain groups of nuclei, whereas mild reaction with patchy astrocytosis was observed in the midbrain and in the deep cerebellar nuclei. Notably, the caudate-putamen of FL-PGC-1α -/- mice were free of astroglial reaction even at this age (Figure 11), supporting our prior observations [115].

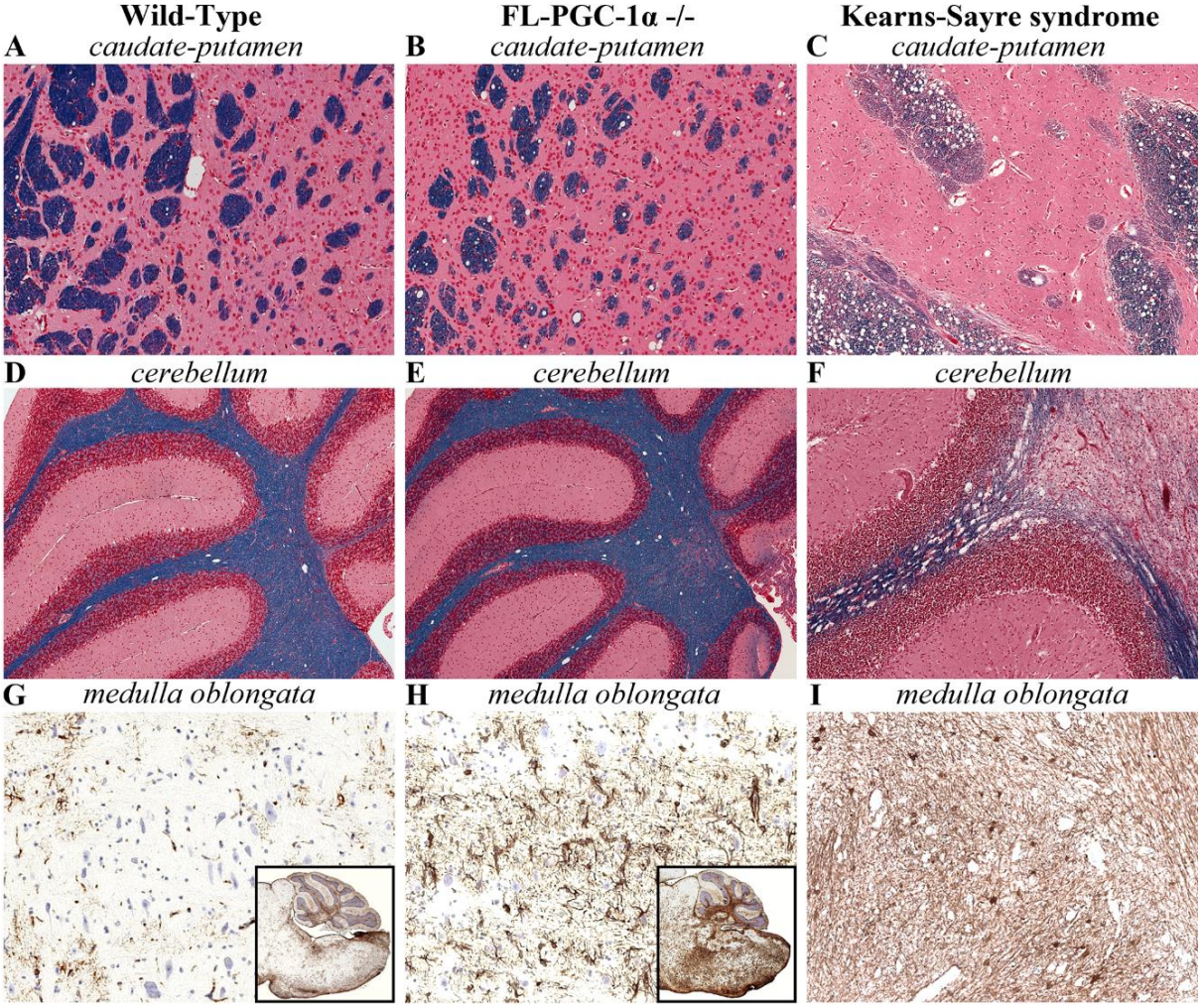

Figure 10. Vacuolation and astroglial reaction. Aged FL-PGC-1α-deficient mice develop moderate-to-severe vacuolation (**B** and **E**) in areas corresponding to severe-to-devastating vacuolation in KSS (**C** and **F**) and mild vacuolation in aged wild-type mice (**A** and **D**; Klüver-Barrera). Vacuoles are in association with WM structures. Note the patchy areas of myelin pallor in the pencil fibers of the caudate-putamen (**C**) and the demyelinated cystic-necrotic lesion in the cerebellar WM in KSS (**F**). Reactive astrogliosis indicative of neuronal degeneration is present in the brainstem of FL-PGC-1α-deficient mice and the KSS case (**H** and **I**; GFAP). Original publication in: [117].

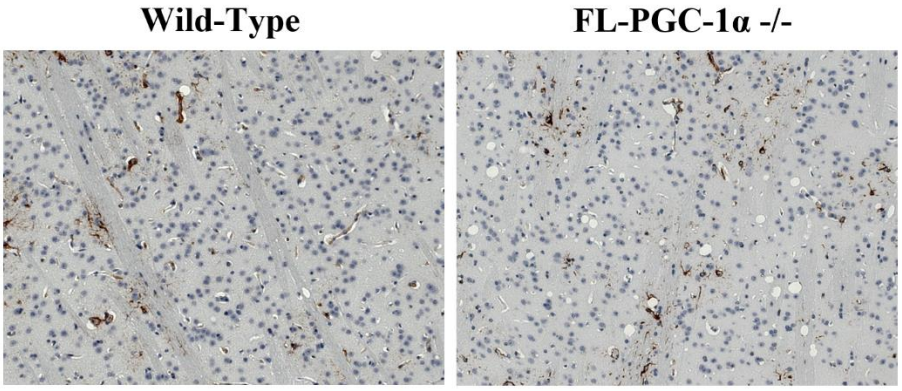

Figure 11. The caudate-putamen of FL-PGC-1α -/- mice is free of reactive astrogliosis (GFAP) even at 70-75 weeks of age, suggesting that this murine strain is not a model for HD, as it was previously suggested. Original publication in: [117].

Axonal pathology

The intensity of axonal destruction in the KSS brain was variable and generally followed that of the vacuolar change. Only the severely affected cystic-necrotic lesions showed axonal loss or swelling, whereas most of the moderately vacuolated areas were devoid of axonal pathology (Figure 12C), except for scattered swellings and APP-positive spheroids indicating acute-subacute impairment of axonal transport (Figure 12F). Regions with severe axonal involvement were accompanied by reactive microgliosis (Figure 12I). The axons in FL-PGC-1α -/- mice were generally well preserved with patterns of APP, neurofilament and microglia stainings being similar to that seen in wild-type (Figure 12A-B, D-E, and G-H).

Characterization of white matter vacuoles

Vacuoles within the WM were surrounded by rings of MBP-positive myelin in both the KSS and FL-PGC-1α-deficient brains (Figure 13A). The vacuoles were usually ovoid with their longitudinal axis paralleling the direction of axons. They often formed chain-like structures in longitudinal or sieve-like lesions in transverse sections. No apparent macrophage activity was present even in the areas of severe vacuolation, and no signs of active myelin degradation could be detected by Oil Red O (not shown).

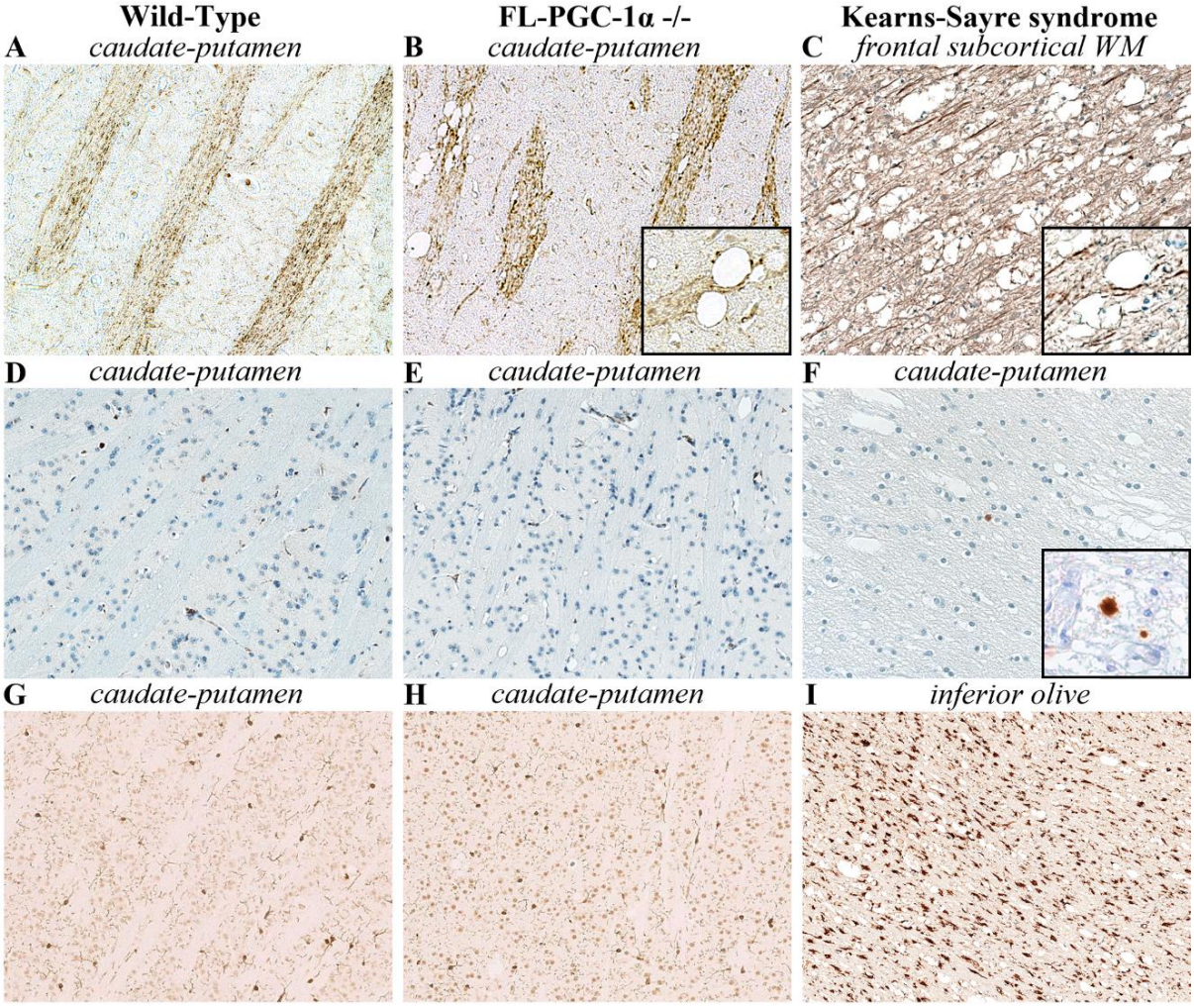

Figure 12. Axonal pathology. Axons in moderately vacuolated areas in KSS are relatively intact with scattered appearance of axonal swellings (**C**; SMI-31) and APP+ axonal spheroids indicative of subacute axonal transport impairment (**F**; APP). Regions with cystic-necrotic lesions show robust microglia accumulation in KSS (**I**; Iba). These pathologies are virtually absent in the FL-PGC-1α -/- mice and the immunohistochemical patterns are rather similar to that in aged wild-types (**A-B**, **D-E**, and **G-H**). Note the well-preserved axons dodging between multiple vacuoles (**B**), similarly to that seen in KSS (**C**). Original publication in: [117].

Electron microscopy revealed that myelin ‘bubbles’ in the WM were formed mostly by splitting at the intraperiod lines (Figure 14A-B), and in the KSS material (where countless vacuoles could be analyzed) occurrences between the axons and the innermost myelin lamellae (adaxonal vacuoles) could also be noticed (Figure 15). The vacuoles were ‘empty’ or contained various amount of debris with myelin-like figures (Figure 14A-B, Figure 15).

Characterization of neuropil vacuoles

Staining for neuronal (MAP-2 and SMI-32) or astrocytic (GFAP) antigens sparsely revealed associations of neuropil vacuoles with these cell-types in either the KSS or the FL-PGC-1α-deficient brains (Figure 16). Staining for MBP, however, unveiled that the vast majority of neuropil vacuoles were clearly encompassed by a myelin-positive rim, suggesting a same intramyelinic localization as for vacuoles within the WM (Figure 13B). Neuropil vacuoles were also frequently associated with oligodendrocytes in sections immunostained for TPPP/p25 (Figure 13C). Such close contacts of oligodendrocytes and vacuoles were also observed by electron microscopy. Furthermore, vacuoles (sometimes multiloculated) could frequently be identified within the cytoplasm of glial cells, which, due to the lack of glial fibers observed in the cytoplasm, also appeared to be oligodendrocytic (Figure 14C). Likewise myelin ‘bubbles’, these membrane-bound vacuoles often contained myelin-like figures, and they occasionally coalesced occupying most of the oligodendroglial cytoplasm and led to the swelling of the cells (Figure 14C). These observations were seen in both the KSS and the FL-PGC-1α-deficient brains.

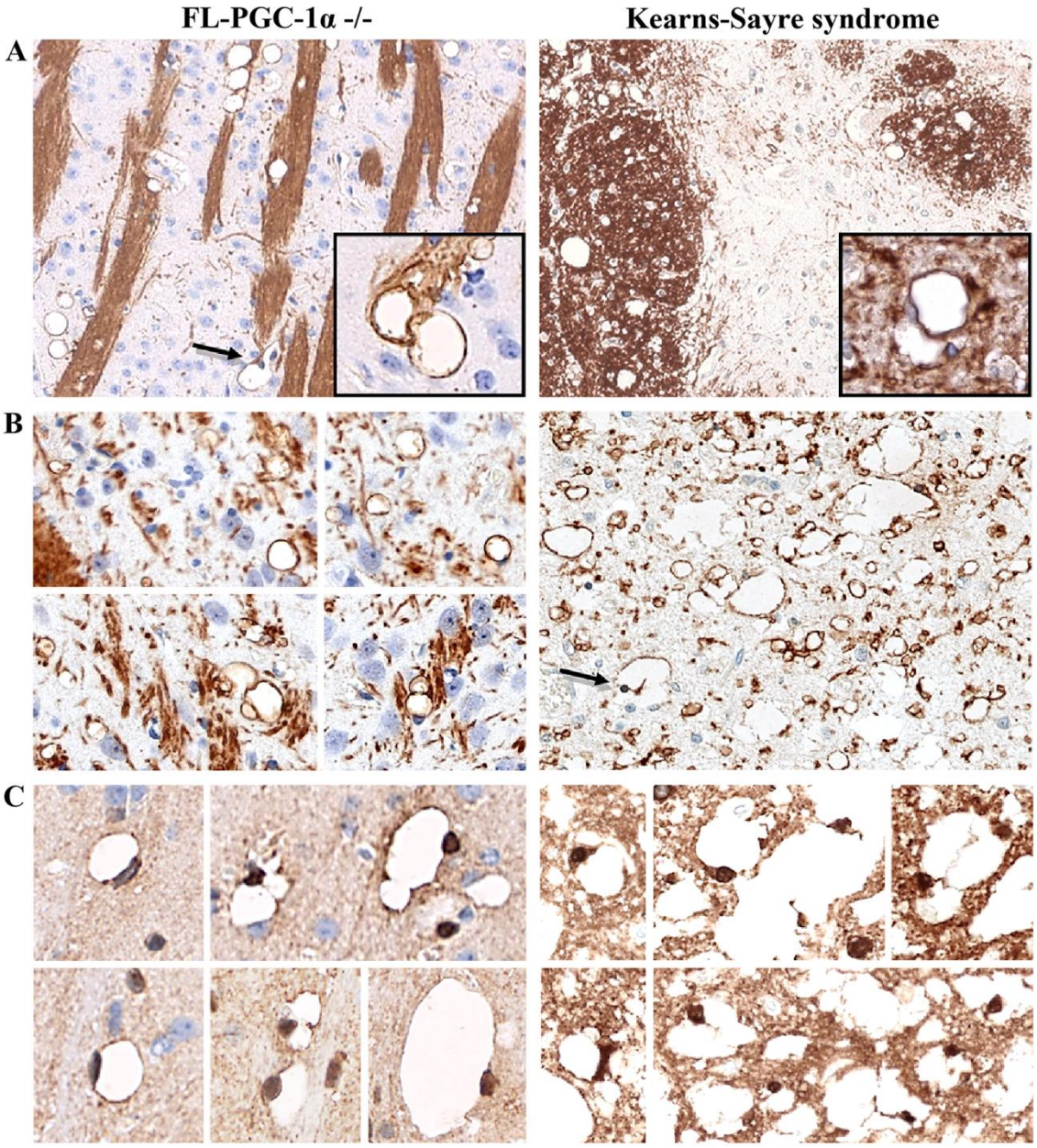

Figure 13. Immunohistochemical characterization of vacuoles. Vacuoles within the WM are surrounded by rings of myelin (**A**; MBP). Likewise WM myelin 'bubbles', vacuoles within the GM neuropil are also encompassed by a myelin-positive rim, suggesting an identical origin (**B**; MBP). Oligodendroglial cells in close contact with and/or localized within the inner edge of either single or multiloculated vacuoles can frequently be detected by immunohistochemistry (**C**; TPPP/p25), suggesting an important role of oligodendrocytes in vacuole formation. Note some of the larger vacuoles being separated to multiple chambers by oligodendroglial processes (**C**). Note also the glial cell encompassing a vacuole with its MBP+ process (indicated by arrows, **A** – left, **B** – right). Original publication in: [117].

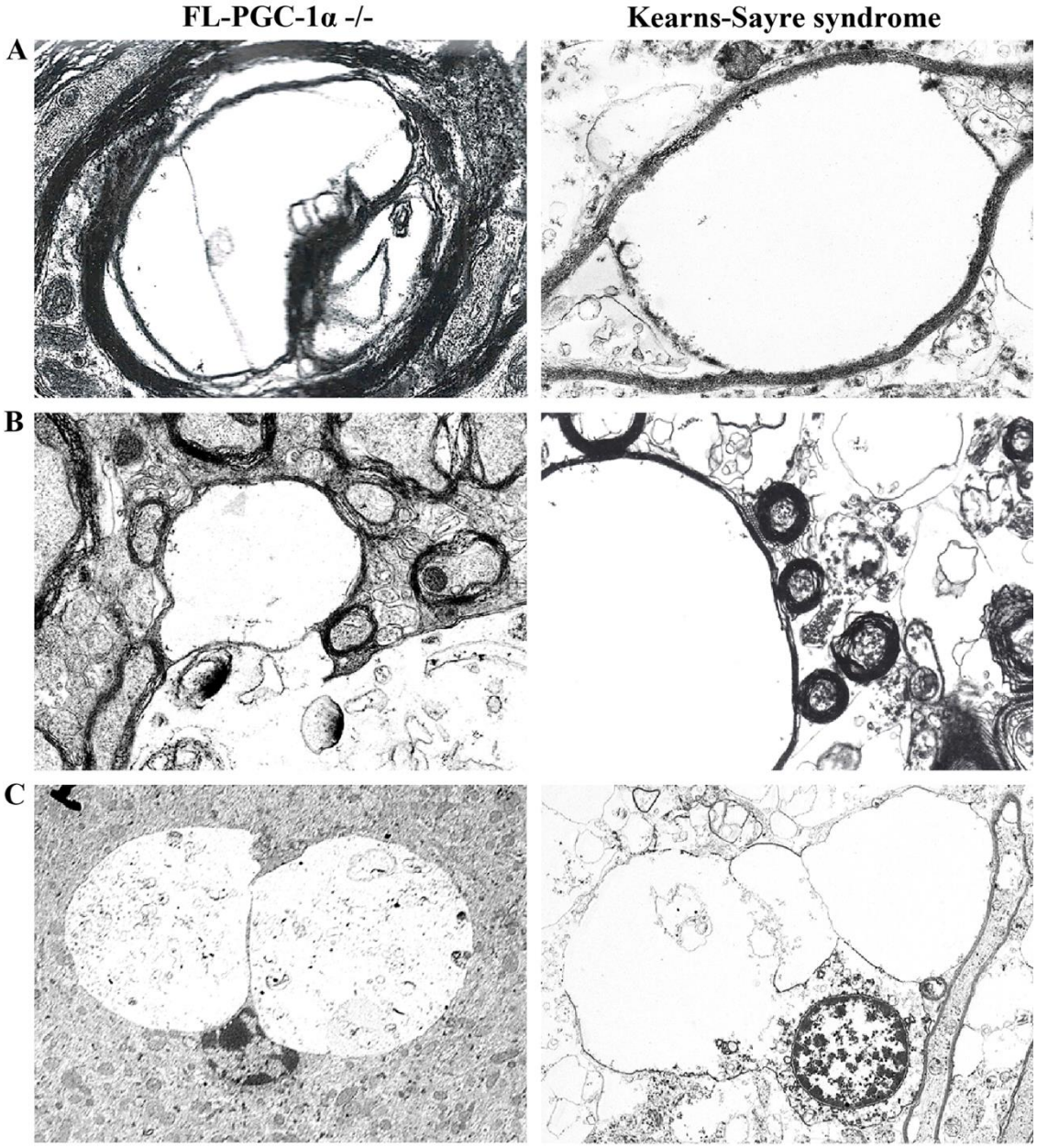

Figure 14. Ultrastructural characterization of vacuoles. Intramyelin vacuolation can be identified also by electron microscopy (longitudinal section, **A**; transverse section, **B**). In accordance with immunohistochemical findings, vacuoles, often multiloculated, can frequently be identified within the cytoplasm of oligodendroglial cells by electron microscopy (**C**). Oligodendroglial vacuoles have not been reported previously in mitochondrial encephalopathies, and their presence as a potential source of intramyelin vacuoles might underlie their focal appearance. Original publication in: [117].

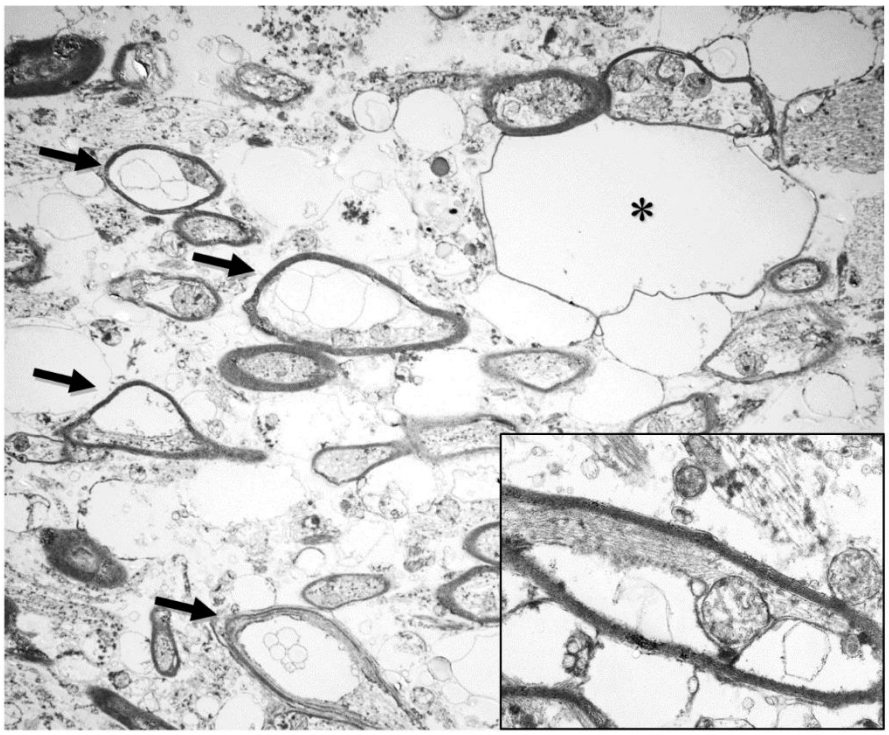

Figure 15. Distinct types of intramyelin vacuoles in KSS. Asterisk denotes a huge 'classical' intramyelin vacuole originating from splitting at the intraperiod line. Arrows indicate the multiple presence of adaxonal vacuoles, some of which appear to originate from splitting between the two innermost myelin lamellae at higher power. The frequent appearance of adaxonal vacuoles may underpin the role of intraoligodendroglial vacuolar change in the development of intramyelin vacuoles in mitochondrial encephalopathies such as KSS, as discussed later. Original publication in: [117].

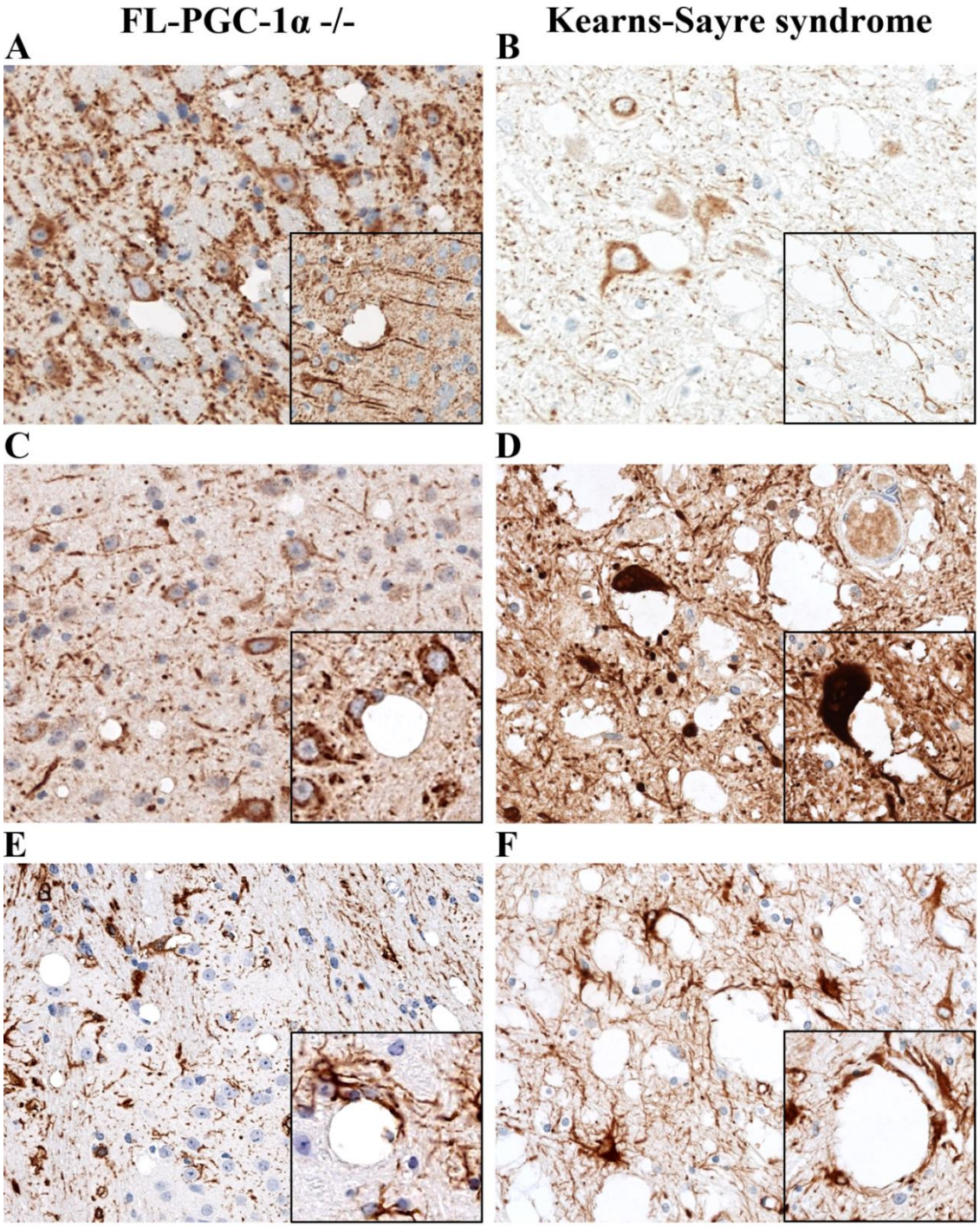

Figure 16. Rare findings of vacuoles being in close contact with structures positive for staining against neuronal (A–B, MAP-2**; C–D,** SMI-32**) or astroglial (E–F,** GFAP**) antigens.** Notably, the vast majority of GM neuropil vacuoles have no immunohistochemically detectable association with such structures, which are often merely pushed by vacuoles of most probably distinct origin. Original publication in: [117].

V. DISCUSSION

The contribution of dysfunctional PGC-1α axis to the pathogenesis of mitochondrial dysfunction in neurodegenerative diseases is supported by the findings of an emerging number of experimental and human examinations. With multiple links associating PGC-1α dysfunction with neurodegenerative diseases, especially HD, PGC-1α-deficient mice with consistent early reports on striatal vacuolation and astrocytosis are frequently referred to as animals exhibiting HD-like pathology and phenotype. This was proposed to be in correspondence with hyperactive behavior of complete PGC-1α -/- mice, a finding that has, however, been contrasted by subsequent reports on various types of PGC-1α-deficient animals. Prompted by the facts that mitochondrial dysfunction and, though only at *in vitro* levels, PGC-1α deficiency has been causatively linked to pathological neuronal protein accumulation as a sign of impaired protein processing characteristic of neurodegenerative diseases, as well as by the notion that neither hyperactive behavior nor CNS vacuolation are typical features of mammalians with HD, our first pillar of investigations targeted at the lesion profiling of the brain of FL-PGC-1α -/- animals bred in our institute, with special focus on the accumulation of proteins associated with different neurodegenerative diseases.

Indeed, based on evidences suggesting that mitochondrial dysfunction and impaired protein processing are interrelated, together with *in vitro* findings associating PGC-1α deficiency with protein accumulation [30, 55], we hypothesized that the ablation of FL-PGC-1α could evoke the accumulation of neurodegeneration-related proteins *in vivo*. This concept seemed feasible as disturbances in protein processing and formation of aggregates have been demonstrated in rodents without a transgenic background for neurodegeneration-associated proteins (*i.e.*, neurotoxin models [50, 118, 119] and aged animals [120]). However, our systematic immunohistochemical analysis revealed no protein depositions, with a complete lack of tangle-like structures, extracellular plaques, and ubiquitin-immunopositive inclusion bodies throughout the 30-weel-old murine brains, and the patterns of immunoreactivity were similar in FL-PGC-1α -/- and wild-type animals (Figure 7). Notably, the same set of immunohistochemical stainings performed on remarkably more aged, 70-75-week-old, FL-PGC-1α -/- mice, revealed identical physiological patterns. These results indicate that despite its widely established contribution in neurodegenerative diseases, the absence of FL-PGC-1α has no major influence on protein processing in mice, suggesting a more complex scenario for

the interaction of mitochondrial dysfunction and protein aggregation in such conditions, and is consistent with the findings that neither the ablation [121] nor the overexpression [56] of PGC-1α is associated with alterations in autophagy in nervous tissues. However, we cannot exclude the supplementary effect of NT-PGC1α^{254} in this context, which question could be answered by studying the complete PGC-1α -/- mice in a similar immunohistochemical paradigm.

On the comprehensive neuropathological work-up, the observed alterations in FL-PGC-1α -/- mice consisted of widespread spongiform vacuolation and circumscribed astrogliosis. The most striking spongiform alterations were observed in the long WM bundles of the internal capsule and the retroflex fascicle. The most severely affected GM regions included the thalamus and the caudate-putamen; however, basic myelin histology suggested the massive involvement of the pencil fibers streaming across the caudate-putamen, raising the possibility that the abundant vacuolation in this region is at least in part attributable to myelin degeneration. Notably, the whole forebrain, including the caudate-putamen, was free of gliosis, which contrasted with the robust and confluent astrogliotic reaction in the central pontomedullary brainstem, and the patchy reactions in the midbrain and the cerebellar nuclei. Collectively, this pattern is highly reminiscent of human mitochondrial spongiform encephalopathies, particularly of KSS and, in some aspects, LS (Table 2), but not of HD or any other 'classical' neurodegenerative disorders. This impression was further supported by the ovoid shape and unidirectional longitudinal axes of the vacuoles in the WM, the deep occipito-parietal preference of the cortical vacuoles, the median predominance of brainstem involvement, along with the seemingly preserved axons in the vacuolated fibers, all being typical features of mitochondrial status spongiosus.

The relevance of the characterization of a viable animal model with mitochondrial encephalopathy is potentially striking, as the continuous attempts to create valid animal tools to model such diseases have been facing difficulties. Indeed, most of the developed genetic modifications resulted in embryonic or early postnatal lethality (*e.g.*, knockouts of CREB [122], ERRγ [123], NRF-1 [124], NRF-2 [125], Tfam [126], mtDNA polymerase γ 1 (POLG1) [127], optic atrophy 1 (OPA1) [128] and synthesis of cytochrome *c* oxidase 2 (SCO2) [129]), whereas a great proportion of viable strains, surprisingly, exhibit no neuropathology (*e.g.*, knockouts of ANT-1 [130], PPARγ [131], ERRα [132], and SURF1 [133]; Twinkle mutant 'Deletor' mice [134], and ΔmtDNA Mito-Mice [135]). To our knowledge, murine knockouts of AIF [136,

137], SOD2 [138], thymidine phosphorylase and uridine phosphorylase (TP/UP) [139], and NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 (NDUFS4) [140] are the only ones that display a neuropathology resembling that of human mitochondrial encephalopathies (Table 2). Our finding that the anatomical pattern of the lesions in this model overlaps with multiple human mitochondrial encephalopathies is not that surprising, considering that FL-PGC-1α contributes to the proper functioning of several downstream proteins, which practically cover all levels of mitochondrial function that have been associated with mitochondrial diseases [100]. More surprisingly, PGC-1α-deficient mice are not only free of embryonic lethality, but have normal longevity and acceptable reproduction [111, 112], suggesting the existence of potent complementary pathways, probably within the PGC family itself. It is possible that the presence of intact PGC-1β and PGC-1-related coactivator (PRC) is sufficient to maintain an acceptable level of mitochondrial function, as both possess functional domains capable of interacting with nuclear hormone receptors and NRFs [141, 142]. Indeed, though PGC-1β expression was found unchanged in PGC-1α-deficient conditions [17, 111, 112, 143], the combined knockout mice of both PGC-1α and PGC-1β succumb to perinatal lethality due to heart failure [144].

Of note, the directly upstream CREB, and a number of downstream factors (ERRγ, NRF-1, NRF-2, Tfam, and POLG1) were found indispensable for embryonic or early postnatal development. On the other hand, the murine knockouts of the directly coactivated PPARγ and the PGC-1α-dependent ANT1 are viable, but display no neuropathological alterations. The most closely reminiscent brain pathology of that observed in this study was reported in the murine knockout of SOD2 (also acting downstream of PGC-1α), indicating that oxidative damage may play an important role in the development of the spongiform leukoencephalopathy. Although the lifespan of the SOD2 null model can be somewhat increased by antioxidant protection, these animals die within the first 3–4 weeks [138].

Disease	*Vacuolation*	*Anatomical predominance of vacuolation*	*Astrogliosis*	*Anatomical predominance of astrogliosis*	*Reference*
Human disorders					
KSS	+	cerebral WM thalamus basal ganglia brainstem cerebellum	+	brainstem cerebellum	
LS	+	thalamus basal ganglia brainstem cerebellar nuclei	+	thalamus basal ganglia brainstem cerebellar nuclei	
MELAS	+	cerebrum cerebellum	+	cerebrum thalamus basal ganglia brainstem cerebellum	[101-104]
MERRF	+	cerebrum brainstem	+	basal ganglia brainstem cerebellar cortex cerebellar nuclei	
LHON	+	optic nerve	+	optic nerve retinal ganglia	
Experimental models					
FL-PGC-1α -/- mice	+	cerebrum thalamus basal ganglia brainstem cerebellar nuclei	+	brainstem cerebellar nuclei	
Ndufs4 -/- mice	+	brainstem cerebellar nuclei	+	brainstem cerebellar nuclei	[140]
TP/UP double -/- mice	+	cerebral WM thalamus basal ganglia cerebellar WM cerebellar nuclei	No data available		[139]
SOD-2 -/- mice	+	cerebral cortex brainstem	+	cerebral cortex brainstem	[138]
AIF -/- (Harlequin) mice	-		+	thalamus basal ganglia cerebellar nuclei retinal ganglia optic tract	[136, 137]

Table 2. Comparative summary of the typical neuropathological findings and their distribution in human mitochondrial spongiform encephalopathies and their most reminiscent murine models. Original publication in: [115].

The possibility that PGC-1α-deficient mice with their normal lifespan and some forms of measurable neurological deficits (though with contrasting reports) could represent a useful animal model to investigate these currently intractable group of diseases drove us to further characterize the neuropathological alterations of FL-PGC-1α -/- animals by a step-by-step comparison of aged 70-75-week-old mice and a human case of KSS, with special focus on the origin of vacuoles.

Our immunohistochemical and ultrastructural analysis revealed that vacuolation in FL-PGC-1α -/- mice is predominantly localized within the myelin sheath in the absence of morphological signs of axonal and scattered indications of astroglial or neuronal involvement. The findings were altogether strikingly similar to that seen in KSS, with a remarkable difference being that in KSS, the devastatingly vacuolated (cystic-necrotic) lesions presented also with myelin pallor and axonal disintegration. The sparing of axons in FL-PGC-1α -/- mice contrasts the concept proposed by Lin et al. during the initial characterization of the complete whole body PGC-1α -/- strain suggesting that the spongiform lesions in the caudate-putamen might arise from axonal degeneration [111], but corresponds with earlier findings on young (postnatal day 10) FL-PGC-1α -/- animals using a semi-quantitative immunohistochemical assessment of striatal neurofilament-positive bundles [145]. Furthermore, the consistent lack of reactive astrocytes in this region even at an old age repeatedly questions the concept that PGC-1α-deficient animals might indeed be models of HD. Notably, an independent group performing parallel examinations on the complete PGC-1α -/- strain recently reported no significant reduction in the number of MSNs in the knockout animals [146], supporting our observations. By contrast, during the preparation of our manuscript, the same group reported a significant loss of cerebellar Purkinje neurons in PGC-1α -/- mice by stereological methods [147], which is striking, as this feature is virtually pathognomonic of KSS. This observation independently (and unintentionally) confirms our concept. Unfortunately, we could not address this question due to technical issues.

In addition to being the first side-by-side systematic comparison of human and animal mitochondrial encephalopathy to our knowledge published to date, this part of our study provided two main novelties. A first major finding of our study is that it demonstrates commonalities of vacuolation in the WM and GM, placing oligodendrocytes in the center of disease pathogenesis. Indeed, though the exact mechanism of vacuole formation in

mitochondrial encephalopathies has not yet been revealed, WM vacuoles are generally presumed to develop due to intramyelin edema secondary to mitochondrial dysfunction of oligodendrocytes, manifesting in splitting of the myelin sheath at the intraperiod line. On the other hand, GM neuropil vacuoles are presumed to develop due to ion transport disorder of astrocytic membranes [148-150]. Contrastingly, however, our observation that the vast majority of GM neuropil vacuoles are clearly myelin-bound in both the KSS and FL-PGC-1α-deficient brains indicates that the cellular localization and thus the mechanism of vacuole formation is most likely similar, irrespective of whether the vacuoles are in the WM or GM. This observation can explain the phenomenon widely observed in mitochondrial encephalopathies, including PGC-1α-deficient mice, that cortical vacuoles show a predilection toward the deeper layers of the neocortex, as the density of myelinated fibers apparently gradually decreases by approaching the superficial layers. Importantly, this could also explain the predilection of GM neuropil vacuoles toward the reticular area of the brainstem, thalamus, deep cerebellar nuclei, and basal ganglia adjacent to the internal capsule; as these GM regions are intermingled with the WM. Interestingly, these regions are the predilection areas for the development of GM vacuolation in hepatic encephalopathy as well [151]. Considering this overlapping predilection of spongy change between hepatic and mitochondrial encephalopathies, and that mitochondrial disorders, including PGC-1α deficiency [112], also commonly present with hepatic involvement, the contribution of liver insufficiency to the development of mitochondrial status spongiosus cannot be excluded either. Notably, intramyelin vacuolation has been frequently described in other experimental, veterinary and human metabolic conditions (Table 3), suggesting that this change might be a general response to various insults that compromise the metabolism of the myelin sheath and/or oligodendrocytes.

As a second novelty, the ultrastructural analysis revealed the common appearance of intracellular, often multiloculated formation of vacuoles within oligodendroglial cells both in the KSS case and FL-PGC-1α-deficient brains. This finding was supported by the immunohistochemical observation of TPPP/p25-positive oligodendrocytes directly attaching to and sometimes bulging into vacuoles within the neuropil. The finding, however, that some vacuoles were observed closely attached to and partly encompassed by neuronal structures in MAP-2 and SMI-32 stainings suggests that at least a small proportion of neuropil vacuoles can be of neuronal origin, and that the mechanisms which lead to excessive intracellular membrane-

bound fluid accumulation may affect different cell-types within the CNS, though to different extents. This hypothesis is supported by the report on CNS vacuoles observed also in neuron-specific PGC-1α -/- mice [121].

Interestingly, though intracellular oligodendroglial vacuoles have not been previously described in mitochondrial diseases, their presence is not unprecedented in pathologies associated with mitochondrial dysfunction and/or severe cellular stress. Indeed, chronic feeding of mice with cuprizone, a copper-chelating mitochondrial toxin associated with megamitochondria [152] and alterations in complex IV and SOD activity [153], evokes a CNS pathology comprising vacuole formation in the pons, midbrain, thalamus, cerebral and cerebellar WM, as well as in deep cortical layers [154]. It was described that cuprizone-induced intramyelin vacuolation was due to splitting at the intraperiod line and was not stained by Sudan IV (equivalent with Oil Red O in our study) [154]. Additionally, the authors reported the presence of oligodendrocytes with enlarged cytoplasm containing multiloculated vesicles, and that some of these cells were juxtaposed to myelin sheaths, where a thin myelin layer appeared to form the glial membrane [154]. These findings are strikingly similar to those observed in our study. A further similarity between cuprizone-induced and mitochondrial status spongiosus is that cuprizone toxicity in doses evoking demyelination associates with oligodendroglial apoptosis [153], a phenomenon recently described in KSS [155]. Our observation of intraoligodendroglial vacuolation in mitochondrial encephalopathy expands the spectrum of disorders where this has been described (Table 4).

Experimental pathologies	***Reference***
Intoxication by	
actinomycin D	[156]
copper	[157]
cuprizone	[154, 158]
ethidium bromide	[159]
hexachlorophene	[160]
isonicotinic acid hydrazide	[161]
lysolecithin	[162]
triethyltin	[163]
Genetic models of	
mitochondrial dysfunction	reviewed in [115]
altered proteolipid expression	[164]
Models of scrapie	[158, 165]
Veterinary pathologies of various etiology	
Helichrysum poisoning in sheep and a goat	[166]
Tetrapterys poisoning in sheep	[167]
Maple syrup urine disease in calves	[168]
Hereditary spongy degeneration of dogs	[169, 170]
Hereditary spongy degeneration of silver foxes	[171]
Human pathologies	
Aging	reviewed in [172]
Aspartoacylase deficiency (Canavan's disease)	reviewed in [173]
Hepatic encephalopathy	reviewed in [173]
Heroin-induced spongiform leukoencephalopathy	[174]
Maple syrup urine disease	reviewed in [173]
Mitochondrial disorders	reviewed in [173]
Urea cycle disorders	reviewed in [173]

Table 3. Animal and human pathologies with intramyelin vacuolation. Original publication in: [117].

Experimental and pathologies	*Reference*
Cuprizone intoxication	[154]
Ionizing radiation	[175]
Methionine sulfoximine intoxication	[176]
Triethyltin intoxication in quaking mice	[177]
Twitcher mouse	[178]
Zitter rat	[179]
Veterinary pathologies	
Hereditary ataxia of the rabbit	[180]
Hereditary spongy degeneration of silver foxes	[171]

Table 4. Experimental and veterinary pathologies with previously reported oligodendroglial vacuolation. Original publication in: [117].

An interesting interrelation between mitochondrial leukoencephalopathies and multiple sclerosis (MS), a pathology where a potential central role of oligodendroglia is also suggested [116], is represented by the fact that the above mentioned cuprizone intoxication, presenting with a highly similar neuropathology to that observed in KSS and FL-PGC-1α-deficient mice in our study, is in fact a widely applied toxin model of MS [153, 181].

Besides dem3yelinating WM disease, our study also has implications for understanding WM lesions in the aging brain, a frequent pathology which includes the formation of intramyelin 'balloons' due to intraperiod line splitting [172], similar to that seen in mitochondrial disorders. Our observation that aged wild-type mice also developed vacuoles to an apparently slighter extent but at the same predilection areas as their FL-PGC-1α-deficient counterparts recapitulates previous observations [182]. Based on these, we propose that vacuole formation in mitochondrial encephalopathies and their representative animal models (*e.g.*, PGC-1α-deficient mice) might also be regarded as an accelerated form of 'normal' WM degeneration, which underpins the role of (oligodendroglial) mitochondrial dysfunction in aging.

Although the potential role of oligodendrocytes in WM vacuolation in mitochondrial encephalopathies has already been suggested, the fundamental concepts included 1) a disrupted ion-homeostasis of the sheath, 2) a dysfunction of the blood-brain barrier, in both cases with consequent development of 'intramyelin edema' [148-150]. These hypotheses, however, do not explain why vacuoles develop focally and how multiple vacuoles can be found within the same

internode, instead of a complete splitting and diffuse loosening of the sheath between all lamellae. We propose that chronic mitochondrial dysfunction in a yet unknown pathway leads to the formation of multiple intraoligodendroglial fluid-filled vacuoles. The increased intracellular content might provoke splitting between the intracellular surfaces of the myelin sheath (major dense line). Due to their firm connections at the macromolecular level, this would cause tears and focal myelin disruptions, allowing the vacuolar content to access into the virtual space between the loosely attached extracellular surfaces (intraperiod lines). Consequently, this could evoke the formation of focal splits and eventually myelin bubbles. Accordingly, disruption of the lateral loops or probably of developmental remnants of the cytoplasmic incisures would result in intraperiod line splitting at the corresponding levels, whereas leakage from the inner tongue would cause adaxonal swelling between the axolemma and the innermost myelin lamellae or between the two innermost layers. Supporting this theoretical consideration, such distinct types of intramyelin vacuoles have indeed been described in experimental status spongiosus [156, 159], and could also be observed in our study (Figure 15).

Our observations, therefore, place oligodendrocytes in the center of the pathogenesis of CNS lesioning in association with chronic mitochondrial dysfunction in both the WM and GM, which is in line with the recognition that oligodendrocytes, contrasting with astrocytes [21], are most sensitive to mitochondrial stress, exceeding the vulnerability of neurons [22]. This may serve as rationale for cytoprotective targeting of oligodendrocytes in mitochondrial encephalopathies as well as in other disorders with vacuole formation and myelin degeneration.

VI. CONCLUSION

In our studies, we systematically characterized the neuropathological alterations of a murine strain deficient in the expression of full-length PGC-1α protein, a mitochondrial master regulator of biogenesis, respiration, and antioxidant responses. Providing multiple lines of evidence that this animal strain does not associate with morphological features of neurodegenerative diseases, including HD, we reported the characterization of a phenotype strikingly reminiscent of that seen in mitochondrial encephalopathies, especially in KSS. Extending our investigations to further immunohistochemical and ultrastructural levels on a systematic comparison with the human disease, we identified novel pathological alterations present in both human KSS and FL-PGC-1α -/- animals, providing basis of a novel generalized

theory for vacuole formation in mitochondrial and probably other metabolic diseases. We propose that PGC-1α-deficient mice can be an appropriate animal model for this yet incurable group of diseases, and should be subjected to examination with therapeutic aim.

VII. ACKNOWLEDGEMENTS

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