Rational therapeutic approaches to progressive supranuclear palsy

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Progressive supranuclear palsy is a sporadic and progressive neurodegenerative disease, most often presenting as a symmetric, akine\-tic-rigid syndrome with postural instability, vertical supranuclear gaze palsy and frontal lobe deficits. It belongs to the family of tauopathies and involves both cortical and subcortical structures. Although the exact pathophysiology is not yet fully understood, several lines of evidence point to a crucial contribution from both genetic predisposition and mitochondrial dysfunction. Recently gained insights into the pathophysiology of this disease have led to several hypothesis-driven therapeutic approaches aiming at disease-modification rather than mere symptomatic neurotransmitter-replacement therapy. Agents targeting mitochondrial dysfunction have already shown a positive effect in a phase II study and further studies to verify and expand these results are ongoing. Clinical studies with agents targeting tau dysfunction such as tau-kinase inhibitors, tau-aggregation inhibitors and microtubule stabilizers are in preparation or ongoing. This review presents the current pathophysiological concepts driving these exciting therapeutic developments.

Keywords: progressive supranuclear palsy; aetiology; mitochondrial respiratory chain; complex I; microtubule associated protein tau

Abbreviations: FDG-PET = 18F-fluoro-2-deoxy-glucose-positron emission tomography; GSK = glycogen synthase kinase; HSP = heat shock protein; MAPT = microtubule associated protein tau; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRS = magnetic resonance spectroscopy; PSP = progressive supranuclear palsy; UBB+1 = ubiquitin B +1

Progressive supranuclear palsy

Progressive supranuclear palsy (PSP, Steele–Richardson–Olszewski syndrome) is a sporadic and progressive neurodegenerative disease (Litvan et al., 1996; Burn and Lees, 2002; Williams and Lees, 2009). PSP has an average prevalence of 5.3 per 100 000 (Bower et al., 1997; Schrag et al., 1999; Nath et al., 2001). The mean age at onset is approximately 63 years with a mean survival of 9 years from the onset of symptoms (Rajput and Rajput, 2001; Burn and Lees, 2002; Litvan et al., 2003; Golbe and Ohman-Strickland, 2007). No effective symptomatic, disease-modifying or neuroprotective therapy is presently available. As a first step towards the development of effective treatments, a detailed understanding of the underlying pathophysiology is needed.
Clinical presentation

The classical clinical picture of PSP, presenting most frequently with a symmetric, akinetic-rigid syndrome, vertical supranuclear gaze palsy, frontal deficits, prominent postural instability and falls, is now referred to as Richardson’s syndrome (Litvan et al., 1996; Williams et al., 2005; 2007) and seems to comprise about half of the patients. However, several variants in the clinical presentation of pathologically confirmed PSP have been described recently, complicating the early differential diagnosis.

PSP-parkinsonism is characterized by asymmetric onset, tremor and moderate initial therapeutic response to levodopa, and it is estimated to comprise about one third of the patients (Williams et al., 2005, 2007). Early gait disturbance with freezing, micrographia and hypophonia constitute another variant called pure akinesia with gait freezing (Williams et al., 2007). Progressive, asymmetric dystonia, apraxia and cortical sensory loss (PSP–corticobasal syndrome) and progressive non-fluent aphasia are additional clinical phenotypes, which present with more pronounced cortical pathology (Josephs et al., 2005; Tsuboi et al., 2005). Further clinical variants are PSP with pallido-nigro-luysian-atrophy (Ahmed et al., 2008), with frontotemporal dementia (Kaat et al., 2007) and with semantic dementia (Josephs et al., 2006).

The heterogeneity of the clinical presentation appears to follow variations in the anatomical distribution of distinct, PSP-specific histopathological alterations (Ahmed et al., 2008; Williams and Lees, 2009). These clinical presentations have been described based on retrospective neuropathological studies. To date it is unknown whether and to what extent they apply to the clinically relevant spectrum of PSP and if it will be possible to diagnose them reliably in vivo in absence of validated diagnostic criteria or biomarkers.

Neuropathology

Neuronal cell loss involves both cortical and subcortical structures, in particular the subthalamic nuclei, globus pallidus, superior colliculi, pretectal regions, periaqueductal grey matter, substantia nigra, thalamus, cerebellum, the entire tegmentum and the spinal cord (Hauw et al., 1994; Daniel et al., 1995; Rajput and Rajput, 2001; Iwasaki et al., 2007).

Histopathological examinations demonstrate intracellular, somatodendritic tau-aggregates revealed by silver staining or by immunohistochemistry with antibodies against phosphorylated tau protein (e.g. AT8 or AD2 antibodies) (Dickson, 1999) (Fig. 1). Neurofibrillary tangles in neurons, neuropil threads in neuronal processes, coiled bodies in oligodendrocytes, tufted astrocytes in the basal ganglia, amygdala and motor cortex and the absence of neuritic plaques help to differentiate PSP from other tauopathies. Ultrastructurally, the tau-aggregates present as paired helical filaments and spherical filaments (Dickson, 1999).

Biochemical examinations in the affected regions demonstrate an abnormal accumulation of insoluble, hyperphosphorylated specimen of the microtubule associated protein tau (MAPT) and an altered ratio of the tau isoforms (3R-tau and 4R-tau) in favour of 4R-tau (Fig. 2).

Aetiology

The development of an effective, disease-modifying therapy requires a detailed understanding of the disease mechanisms. Recent studies provided evidence for mitochondrial dysfunction in PSP (Stamelou et al., 2008; 2009). Yet, there is also a strong genetic component in the aetiology of PSP, since some rare cases follow an autosomal dominant mode of inheritance, and sporadic patients with PSP have more first-degree relatives with parkinsonism compared to controls (odds ratio 3.9; Kaat et al., 2009). Such observations suggest that rare variants of PSP are caused by either genetic or environmental factors, whereas the vast majority of cases result from an interaction of genetic and environmental factors.

Apart from mitochondrial dysfunction and genetic predisposition, other possible aetiologic factors have also been implicated in the pathophysiology of PSP, but strong evidence supporting their contribution is still lacking. The way and sequence in which the putative aetiologic factors cooperate to mediate increased levels of 4R-tau, abnormal tau hyperphosphorylation, formation of neurofibrillary tangles and ultimately cell death remains uncertain. Thus, we review here the evidence for genetic predisposition and mitochondrial dysfunction and their interplay as core phenomena in the pathophysiology of the disease, which may be the basis of a future effective, disease-modifying therapy (Table 1).

Figure 1 The histopathological hallmarks of PSP. Immunohistochemical examination of the striatum of a deceased patient with PSP, using an antibody (AD2) raised against the human tau protein phosphorylated on serine residues 396 and 404, demonstrates cytosolic aggregates in oligodendrocytes (coiled bodies, A), in astrocytes (astrocytic tuffs, B), in neuronal processes (neuropil threads, C) and in neurons (pre-tangles, D and neurofibrillary tangles, E). Scale bar = 50 μm.
Genetic factors

PSP can be transmitted as an autosomal dominant trait in rare instances. Linkage studies in one large Spanish family (de Yebenes et al., 1995) assigned a disease locus to a 3.4 Cm interval on chromosome 1 (1q13.1) (Ros et al., 2005b). However, the underlying gene has not been identified yet because the region is devoid of known genes. In other rare autosomal dominant variants of PSP, several mutations (RS1, ΔN296, G303V) of the gene coding for MAPT have been reported (Stanford et al., 2000; Pastor et al., 2001; Poorkaj et al., 2002; Rossi et al., 2004; Ros et al., 2005a, b) (Fig. 2).

Despite these few cases with Mendelian inheritance, PSP is a predominantly sporadic disease. In most cases of PSP, MAPT plays a predisposing rather than a causative role, since the best-established genetic risk factor for sporadic PSP is the H1 haplotype covering the MAPT gene (Conrad et al., 1997; Oliva et al., 1998; Baker et al., 1999; Higgins et al., 2000; de Silva et al., 2001; Houlden et al., 2001; Poorkaj et al., 2002; Morris et al., 2003).

The possible molecular basis of the association of the H1 haplotype has been identified by detailed genetic mapping of the MAPT region on chromosome 17 in PSP (Pittman et al., 2005; Rademakers et al., 2005). The dichotomy of the non-recombining H1 and H2 haplotypes is found only in European Caucasian populations (Evans et al., 2004), and originated from a large ~900 kb inversion of this region (Hardy et al., 2005; Stefansson et al., 2005). Normal variation within the H1 clade resulted in multiple sub-haplotypes, and only one of the common (>10% frequency) sub-haplotypes, designated H1c, is associated with PSP, together with a strong negative (i.e. protective) association of the H2 haplotype (Pittman et al., 2005).

A predisposing role of the H1c-sub-haplotype appears to be mainly mediated by a single nucleotide polymorphism within the 5’ promoter region of MAPT between the untranslated exon-1 (Andreadis et al., 1996) and the coding exon 1 (Ezquerra et al., 1999; de Silva et al., 2001; Pittman et al., 2005; Rademakers et al., 2005). This polymorphism, referred to as single nucleotide polymorphism-rs242557 (A/G) has strong allele-specific effects on MAPT transcription (Rademakers et al., 2005; Myers et al., 2007). Its risk allele (A) is associated with significantly increased MAPT transcription (Myers et al., 2007). This allele-specific effect on transcription is combined with increased incorporation of the alternatively spliced MAPT exon 10 in transcripts of the H1c allele (Caffrey et al., 2006, 2008; Myers et al., 2007). Exon 10 codes for one of the four microtubule-binding repeat domains and exclusion or inclusion result in the 3R- or 4R-tau isoforms, respectively. The H1 allele, therefore, could be priming the neurodegenerative process via a combined effect of increased MAPT transcription and exon 10 splicing. This appears to result in increased levels of the more fibrillogenic 4R-tau isoform, thus providing a plausible molecular basis for the increased risk of developing the 4R-tau-dominant pathology characteristic of PSP (Pittman et al., 2006).

Since the H1 haplotype also has a rather high prevalence among healthy controls, it appears to be a predisposing condition requiring additional genetic or environmental co-factors to trigger the disease. The question of the existence of further genetic factors
was addressed in a genome-wide association study (Melquist et al., 2007). This analysis was carried out on a 500K single nucleotide polymorphism array with pooled DNAs from 288 patients with PSP with clinical or pathological diagnosis. The association of the MAPT H1 haplotype as a risk factor for PSP was confirmed. Moreover, the study identified two single nucleotide polymorphisms and a single haplotype block on the short arm of chromosome 11 (11p12-p11) associated with PSP. The CurePSP+ Charles D. Peebler Jr. PSP and CBD Genetics Program is presently conducting a more comprehensive genome-wide association study. DNA from more than 1100 patients with PSP with a diagnosis confirmed by autopsy is being analysed with higher density of genotyping (620 K single nucleotide polymorphisms). Furthermore, two independent control groups will be studied using the Eigen vector method, a statistical approach to correct for ethnic population admixture. The findings will be verified in an independent cohort of more than 1100 patients. This study is expected to have a tremendous impact on the understanding of the genetic factors implicated in PSP.

Mitochondrial dysfunction

Mitochondria in advanced age and disease

The inner membrane of mitochondria contains the respiratory chain (complexes I–V) (Fig. 3A), whose function is to maintain adequate cellular concentrations of ATP by aerobic oxidative phosphorylation. In situations of high energy demand, phosphorylated creatine, which is a rapidly accessible energy storage compound, is dephosphorylated to unphosphorylated creatine, thereby charging adenosine–diphosphate (ADP) by phosphorylation to yield ATP. Each mitochondrion has its own DNA (mitochondrial DNA) comprising 37 genes, 22 of which code for tRNA molecules and 2 for mitochondrial rRNA. The remaining 13 genes encode components of the oxidative phosphorylation system. However, the majority of mitochondrial proteins are encoded by the nuclear genome (Yakes et al., 1997).

Mitochondria are the major cellular source for generation of reactive oxygen species. Complex I is considered to be the major enzyme emitting reactive oxygen species (Lenaz et al., 2006). Reactive oxygen species extract electrons from neighbouring molecules to complete their own orbital, thereby leading to the oxidation of cellular macromolecules including proteins, lipids and DNA (Lenaz et al., 2006). According to the mitochondrial theory of ageing, random mitochondrial DNA alterations induced by reactive oxygen species cause errors in the proteins of oxidative phosphorylation, leading to bioenergetic deficits, which in turn further increase the production of reactive oxygen species, thereby establishing a vicious cycle (Mecocci et al., 1993; Bender et al., 2006).

The molecular and cellular processes occurring during ageing and in neurodegenerative disorders may be similar. However, while in the ageing brain these processes appear to spread out in a random way regarding both anatomical and molecular

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<th>Target</th>
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<th>Tau dysfunction</th>
<th>GSK-3β inhibitors</th>
<th>Multifunctional cocktail</th>
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<td>Complex I cofactor</td>
<td>Substrate</td>
<td>Coenzyme Q10</td>
<td>Parvcl, creatine, NPD</td>
<td>Active (NCT00992580)</td>
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<td>Phase I trial</td>
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The numbers in the brackets indicate registration numbers at http://www.clinicaltrials.gov.
Figure 3 Pathophysiological concept of mitochondrial dysfunction in PSP. (A) The inner mitochondrial membrane contains five protein complexes (complexes I–V), which constitute the respiratory chain. The function of the mitochondrial respiratory chain is to maintain adequate cellular concentrations of ATP by aerobic oxidative phosphorylation. Several lines of evidence point to a dysfunction of the respiratory chain, particularly affecting complex I, in PSP. Dysfunction of the respiratory chain leads to reduced ATP levels and increased generation of reactive oxygen species. Coenzyme Q10 (Q10) serves as a physiological electron (e\textsuperscript{−}) shuttle from complexes I and II to complex III, as well as a potent antioxidant. Coenzyme Q10 supplementation enhances the ATP production upon exposure to complex I inhibitors. (B) In cultured neurons of rat striatum, nuclei were visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining (blue), the cytoplasm by neurofilament immunostaining (green) and phosphorylated tau by immunostaining with the AD2 antibody (red). Treatment with the complex I inhibitor annonacin induces a redistribution of AD2\textsuperscript{+} tau from the physiological location in axons to the pathological location in the cell body in individual neurons (white arrow). (C) The pathophysiological model assumes that mitochondrial neurotoxins, particularly affecting complex I (CX I) of the mitochondrial respiratory chain (1), induce ATP-depletion (2) and production of reactive oxygen species (ROS, 3). ATP-depletion causes the retrograde transport of mitochondria and phosphorylated tau to the neuronal soma (4). Reactive oxygen species cause damage to structural proteins including microtubules, neurofilaments and tau, which in turn causes further mitochondrial dysfunction (5), which may lead to loss of physiological function. In a chronic condition, elevated levels of phosphorylated tau in the cytoplasm may result in tau-aggregates (6).
structures, in neurodegenerative disease specific causes and cellular disturbances seem to affect specific structures overlaying the age-dependent decrease of the homeostatic reserve. These disease-specific alterations may have both genetic and non-genetic causes (Sas et al., 2007). In PSP, there are several lines of evidence to suggest a failure in mitochondrial energy production as an upstream event in the chain of pathological events leading to tau aggregation and neuronal cell death.

**Indirect evidence of mitochondrial dysfunction**

Post-mortem immunochemical studies provided evidence for oxidative stress in PSP brains (Dexter et al., 1992; Sian et al., 1994; Smith et al., 1994; Loeffler et al., 1996; Jenner et al., 1996; Albers et al., 1999; Odetti et al., 2000; Cantuti-Castelvetri et al., 2002). Cytoplasmic hybrid cells (cybrids) are created from a human neuroblastoma or osteosarcoma cell line lacking mitochondrial DNA by introducing mitochondria from a patient’s platelets, and are used to determine whether defects in oxidative phosphorylation are caused by the donor patient’s mitochondria (Albers et al., 2002). Cybrids with mitochondria from patients with PSP showed reduced activity of complex I, ATP-production and oxygen consumption, as well as traces indicating oxidative damage, e.g. increased activities of antioxidant enzymes and oxidative damage to lipids (Swerdlow et al., 2000; Albers et al., 2001; Chirichigno et al., 2002). These data provided the first indirect evidence that mitochondrial dysfunction occurs as a primary phenomenon in PSP. However, it remains unclear whether mitochondrial dysfunction is caused by sequence changes in mitochondrial DNA, by mitochondrial toxins, or even by tau oligomers damaging mitochondrial membranes.

**Imaging studies indicating mitochondrial dysfunction**

$^{18}$F-fluoro-2-deoxy-glucose-positron emission tomography (FDG-PET) demonstrated hypometabolism in PSP compared to healthy controls, affecting mainly the frontal lobes, cingulate gyri, basal ganglia, thalamus, midbrain and pons (Blin et al., 1990; Johnson et al., 1992; Karbe et al., 1992; Burn et al., 1994; Santens et al., 1997; Hosaka et al., 2002; Juh et al., 2004, 2005). Still, FDG-PET does not distinguish whether hypometabolism is a primary phenomenon leading to cell death or a secondary phenomenon following cell death. The hypometabolism found in the aforementioned PSP studies is likely to be secondary as a consequence of neuronal cell loss, since the stage of the patients studied was rather advanced to ensure sufficient diagnostic certainty (Golbe and Ohman-Strickland, 2007). Of interest, in regions without pronounced cell loss like the parietal lobes, hypermetabolism has been reported, which could be compensatory to a primary mitochondrial dysfunction (Eckert et al., 2005, 2008). Nevertheless, it cannot be ruled out that these observations may be simply compensatory for some other disease-related process.

A more reliable technique to study the energy metabolism in vivo is proton and phosphorus magnetic resonance spectroscopy ($^1$H- and $^{31}$P-MRS). High-energy metabolites (ATP and phosphorylated creatine) and inorganic phosphate can be quantified in vivo by $^{31}$P-MRS. Lactate, an indicator of anaerobic glycolysis and N-acetylaspartate, a marker of neuronal integrity, can be quantified by $^1$H-MRS (Moffett et al., 2007; Rango et al., 2007). In a recent study, combined $^{31}$P- and $^1$H-MRS in 21 clinically probable PSP patients at early stages showed decreased concentrations of high-energy phosphates in the basal ganglia and frontal lobes, without N-acetylaspartate alterations, suggesting that this decrease is unlikely to be a consequence of neuronal death only and that mitochondrial dysfunction could be an upstream phenomenon in the sequence of events leading to neurodegeneration in PSP (Stamelou et al., 2009).

**Mitochondrial neurotoxins**

An environmental factor has been implicated in the aetiology of PSP by the description of a sporadic PSP-like disease that has been linked to chronic consumption of plants of the Annonaceae family, in particular *Annona muricata* in Guadeloupe (Caparros-Lefebvre and Elbaz, 1999; Lannuzel et al., 2007), but also in other regions (Angibaud et al., 2004). One third of the patients manifesting parkinsonism presented with clinical signs and symptoms resembling PSP (Caparros-Lefebvre and Elbaz, 1999; Caparros-Lefebvre et al., 2002, 2005, 2006; Lannuzel et al., 2007). Autopsy was only performed in three cases that were neuropathologically confirmed as PSP. However, it is still possible that these were sporadic PSP cases unrelated to the other cases (Caparros-Lefebvre et al., 2002).

Annonaceae plants contain acetogenins, which are highly lipophilic, stable and extremely potent inhibitors of complex I at nanomolar concentrations in vitro (Zafra-Polo et al., 1996). Annonacin, the most abundant acetogenin in *A. muricata*, inhibits complex I with an IC$_{50}$ of about 30 nM and kills neurons by ATP-depletion. When administered intravenously to rats for 28 days, annonacin decreases brain ATP levels and induces pronounced neurodegeneration in basal ganglia and brainstem nuclei (Champy et al., 2004), which mimics the distribution seen in brains from patients with atypical parkinsonism in Guadeloupe (Caparros-Lefebvre et al., 2002).

Based on these data, further environmental lipophilic complex I inhibitors have been studied and were found to cause decreased ATP levels, neuronal cell death and somatodendritic redistribution of phosphorylated tau protein from axons to the cell body in primary cultures of foetal rat striatum (Escobar-Khondiker et al., 2007; Höllerhage et al., 2009). Their potency to decrease ATP levels correlated with their potency to induce tau redistribution, suggesting that ATP depletion is the main underlying cause of tau redistribution.

**Association between mitochondrial dysfunction and tau aggregation**

In primary cultures of rat striatal neurons treated with annonacin for 48 h, a concentration-dependent decrease in ATP levels, a redistribution of tau from the axons to the cell body and cell death was observed (Fig. 3B) (Escobar-Khondiker et al., 2007;
Höllerhage et al., 2009). Redistribution of tau and cell death were prevented by forced expression of the ND11-NADH-quinone-oxidoreductase of Saccharomyces cerevisiae, which can restore NADH-oxidation in complex I-deficient mammalian cells, as well as by stimulation of energy production via anaerobic glycolysis. Other ATP-depleting neurotoxins reproduced the somatic redistribution of tau, whereas toxins that did not decrease ATP levels did not cause redistribution of tau, suggesting that ATP-depletion may be a potent trigger of somatic accumulation of the axonal protein tau (Fig. 3C) (Escobar-Khondiker et al., 2007; Höllerhage et al., 2009).

Additionally, dysfunctional complex I is a major source of cellular reactive oxygen species (Fig. 3C). Increased oxidative stress and reactive oxygen species activate many kinase-based signal transduction pathways (tau-kinases), all of which were found activated in PSP-neurons and glial cells (Ferrer et al., 2001a, b, 2002; Hartzier et al., 2002) and cause hyperphosphorylation of tau (Albers et al., 1999, 2000). Hyperphosphorylated tau is more prone to aggregation than dephosphorylated tau (Iqbal et al., 2008). Consistent with these observations, upregulation and phosphorylation of tau have previously been detected in vivo in the substantia nigra of mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the precursor of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) (Smith et al., 2003; Miller et al., 2004) and with complex I inhibitor rotenone (Höglinger et al., 2005). In conditions of chronic complex I inhibition, the combination of somatodendritic redistribution and hyperphosphorylation is likely to result in tau aggregation (Fig. 3C), which may in turn cause further mitochondrial dysfunction.

Complex I dysfunction is also believed to be an upstream event in Parkinson's disease (Schapira et al., 2008), which is characterized by aggregation of α-synuclein rather than by tau-pathology. Chronic generalized complex I inhibition with rotenone caused selective nigral dopaminergic cell death and α-synuclein deposition in rats (Betarbet et al., 2000), reproducing features of Parkinson's disease. However, other studies suggested that generalized complex I inhibition causes tau-pathology and kills neurons in a pattern more reminiscent of PSP (Höglinger et al., 2003; Champy et al., 2004). How could a common mechanism be relevant for such different diseases as Parkinson's disease and PSP?

We found that mild cerebral complex I inhibition in rotenone-treated rats caused aggregates of both α-synuclein and tau and moderate neuronal cell loss, whereas more severe complex I inhibition caused a predominant tauopathy and severe neuronal loss (Höglinger et al., 2005). Thus, the pathological consequences of complex I inhibition may crucially depend on the severity of the metabolic defect. Other co-variables, such as cell-type specific uptake or elimination mechanisms for mitochondrial neurotoxins (Javitch et al., 1985), the location of the damage within the mitochondrial respiratory chain or genetically determined susceptibility factors (Melquist et al., 2007) might also strongly impact the neuropathological consequences of mitochondrial dysfunction. This could explain why mitochondrial dysfunction is aetiologically implicated in diseases with such different pathology as in PSP, Parkinson's disease, primary mitochondrial diseases, Alzheimer's disease and Huntington's disease (Quintanilla et al., 2009; Quefrth et al., 2010).

With regard to this concept, it will be intriguing to compare the genetically determined susceptibility factors differing between PSP and Parkinson's disease and other neurodegenerative disorders with mitochondrial failure on a genome wide basis. To date, several studies have shown that the MAPT H1 haplotype (see Zhang et al., 2005a and references therein) and specific H1 sub-haplotypes are associated with Parkinson's disease (Skipper et al., 2004; Fidani et al., 2006; Edwards et al., 2010), and these sub-haplotypes seem to be different from the sub-haplotypes associated with PSP (Cruchaga et al., 2009). However, one study with pathologically confirmed Parkinson's disease cases showed that the association of Parkinson's disease is due to the H1/H2 dichotomy alone as none of the H1 specific sub-haplotypes were associated with Parkinson's disease (Vandrovcova et al., 2009).

Other possible disease mechanisms

Ubiquitin B+1 (UBB+1) is an aberrant ubiquitin protein that was first described in Alzheimer's disease and Down's syndrome brains (van Leeuwen et al., 1998). It was later shown that pontine nuclei of PSP brains contain some neurofibrillary tangles that are immunoreactive for UBB+1 (Fergusson et al., 2000). UBB+1 is the product of misreading of the mRNA derived from the ubiquitin-B gene (UBB), leading to an ubiquitin product with a deleted carboxy-terminal glycine-76 and additional 20 frameshift amino acids. UBB+1 inhibits the 26S proteasome and this results in the protection of cells from oxidative stress as with proteasomal inhibition with MG132 and lactacystin (Hope et al., 2003). UBB+1 also causes increased expression of the heat shock proteins, HSP40 and HSP70 (Hope et al., 2003). It is possible that this is responsible for protecting the cells from oxidative stress (Hope et al., 2003). In pontine neurons of PSP brains, there is no co-localization of UBB+1 and HSP40 or HSP70, which could suggest that in the vulnerable neurons in PSP associated with UBB+1, there is the failure to upregulate these chaperones (Hope et al., 2004). In vitro experiments show that the chaperones HSP70 and HSP90 promote tau solubility, enhance tau binding to microtubules, cause reduced tau-phosphorylation and thereby prevent tau-aggregation (Dou et al., 2003). Thus, a failure of HSP upregulation has been proposed to play an important role in tau dysfunction, hyperphosphorylation and accumulation in PSP (Hope et al., 2003). These findings would therefore suggest that HSPs play an important role in tau-pathophysiology and might be a possible target for future therapy.

A further mechanism with possible relevance to PSP is a dysregulation of several tau-kinases. Two kinases, the c-Jun N-terminal kinase and p38 kinase, both members of the family of mitogen-activated protein kinase kinases, have been proposed to be of special interest in PSP. In vitro both kinases phosphorylate tau only at sites that are pathologically phosphorylated in tauopathies, but not in healthy individuals (Reynolds et al., 1997, 2000). Interestingly both kinases induce apoptosis in several experimental paradigms (Xia et al., 1995; Le-Niculescu et al., 1999; Chang and Karin, 2001). However the absence of caspase-3 in PSP brains...
with c-Jun N-terminal kinase expression suggests that instead of apoptosis, the phosphorylation of tau might be the critical process that leads to neuronal dysfunction and death (Atzori et al., 2001).

Therapeutic approaches

Targeting tau dysfunction

Both neuropathological and human genetic observations and experimental results from transgenic models point to a central role of dysfunction or dysregulation of the tau protein in the pathophysiology of PSP. The presently available data suggest a pathophysiological concept, according to which tau is being hyperphosphorylated by kinases, which leads to detachment of tau from microtubules. Unbound phosphorylated tau, particularly the 4R-tau isoforms containing exon 10, has an intrinsic propensity to aggregate causing toxicity by a variety of different mechanisms (toxic gain of function). In turn, microtubules are destabilized after detachment of tau and are depolymerized to tubulin-monomers, thereby losing their vital function as railways for intracellular vesicle- and organelle-trafficking (loss of function). This model (Fig. 4) offers various options for therapeutic interventions.

Since the increased 4R/3R-tau ratio, caused by augmented inclusion of exon 10, appears to facilitate the propensity of tau to aggregate, strategies to modify tau mRNA splicing could be a target for causal therapy (Fig. 2). An increased stability of the tau stem loop results in decreased inclusion of exon 10 and increased expression of 3R-tau (Donahue et al., 2006). In PSP, stabilization of the tau stem loop by splice modifiers might reverse the altered 4R/3R ratio. The aminoglycoside neomycin, for example, can bind and stabilize the tau stem loop via electrostatic interactions (Varani et al., 2000). However, this effect is rather non-specific. Mitoxantrone has recently been identified as another stem loop stabilizer (Liu et al., 2009; Zheng et al., 2009).

Modifying splicing and translation by small molecules binding directly to mRNA is currently of substantial interest (Tor et al., 2003; Zaman et al., 2003; Thomas et al., 2006; Donahue et al., 2007).

*In vitro* and *in vivo* studies also implicate increased allele-specific total *MAPT* transcriptional levels associated with the H1c risk sub-haplotype, compared to all the other haplotypes (Myers et al., 2007). Furthermore, there is clear evidence of a mechanistic connection between transcription and splicing whereby rate of transcription influences alternative splicing choices (Kornblith, 2007). As has been proposed recently (Dickey et al., 2006), a possible therapeutic approach could be the pharmacological reduction of tau levels either at the level of gene expression or clearance of the protein so as to reduce the apparent toxic effect of elevated levels of fibrillogenic protein species within the cytoplasm. This could apply to other proteopathies where increased risk conferred by genetic variation is manifested by increased levels of the pathological proteins that define the respective disorders.

Hyperphosphorylation of tau in tauopathies is regulated by several kinases that phosphorylate specific serine or threonin residues of the tau protein (Fig. 4B). Glycogen synthase kinase (GSK)-3 is a serine/threonine protein kinase involved in the pathogenesis of

![Figure 4](https://academic.oup.com/brain/article-abstract/133/6/1578/357248) Pathophysiological concept of tau-pathology in PSP. (A) Under physiological conditions, unphosphorylated tau (red) binds and thereby stabilizes microtubules, composed of tubulin monomers (blue and green), which are the railways for intraneuronal vesicle- and organelle-trafficking. (B) Under pathological conditions, presently available data from human post-mortem brains and experimental systems suggest a pathophysiological concept, according to which tau is hyperphosphorylated (lilac) by kinases, leading to detachment of tau from microtubules. Unbound phosphorylated tau, particularly as the 4R isoform, has an intrinsic propensity to form cytotoxic aggregates (toxic gain of function). Microtubules, in turn, are destabilized after detachment of tau and become depolymerized to tubulin-monomers, thereby losing their vital functions (loss of physiological function). Kinase inhibitors or tau-phosphatases, aggregation-inhibitors or tau-degradation enhancers and microtubule stabilizers may interfere with these pathological events at different levels.
several neurodegenerative diseases. It phosphorylates 95% of all phosphorylation sites on the tau protein and co-purifies with microtubules in biochemical assays (Jope and Johnson, 2004). It exists in two similar isoforms (α and β) encoded by two different genes. GSK-3 co-localizes with neurofibrillary tangle-like brain lesions in transgenic mice (Ishizawa et al., 2003) as well as with hyperphosphorylated tau deposits in patients with PSP (Ferrer et al., 2002). Lithium (Engel et al., 2006) and other GSK-3β inhibitors block tau pathology in transgenic mice in vivo (Serénö et al., 2009). These data suggest that the inhibition of GSK-3β might be a potential therapeutic target in PSP. Presently, several clinical trials with GSK-3β inhibitors have been initiated or are ongoing in patients with PSP [lithium, valproic acid, NPI031112 (generic name, nypta)]. The clinical study with lithium has been stopped because of poor drug tolerability. Substances activating tau-phosphatases (Le Corre et al., 2006) may also be of therapeutic interest.

There is also experimental evidence in vivo and in vitro suggesting a therapeutic benefit from small molecules acting as tau-aggregation inhibitors (Fig. 4C; for review see Bulic et al., 2009). Recently, methylthioninium chloride was orally administered for 84 weeks in a double-blind, randomized phase II trial (Serénö et al., 2009). These data suggest that the inhibition of GSK-3β might be a potential therapeutic target in PSP. Presently, several clinical trials with GSK-3β inhibitors have been initiated or are ongoing in patients with PSP (lithium, valproic acid, NPI031112 (generic name, nypta)). The clinical study with lithium has been stopped because of poor drug tolerability. Substances activating tau-phosphatases (Le Corre et al., 2006) may also be of therapeutic interest.

In an attempt to prevent the loss of microtubular function, molecules binding and stabilizing microtubules are being evaluated (Fig. 4D). For example, in a transgenic mouse model of a tauopathy, the microtubule-stabilizing drug paclitaxel (TAXOL), an alkaldoid of Taxus brevifolia (Samsonov et al., 2004), has been shown to reverse fast axonal transport deficits by functionally substituting tau (Zhang et al., 2005b). However, this approach did not successfully prevent neuronal cell death in a toxin-induced model of a tauopathy (Escarob-Khondiker et al., 2007). NAP (NAPVSIPQ, generic name, davalnetide) is a neuroprotective peptide (Gozes, 2007) interacting with the microtubule cytoskeleton to protect microtubule function (Divinski et al., 2004; 2006; Gozes and Divinski, 2004; Matsuoka et al., 2007). In a double-blind, randomized, placebo-controlled, multiple-dose phase II trial to evaluate the safety, tolerability and effect on cognitive function in patients with mild cognitive impairment, 12 weeks of intranasal administration of davalnetide resulted in a statistically significant improvement in a psychometric test termed delayed-match-to-sample task by Week 16 (62.4% change from baseline, P = 0.038 versus placebo) (http://www.allontherapeutics.com). A clinical trial to test the effects of davalnetide in PSP is presently being initiated.

Another promising therapeutic approach is the induction of increased degradation of molecules resulting in pathological conformation of tau and the resulting neuronal death. Novel chemical compounds aimed to reduce noxious tau species (http://www.remynd.com) have demonstrated robust efficacy in transgenic tau mouse models of Alzheimer’s disease (Dr Griffioen, personal communication). A drug candidate is being geared up for phase I clinical testing in 2011.

**Targeting mitochondrial dysfunction**

Coenzyme Q10 is a lipophilic substance occurring in every cell of the human body. It serves to transport electrons from the complexes I and II to complex III (Fig. 3A). Its reversible oxidation is the basis for its function as electron carrier. Reduced coenzyme Q10 additionally functions as an intracellular antioxidant (Lagendijk et al., 1996). In cultured neurons, coenzyme Q10 attenuates the toxicity of rotenone by preserving the mitochondrial membrane potential (Menke et al., 2003; Moon et al., 2005). In vivo, oral administration of coenzyme Q10 restored striatal complex I activity and ATP levels in a dose-dependent manner. Furthermore, it reduced neuronal apoptosis in rotenone-treated rats (Abdin et al., 2008) and protected from neurodegeneration induced by MPTP (Cleren et al., 2008). In patients with PSP, a regimen of 5 mg coenzyme Q10/kg/day for 6 weeks was studied in a double-blind, randomized, placebo-controlled, phase II trial, including 21 patients with clinically probable PSP. Compared to placebo, this treatment significantly increased the ratio of high-energy metabolites to low-energy metabolites (ATP/ADP, phosphorylated creatine/unphosphorylated creatine), as measured in the living brain by 31P- and 1H-MRS (Stamelou et al., 2008). Concomitantly, the PSP rating scale and the frontal assessment battery, which are the most specific disease-related clinical scales, improved significantly upon coenzyme Q10 treatment compared to placebo. In this short-term coenzyme Q10 treatment, a restoration of higher energy levels may lead to a restoration of lost functions in individual neurons, thereby leading to mild clinical improvement. During long-term treatment, restoration of higher energy levels may prevent neurons from dying, thereby retarding disease progression. Thus, the main outcome of this study may lie in the fact that it provides a strong rationale for a phase III trial to study the disease-modifying, neuroprotective potential of coenzyme Q10 in PSP. A phase III trial of coenzyme Q10 for 12 months in patients with PSP is currently recruiting (NCT00382824).

Pyruvate is a free radical scavenger, niacinamide boosts the mitochondrial cofactor NAD+ and creatine is a rapidly accessible cellular energy buffer. These nutrients have been suggested to be neuroprotective in various animal models of brain injury or degeneration (Matthews et al., 1998, 1999; Sullivan et al., 2000; Fernandez-Gomez et al., 2006; Wang et al., 2007). On the basis of the mitochondrial hypothesis of PSP, a randomized, double-blind, placebo-controlled phase I pilot study to examine safety and tolerability of a cocktail of these nutrients over 6 months in patients with PSP is currently ongoing (NCT00605930).

**Conclusions**

In recent years, much research has been done to elucidate the aetiology of this devastating disorder. Several lines of evidence point to a strong contribution of genetic predisposition and mitochondrial failure, possibly caused by environmental factors. Studies
are well underway to further define the precise identity of the genetic and environmental factors and their interplay to trigger the PSP-specific sequence of pathological events. Present knowledge has already facilitated the implementation of a series of rational clinical studies (Table 1). These studies are likely to reveal whether interference with the identified mechanisms will result in the development of urgently needed disease-modifying therapies.

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