Review: Multiple system atrophy: emerging targets for interventional therapies

N. Stefanova and G. K. Wenning

Division of Neurobiology, Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria

N. Stefanova and G. K. Wenning (2016) *Neuropathology and Applied Neurobiology* **42**, 20–32
Multiple system atrophy: emerging targets for interventional therapies

Multiple system atrophy (MSA) is a fatal orphan neurodegenerative disorder that manifests with rapidly progressive autonomic and motor dysfunction. The disease is characterized by the accumulation of α-synuclein fibrils in oligodendrocytes that form glial cytoplasmic inclusions, a neuropathological hallmark and central player in the pathogenesis of MSA. Here, we summarize the current knowledge on the etiopathogenesis and neuropathology of MSA. We discuss the role of α-synuclein pathology, microglial activation, oligodendroglial dysfunction and putative cell death mechanisms as candidate therapeutic targets in MSA.

Keywords: α-synuclein, aetiology, clinical trial, multiple system atrophy, neuropathology, pathogenesis

Introduction

Multiple system atrophy (MSA) is a rare neurodegenerative disorder that presents with a variable combination of autonomic, cerebellar, parkinsonian and pyramidal features [1]. Depending on the predominant motor phenotype the disease is clinically sub-classified in parkinsonian (MSA-P) or cerebellar (MSA-C) variant. MSA is an orphan disease with an incidence of up to 2.4 cases per 100 000 persons per year [2], while the prevalence may reach up to 7.8 patients per 100 000 population over the age of 40 [3]. The MSA-P variant seems to be more common in the western hemisphere [4,5], whereas MSA-C appears to be more frequent in Asia [6]. The motor symptom onset is usually in the fifth or sixth decade of life [5,7]. However, non-motor features including cardiovascular autonomic failure, urogenital dysfunction, or respiratory and sleep disorders may precede the motor presentation by some years [7–9]. Cognitive impairment with frontal lobe dysfunction and depression seem to be more common than originally considered [10,11]. The disease duration after clinical diagnosis is usually up to 9 years and disability milestones are reached much earlier as compared to Parkinson's disease (PD) [8].

Aetiology

Multiple system atrophy is a predominantly sporadic disorder. Genetic studies provide controversial data possibly related to geographical and intra-group heterogeneity. A recent genome-wide estimate in 907 MSA pooled cases and 3866 controls defined MSA heritability at 2.09–6.65% which was explained by the presence of misdiagnosed cases in the analysed subgroups [12]. Genetic mutations of the COQ2 gene have been linked to MSA as identified in Japanese families [13], but the link between the COQ2 gene and MSA risk was not confirmed in other patient populations [14–16]. Recently, Gaucher disease-causing GBA variants were associated with MSA [17]. Similarly, SNCA polymorphism was proposed to be associated with increased risk for MSA

Correspondence: Gregor K. Wenning, Department of Neurology, Anichstr. 35, 6020 Innsbruck, Austria. E-mail: gregor.wenning@i-med.ac.at, Telephone: +43-512-50423933; Fax: +43-512-504-24230

[18,19], but not confirmed in different patient cohorts [20–22]. Growing, but as yet sparse, evidence supports the notion that epigenetic factors may play a role in MSA [23]. Dysregulation of miR-202 and miR-96 was associated with MSA [24,25]. Circulating miRNAs were identified to be differentially expressed in MSA patients [26]. Finally, environmental toxins were associated with the risk of developing MSA in a few limited epidemiological studies [27,28]. An occupational history of farming was significantly related to higher MSA risk, whereas history of smoking was less common in MSA patients [28].

In summary, all studies on the aetiology of MSA suffer from the limited number of cases as the disease is rare and underdiagnosed. Application of the revised consensus criteria [7] increases diagnostic accuracy but sensitivity remains limited, particularly at first neurological visit. In addition, pathologically proven PD, dementia with Lewy bodies (DLB) and progressive supranuclear palsy may mimic the presentation of MSA, reducing diagnostic accuracy [29]. The definite diagnosis of MSA can currently be only made at *post mortem* examination. However, that is missing in the majority of the cases involved in genetic or epidemiological studies, which may contribute to their major limitations and inconclusive results.

Neuropathology

The pathological diagnostic hallmark of MSA is the ectopic aggregation of α-synuclein in the cytoplasm of oligodendrocytes, forming wide-spread glial cytoplasmic inclusions (GCIs) [30–32]. GCIs are typical for MSA and do not seem to commonly occur in the brains of patients with other synucleinopathies like PD or DLB [33]. However, neuronal cytoplasmic inclusions of α-synuclein may be also identified in MSA, but have different characteristics from the classical LBs seen in PD and DLB [34]. Furthermore, α-synuclein aggregation may be found intranuclearly in oligodendrocytes and neurons of MSA brains, but these inclusions seem to be less common than GCIs. [35] The structure of GCIs consists primarily of loosely packed α-synuclein fibrils [36], but multiple other components can be detected as well [37].

Parallel to the inclusion pathology, distinctive patterns of neuronal loss can be observed in MSA brains. Striatonigral degeneration (SND) underlies MSA-P and

is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) as well as projection medium-spiny neurons GABAergic neurons in the caudate-putamen. The symptomatology of MSA-C is linked to olivopontocerebellar atrophy (OPCA) which is characterized by loss of Purkinje neurons in the cerebellar cortex (along with preservation of the neurons in the deep cerebellar nuclei), as well as loss of neurons in the pontine nuclei and the inferior olivary complex [35,38,39]. Recent stereological analysis indicated neocortical neuronal loss that might underlie cognitive impairment in MSA cases [40]. Non-motor symptoms are strongly associated with neurodegeneration in the brainstem and spinal cord. Typically affected regions are the locus ceruleus, the ventrolateral tegmental nucleus, the pedunculopontine tegmental area [41–43], catecholaminergic neurons of the rostral ventrolateral medulla (C1 group) and noradrenergic neurons of the caudal ventrolateral medulla (A1 group) [44], neurons in the pontine micturition area [45], serotonergic neurons in the nucleus raphe magnus, raphe obscurus, raphe pallidus and ventrolateral medulla [46], as well as neuronal loss in the dorsal vagal nucleus, the ventrolateral nucleus ambiguus [47] and the periaqueductal grey [43,48]. Spinal cord pathology is characterized by neuronal loss in the intermediolateral columns and the Onuf's nucleus in the lumbosacral region [49,50]. Recent high-definition optical coherence tomography analysis suggested progressive retinal changes in MSA patients with reduction in the thickness of the retinal nerve fibre layer and in the macular ganglion cell complex [51].

Demyelination with variable severity is observed in MSA white matter, but severe myelin lesions seem to be present in only 50% of cases, predominantly in the MSA-C variant [52]. Myelin proteins including sphingomyelin, sulfatide and galactosylceramide were reported decreased by about 50% in degenerating MSA white matter [53].

Gliosis is invariably described in the degenerating areas of the MSA brain [54]. Region specific astroglial activation was reported to positively correlate with the α-synuclein pathology in MSA cases in contrast to PD [55]. Microglial activation is prominent in the degenerating regions of MSA brains and accompanies GCI pathology [52,56]. Upregulation of TLR4 [57] and myeloperoxidase [58] has been shown in activated MSA microglia.

The peripheral nervous system and the autonomic nervous system in MSA have been the focus of studies recently, but the outcomes are still controversial and under debate. In skin biopsies phosphorylated α-synuclein accumulation was shown in unmyelinated somatosensory fibres of 67% of MSA patients, whereas in a PD cohort phosphorylated α-synuclein seemed to accumulate in autonomic fibres [59]. However, other studies indicate lack of phosphorylated α-synuclein immunoreactivity in dermal nerve fibres in MSA in contrast to PD patients [60,61]. Importantly, recent work showed α-synuclein pathology affecting Schwann cells of cranial, spinal and autonomic nerves in MSA cases [62]. Furthermore, α-synuclein pathology in the enteric nervous system of MSA patients may occur [63].

Pathogenesis

The pathogenesis of MSA remains largely unknown. It is currently accepted that the oligodendroglial α-synuclein accumulation plays a central role in the disease process. A correlation between the GCI load and the degree of neuronal loss was reported in both the striatonigral and the olivopontocerebellar regions [39]. In the white matter, both GCI burden as well as microglial activation were shown to be greater in tissue with mild to moderate demyelination, and to decrease when demyelination became severe [52]. The leading role of GCI pathology in MSA was further supported by cases of so-called minimal change MSA. In such cases severe GCI burden associated with less severe neuronal loss triggered rapidly progressive clinical MSA profile at a younger age, with significantly shorter duration as compared to 'classical' MSA cases [64].

Neuronal α-synuclein-positive cytoplasmic inclusions (NCIs) seem to be much more widespread than previously assumed [34]. However, NCIs can be composed of non-fibrillar α-synuclein and show hierarchical pattern of neuronal involvement related with the duration of the disease, but rather independent of the pattern of neuronal destruction suggesting that other factors play a leading role in the subtype-dependent neuronal loss [34]. Although it is tempting to speculate that primary neuronal pathology leads to secondary oligodendroglial α-synuclein accumulation as suggested by the finding that NCIs may exist in areas that lack GCIs [34], the robust observation that distribution and severity of neurodegeneration reflect subregional GCI densities supports the hypothesis of a primary oligodendrogliopathy.

The causative role of GCI-like pathology for the induction of neuronal loss was confirmed experimentally in transgenic mice overexpressing human α-synuclein in oligodendrocytes under various oligodendroglia-specific promoters [65–69]. The selectivity of neurodegeneration in these models as well as in the human disease is still unresolved. In the 2,′3′-cyclic nucleotide 3′-phosphodiesterase (CNP)-α-synuclein mouse the neuronal loss related to the GCI pathology mostly affected cortical and spinal cord regions linked to secondary axonal degeneration [65]. When overexpressed under the myelin basic protein (MBP) promoter, α-synuclein in oligodendrocytes triggered dose-dependent neuronal loss in the neocortex, and fibre degeneration in the basal ganglia without loss of nigral neurons as well as demyelination and astrogliosis in the white matter tracts [66]. The proteolipid (PLP)-α-synuclein transgenic mouse [67] modelled to a great extent [70] the specific neuropathology of MSA, including progressive nigrostriatal neuronal loss [68,71] as well as loss of neurons in autonomic centres relevant to the human disease [69,72,73]. The PLP-α-synuclein transgenic mouse is the only one that replicates microglial activation accompanying the neurodegeneration of MSA type [57]. Whether the specificity of distribution of the promoters used (CNP *vs.* MBP *vs.* PLP) or the triggering of different disease cascades, that is (i) GCIs and secondary axonal degeneration in the CNP-α-synuclein model resulting in spinal cord and cortical degeneration; (ii) GCIs and secondary demyelination and astrogliosis in the MBP-α-synuclein model resulting in neocortical degeneration or (iii) GCIs and microglial activation resulting in nigral neuronal loss and degeneration in autonomic centres of PLP-α-synuclein mice is under debate. Intriguingly the presence of GCIs and microglial activation in PLP-α-synuclein mice makes them more susceptible to exogenous oxidative or proteolytic stress and moreover triggers MSA-like selective SND and OPCA not observed in wild-type mice exposed to the same stress factors [68,74]. In conclusion, it seems that the selectivity of neurodegeneration in MSA is determined by the concerted interaction of multiple factors, among them the ectopic α-synuclein accumulation in oligodendrocytes, microglial activation, oxidative stress and proteolytic dysbalance.

The source of α-synuclein in GCIs is debatable. Earlier studies suggested that α-synuclein is an exclusively neuronal protein that is not expressed in healthy adult oligodendroglia [75–77]. These data suggested that the source of α-synuclein that accumulated in MSA oligodendroglia was the diseased neurons (Figure 1). In light of the recent findings on α-synuclein cell-to-cell transmission, this hypothesis became more plausible [78]. Prusiner and co-workers suggested that MSA- and not PD-derived α-synuclein may induce α-synuclein-positive inclusion propagation in a transgenic model of PD [79,80]. However, the same studies failed to provide evidence for propagation of MSA-derived α-synuclein inclusions in wild-type/healthy mouse brain and furthermore no oligodendroglial α-synuclein aggregation was reported, therefore failing to reproduce the core pathology of MSA. Experimental studies showed that oligodendrocytes may take up α-synuclein from the extracellular space [81], but in none of these cases was typical GCI-like aggregation reported. It seems that healthy oligodendrocytes are normally able to cope with the uptaken α-synuclein and successfully 'digest' it without forming GCIs. Primary oligodendroglial dysfunction may be therefore result in ectopic accumulation of α-synuclein in oligodendrocytes [82]. Alternatively, specific α-synuclein conformational strains were proposed to be responsible for the generation of PD- and MSA-like α-synuclein seeding traits [83]. However, this hypothesis needs further clarification and support, as inoculation of MSA-derived α-synuclein into the brains of healthy non-transgenic mice did not induce GCI-like pathology, nor did. PD-derived α-synuclein show prion-like behaviour in any of the models (transgenic or non-transgenic) used [80]. In summary, the existing data indicate that neuron-derived α-synuclein with certain conformational changes may contribute to the formation of GCIs, and that primary oligodendroglial dysfunction of as yet unknown origin is permissive and may be obligatory for α-synuclein fibrils to accumulate in the cytoplasm of oligodendrocytes in MSA. In support of the notion of a primary oligodendrogliopathy p25α/TPPP dislocation and accumulation in MSA oligodendroglial soma were shown to precede the accumulation of α-synuclein [84] (Figure 1).

Recent studies based on modern technologies like laser dissection and iPSCs technology have cast doubt upon the classical dogma that α-synuclein or SNCA mRNA are not expressed by oligodendrocytes. Asi *et al.* [85] showed that laser-dissected oligodendrocytes from healthy or MSA brains do show α-synuclein expression, and even identified a tendency to increase in the signals in MSA oligodendrocytes. Djelloul *et al.* [86] proposed that oligodendrocytes differentiated from MSA-derived iPSCs express α-synuclein in contrast to those derived from healthy controls or PD patients. Although having some methodological limitations, these studies raise the possibility that an endogenous intra-oligodendroglial α-synuclein source may contribute to the GCI formation in MSA, and thus play a primary role in triggering MSA neurodegeneration.

As already mentioned, microglial activation is a prominent finding in the degenerating brain areas in MSA, and can be visualized both neuropathologically [56] as well as by *in vivo* PET imaging [87]. The association of activated microglial cells and GCI burden [56] suggests that pathological α-synuclein triggers neuroinflammatory responses in the MSA brain. This hypothesis was corroborated by a number of experimental studies both *in vitro* and *in vivo* [54,88,89]. Furthermore, observations in PLP-α-synuclein transgenic mice suggested that microglial activation occurred parallel to the dopaminergic neuronal loss in SNc between the age of 2 and 4 months. These changes could be reversed by suppression of microglial activation using minocycline [57]. TLR4 dysfunction in microglia of PLP-α-synuclein transgenic mice led to accumulation of α-synuclein in the brains and resulted in aggravated functional phenotype and increased nigral neuronal loss, suggesting that a subpopulation of microglia might play an important role in the clearance of α-synuclein and neuroprotection [88]. In summary, compelling evidence supports the notion that microglial activation may contribute to the progression of the degenerative process in MSA, like in other neurodegenerative diseases [90,91], and although non-specific this mechanism may be exploited for therapeutic interventions.

Oligodendroglial dysfunction may also be a primary event in MSA pathogenesis [82], but it is plausible that the accumulation of α-synuclein in oligodendrocytes may deepen and broaden this dysfunction resulting in reduced trophic support and demyelination, as suggested by findings in the MBP-α-synuclein transgenic mouse model of MSA [66,92]. Furthermore, changes in sphingomyelin, sulfatide and galactosamide levels
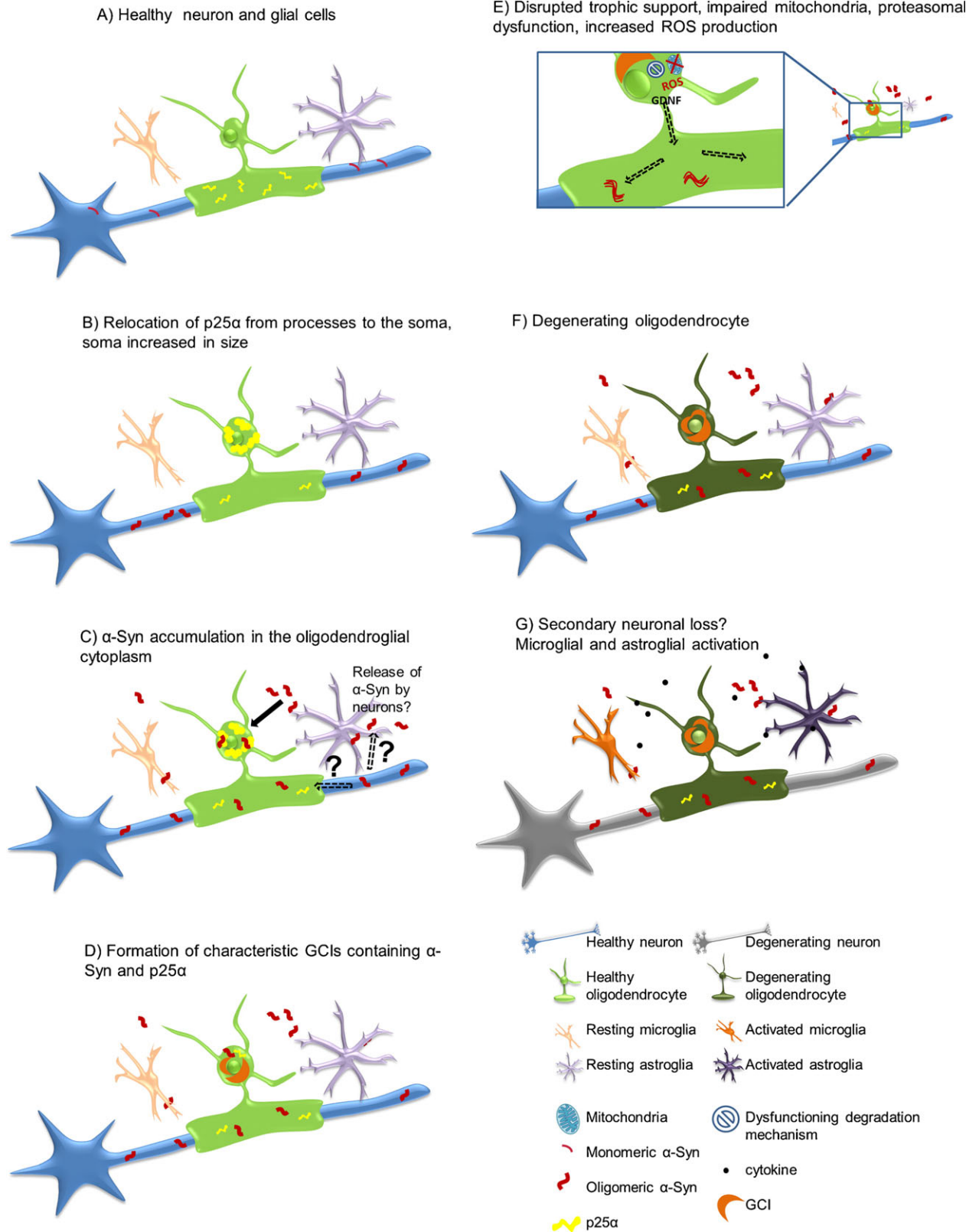
A) Healthy neuron and glial cells

E) Disrupted trophic support, impaired mitochondria, proteasomal dysfunction, increased ROS production

B) Relocation of p25α from processes to the soma, soma increased in size

F) Degenerating oligodendrocyte

C) α-Syn accumulation in the oligodendroglial cytoplasm

Release of α-Syn by neurons?

G) Secondary neuronal loss? Microglial and astroglial activation

D) Formation of characteristic GCIs containing α-Syn and p25α

Healthy neuron   Degenerating neuron
Healthy oligodendrocyte   Degenerating oligodendrocyte
Resting microglia   Activated microglia
Resting astroglia   Activated astroglia
Mitochondria   Dysfunctioning degradation mechanism
Monomeric α-Syn   cytokine
Oligomeric α-Syn   GCI
p25α

Figure 1. Possible pathological α-synuclein-spreading and accumulation mechanism leading to neurodegeneration. (**A**) Healthy neuron, oligodendrocyte, microglia and astrocyte, p25α mainly located in the myelinating oligodendroglial processes, monomeric α-synuclein present in presynaptic nerve terminals. (**B**) Relocalization of p25α from the processes to the soma, inclusion formation and swelling of the oligodendroglial soma. (**C**) Oligomeric α-synuclein accumulation in the oligodendroglial cytoplasm-(the exact source of α-synuclein remains to be determined). Possible hypotheses include exocytosed α-synuclein from neurons and uptake into oligodendrocytes by cell-to-cell propagation or upregulation of α-synuclein expression in oligodendrocytes themselves. In addition, axonal α-synuclein may be taken up by the dysfunctional oligodendroglial myelin compartment. (**D**) α-synuclein aggregates form insoluble half-moon shaped glial cytoplasmic inclusions (GCIs) characteristic for the disease. (**E**) Disruption of trophic support (e.g. GDNF), mitochondrial failure, increased production of reactive oxygen species (ROS) and proteasomal dysfunction. (**F**) Oligodendrocytes suffer from severe distress and will eventually degrade. (**G**) Activation of micro/astroglial cells by cytokines released from the damaged oligodendrocytes, and proposed secondary neuronal loss potentially due to lack of trophic support, ROS production, proteasomal failure and pro-inflammatory environment. Reproduced from Kuzdas-Wood *et al.* [106], doi:10.1016/j.pneurobio.2014.02.007, available under the terms of the Creative Commons Attribution License (CC BY).

in MSA brains suggested myelin lipid dysfunction and instability [53,93]. The oligodendroglial precursor cells are responsible for remyelination and show increased density in MSA white matter suggesting disease-associated repair efforts. [94]. Taken together the data on oligodendroglial dysfunction in MSA support the possible scenario that neurodegeneration may occur secondarily to the demyelination and lack of trophic support by oligodendrocytes bearing GCIs.

Finally, the cell death mechanisms in MSA are to a great extent unresolved. Early studies suggested increased iron levels in the degenerating areas [95], supporting the notion that oxidative stress might play a significant role in the selective neuronal loss in MSA. This idea was strongly supported by the report on COQ2 mutations linked to mitochondrial dysfunction in MSA cases [13]. Microglial activation might contribute to the increased levels of reactive oxygen species in the degenerating areas [58]. In addition to oxidative stress α-synuclein oligomers and fibrils might directly exert neurotoxicity. Experimental studies demonstrated that α-synuclein oligomers might disintegrate the cellular membranes by forming pores [96], however, this hypothesis was never proven in MSA. Alternatively α-synuclein fibril accumulation in cells might induce metabolic imbalance which might in turn promote cell death. Intriguingly, exclusive oligodendroglial apoptosis was reported in MSA brains [97,98]. Phosphoinositide 3-kinase upregulation was found in neurons and oligodendrocytes in MSA, suggesting a possible response to apoptotic signals in these cells [99]. Furthermore, the X-linked inhibitor of apoptosis protein, which selectively binds to caspases-3, -7 and -9, and inhibits their activities, was found to be upregulated in GCI- and NCI-bearing oligodendrocytes and neurons respectively

[100]. The expression of the calcium binding proteins calbindin and parvalbumin in Purkinje cells was found to be significantly reduced in MSA, whereas the apoptosis modulating proteins Bax, and Bcl-x were increased, suggesting that a diminished calcium binding capacity might lead to the pathological initiation of apoptosis in the affected areas [98]. Other mechanisms that have been discussed to relate to the cell death in MSA include proteasomal [101] or autophagosomal dysfunction [102] and excitotoxicity [103]. While experimental studies support the involvement of the proteasome and autophagosome dysfunction in oligodendroglial α-synucleinpathy [74,104], excitotoxic cell death was not aggravated by GCI pathology [105].

Target validation – where are we now?

Although the exact sequence of events in the pathogenesis of MSA is still hypothetical, there are several main players that definitely contribute to the disease process and its progression and may serve as prominent targets for disease therapy. These include (i) pathological α-synuclein species accumulation, (ii) microglial activation and neuroinflammatory responses, (iii) oligodendroglial dysfunction and (iv) cell death (Table 1). Most of the preclinical screening studies in α-synuclein transgenic models confirm the feasibility of the above-mentioned targets for the treatment of MSA. However, in a clinical setting the same therapeutic approaches appeared insufficient to provide disease modification and reduce the progression of symptoms. A major difference between the preclinical studies and the clinical trials relates to the disease stage when the therapies are initiated. The clinical diagnosis of MSA is possible only after the motor symptoms

Table 1. Experimental therapies for MSA

Therapy	Target(s)	α-Synuclein MSA model	Efficacy in α-Synuclein MSA model(s)	Clinical trial
Recombinant human growth hormone	Neuronal and glial proliferation	n.a.	n.a.	**Design:** Randomized, double-blind, placebo-controlled; **Primary end-point:** UPDRS (Unified Parkinson's Disease Rating Scale)-total, autonomic testing; **Result:** Ineffective [107]
Minocycline	Microglial activation	PLP-α-synuclein [57]	Suppression of microglial activation, neuroprotection in early disease stage	**Design:** Randomized, double-blind, placebo-controlled; phase III study **Primary end-point:** Change of UMSARS (Unified Multiple System Atrophy Rating Scale)-II **Result:** Suppression of microglial activation, but no change of symptom severity [108]
Riluzole	Cell death	n.a.	n.a.	**Design:** Randomized, double-blind, placebo-controlled; phase III study **Primary end-point:** survival **Result:** No effect on survival and motor decline [109]
Autologous MSCs (intravenous and intraarterial)	Unclear	PLP-α-synuclein [110]	Modulation of neuroinflammatory responses; mild nigral neuroprotection	**Design:** Randomized, double-blind, placebo-controlled; phase II study **Primary end-point:** Change of UMSARS-total **Result:** Slowed disease progression [111], safety concerns
Lithium	α-synuclein	n.a.	n.a.	**Design:** Randomized, double-blind, placebo-controlled; phase II study **Primary end-point:** frequency of severe AE **Result:** Discontinued due to safety concerns [112]
Rifampicin	α-synuclein	MBP-α-synuclein [113]	Reduced α-synuclein load, reduced astrogliosis, neuroprotection	**Design:** Randomized, double-blind, placebo-controlled; phase III study **Primary end-point:** UMSARS, MR parameters, BDI-II, EQ-5D scale **Result:** Study terminated, ineffective [114]
Rasagiline	Cell death	PLP-α-synuclein + 3NP [115]	Neuroprotection and functional improvement in early disease stage	**Design:** Randomized, double-blind, placebo-controlled; phase II study **Primary end-point:** Change of UMSARS-total **Result:** No change in outcome measures [116]
Fluoxetine	Neuroinflammation, Oligodendroglial dysfunction	MBP-α-synuclein [117]	Modulation of trophic factor support, improved neurogenesis, reduced astrogliosis, ameliorated demyelination, reduced	**Design:** Randomized, double-blind, placebo-controlled; phase II study **Primary end-point:** Change

Table 1. (*Continued*)

Therapy	Target(s)	α-Synuclein MSA model	Efficacy in α-Synuclein MSA model(s)	Clinical trial
			α-synuclein aggregation, neuroptotection and functional improvement	of UMSARS-II **Result:** pending
MPO inhibitor (AZD3241)	Microglial activation	PLP-α-synuclein + 3NP [58,118]	Suppression of microglial activation, reduced α-synuclein aggregation, neuroprotection and functional improvement in early disease stage Suppression of microglial activation but no neuroprotection nor functional improvement in advanced disease	**Design:** Randomized, double-blind, parallel group; phase II study **Primary end-point:** safety, tolerability and effect on microglia activation via PET imaging **Result:** Ongoing
Active immunization (AFFITOPE PD01A; PD03A)	α-synuclein	MBP-α-synuclein [119]	Reduced α-synuclein load, reduced demyelination, neuroprotection, functional improvement	**Design:** A randomized, placebo-controlled, parallel group, patient-blind, phase I study; **Primary end-point:** Safety and immunological/ therapeutic activity **Result:** Ongoing
Autologous MSCs (intrathecal)	Unclear	PLP-α-synuclein [110]	Modulation of neuroinflammatory responses; mild nigral neuroprotection	**Design:** Open label; Single group assignment, phase I study **Primary end-point:** Safety/ Efficacy **Result:** Ongoing

MSA, multiple system atrophy; 3NP, 3-nitropropionic acid; MBP, myelin basic protein; MPO, myeloperoxidase, MSCs mesenchymal stem cells; PLP, proteolipid protein; AE, adverse events.

become overt [7]. This disease stage may represent a rather late stage of the pathogenic process in MSA with a significant degree of neuronal loss that is hardly reversible. As it is likely that the disease onset precedes the motor presentation by many years, identification of early biomarkers to determine at risk individuals in selected cohorts of patients with neurogenic orthostatic hypotension, urogenital dysfunction or REM sleep behaviour disorder may significantly improve the outcomes in the treatment of MSA.

In summary, future studies on MSA will need to focus on better understanding of the triggers of the disease process, as well as on improved diagnosis, with identification of early biomarkers that may allow the timely initiation of disease modifying therapies with beneficial effects on disease progression.

Acknowledgement

References

1 Fanciulli A, Wenning GK. Multiple-system atrophy. *N Engl J Med* 2015; **372**: 249–63.

2 Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Incidence of progressive supranuclear palsy and multiple system atrophy in Olmsted County, Minnesota, 1976 to 1990. *Neurology* 1997; **49**: 1284–8.

3 Schrag A, Ben Shlomo Y, Quinn NP. Prevalence of progressive supranuclear palsy and multiple system atrophy: a cross-sectional study. *Lancet* 1999; **354**: 1771–5.

4 Low PA, Reich SG, Jankovic J, Shults CW, Stern MB, Novak P, Tanner CM, Gilman S, Marshall FJ, Wooten F, Racette B, Chelimsky T, Singer W, Sletten DM, Sandroni P, Mandrekar J. Natural history of multiple system atrophy in the USA: a prospective cohort study. *Lancet Neurol* 2015; **14**: 710–19.

5 Wenning GK, Geser F, Krismer F, Seppi K, Duerr S, Boesch S, Kollensperger M, Goebel G, Pfeiffer KP, Barone P, Pellecchia MT, Quinn NP, Koukouni V, Fowler CJ, Schrag A, Mathias CJ, Giladi N, Gurevich T, Dupont E, Ostergaard K, Nilsson CF, Widner H, Oertel W, Eggert KM, Albanese A, del SF, Tolosa E, Cardozo A, Deuschl G, Hellriegel H, Klockgether T,

Dodel R, Sampaio C, Coelho M, Djaldetti R, Melamed E, Gasser T, Kamm C, Meco G, Colosimo C, Rascol O, Meissner WG, Tison F, Poewe W. The natural history of multiple system atrophy: a prospective European cohort study. *Lancet Neurol* 2013; **12**: 264–74.

6 Watanabe H, Saito Y, Terao S, Ando T, Kachi T, Mukai E, Aiba I, Abe Y, Tamakoshi A, Doyu M, Hirayama M, Sobue G. Progression and prognosis in multiple system atrophy: an analysis of 230 Japanese patients. *Brain* 2002; **125**: 1070–83.

7 Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Durr A, Fowler CJ, Kaufmann H, Klockgether T, Lees A, Poewe W, Quinn N, Revesz T, Robertson D, Sandroni P, Seppi K, Vidailhet M. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* 2008; **71**: 670–6.

8 Stefanova N, Bucke P, Duerr S, Wenning GK. Multiple system atrophy: an update. *Lancet Neurol* 2009; **8**: 1172–8.

9 Jecmenica-Lukic M, Poewe W, Tolosa E, Wenning GK. Premotor signs and symptoms of multiple system atrophy. *Lancet Neurol* 2012; **11**: 361–8.

10 Siri C, Duerr S, Canesi M, Delazer M, Esselink R, Bloem BR, Gurevich T, Balas M, Giladi N, Santacruz P, Marti F, Tolosa E, Rubino A, Meco G, Poewe W, Pezzoli G, Wenning G, Antonini A. A cross-sectional multicenter study of cognitive and behavioural features in multiple system atrophy patients of the parkinsonian and cerebellar type. *J Neural Transm (Vienna)* 2013; **120**: 613–18.

11 Cao B, Zhao B, Wei QQ, Chen K, Yang J, Ou R, Wu Y, Shang HF. The global cognition, frontal lobe dysfunction and behavior changes in chinese patients with multiple system atrophy. *PLoS ONE* 2015; **10**: e0139773.

12 Federoff M, Price TR, Sailer A, Scholz S, Hernandez D, Nicolas A, Singleton AB, Nalls M, Houlden H. Genome-wide estimate of the heritability of Multiple System Atrophy. *Parkinsonism Relat Disord* 2016; **22**: 35–41.

13 The Multiple-System Atrophy Research Collaboration. Mutations in COQ2 in familial and sporadic multiple-system atrophy. *N Engl J Med* 2013; **369**: 233–44.

14 Ogaki K, Fujioka S, Heckman MG, Rayaprolu S, Soto-Ortolaza AI, Labbe C, Walton RL, Lorenzo-Betancor O, Wang X, Asmann Y, Rademakers R, Graff-Radford N, Uitti R, Cheshire WP, Wszolek ZK, Dickson DW, Ross OA. Analysis of COQ2 gene in multiple system atrophy. *Mol Neurodegener* 2014; **9**: 44.

15 Schottlaender LV, Houlden H. Mutant COQ2 in multiple-system atrophy. *N Engl J Med* 2014; **371**: 81.

16 Sharma M, Wenning G, Kruger R. Mutant COQ2 in multiple-system atrophy. *N Engl J Med* 2014; **371**: 80–81.

17 Mitsui J, Matsukawa T, Sasaki H, Yabe I, Matsushima M, Durr A, Brice A, Takashima H, Kikuchi A, Aoki M, Ishiura H, Yasuda T, Date H, Ahsan B, Iwata A, Goto J, Ichikawa Y, Nakahara Y, Momose Y, Takahashi Y, Hara K, Kakita A, Yamada M, Takahashi H, Onodera O, Nishizawa M, Watanabe H, Ito M, Sobue G, Ishikawa K, Mizusawa H, Kanai K, Hattori T, Kuwabara S, Arai K, Koyano S, Kuroiwa Y, Hasegawa K, Yuasa T, Yasui K, Nakashima K, Ito H, Izumi Y, Kaji R, Kato T, Kusunoki S, Osaki Y, Horiuchi M, Kondo T, Murayama S, Hattori N, Yamamoto M, Murata M, Satake W, Toda T, Filla A, Klockgether T, Wullner U, Nicholson G, Gilman S, Tanner CM, Kukull WA, Stern MB, Lee VM, Trojanowski JQ, Masliah E, Low PA, Sandroni P, Ozelius LJ, Foroud T, Tsuji S. Variants associated with Gaucher disease in multiple system atrophy. *Ann Clin Transl Neurol* 2015; **2**: 417–26.

18 Scholz SW, Houlden H, Schulte C, Sharma M, Li A, Berg D, Melchers A, Paudel R, Gibbs JR, Simon-Sanchez J, Paisan-Ruiz C, Bras J, Ding J, Chen H, Traynor BJ, Arepalli S, Zonozi RR, Revesz T, Holton J, Wood N, Lees A, Oertel W, Wullner U, Goldwurm S, Pellecchia MT, Illig T, Riess O, Fernandez HH, Rodriguez RL, Okun MS, Poewe W, Wenning GK, Hardy JA, Singleton AB, Gasser T. SNCA variants are associated with increased risk for multiple system atrophy. *Ann Neurol* 2009; **65**: 610–14.

19 Al-Chalabi A, Durr A, Wood NW, Parkinson MH, Camuzat A, Hulot JS, Morrison KE, Renton A, Sussmuth SD, Landwehrmeyer BG, Ludolph A, Agid Y, Brice A, Leigh PN, Bensimon G. Genetic variants of the alpha-synuclein gene SNCA are associated with multiple system atrophy. *PLoS ONE* 2009; **4**: e7114.

20 Ross OA, Vilarino-Guell C, Wszolek ZK, Farrer MJ, Dickson DW. Reply to: SNCA variants are associated with increased risk of multiple system atrophy. *Ann Neurol* 2010; **67**: 414–15.

21 Sun Z, Xiang X, Tang B, Chen Z, Peng H, Xia K, Jiang H. SNP rs11931074 of the SNCA gene may not be associated with multiple system atrophy in Chinese population. *Int J Neurosci* 2015; **125**: 612–15.

22 Yun JY, Lee WW, Lee JY, Kim HJ, Park SS, Jeon BS. SNCA variants and multiple system atrophy. *Ann Neurol* 2010; **67**: 554–5.

23 Sturm E, Stefanova N. Multiple system atrophy: genetic or epigenetic? *Exp Neurobiol* 2014; **23**: 277–91.

24 Lee ST, Chu K, Jung KH, Ban JJ, Im WS, Jo HY, Park JH, Lim JY, Shin JW, Moon J, Lee SK, Kim M, Roh JK. Altered expression of miR-202 in cerebellum of multiple-system atrophy. *Mol Neurobiol* 2015; **51**: 180–6.

25 Ubhi K, Rockenstein E, Kragh C, Inglis C, Spencer B, Michael S, Mante M, Adame A, Galasko D, Masliah E. Widespread microRNA dysregulation in multiple system atrophy - disease-related alteration in miR-96. *Eur J Neurosci* 2014; **39**: 1026–41.

26 Vallelunga A, Ragusa M, Di MS, Iannitti T, Pilleri M, Biundo R, Weis L, Di PC, De IA, Nicoletti A, Zappia

M, Purrello M, Antonini A. Identification of circulating microRNAs for the differential diagnosis of Parkinson's disease and Multiple System Atrophy. *Front Cell Neurosci* 2014; **8**: 156.

27 Vanacore N. Epidemiological evidence on multiple system atrophy. *J Neural Transm* 2005; **112**: 1605–12.

28 Vanacore N, Bonifati V, Fabbrini G, Colosimo C, De Michele G, Marconi R, Stocchi F, Nicholl D, Bonuccelli U, De MM, Vieregge P, Meco G. Case-control study of multiple system atrophy. *Mov Disord* 2005; **20**: 158–63.

29 Koga S, Aoki N, Uitti RJ, van GJ, Cheshire WP, Josephs KA, Wszolek ZK, Langston JW, Dickson DW. When DLB, PD, and PSP masquerade as MSA: an autopsy study of 134 patients. *Neurology* 2015; **85**: 404–12.

30 Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). *J Neurol Sci* 1989; **94**: 79–100.

31 Goedert M, Spillantini MG. Lewy body diseases and multiple system atrophy as alpha-synucleinopathies. *Mol Psychiatry* 1998; **3**: 462–5.

32 Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M. Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neurosci Lett* 1998; **251**: 205–8.

33 Spillantini MG, Goedert M. The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Ann N Y Acad Sci* 2000; **920**: 16–27.

34 Cykowski MD, Coon EA, Powell SZ, Jenkins SM, Benarroch EE, Low PA, Schmeichel AM, Parisi JE. Expanding the spectrum of neuronal pathology in multiple system atrophy. *Brain* 2015; **138**: 2293–309.

35 Yoshida M. Multiple system atrophy: alpha-synuclein and neuronal degeneration. *Neuropathology* 2007; **27**: 484–93.

36 Arima K, Murayama S, Mukoyama M, Inose T. Immunocytochemical and ultrastructural studies of neuronal and oligodendroglial cytoplasmic inclusions in multiple system atrophy. 1. Neuronal cytoplasmic inclusions. *Acta Neuropathol* 1992; **83**: 453–60.

37 Jellinger KA. Neuropathology of multiple system atrophy: new thoughts about pathogenesis. *Mov Disord* 2014; **29**: 1720–41.

38 Ahmed Z, Asi YT, Sailer A, Lees AJ, Houlden H, Revesz T, Holton JL. The neuropathology, pathophysiology and genetics of multiple system atrophy. *Neuropathol Appl Neurobiol* 2012; **38**: 4–24.

39 Ozawa T, Paviour D, Quinn NP, Josephs KA, Sangha H, Kilford L, Healy DG, Wood NW, Lees AJ, Holton JL, Revesz T. The spectrum of pathological involvement of the striatonigral and olivopontocerebellar sys-tems in multiple system atrophy: clinicopathological correlations. *Brain* 2004; **127**: 2657–71.

40 Salvesen L, Ullerup BH, Sunay FB, Brudek T, Lokkegaard A, Aganvder TK, Winge K, Pakkenberg B. Changes in total cell numbers of the basal ganglia in patients with multiple system atrophy – a stereological study. *Neurobiol Dis* 2015; **74**: 104–13.

41 Benarroch EE, Schmeichel AM, Parisi JE. Depletion of mesopontine cholinergic and sparing of raphe neurons in multiple system atrophy. *Neurology* 2002; **59**: 944–6.

42 Schmeichel AM, Buchhalter LC, Low PA, Parisi JE, Boeve BW, Sandroni P, Benarroch EE. Mesopontine cholinergic neuron involvement in Lewy body dementia and multiple system atrophy. *Neurology* 2008; **70**: 368–73.

43 Benarroch EE, Schmeichel AM, Low PA, Parisi JE. Differential involvement of the periaqueductal gray in multiple system atrophy. *Auton Neurosci* 2010; **158**: 111–17.

44 Benarroch EE. New findings on the neuropathology of multiple system atrophy. *Auton Neurosci* 2002; **96**: 59–62.

45 Benarroch EE. Brainstem in multiple system atrophy: clinicopathological correlations. *Cell Mol Neurobiol* 2003; **23**: 519–26.

46 Benarroch EE, Schmeichel AM, Low PA, Parisi JE. Involvement of medullary serotonergic groups in multiple system atrophy. *Ann Neurol* 2004; **55**: 418–22.

47 Benarroch EE, Schmeichel AM, Sandroni P, Low PA, Parisi JE. Involvement of vagal autonomic nuclei in multiple system atrophy and Lewy body disease. *Neurology* 2006; **66**: 378–83.

48 Benarroch EE. Neural control of the bladder: recent advances and neurologic implications. *Neurology* 2010; **75**: 1839–46.

49 Wenning GK, Tison F, Ben Shlomo Y, Daniel SE, Quinn NP. Multiple system atrophy: a review of 203 pathologically proven cases. *Mov Disord* 1997; **12**: 133–47.

50 Yamamoto T, Sakakibara R, Uchiyama T, Liu Z, Ito T, Awa Y, Yamamoto K, Kinou M, Yamanishi T, Hattori T. When is Onuf's nucleus involved in multiple system atrophy? A sphincter electromyography study. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1645–8.

51 Mendoza-Santiesteban CE, Palma JA, Martinez J, Norcliffe-Kaufmann L, Hedges TR III, Kaufmann H. Progressive retinal structure abnormalities in multiple system atrophy. *Mov Disord* 2015; **30**: 1944–53.

52 Ishizawa K, Komori T, Arai N, Mizutani T, Hirose T. Glial cytoplasmic inclusions and tissue injury in multiple system atrophy: a quantitative study in white matter (olivopontocerebellar system) and gray matter (nigrostriatal system). *Neuropathology* 2008; **28**: 249–57.

53 Don AS, Hsiao JH, Bleasel JM, Couttas TA, Halliday GM, Kim WS. Altered lipid levels provide evidence for

myelin dysfunction in multiple system atrophy. *Acta Neuropathol Commun* 2014; **2**: 150.

54 Fellner L, Jellinger KA, Wenning GK, Stefanova N. Glial dysfunction in the pathogenesis of alpha-synucleinopathies: emerging concepts. *Acta Neuropathol* 2011; **121**: 675–93.

55 Tong J, Ang LC, Williams B, Furukawa Y, Fitzmaurice P, Guttman M, Boileau I, Hornykiewicz O, Kish SJ. Low levels of astroglial markers in Parkinson's disease: relationship to alpha-synuclein accumulation. *Neurobiol Dis* 2015; **82**: 243–53.

56 Ishizawa K, Komori T, Sasaki S, Arai N, Mizutani T, Hirose T. Microglial activation parallels system degeneration in multiple system atrophy. *J Neuropathol Exp Neurol* 2004; **63**: 43–52.

57 Stefanova N, Reindl M, Neumann M, Kahle PJ, Poewe W, Wenning GK. Microglial activation mediates neurodegeneration related to oligodendroglial alpha-synucleinopathy: implications for multiple system atrophy. *Mov Disord* 2007; **22**: 2196–203.

58 Stefanova N, Georgievska B, Eriksson H, Poewe W, Wenning GK. Myeloperoxidase inhibition ameliorates multiple system atrophy-like degeneration in a transgenic mouse model. *Neurotox Res* 2012; **21**: 393–404.

59 Doppler K, Weis J, Karl K, Ebert S, Ebentheuer J, Trenkwalder C, Klebe S, Volkmann J, Sommer C. Distinctive distribution of phospho-alpha-synuclein in dermal nerves in multiple system atrophy. *Mov Disord* 2015; **30**: 1688–92.

60 Zange L, Noack C, Hahn K, Stenzel W, Lipp A. Phosphorylated alpha-synuclein in skin nerve fibres differentiates Parkinson's disease from multiple system atrophy. *Brain* 2015; **138**: 2310–21.

61 Haga R, Sugimoto K, Nishijima H, Miki Y, Suzuki C, Wakabayashi K, Baba M, Yagihashi S, Tomiyama M. Clinical utility of skin biopsy in differentiating between parkinson's disease and multiple system atrophy. *Parkinsons Dis* 2015; **2015**: 167038.

62 Nakamura K, Mori F, Kon T, Tanji K, Miki Y, Tomiyama M, Kurotaki H, Toyoshima Y, Kakita A, Takahashi H, Yamada M, Wakabayashi K. Filamentous aggregations of phosphorylated alpha-synuclein in Schwann cells (Schwann cell cytoplasmic inclusions) in multiple system atrophy. *Acta Neuropathol Commun* 2015; **3**: 29.

63 Pouclet H, Lebouvier T, Coron E, Rouaud T, Flamant M, Toulgoat F, Roy M, Vavasseur F, Bruley V, Neunlist M, Derkinderen P. Analysis of colonic alpha-synuclein pathology in multiple system atrophy. *Parkinsonism Relat Disord* 2012; **18**: 893–5.

64 Ling H, Asi YT, Petrovic IN, Ahmed Z, Prashanth LK, Hazrati LN, Nishizawa M, Ozawa T, Lang A, Lees AJ, Revesz T, Holton JL. Minimal change multiple system atrophy: an aggressive variant? *Mov Disord* 2015; **30**: 960–7.

65 Yazawa I, Giasson BI, Sasaki R, Zhang B, Joyce S, Uryu K, Trojanowski JQ, Lee VM. Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. *Neuron* 2005; **45**: 847–59.

66 Shults CW, Rockenstein E, Crews L, Adame A, Mante M, Larrea G, Hashimoto M, Song D, Iwatsubo T, Tsuboi K, Masliah E. Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. *J Neurosci* 2005; **25**: 10689–99.

67 Kahle PJ, Neumann M, Ozmen L, Muller V, Jacobsen H, Spooren W, Fuss B, Mallon B, Macklin WB, Fujiwara H, Hasegawa M, Iwatsubo T, Kretzschmar HA, Haass C. Hyperphosphorylation and insolubility of alpha-synuclein in transgenic mouse oligodendrocytes. *EMBO Rep* 2002; **3**: 583–8.

68 Stefanova N, Reindl M, Neumann M, Haass C, Poewe W, Kahle PJ, Wenning GK. Oxidative stress in transgenic mice with oligodendroglial alpha-synuclein overexpression replicates the characteristic neuropathology of multiple system atrophy. *Am J Pathol* 2005; **166**: 869–76.

69 Stemberger S, Poewe W, Wenning GK, Stefanova N. Targeted overexpression of human alpha-synuclein in oligodendroglia induces lesions linked to MSA -like progressive autonomic failure. *Exp Neurol* 2010; **224**: 459–64.

70 Flabeau O, Meissner WG, Ozier A, Berger P, Tison F, Fernagut PO. Breathing variability and brainstem serotonergic loss in a genetic model of multiple system atrophy. *Mov Disord* 2014; **29**: 388–95.

71 Stefanova N, Wenning GK. Animal models of multiple system atrophy. *Clin Auton Res* 2015; **25**: 9–17.

72 Boudes M, Uvin P, Pinto S, Voets T, Fowler CJ, Wenning GK, De RD, Stefanova N. Bladder dysfunction in a transgenic mouse model of multiple system atrophy. *Mov Disord* 2013; **28**: 347–55.

73 Kuzdas D, Stemberger S, Gaburro S, Stefanova N, Singewald N, Wenning GK. Oligodendroglial alpha-synucleinopathy and MSA-like cardiovascular autonomic failure: experimental evidence. *Exp Neurol* 2013; **247**: 531–6.

74 Stefanova N, Kaufmann WA, Humpel C, Poewe W, Wenning GK. Systemic proteasome inhibition triggers neurodegeneration in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. *Acta Neuropathol* 2012; **124**: 51–65.

75 Solano SM, Miller DW, Augood SJ, Young AB, Penney JB Jr. Expression of alpha-synuclein, parkin, and ubiquitin carboxy-terminal hydrolase L1 mRNA in human brain: genes associated with familial Parkinson's disease. *Ann Neurol* 2000; **47**: 201–10.

76 Miller DW, Johnson JM, Solano SM, Hollingsworth ZR, Standaert DG, Young AB. Absence of alpha-synuclein mRNA expression in normal and multiple system atrophy oligodendroglia. *J Neural Transm* 2005; **112**: 1613–24.

77 Ozawa T, Okuizumi K, Ikeuchi T, Wakabayashi K, Takahashi H, Tsuji S. Analysis of the expression level of alpha-synuclein mRNA using postmortem brain samples from pathologically confirmed cases of multiple system atrophy. *Acta Neuropathol (Berl)* 2001; **102**: 188–90.

78 Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 2012; **338**: 949–53.

79 Watts JC, Giles K, Oehler A, Middleton L, Dexter DT, Gentleman SM, DeArmond SJ, Prusiner SB. Transmission of multiple system atrophy prions to transgenic mice. *Proc Natl Acad Sci U S A* 2013; **110**: 19555–60.

80 Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, Patel S, Oehler A, Lowe JK, Kravitz SN, Geschwind DH, Glidden DV, Halliday GM, Middleton LT, Gentleman SM, Grinberg LT, Giles K. Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc Natl Acad Sci U S A* 2015; **112**: E5308–17.

81 Reyes JF, Rey NL, Bousset L, Melki R, Brundin P, Angot E. Alpha-synuclein transfers from neurons to oligodendrocytes. *Glia* 2014; **62**: 387–98.

82 Wenning GK, Stefanova N, Jellinger KA, Poewe W, Schlossmacher MG. Multiple system atrophy: a primary oligodendrogliopathy. *Ann Neurol* 2008; **64**: 239–46.

83 Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, Van den Haute C, Melki R, Baekelandt V. Alpha-synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* 2015; **522**: 340–4.

84 Song YJ, Lundvig DM, Huang Y, Gai WP, Blumbergs PC, Hojrup P, Otzen D, Halliday GM, Jensen PH. p25alpha relocalizes in oligodendroglia from myelin to cytoplasmic inclusions in multiple system atrophy. *Am J Pathol* 2007; **171**: 1291–303.

85 Asi YT, Simpson JE, Heath PR, Wharton SB, Lees AJ, Revesz T, Houlden H, Holton JL. Alpha-synuclein mRNA expression in oligodendrocytes in MSA. *Glia* 2014; **62**: 964–70.

86 Djelloul M, Holmqvist S, Boza-Serrano A, Azevedo C, Yeung MS, Goldwurm S, Frisen J, Deierborg T, Roybon L. Alpha-synuclein expression in the oligodendrocyte lineage: an in vitro and in vivo study using rodent and human models. *Stem Cell Reports* 2015; **5**: 174–84.

87 Gerhard A, Banati RB, Goerres GB, Cagnin A, Myers R, Gunn RN, Turkheimer F, Good CD, Mathias CJ, Quinn N, Schwarz J, Brooks DJ. [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. *Neurology* 2003; **61**: 686–9.

88 Stefanova N, Fellner L, Reindl M, Masliah E, Poewe W, Wenning GK. Toll-like receptor 4 promotes alpha-synuclein clearance and survival of nigral dopaminergic neurons. *Am J Pathol* 2011; **179**: 954–63.

89 Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, Wenning GK, Stefanova N. Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. *Glia* 2013; **61**: 349–60.

90 Fellner L, Stefanova N. The role of glia in alpha-synucleinopathies. *Mol Neurobiol* 2013; **47**: 575–86.

91 von BR, Eugenin-von BL, Eugenin J. Microglial cell dysregulation in brain aging and neurodegeneration. *Front Aging Neurosci* 2015; **7**: 124.

92 Ubhi K, Rockenstein E, Mante M, Inglis C, Adame A, Patrick C, Whitney K, Masliah E. Neurodegeneration in a transgenic mouse model of multiple system atrophy is associated with altered expression of oligodendroglial-derived neurotrophic factors. *J Neurosci* 2010; **30**: 6236–46.

93 Wong JH, Halliday GM, Kim WS. Exploring myelin dysfunction in multiple system atrophy. *Exp Neurobiol* 2014; **23**: 337–44.

94 Ahmed Z, Asi YT, Lees AJ, Revesz T, Holton JL. Identification and quantification of oligodendrocyte precursor cells in multiple system atrophy, progressive supranuclear palsy and Parkinson's disease. *Brain Pathol* 2013; **23**: 263–73.

95 Dexter DT, Jenner P, Schapira AH, Marsden CD. Alterations in levels of iron, ferritin, and other trace metals in neurodegenerative diseases affecting the basal ganglia. The Royal Kings and Queens Parkinson's Disease Research Group. *Ann Neurol* 1992; **32** (Suppl): S94–100.

96 Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, Keller SH, Platoshyn O, Yuan JX, Masliah E. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. *FEBS J* 2007; **274**: 1862–77.

97 Probst-Cousin S, Rickert CH, Schmid KW, Gullotta F. Cell death mechanisms in multiple system atrophy. *J Neuropathol Exp Neurol* 1998; **57**: 814–21.

98 Wullner U, Weller M, Kornhuber J, Bornemann A, Schulz JB, Riederer P, Klockgether T. Altered expression of calcium- and apoptosis-regulating proteins in multiple system atrophy Purkinje cells. *Mov Disord* 2000; **15**: 269–75.

99 Nakamura S, Kawamoto Y, Kitajima K, Honjo Y, Matsuo A, Nakano S, Akiguchi I. Immunohistochemical localization of phosphoinositide 3-kinase in brains with multiple system atrophy. *Clin Neuropathol* 2001; **20**: 243–7.

100 Kawamoto Y, Ito H, Ihara M, Takahashi R. XIAP immunoreactivity in glial and neuronal cytoplasmic inclusions in multiple system atrophy. *Clin Neuropathol* 2014; **33**: 76–83.

101 Furukawa Y, Vigouroux S, Wong H, Guttman M, Rajput AH, Ang L, Briand M, Kish SJ, Briand Y. Brain proteasomal function in sporadic Parkinson's disease and related disorders. *Ann Neurol* 2002; **51**: 779–82.

102 Tanji K, Odagiri S, Maruyama A, Mori F, Kakita A, Takahashi H, Wakabayashi K. Alteration of autophagosomal proteins in the brain of multiple system atrophy. *Neurobiol Dis* 2013; **49**: 190–8.

103 Greenamyre JT. The role of glutamate in neurotransmission and in neurologic disease. *Arch Neurol* 1986; **43**: 1058–63.

104 Schwarz L, Goldbaum O, Bergmann M, Probst-Cousin S, Richter-Landsberg C. Involvement of macroautophagy in multiple system atrophy and protein aggregate formation in oligodendrocytes. *J Mol Neurosci* 2012; **47**: 256–66.

105 Kuzdas-Wood D, Fellner L, Premstaller M, Borm C, Bloem B, Kirik D, Wenning GK, Stefanova N. Overexpression of alpha-synuclein in oligodendrocytes does not increase susceptibility to focal striatal excitotoxicity. *BMC Neurosci* 2015; **16**: 86.

106 Kuzdas-Wood D, Stefanova N, Jellinger KA, Seppi K, Schlossmacher MG, Poewe W, Wenning GK. Towards translational therapies for multiple system atrophy. *Prog Neurobiol* 2014; **118**: 19–35.

107 Holmberg B, Johansson JO, Poewe W, Wenning G, Quinn NP, Mathias C, Tolosa E, Cardozo A, Dizdar N, Rascol O, Slaoui T. Safety and tolerability of growth hormone therapy in multiple system atrophy: a double-blind, placebo-controlled study. *Mov Disord* 2007; **22**: 1138–44.

108 Dodel R, Spottke A, Gerhard A, Reuss A, Reinecker S, Schimke N, Trenkwalder C, Sixel-Doring F, Herting B, Kamm C, Gasser T, Sawires M, Geser F, Kollensperger M, Seppi K, Kloss M, Krause M, Daniels C, Deuschl G, Bottger S, Naumann M, Lipp A, Gruber D, Kupsch A, Du Y, Turkheimer F, Brooks DJ, Klockgether T, Poewe W, Wenning G, Schade-Brittinger C, Oertel WH, Eggert K. Minocycline 1-year therapy in multiple-system-atrophy: effect on clinical symptoms and [(11)C] (R)-PK11195 PET (MEMSA-trial). *Mov Disord* 2010; **25**: 97–107.

109 Bensimon G, Ludolph A, Agid Y, Vidailhet M, Payan C, Leigh PN. Riluzole treatment, survival and diagnostic criteria in Parkinson plus disorders: the NNIPPS study. *Brain* 2009; **132**: 156–71.

110 Stemberger S, Jamnig A, Stefanova N, Lepperdinger G, Reindl M, Wenning GK. Mesenchymal stem cells in a transgenic mouse model of multiple system atrophy: immunomodulation and neuroprotection. *PLoS ONE* 2011; **6**: e19808.

111 Lee PH, Lee JE, Kim HS, Song SK, Lee HS, Nam HS, Cheong JW, Jeong Y, Park HJ, Kim DJ, Nam CM, Lee JD, Kim HO, Sohn YH. A randomized trial of mesenchymal stem cells in multiple system atrophy. *Ann Neurol* 2012; **72**: 32–40.

112 Sacca F, Marsili A, Quarantelli M, Brescia Morra V, Brunetti A, Carbone R, Pane C, Puorro G, Russo CV, Salvatore E, Tucci T, De MG, Filla A. A randomized clinical trial of lithium in multiple system atrophy. *J Neurol* 2013; **260**: 458–61.

113 Ubhi K, Rockenstein E, Mante M, Patrick C, Adame A, Thukral M, Shults C, Masliah E. Rifampicin reduces alpha-synuclein in a transgenic mouse model of multiple system atrophy. *NeuroReport* 2008; **19**: 1271–6.

114 Low PA, Robertson D, Gilman S, Kaufmann H, Singer W, Biaggioni I, Freeman R, Perlman S, Hauser RA, Cheshire W, Lessig S, Vernino S, Mandrekar J, Dupont WD, Chelimsky T, Galpern WR. Efficacy and safety of rifampicin for multiple system atrophy: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol* 2014; **13**: 268–75.

115 Stefanova N, Poewe W, Wenning GK. Rasagiline is neuroprotective in a transgenic model of multiple system atrophy. *Exp Neurol* 2008; **210**: 421–7.

116 Poewe W, Seppi K, Fitzer-Attas CJ, Wenning GK, Gilman S, Low PA, Giladi N, Barone P, Sampaio C, Eyal E, Rascol O. Efficacy of rasagiline in patients with the parkinsonian variant of multiple system atrophy: a randomised, placebo-controlled trial. *Lancet Neurol* 2015; **14**: 145–52.

117 Ubhi K, Inglis C, Mante M, Patrick C, Adame A, Spencer B, Rockenstein E, May V, Winkler J, Masliah E. Fluoxetine ameliorates behavioral and neuropathological deficits in a transgenic model mouse of alpha-synucleinopathy. *Exp Neurol* 2012; **234**: 405–16.

118 Kaindlstorfer C, Sommer P, Georgievska B, Mather RJ, Kugler AR, Poewe W, Wenning GK, Stefanova N. Failure of neuroprotection despite microglial suppression by delayed-start myeloperoxidase inhibition in a model of advanced multiple system atrophy: clinical implications. *Neurotox Res* 2015; **28**: 185–94.

119 Mandler M, Valera E, Rockenstein E, Mante M, Weninger H, Patrick C, Adame A, Schmidhuber S, Santic R, Schneeberger A, Schmidt W, Mattner F, Masliah E. Active immunization against alpha-synuclein ameliorates the degenerative pathology and prevents demyelination in a model of multiple system atrophy. *Mol Neurodegener* 2015; **10**: 10.