Multiple system atrophy: cellular and molecular pathology

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Abstract

Multiple system atrophy is an adult onset neurodegenerative disease, featuring parkinsonism, ataxia, and autonomic failure, in any combination. The condition is relentlessly progressive and responds poorly to treatment. Death occurs on average six to seven years after the onset of symptoms. No familial cases of multiple system atrophy have been reported, and no environmental factors have been robustly implicated as aetiological factors. However, analytical epidemiological studies are hampered because the condition is relatively rare. The discovery of the glial cytoplasmic inclusion (GCI) in 1989 helped to define multiple system atrophy as a clinicopathological entity, and drew attention to the prominent, if not primary, role played by the oligodendrocyte in the pathogenesis of the condition. Subsequently, GCIs were shown to be positive for α-synuclein, with immunostaining for α-synuclein (GNCIs), and in neuronal nuclei (nuclear inclusions (NNIs), respectively), and also in axons, although at lower frequencies than the GCIs. However, the mechanisms underlying inclusion body formation, and how it relates to glial and neuronal loss in MSA, remain to be determined. Understanding these processes will be essential before an effective treatment to halt or reverse the disease can be developed.

Recently, an abnormally insoluble filamentous/tubular form of α-synuclein protein was shown to be a major component of GCIs, NCIs, and NNIs, but not of GNIs (fig 1). The accumulation of this synaptic protein in MSA provides an unexpected and as yet unexplained link with Parkinson’s disease, dementia with Lewy bodies, and neurodegeneration with brain iron accumulation, type 1 (NBIA 1 or Hallervorden-Spatz syndrome), in which α-synuclein is an important component of the Lewy bodies. MSA, Parkinson’s disease, dementia with Lewy bodies, and NBIA 1 are thus sometimes referred to as “synucleinopathies”.

Presentation and natural history of MSA

Although parkinsonism develops in most patients with MSA (84–100%), “pure” cerebellar forms of the disorder are uncommon (0–16%). Autonomic failure was reported in 78% of 188 pathologically confirmed cases of MSA. Symptomatic orthostatic hypotension occurring within one year of onset of parkinsonism has recently been shown to predict MSA in 75% of cases, although this study was retrospective, and the numbers involved were small. The parkinsonism of MSA is more variably responsive to levodopa than Parkinson’s disease itself. Up to one third of patients may show a moderate or good response to this...
drug, but this usually declines over the first one to two years of treatment.14

A male predominance of 1.4 : 1 was reported by Quinn in a review of 231 pathologically confirmed MSA cases.14 If confirmed, this observation may have aetiological relevance. For example, there may be greater environmental exposure to putative toxins in men, or endogenous protective factors (hormonal perhaps) in women.

The mean age at onset in 203 pathologically confirmed cases of MSA was 54.3 (range, 33–78) years.16 The upper limit of the age range must be viewed with a degree of caution, however, because clinicopathological series are prone to bias, and older patients are less likely to undergo postmortem examination.17 A population based study is necessary to confirm the age range and mean age of disease onset. The mean disease duration was only 6.2 (range, 0.5–24) years in a recent meta-analysis of 433 pathologically confirmed cases,18 indicative of a relentlessly progressive illness. This review was retrospective, however, and may have been biased towards the most severe cases. Cerebellar features were associated with marginally increased survival in this review, but this did not reach significance.

Neuropathology of MSA and clinicopathological correlation
Macroscopically, the brain in MSA shows varying degrees of atrophy of the cerebellum, cerebellar peduncles (especially the middle and inferior peduncles), pons, medulla, and also the posterolateral putamen. There may be loss of pigment in the substantia nigra and also discoloration of the striatum (notably the putamen). Excessive iron accumulation has been demonstrated within the striatum to account for this pigmentary change.19

Oligodendrocyte GCIs and GNIs have a so called “system bound” distribution in the suprasegmental motor systems (primary motor, and higher motor areas of the cerebral cortex, pyramidal, extrapyramidal, and corticocerebellar systems), in the supraspinal autonomic systems, and in their targets.20–22 Neuropathological changes in neurones follow a similar system bound distribution and include variable neuronal loss, and densities of NCIs and NNIs in the striatum, substantia nigra, locus ceruleus, inferior olives, pontine nuclei, cerebellar Purkinje cells, dorsal motor nucleus of vagus, nucleus vestibularis, intermediolateral cell column of the spinal cord, and Onuf’s nucleus.23 Rare MSA cases showing

Figure 1 α-Synuclein pathology in multiple system atrophy. (A) Glial cytoplasmic inclusions (GCIs) (arrows) in cerebellar white matter with cerebellar granule cells in the lower right corner. (B) Neuronal cytoplasmic inclusions (NCIs) (double arrows) and an early formation of a neuronal nuclear inclusion (NNI) (triple arrow) in neurones of pontine nuclei with neurites in the neuropil (arrowheads). (C) A GCI (arrow) and the early formation of an NCI (double arrow) and NNI (triple arrow) in a neurone of the pontine nuclei with neurites in the neuropil (arrowheads). (D) A GCI (arrow) and an NNI (triple arrow) in the pontine nuclei. Immunohistochemistry was performed using monoclonal antibodies to α-synuclein (Novocastra Laboratories, Newcastle upon Tyne, UK) on formalin fixed, paraffin wax embedded sections that had been pretreated with formic acid; Vectastain Elite ABC peroxidase kit (Vector, Peterborough, UK); DAB; Haematoxylin counterstain. Scale bars in A to D, 30 μm.
additional involvement of frontal or temporal lobes, including atrophy, and the presence of GCIs and NCIs have also been reported. White matter pathology is also increasingly recognised in MSA, with the fibre tracts of the suprasegmental motor and supraspinal autonomic systems (see above) bearing the brunt of demyelination. Furthermore, using monoclonal and polyclonal antibodies that recognise epitope QDENVV of human myelin basic protein, accessible only in areas of myelin degeneration, Matsue and colleagues have demonstrated that in MSA myelin degeneration and abnormal oligodendrocytes were widespread, even in areas where GCIs were not detectable, and where myelin appeared intact with standard myelin stains.

A correlation has been established between akinesia and the degree of nigral and putaminal cell loss, although rigidity relates only to this last feature. Araxia correlates with the degree of olivopontocerebellar atrophy and pyramidal signs with pyramidal tract pallor. Recently, a loss of Betz cells was documented in all of seven patients with MSA studied, six of whom had pyramidal signs documented before death. Some groups have found an association between postural hypotension and intermediolateral cell column degeneration, but this finding has not been confirmed by others. A severe loss of catecholaminergic neurones in the rostral ventrolateral medulla has been noted in patients with MSA. This area is involved in the control of sympathetic cardiovascular outflow.

There is limited information available about striatal dopamine receptors in MSA and their correlation with extrapyramidal features. A relative preservation of putaminal cell counts in patients with MSA responding to levodopa has been reported, and resistance to levodopa might be the result of a loss of putaminal dopamine D2 receptors. Patients with MSA who are not responsive to levodopa may have more severe topographical degeneration of the putaminal efferent terminals in the ventrolateral portion of the globus pallidus. In contrast, a case of MSA has been reported where there was no significant response to dopaminergic treatment, yet there was no evidence of putaminal cell loss at necropsy.

Furthermore, in vivo positron emission tomography (PET) studies using the dopamine D2 receptor ligand [11C]-raclopride have demonstrated only a modest 15% reduction in striatal D2 sites. The failure to respond to levodopa was thought to reflect “loss of other basal ganglia connections”.

**Genes, polymorphisms, and MSA**

MSA, as reflected in its current definition, is regarded as a sporadic disease. Familial cases have not been described, although clinical symptoms of MSA were reported by a significantly larger group of patients’ relatives than controls in one study. However, a self-administered questionnaire was used to elicit symptoms from the relatives in this series, leading to potential bias.

Several studies have looked for polymorphisms or mutations in candidate genes, which may predispose an individual towards developing MSA. The apolipoprotein e4 allele is not over-represented in MSA when compared with controls, and there have been conflicting reports of the association of a cytochrome P-450-2D6 polymorphism with MSA. There is no evidence to suggest that patients with MSA have expansions at the SCA1 and SCA3 alleles. Furthermore, there is no evidence to support an association between polymorphisms in the H5 pore region of the human homologue of the weaver mouse gene, hiGIRK2, the insulin-like growth factor 1 receptor gene (linked with a decreased intracellular response to insulin-like growth factor 1 in the cerebellar cortex of lurcher mice), or the ciliary neurotrophic factor gene. It seems improbable that a mutation in the α-synuclein gene underlies protein accumulation in MSA. Recent studies have not found a mutation in the entire coding region of the α-synuclein gene in patients with pathologically confirmed MSA. However, mutations in the regulatory or intronic regions of the gene have not been ruled out.

Polymorphisms in the α-synuclein gene might increase the risk of developing MSA, by promoting α-synuclein protein aggregation. To date, polymorphisms have been identified in the promoter sequence, and in the intron 4 sequence of the α-synuclein gene. A combination of allele 1 of the α-synuclein promoter polymorphism and the ApoE4 allele has been reported to increase the relative risk for developing sporadic Parkinson’s disease 12.8 fold. Polymorphisms in codons 1 to 39 of the α-synuclein gene, a domain related to interaction with the recently identified protein, synphilin-1, or polymorphisms in the synphilin-1 gene itself, or in the genes of other protein interacting partners of α-synuclein, may also need to be considered. The number of α-synuclein protein interacting partners has expanded to include 14-3-3 protein chaperones, protein kinase C, extracellular regulated kinase, and BAD, a Bcl-2 homologue that regulates cell death.

Increased expression of a brain specific protein called ZNF231 in cerebellar neurones has been reported to occur in patients with MSA. The gene is located on chromosome 3p21 and encodes a neuronal double zinc finger protein. The importance of this finding is as yet uncertain, but it is possible that patients with MSA differ from unaffected individuals by sequence polymorphisms within, and flanking, the putative functional motifs of the ZNF231 gene.

**Gliarial cytoplasmic inclusions: characteristics and composition**

Argyrophilic oligodendroglial inclusions were first described in the brains of patients with MSA in 1989 and became known as gliarial cytoplasmic inclusions. Subsequent studies have confirmed the sensitivity and specificity of
The small heat shock protein and molecular chaperone, αB-crystallin, is a normal component of the central nervous system, where it is expressed primarily in oligodendrocytes and to a lesser degree in astrocytes. It is also an important protein component of GCIs. αB-crystallin binds to 20S proteasome, thereby regulating its proteolytic activity, in addition to binding to intermediate filaments. Ubiquitin is also involved in the 26S proteasome dependent proteolytic process and may play a protective role against neurodegeneration. Although the inclusions of MSA may be identified by ubiquitin immunostaining, results on isolated GCI proteins suggest that they are poorly ubiquitinated.

In sections of MSA brain, antibodies to α-synuclein immunolabel a greater number of GCIs than do anti-ubiquitin antibodies, indicating that α-synuclein is a major component of GCIs, and that the accumulation of α-synuclein precedes its ubiquitination. α-Synuclein, also referred to as the precursor of the non-amyloid component of plaques (NACP), is a 140 amino acid protein that is normally localised in the human brain to presynaptic nerve terminals. It is natively unfolded and highly soluble, but can polymerise into filaments under a variety of in vitro conditions, including increased temperature and concentration, acidic pH conditions, longer time lag, and increased iron concentrations. Hence, the formation and accumulation of α-synuclein filaments in GCIs, and in Lewy bodies, has been speculated to result from altered intracellular conditions. It is of interest that in the basal ganglia of patients with MSA, total iron concentrations are raised, and GCIs have been found within oligodendroglial cells containing iron pigment, although inclusions have also been found in cells with no evidence of pigment accumulation. Oligodendrocytes are the predominant iron regulatory cells in the brain, but it is not known whether oligodendrocytes in patients with MSA show abnormal concentrations or activities of ferritin (the iron sequestration protein) or transferrin (the iron transport protein).

Full length α-synuclein is present in GCIs and NCIs, although more vigorous antigen retrieval is required for immunohistochemical detection of other than C-terminal epitopes. In contrast, very little full length α-synuclein appears to be present in immunosolated GCIs, and the C-terminal truncated form of α-synuclein may predominate. In addition to the formation of GCIs, there is evidence for a more widespread modification of α-synuclein solubility in MSA than is obvious from the GCI distribution. In particular, in MSA, and also in preliminary studies with Lewy body disease, an increased ratio of sodium dodecyl sulphate soluble to buffer soluble α-synuclein has been seen, leading to the proposal that this property may be a biochemical “fingerprint” for the synucleinopathies, even in the absence of inclusion bodies.

Using immunoelectron microscopy on isolated sacrosyl insoluble filaments extracted from MSA brains, α-synuclein was identified by ubiquitin immunostaining, results on isolated GCI proteins suggest that they are poorly ubiquitinated.
from MSA brains, and PER4 antiserum to the C-terminus of α-synuclein, some filaments appear to be twisted, with a width alternating between 5 and 18 nm, and an apparent period of 70–90 nm, whereas other filaments appear to be straight, with a uniform width of 10 nm. The differences in morphology and diameter between the isolated α-synuclein filaments and the aggregated filamentous/tubular structures seen in sections of GCIs (see above) are thought to indicate that although α-synuclein may play a key role in fibrillogenesis, other cytoskeletal proteins (for instance α-tubulin and β-tubulin) are involved in filament formation. Furthermore, non-cytoskeletal elements also appear to be involved, as demonstrated most recently with antibodies to midkine, a new neurotrophic factor found to label most GCIs intensely. With immunoelectron microscopy, midkine positive, granule coated fibrils appear to be essential constituents of GCIs. Midkine is a heparin binding growth factor, implicated in various biological phenomena such as neuronal survival, differentiation, and migration, angiogenesis, and carcinogenesis. Midkine is strongly expressed in the nervous system during the midgestation period, probably by astrocytes. It is absent from the nervous system during the midgestation period.7-9α-synuclein in glial cells could occur in inactive upregulation in the expression of α-synuclein mRNA and protein is developmentally regulated,71 hence the accumulation is more likely to be a consequence of altered rather than de novo expression. One possibility is that oligodendrocytes in MSA brains may have an impaired ability to degrade α-synuclein, which they normally produce at very low concentrations. Alternatively, selective upregulation in the expression of α-synuclein in glial cells could occur in response to certain pathologies. The accumulation in GCIs of tau and MAP2 also appears to be a consequence of altered rather than de novo synthesis because both of these principally neuronal proteins are expressed in immature oligodendrocytes grown without axonal contact in tissue culture. The aberrant expression of the neuronal kinases cdk5 and MAPK (see above), of the neuronal endocytosis regulatory proteins Rab5 and Rabaptin-5, and of the neuronal survival and differentiation factor midkine (see above), normally expressed by astrocytes, suggests even more profound alterations in the phenotype of the MSA oligodendrocytes. Whether this is indicative of the existence of a repair mechanism or of a more profound defect in the oligodendrocyte–axon–neurone communication, involving oligodendroglial/neuronal trophic factors, remains to be established.

The neuronal inclusions (NCI and NNI) in MSA are less frequent than GCIs, and their ultrastructure reveals a composition of granules and filaments that tend to be associated with a more diverse range of cellular organelles, when compared with GCIs. In common with GCIs, however, neuronal inclusions stain positively both for ubiquitin and α-synuclein. Antibodies to α-synuclein, but not to ubiquitin, also reveal numerous degenerating neurites in the white matter of patients with MSA. This suggests that a hitherto unrecognised degree of pathology may be present in the axons of patients with MSA, although whether neuronal/axonal α-synuclein pathology preceeds glial α-synuclein pathology or myelin degeneration (see above) has not yet been determined.

Cell death of oligodendrocytes and neurones in MSA
It is something of a paradox that although MSA produces clinical symptoms typical of grey matter dysfunction, the hallmark pathological lesion affects myelin producing cells. How oligodendroglial dysfunction might lead to regional neuronal loss remains unexplained. There is no evidence to suggest that oligodendrocytes can be subtyped according to the neuronal populations they subserve. Indeed, single oligodendrocytes may myelinate axons of different anatomical tracts. Furthermore, GCIs involve all morphological types of oligodendrocytes (peri-vascular, perifascicular, and perineuronal) with varying frequency in different anatomical regions. Therefore no clues to point towards a “selective vulnerability” of a particular subgroup of oligodendrocytes. Nevertheless, because GCIs and oligodendroglial loss show a pronounced preponderance over NCI and neuronal loss, it has been suggested that oligodendroglial pathology may be the primary lesion of MSA, rather than an epiphenomenon. It has also been shown that in MSA pronounced DNA fragmentation, characteristic of apoptotic cell death, occurs almost exclusively in oligodendrocytes in a distribution pattern similar to that of GCIs. However, it appears that GCI formation may represent a different stage in oligodendroglial pathology because only oligodendrocytes lacking GCIs express Bax, the apoptosis promoting protein. In contrast, the GCI containing oligodendrocytes express Bcl-2, the apoptosis suppressor protein, indicative of an activated repair mechanism. This raises the possibility that in MSA regional neuronal/axonal pathology and withdrawal of axon derived trophic factors normally preventing apoptosis (see below) preceede the dysfunction of oligodendrocytes, and demyelination in the related white fibre tracts.

The absence of DNA fragmentation in MSA neurones may indicate that they are destroyed either by necrosis or by a form of programmed cell death other than apoptosis. Nonetheless, increased p53 immunoreactivity in the striatal and midbrain neurones of patients with MSA has been interpreted as evidence of neuronal apoptosis because p53 gene upregulation is known to precede apoptosis. More recently, immunoreactivity for the calcium binding proteins calbindin and parvalbumin has been shown to be greatly decreased in the cerebellar Purkinje cells of patients with MSA, whereas immunoreactivity for both Bax, the apoptosis
promoting protein, and the Bcl-x, the apoptosis suppressor protein, was increased. The expression of Bcl-2, another apoptosis suppressor protein, was restricted to a subpopulation of granule neurones. It was suggested that a diminished calcium binding capacity of MSA Purkinje cells could lead to a change in the regulation of proteins of the Bcl-2 family, favouring the initiation of apoptosis.

A further clue to the regional selectivity seen in MSA and apoptosis may lie in the study of a family of cysteine proteases called caspases, which act as the executioners of apoptosis. Cytoskeletal proteins and enzymes essential for cell repair may be substrates for caspase processing in several neurodegenerative diseases. Caspase-3 cleaves the antiapoptotic protein Bcl-2. Peptide caspase inhibitors have been shown to protect against 1-methyl-4-phenylpyridinium induced apoptosis in cultured cerebellar granule neurones. Furthermore, the distribution of caspase-3 may contribute towards the regional vulnerability seen in the substantia nigra in idiopathic Parkinson’s disease, with dopaminergic neurones expressing caspase-3 degenerating preferentially. Similar studies in MSA have not yet been reported.

The activation of microglial cells may be the final common pathway, contributing both to demyelination and neuronal removal, irrespective of the mode of cell death. Microglia express proinflammatory peptides, which may be a result of activation of nuclear factor κB (NF-κB). Affected brain areas of patients with MSA show strong immunoreactivity for nuclear Rel A p65 (a subunit of the NF-κB/Rel family), which is almost exclusively localised in activated microglia. Nuclear translocation of Rel A is not detected in striatal tissue of normal controls and patients with Parkinson’s disease. This suggests that NF-κB/Rel A complexes may play a role in mediating microglial activation in MSA. Finally, PK 11195 selectively binds to benzodiazepine sites on activated microglia. Inhibitors of cytoskeletal processes, synapses, and dendritic spines in limbic neuronal populations, whereas the γ1 chain is found in cell bodies of essentially all neurones in the central nervous system. Based on the restricted distribution of the α2 chain to the limbic areas, it has been proposed that isoforms of the laminin α chains, other than the already known α1 and α2 chains, are expressed by different neuronal systems. Thus, it is conceivable that polymorphisms in the genes encoding laminin α chains, which are shared by the suprasegmental motor system, the supraspinal autonomic systems, and their targets, could be risk factors for MSA, and may underlie the system bound distribution of the oligodendroglial and neuronal pathology in this disease.

**Conclusion**

The aetiology of MSA remains elusive. The discovery of α-synuclein within GCIIs has provided a recent impetus to research, and also an elusive link with Parkinson’s disease. Nevertheless, MSA is distinguished from other neurodegenerative diseases by the prominent, if not primary, involvement of the glial cell. In contrast with Parkinson’s disease, dementia with Lewy bodies, and a host of other neurodegenerative tauopathies, familial MSA has never been documented, and there are no clues as yet to a genetic predisposition. It is likely that future progress in unravelling the cause of MSA will need to include large multicentre clinical studies, applying validated
diagnostic criteria to yield a sizeable cohort of well characterised MSA cases. This cohort will form the basis for genetic and pathologic studies. The potentially central role of alpha-synuclein in fibrillogeny, its interaction with other cytoskeletal proteins within glial cells is likely to provide a fruitful avenue for further research. At the same time, the temporal and spatial involvement of the pathologic process in MSA needs to be investigated in more detail, and potentially disruptive factors of the oligodendrocyte–neurone–axon functional unit should be explored further.

be distinguished from abnormal tau in Alzheimer’s disease.


