

Evidence for α -synuclein prions causing multiple system atrophy in humans with parkinsonism

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Prions are proteins that adopt alternative conformations that become self-propagating; the PrP^{Sc} prion causes the rare human disorder Creutzfeldt–Jakob disease (CJD). We report here that multiple system atrophy (MSA) is caused by a different human prion composed of the α -synuclein protein. MSA is a slowly evolving disorder characterized by progressive loss of autonomic nervous system function and often signs of parkinsonism; the neuropathological hallmark of MSA is glial cytoplasmic inclusions consisting of filaments of α -synuclein. To determine whether human α -synuclein forms prions, we examined 14 human brain homogenates for transmission to cultured human embryonic kidney (HEK) cells expressing full-length, mutant human α -synuclein fused to yellow fluorescent protein (α -syn¹⁴⁰*A53T–YFP) and TgM83^{+/-} mice expressing α -synuclein (A53T). The TgM83^{+/-} mice that were hemizygous for the mutant transgene did not develop spontaneous illness; in contrast, the TgM83^{+/+} mice that were homozygous developed neurological dysfunction. Brain extracts from 14 MSA cases all transmitted neurodegeneration to TgM83^{+/-} mice after incubation periods of ~120 d, which was accompanied by deposition of α -synuclein within neuronal cell bodies and axons. All of the MSA extracts also induced aggregation of α -syn¹⁴⁰*A53T–YFP in cultured cells, whereas none of six Parkinson's disease (PD) extracts or a control sample did so. Our findings argue that MSA is caused by a unique strain of α -synuclein prions, which is different from the putative prions causing PD and from those causing spontaneous neurodegeneration in TgM83^{+/+} mice. Remarkably, α -synuclein is the first new human prion to be identified, to our knowledge, since the discovery a half century ago that CJD was transmissible.

neurodegeneration | Parkinson's disease | synucleinopathies | strains

Looking back almost 50 y ago, kuru was the first human prion disease to be transmitted to an experimental animal (1). Subsequently, Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease, and fatal familial insomnia were transmitted to nonhuman primates or transgenic (Tg) mice; all of these disorders were eventually found to be caused by PrP^{Sc} prions that were initially discovered in hamsters with experimental scrapie. Attempts to transmit other neurodegenerative diseases, including Alzheimer's and Parkinson's, to monkeys were disappointing; none of the animals developed signs of neurological dysfunction, and none showed recognizable neuropathological changes at autopsy (2).

In 1960, Milton Shy and Glenn Drager described two male patients suffering from orthostatic hypotension, additional forms of autonomic insufficiency, and a movement disorder resembling Parkinson's disease (PD). They also found an additional 40 cases of idiopathic hypotension in the literature, which shared many

features with their patients. Nine years later, Graham and Oppenheimer suggested that Shy–Drager syndrome should be combined with striatonigral degeneration and olivopontocerebellar atrophy and that these three entities should be called multiple system atrophy (MSA) (3). They presciently argued that all three disorders were likely caused by a similar neurodegenerative process. Two decades passed before support for this hypothesis began to emerge when the brains of 11 MSA patients were reported to contain silver-positive accumulations or glial cytoplasmic inclusions (GCIs) primarily in oligodendrocytes (4). The nature of these GCIs remained elusive for another decade until three groups reported that GCIs exhibited positive immunostaining for α -synuclein (5–7). The discovery that MSA is a synucleinopathy followed a study reported 1 y earlier showing that Lewy bodies in PD contain α -synuclein by immunostaining (8). Such investigations were prompted by molecular genetic studies showing genetic linkage between the A53T point mutation in α -synuclein and inherited PD (9).

MSA is a sporadic, adult-onset, progressive neurodegenerative disorder with an annual incidence of ~3 per 100,000 individuals over the age of 50 (10, 11). The duration of MSA is generally 5–10 y and is substantially shorter than most cases of PD, which

Significance

Prions are proteins that assume alternate shapes that become self-propagating, and while some prions perform normal physiological functions, others cause disease. Prions were discovered while studying the cause of rare neurodegenerative diseases of animals and humans called scrapie and Creutzfeldt–Jakob disease, respectively. We report here the discovery of α -synuclein prions that cause a more common neurodegenerative disease in humans called multiple system atrophy (MSA). In contrast to MSA, brain extracts from Parkinson's disease (PD) patients were not transmissible to genetically engineered cells or mice, although much evidence argues that PD is also caused by α -synuclein, suggesting that this strain (or variant) is different from those that cause MSA.

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The authors declare no conflict of interest.

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leads to death in 10–20 y. MSA has been subdivided based on the predominance of Parkinson's symptoms (MSA-P) or cerebellar dysfunction (MSA-C) (12).

The unanticipated results of an earlier study in 2013 showed that two cases of MSA transmitted CNS dysfunction to transgenic (TgM83^{+/-}) mice expressing mutant human α -synuclein*A53T protein (13). In that initial report, brain homogenates prepared from two cases of MSA were intracerebrally (IC) injected into TgM83^{+/-} mice, which resulted in progressive CNS dysfunction after ~120 d. The brains of the Tg mice exhibited extensive phosphorylated α -synuclein deposits in the cytoplasm and axons of neurons.

To determine whether the transmissions of two MSA cases were anomalous, we inoculated TgM83^{+/-} mice with another dozen cases from three different countries: the United Kingdom, Australia, and the United States. We report here that homogenates prepared from each of the additional 12 cases produced an experimental synucleinopathy in all of the IC inoculated TgM83^{+/-} mice with incubation times of ~120 d. The mice developed intraneuronal deposits of aggregated, phosphorylated α -synuclein in their brainstems and some other CNS regions. Using multiple brain regions from some of the MSA cases, a total of 19 homogenates from 14 MSA cases produced CNS dysfunction in TgM83^{+/-} mice and infected human embryonic kidney (HEK) cells expressing α -syn140*A53T-YFP, resulting in cytoplasmic aggregates of the fusion protein that were measured by fluorescence microscopy (14). From these transmission studies in both TgM83^{+/-} mice and cultured cells, we conclude that MSA is a transmissible human neurodegenerative disease caused by α -synuclein prions.

Results

Patient Histories. Brain specimens from 14 deceased patients carrying the clinical and neuropathological diagnosis of MSA, as well as 6 patients with the diagnosis of PD, were obtained from (i) the Parkinson's UK Brain Bank at Imperial College London in London, England; (ii) the Sydney Brain Bank in Sydney, Australia; and (iii) the Massachusetts General Hospital Alzheimer's Disease Research Center in Boston, MA. Clinical descriptions of the 20 synucleinopathy patients are summarized in Table 1 and Table S1.

The MSA patients exhibited autonomic dysfunction manifested as orthostatic hypotension and/or erectile dysfunction with either parkinsonism or cerebellar dysfunction and, thus satisfied the clinical criteria for possible or probable MSA (15). For the majority of the MSA patients, parkinsonism rather than cerebellar symptoms dominated the clinical presentation. Parkinsonism was consistent with the origin of the patient population, as MSA-P is known to be more common in Western countries (16), whereas MSA-C is more frequent in Asian countries (17). The mean age of onset of disease in this MSA patient population was 59 ± 9 y, consistent with previous reports (18). The average duration of disease in this patient population was 7.8 y, consistent with reports of an average duration of disease of ~6–8 y (18, 19). Most of the MSA patients in this study received symptomatic treatment with carbidopa/levodopa or levodopa alone. Although the majority of the patients in this study showed an initial response to levodopa treatment, the MSA patients worsened regardless of this response to therapeutic intervention. The PD patients displayed classical symptoms of the disease, including resting tremor, rigidity, bradykinesia, and postural instability. The mean age of disease onset for the PD patients was 68 ± 5 y, and the average duration of disease was 8.2 y.

Table 1. Demographic, clinical, and neuropathological characteristics of patient samples

Case	Country	Sex	Age at onset (y)	Duration (y)	Cause of death	Clinical diagnosis	Neuropathological diagnosis
C1	USA	M	77	NA	Cardiovascular disease	Nondiseased control brain	NA
PD1	UK	M	65	8.5		Tremulous hemiparkinsonism, REM sleep disorder, MSA questioned	Lewy body disease
PD2	UK	M	65	8	Myocardial infarction, acute renal failure, pneumonia	Hemiparkinsonism with autonomic features	Lewy body disease
PD3*	UK	M	66	9.5		Parkinsonism with drooling	Lewy body disease
PD5	Australia	M	63	9	Myocardial infarction	Parkinson's disease	Parkinson's disease
PD6	Australia	M	73	6	Myocardial infarction	Parkinson's disease	Diffuse Lewy bodies
PD7	Australia	M	74	8	Cerebrovascular accident	Parkinson's disease	Diffuse Lewy bodies
MSA1	UK	M	78	8		Atypical akinetic-rigid syndrome with prominent ataxia, PSP questioned	MSA
MSA2	UK	M	65	5.5		Akinetic-rigid syndrome with autonomic involvement	MSA
MSA3	UK	F	52	6	Bronchopneumonia	MSA-P	MSA
MSA4	UK	M	68	7	Pneumonia	MSA	MSA
MSA5	UK	M	52	8	Respiratory failure, pneumonia	Parkinsonism, MSA questioned	MSA
MSA6	UK	F	48	13	Pneumonia	Akinetic-rigid syndrome with antecollis and camptocormia: MSA vs. PD	MSA
MSA7	UK	M	52	12	Bronchopneumonia	MSA-C	MSA
MSA8	Australia	M	57	4	Aspiration pneumonia	MSA-P	MSA
MSA9	Australia	M	75	7	Cardiorespiratory failure	MSA-C	MSA
MSA10	Australia	M	56	8	Bronchopneumonia	MSA-P with early autonomic dysfunction	MSA
MSA11	Australia	M	59	2	Respiratory failure	MSA-P with early autonomic dysfunction	MSA
MSA12	USA	F	55	11	Acute bronchopneumonia	MSA	MSA
MSA13	USA	M	55	10	Chronic pneumonia	MSA	MSA
MSA14	USA	M	60	8		MSA-C	MSA

NA, not applicable.

*Clinical report for PD3 was incomplete.

Brain Specimens. A definitive diagnosis of MSA requires post-mortem neuropathological microscopic examination, the results of which are summarized in Table 1 and Table S1. On removal of the brains, no gross changes in cortical regions were observed, with gyri and sulci appearing to be normal. On cutting the brains, we found depigmentation of the substantia nigra (Fig. 1A) and atrophy of the putamen/basal ganglia and cerebellum. Histological sections from all 14 MSA brains exhibited GCIs that stained with antiphosphorylated α -synuclein antibodies, and α -synuclein-positive Lewy bodies were found in the six PD patient samples (Fig. 1B). Interestingly, the density of GCIs varied widely among the patients (Table 2). Total α -synuclein was determined from frozen brain samples by ELISA and found to be higher in the nondiseased control brain compared with PD brains and most of the MSA brains (Table 2). Frozen brain samples were fractionated to determine the level of insoluble, aggregated phosphorylated α -synuclein using SDS/PAGE (Fig. 1C). Homogenates from MSA patients contained more (although variable) amounts of phosphorylated α -synuclein in the insoluble fraction compared with the nondiseased control brains.

Interestingly, PD cases 1 and 2 contained very small amounts of phosphorylated α -synuclein, whereas the other four cases contained similar amounts as the MSA patients (Fig. 1C).

Sequencing of the *SNCA* and *COQ2* Genes. Because inherited cases have been identified in all neurodegenerative diseases, we asked if any of the MSA or PD samples contained either a mutant α -synuclein (*SNCA*) or *COQ2* gene. Duplications, triplications, and missense mutations in *SNCA* have been identified in a minority of patients with PD (20); *SNCA* single nucleotide polymorphisms (SNPs) are associated with MSA risk (21); and, recently, novel *SNCA* mutations were reported in cases with mixed MSA and PD pathology (22–24). In addition, an investigation of familial MSA identified coenzyme Q10, specifically the *COQ2* gene, to be associated with MSA in two families (25).

To determine whether any mutations were present in our study population, we sequenced all five coding exons of *SNCA* and all seven exons of *COQ2* using standard Sanger sequencing (primers used are shown in Table S2). We found no missense mutations in any of the samples tested. No *SNCA* SNPs were

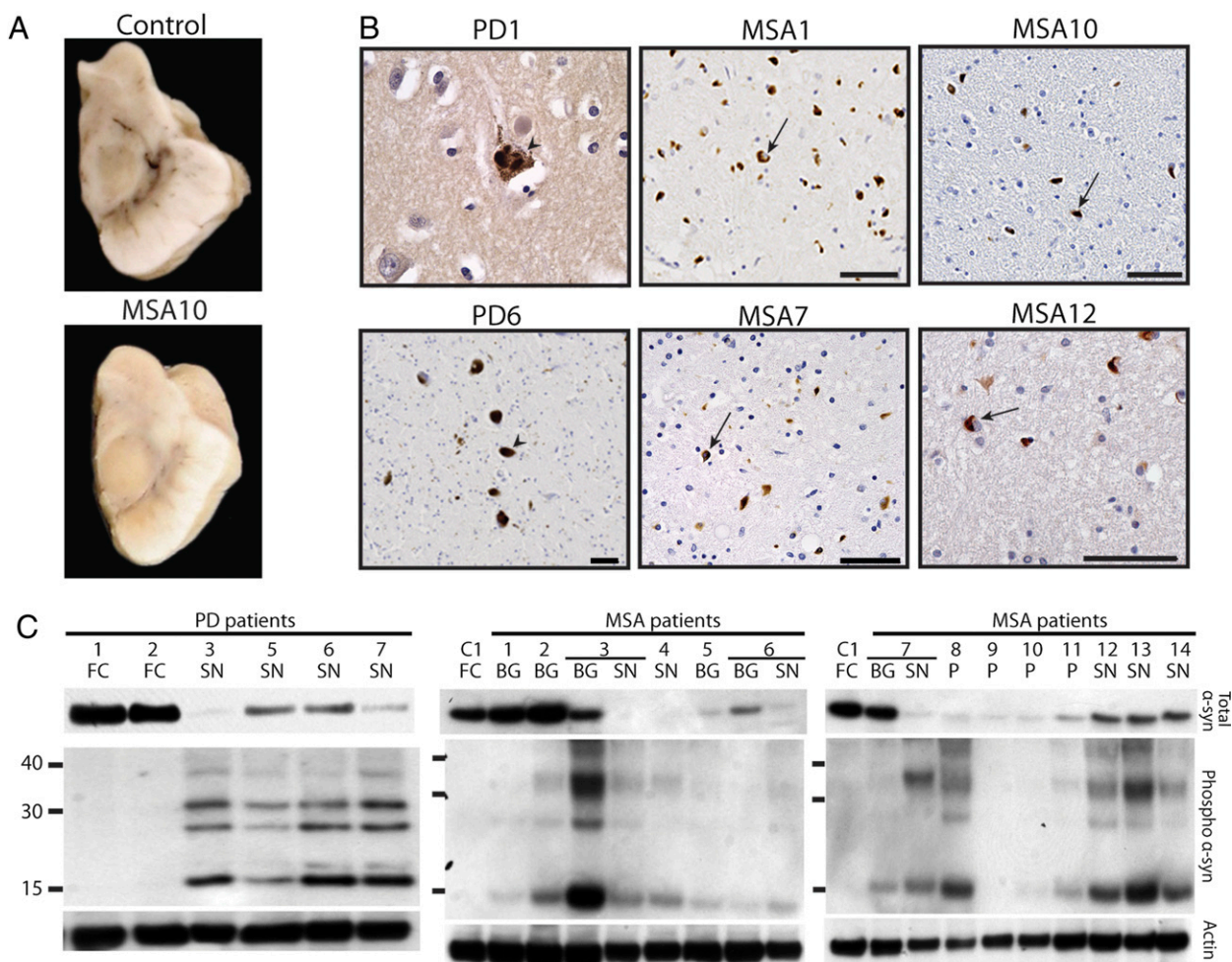


Fig. 1. Neuropathological and biochemical analysis of synucleinopathy cases. (A) Gross pathology of one representative sample (MSA10) demonstrating depigmentation of the substantia nigra compared with nondiseased control. (B) Immunohistochemical detection of α -synuclein deposits in patient samples. Two representative PD samples and four representative MSA samples were stained for α -synuclein using the antibodies clone 42 (BD Biosciences; PD1, PD6, MSA1, MSA7, MSA10) and LB509 (MSA12). Arrowheads point to Lewy bodies, arrows to GCIs. (Scale bar, 50 μ m for all samples.) (C) Immunoblots of brain homogenates of human control (C1), PD, and MSA cases show total human α -synuclein (Top) and detergent-insoluble phosphorylated α -synuclein (Middle). The brain region used for each case is noted: FC (frontal cortex), SN (substantia nigra), BG (basal ganglia), and P (pons). Blots were probed for actin as a loading control (Bottom). Total human α -synuclein was probed using the monoclonal antibody Syn211, and S129-specific, phosphorylated α -synuclein was probed with antibody EP1536Y. Molecular weight markers of migrated protein standards are shown in kilodaltons.

Table 2. Transmission of α -synuclein prions to TgM83^{+/-} mice

Sample	Brain region	Inoculum		Primary transmission				
		α -Synuclein inclusion density (per mm ²)		Total α -synuclein (μ g/mL)	Mean cell infection \pm SEM (%)	Mean incubation time \pm SEM (d)	n/n_0	Mean cell infection \pm SEM (%)
		GICs	Lewy bodies					
C1	FC	0	0	3.0	7 \pm 1 [†]	>360	0/8	2 \pm 0
PD1	FC	0	*	1.9	7 \pm 3	>360	0/8	2 \pm 1
PD2	FC	0	*	2.3	3 \pm 1	>360	0/7	2 \pm 0
PD3	SN	0	3.3	1.6	0	> 243 [‡]	0/8	ND
PD5	SN	0	3.0	2.4	6 \pm 0	> 208 [‡]	0/7	ND
PD6	SN	0	7.3	0.6	15 \pm 1	> 208 [‡]	0/4	ND
PD7	SN	0	6.8	1.5	10 \pm 1	> 208 [‡]	0/6	ND
MSA1	BG	69	0	4.2	11 \pm 1	143 \pm 17 [§]	7/8	29 \pm 4
MSA2	BG	82	0	3.6	30 \pm 1	109 \pm 12 [§]	7/7	31 \pm 3
	SN	99	0	ND	ND	119 \pm 10	7/7	21 \pm 2
MSA3	BG	69	0	2.5	42 \pm 2	114 \pm 14	5/5	30 \pm 2
	SN	20	0	0.3	24 \pm 2	119 \pm 10	7/7	24 \pm 2
MSA4	BG	54	0	0.4	25 \pm 4	135 \pm 13	8/8	47 \pm 3
	SN	25	0	ND	19 \pm 5	134 \pm 7	8/8	33 \pm 2
MSA5	BG	200	0	0.6	47 \pm 2	119 \pm 12	8/8	45 \pm 3
MSA6	BG	50	0	1.6	20 \pm 3	108 \pm 10	8/8	43 \pm 3
	SN	ND	ND	0.1	40 \pm 3	106 \pm 7	6/6	39 \pm 3
MSA7	BG	29	0	4.3	48 \pm 1	106 \pm 10	8/8	24 \pm 1
	SN	21	0	0.2	58 \pm 3	122 \pm 10	8/8	36 \pm 4
MSA8	P	22	0	0.3	31 \pm 1	108 \pm 15	6/6	33 \pm 1
MSA9	P	3.3	0	0.8	24 \pm 1	121 \pm 8	7/7	14 \pm 1
MSA10	P	13	0	0.9	36 \pm 1	108 \pm 8	6/6	42 \pm 2
MSA11	P	12	0	0.8	23 \pm 2	144 \pm 16	6/6	37 \pm 3
MSA12	SN	133	0	0.8	29 \pm 1	117 \pm 10	8/8	51 \pm 3
MSA13	SN	127	0	0.8	40 \pm 2	113 \pm 9	8/8	41 \pm 3
MSA14	SN	17	0	0.9	23 \pm 1	130 \pm 12 [¶]	6/6	45 \pm 2

BG, basal ganglia; FC, frontal cortex; n , number of ill mice; n_0 , number of inoculated mice; ND, not determined; P, pons; SN, substantia nigra.

*A single Lewy body was found in the entire section.

[†]Average value from two alternate age-matched control samples.

[‡]Experiments ongoing.

[§]Data previously reported in ref. 13.

[¶]Data previously reported in ref. 14.

identified in any of the patient cases; however, three *COQ2* SNPs varied among cases (Table S3). In our small sample, there did not seem to be any association between SNP genotype and MSA or PD. Although α -synuclein is the most attractive target for genetic studies of MSA and PD, several other candidate genes have been studied in α -synucleinopathies. These genes have diverse functions, including roles in mitochondrial function, protection against oxidative stress, and inflammatory processes (25–27).

Transmission of MSA Prions to Cultured HEK Cells. We recently described the creation of a cell-based assay for detecting human α -synuclein prions using cultured HEK cells expressing full-length α -synuclein containing the A53T mutation fused to yellow fluorescent protein (α -syn140*A53T-YFP) (14). Using this assay, we selectively precipitated α -synuclein prions from the human patient samples using sodium phosphotungstic acid (PTA). After exposing the cells to the precipitated samples for 4 d in a 384-well plate, we collected four images from each of the six wells for each sample using automated confocal fluorescence microscopy. The images were then analyzed using an algorithm we developed to determine the percentage of cells containing aggregates. On initial analysis of the data, we found 17 of the 18 samples from MSA patients infected HEK cells expressing α -syn140*A53T-YFP fusion protein significantly higher than the control. Conversely, only one of the six PD samples was significantly higher than the control sample (Table 2 and Fig. 24). We

reevaluated these two outliers, patients PD6 and MSA1, by manually determining the percentage of cells with aggregates present in a single representative image from each of the six wells plated. We found that sample PD6 was a false positive, infecting only 5 \pm 2% of cells with aggregates, whereas MSA1 was a false negative, inducing aggregates in 13 \pm 5% of cells (error is reported as SD instead of SEM as presented in Table 2). Notably, the algorithm was developed for high-throughput analysis and cannot consistently distinguish diffuse signals in overlapping cells from small intense aggregates. Importantly, the MSA patient samples were found to induce aggregate formation in the α -syn140*A53T-YFP cells at a significantly higher level than the PD samples (Fig. 2B; $P < 0.0001$). Moreover, a concomitant study of 17 brain samples from 11 deceased males and 6 females, all of whom were between the ages 56 and 88 and without evidence of CNS dysfunction, showed that none of these control brain homogenates induced fluorescent aggregates in α -syn140*A53T-YFP cells (14).

MSA Samples Transmit Disease to TgM83^{+/-} Mice. In a preliminary study, we IC inoculated TgM83^{+/-} mice with basal ganglia samples from two MSA patients. Unexpectedly, those MSA samples transmitted CNS dysfunction in \sim 120 d (13). To determine whether neurological dysfunction could be transmitted with other MSA patient samples, we collected additional brain specimens from a dozen deceased MSA patients and IC inoculated

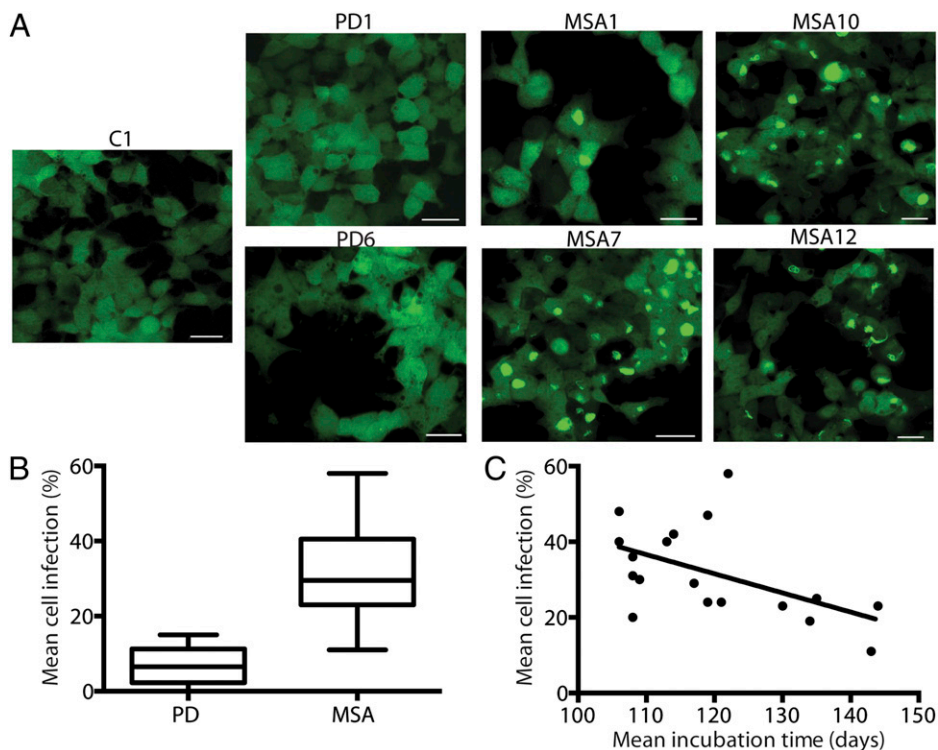


Fig. 2. A cell infectivity assay can quantify infectivity in synucleinopathy tissue samples. (A) Representative images of α -syn140*A53T-YFP-expressing cells infected with PTA-precipitated brain homogenate from control (C1), PD, or MSA patients. YFP is shown in green. (Scale bars, 100 μ m.) (B) Box and whisker plot of cell infectivity from PD and MSA samples shows a significant difference between the two groups ($P < 0.001$). Whiskers indicate maximum and minimum values. (C) For each of the MSA samples tested in both cell assay and mouse bioassay, cell infectivity and incubation time were significantly inversely correlated ($R^2 = 0.27$, $P = 0.026$).

them into TgM83^{+/-} mice. Inoculation with all of the MSA samples caused CNS dysfunction with mean incubation periods of 100–150 d postinoculation (dpi) (Table 2). The most common clinical signs were dysmetria and circling behavior. In contrast, brain homogenates from six PD patients or a control inoculated into TgM83^{+/-} mice failed to produce signs of neurological dysfunction in >360 dpi (Table 2), analogous to our findings when these samples were bioassayed in HEK cells expressing α -syn140*A53T-YFP fusion protein.

Comparing the level of cell infectivity from each of the MSA samples with the incubation times observed from inoculation into TgM83^{+/-} mice, we found a significant inverse correlation (Fig. 2C; $R^2 = 0.27$, $P = 0.026$): the greater the level of infectivity in the cell assay, the shorter the time to disease onset in mice (Fig. 2C).

Neuropathological examination revealed large aggregates of phosphorylated α -synuclein, as well as widespread astrocytic gliosis, in the brains of TgM83^{+/-} mice inoculated with MSA brain homogenates (Fig. 3A). Aggregated α -synuclein was primarily observed as neuronal cytoplasmic inclusions (NCIs) and in neurites. Although some of these NCIs resemble Lewy bodies (Fig. 3A, MSA5, *Inset*), the majority featured a thin rim around the nucleus with α -synuclein-positive immunostaining extending to the proximal part of the neuronal processes. The predominance of neuronal over oligodendroglial inclusions may reflect the transgene expression that is driven by the prion protein promoter. In comparison, the brains of TgM83^{+/-} mice inoculated with PD brain exhibited low and unspecific background signal of phosphorylated α -synuclein after >360 dpi, similar to that seen in the control. The distribution of the phosphorylated α -synuclein throughout multiple brain regions in the mice was also assessed (Fig. S1). Inoculation with either control or PD brain homogenate did not lead to deposition of appreciable

phosphorylated α -synuclein in any brain region. In contrast, mice inoculated with MSA brain homogenate developed phosphorylated α -synuclein deposits in several brain regions. These neuropathological changes were most apparent in the brainstem, especially in the reticular formation, but notably absent from cortical regions. The contralateral hemispheres of TgM83^{+/-} mice inoculated with MSA homogenates contained phosphorylated α -synuclein in the detergent-insoluble fraction, whereas similar brain fractions from mice inoculated with control or PD samples contained low levels of phosphorylated α -synuclein (Fig. 3B and C). TgM83^{+/-} mice inoculated with MSA brain homogenates displayed slowly migrating phosphorylated α -synuclein on a Western blot, whereas those inoculated with control brain or PD brain samples did not. Additionally, we tested the mouse brain homogenates in the HEK cell assay and found that the PTA-precipitated homogenates from mice inoculated with MSA infected the α -syn140*A53T-YFP cells, but the homogenates from TgM83^{+/-} mice inoculated with PD or control patient brain did not infect the cells (Table 2). Our results argue that transmission of MSA to TgM83^{+/-} mice results in the de novo formation of prions in mouse brain.

Propagation of α -Synuclein Prions in TgM83 Mice. Because serial propagation is a characteristic of authentic prions, we prepared brain homogenates from four ill TgM83^{+/-} mice inoculated with either MSA or spontaneously ill TgM83^{+/-} mouse brain. Serial passage in TgM83^{+/-} mice was then compared with passage of an aged TgM83^{+/-} brain, which did not transmit disease (Table 3). Incubation periods for serially passaged MSA prions were slightly shorter than those observed for primary transmissions and ~40% shorter than those for serial transmission of spontaneous TgM83^{+/-} prions, indicating that these represent two different

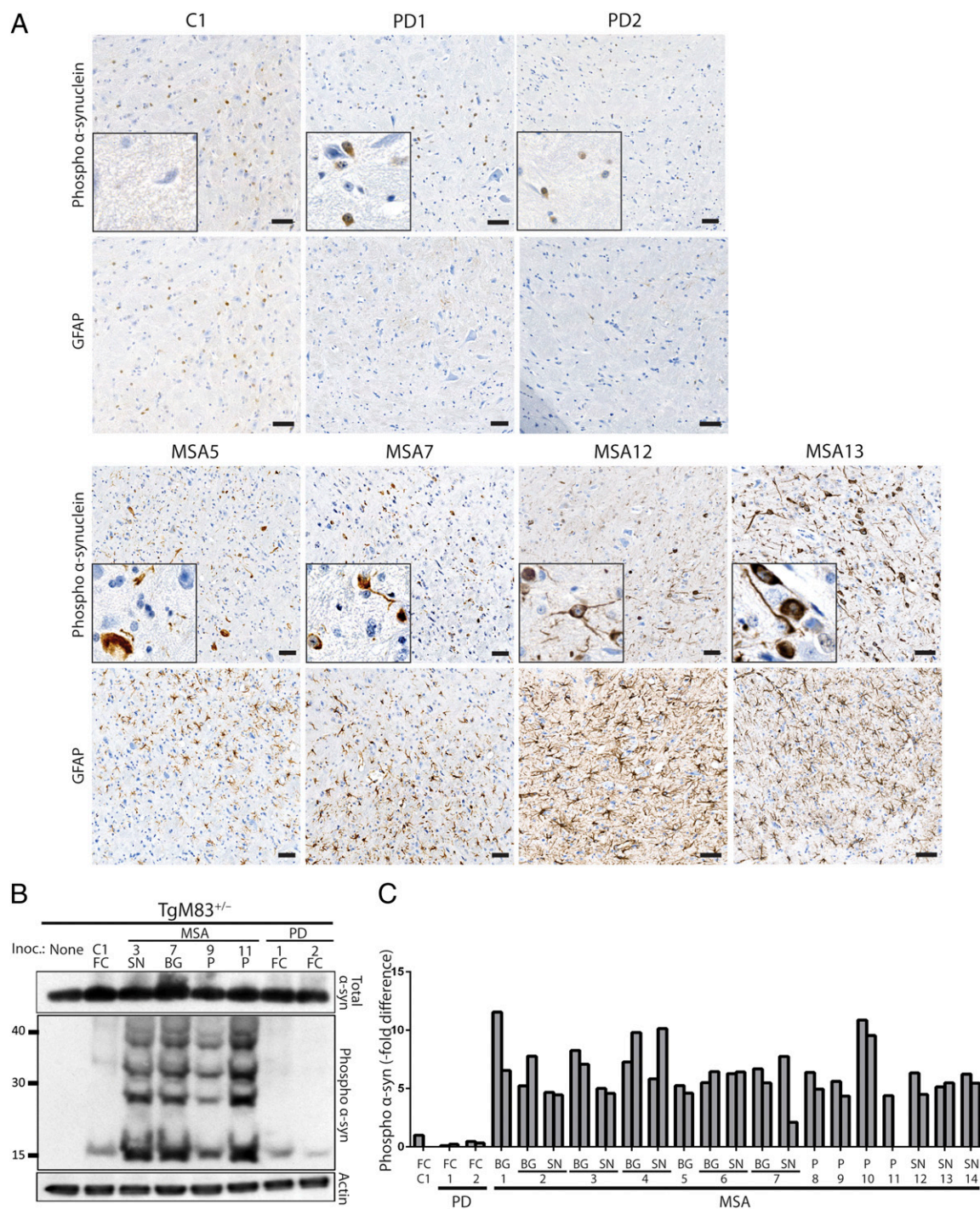


Fig. 3. Inoculation of α -synuclein aggregates from MSA but not PD cases induced deposition of phosphorylated α -synuclein and reactive astrogliosis. (A) Brain homogenates were prepared from control (C1), PD, and MSA patients and IC inoculated into TgM83^{+/-} mice. Mice inoculated with MSA homogenates, but not PD or control homogenates, showed deposition of phosphorylated α -synuclein in the brainstem (top panels, staining with EP1536Y antibody); *Insets* show a 4 \times magnification relative to the main image. These mice also showed prominent reactive astrogliosis, as indicated by GFAP staining (bottom panels). (Scale bars, 50 μ m.) (B) Representative immunoblot shows total α -synuclein (*Top*) and detergent-insoluble phosphorylated α -synuclein (*Middle*) in the brains of TgM83^{+/-} mice inoculated with homogenates from C1, PD, or MSA patients. The brain region for each inoculum is noted: FC (frontal cortex), P (pons), and SN (substantia nigra). Total α -synuclein was detected from the crude brain homogenates using the Syn211 antibody; phosphorylated α -synuclein was probed with the EP1536Y antibody. Actin is shown as a loading control (*Bottom*). Molecular weight markers of migrated protein standards are shown in kilodaltons. (C) Phosphorylated α -synuclein in the brains of TgM83^{+/-} mice inoculated with PD or MSA was quantified by densitometry and expressed as an x-fold difference compared with mice inoculated with nondiseased control brain (C1). The brain-region origin for each inoculum is noted: BG (basal ganglia), FC (frontal cortex), P (pons), and SN (substantia nigra). For each inoculum, data from two animals are shown, except for the MSA11 inoculum, for which only one animal was available for biochemical analysis.

strains of α -synuclein prions. We IC inoculated 30 μ L of a 1% (wt/vol) brain homogenate, equivalent to 0.3 mg of the original

brain weight, which caused CNS dysfunction in \sim 120 d, at which time the mice were killed. For samples from MSA1 and MSA2

Table 3. Effect of transgene and serial transmission on incubation period

Inoculum (brain region)	Mouse line	Primary transmission		Incubation time of mouse brain inoculated (d)	Secondary transmission in M83 ^{+/-}	
		Mean incubation time ± SEM (d)	n/n ₀		Mean incubation time ± SEM (d)	n/n ₀
No inoculum	TgM83 ^{+/-}	>412	0/6	259	>360	0/6
	Tg(SNCA) <i>Snca</i> ^{0/0}	>580	0/9		ND	
	TgM83 ^{+/-}	143 ± 17*	7/8		113 ± 13 [†]	
MSA1 (basal ganglia)	WT	>360	0/7	105	ND	
	Tg(SNCA) <i>Snca</i> ^{0/0}	>360	0/6		ND	
	TgM83 ^{+/-}	109 ± 12*	7/7		91	
MSA2 (basal ganglia)	WT	>360	0/2		ND	
	Tg(SNCA) <i>Snca</i> ^{0/0}	>360	0/5		ND	
9 m.o. spont. TgM83 ^{+/+} (whole brain)	TgM83 ^{+/-}	222 ± 15*	6/6	205	193 ± 19	8/8
11 m.o. spont. TgM83 ^{+/+} (whole brain)	TgM83 ^{+/-}	216 ± 18*	8/8	162	175 ± 8	8/8

n, number of ill mice; n₀, number of inoculated mice; ND, not determined.

*Data previously reported in ref. 13.

[†]Data previously reported in ref. 14.

patients (Table 3), the brains were harvested and passaged a second time, representing more than a 1,000-fold dilution of brain homogenate (0.3 mg from an ~0.5-g brain). The second passage had a similar incubation period, suggesting the MSA prions had replicated to the same level that were present in the human brain before dilution for transmission studies. The more than 1,000-fold amplification per passage implies that in two passages, the MSA prions had multiplied more than 1 × 10⁶-fold, underscoring the infectivity of the α-synuclein prions described here.

To determine whether MSA prions could transmit to Tg mice expressing WT mouse or human α-synuclein, we inoculated MSA1 and MSA2 into additional lines of WT and Tg mice. Neither WT mice nor Tg mice expressing WT human α-synuclein developed CNS dysfunction on inoculation with the MSA samples (Table 3). Presumably, the A53T point mutation facilitated prion replication, as has been observed analogously with some PrP mutations for PrP^{Sc} prions. In support of this posit, MSA-inoculated TgM83^{+/+} mice, which were homozygous for the α-synuclein*A53T transgene array, developed CNS dysfunction in ~90 d (Table S4) compared with the TgM83^{+/-} mice that required ~120 d (Table 2). Because expression of endogenous mouse PrP can interfere with the propagation of human PrP prions (28), we decided to test whether endogenous mouse α-synuclein impacts the propagation of MSA prions. We crossed the TgM83 mice onto an α-synuclein knockout background but found no difference in incubation periods among the Tg mice on the *Snca*^{0/0}, *Snca*^{0/+}, and *Snca*^{+/+} backgrounds (Table S4).

We also investigated alternate routes of inoculation for MSA prions. Recently, it was reported that hind limb intramuscular (IM) inoculations with recombinant human α-synuclein polymerized into fibrils could be as or more efficient than IC inoculation in TgM83^{+/+} mice (29). In other investigations, inoculation of PrP^{Sc} prions into the lingual muscles has been shown to be an effective means of PrP^{Sc} prion transmission (30). We compared MSA inoculations that were performed IC, IM, and intraglossally. Preliminary results suggest that IC and IM inoculations of 5 μL 1% MSA2 brain homogenate produced similar incubation times: IC, 133 ± 6 d (7/8 mice), and IM, 136 ± 10 d (6/8 mice). Both routes were more efficient than intraglossal inoculation, with no transmissions to date, >220 d.

Discussion

The posit that α-synuclein prions cause PD began with speculation that PD, like kuru and CJD, might be caused by slow viruses (31). Although subsequent studies demonstrated that PrP^{Sc} prions, not viruses, cause kuru and CJD, the hypothesis that

some CNS diseases, including AD and PD, are also caused by prions has gained increasing support (32–34). Similar to PrP^{Sc}, α-synuclein was found to assemble into β-sheet-rich amyloid fibrils (35, 36). In 2008, two groups reported Lewy bodies in fetal grafts of substantia nigra tissue, which had been implanted more than 10 y earlier in patients with advanced PD (37, 38). These findings argued that PD is a prion disease and that α-synuclein prions spread to the grafts. As the α-synuclein prions multiplied, they were sequestered into Lewy bodies in the fetal implants (39). Subsequently, α-synuclein prions were shown to arise spontaneously in TgM83^{+/+} mice expressing mutant human α-synuclein by three separate groups (13, 40, 41). Concurrent with those studies, other investigations showed that recombinant WT human α-synuclein could assemble into amyloid fibrils in vitro and initiate CNS lesions after IC or IM injection (29, 41–44).

In 2013, we reported our unexpected finding suggesting that the human synucleinopathy MSA is a prion disease (13). Here, we report that brain homogenates prepared from 14 MSA cases (Table 1 and Table S1) were inoculated into the thalamus of TgM83^{+/-} mice, nearly all of which subsequently developed progressive CNS dysfunction at ~120 dpi (Table 2). Homogenates prepared from the brains of selected MSA-inoculated mice were then inoculated into the thalamus of additional TgM83^{+/-} mice, after which the second group of mice exhibited progressive CNS dysfunction with incubation times similar to those found on initial passage (Table 3) (14). Critical to the interpretation of our results is that TgM83^{+/-} mice that were hemizygous for the human α-synuclein*A53T transgene did not develop CNS dysfunction spontaneously (13); in addition, the brains of these mice did not infect cultured HEK cells expressing α-syn140*A53T fused to YFP (14). In contrast, mouse brain homogenates from both the primary and secondary MSA prion transmissions did infect the cultured HEK cells.

The findings reported here demonstrate that α-synuclein prions exist in at least two different strains: MSA and TgM83^{+/+}. The incubation time for the TgM83^{+/+} strain of α-synuclein prions in TgM83^{+/-} mice was ~80% longer than that for the MSA strain as shown on both primary and secondary passage (Table 3). In previous studies using cultured HEK cells expressing α-syn140*A53T-YFP, we found evidence for three distinct MSA strains based on the quantity of MSA prions in different regions of three human brains (14).

Although there is no evidence that MSA is a naturally occurring transmissible disease among humans, the unequivocal experimental transmission studies reported here clearly warrant classification of MSA as a novel, bona fide α-synuclein prion

disorder. Like CJD, the vast majority of MSA cases are sporadic, with MSA arising spontaneously. All 14 of our MSA patients appear to be sporadic cases, as their *SNCA* and *COQ2* genes showed no missense mutations (Table S3). Additionally, like PrP^{Sc} prions, MSA prions are capable of spreading from cell to cell along the entire neuraxis (Fig. S1) (13). Importantly, the ability of MSA to induce progressive neurological disease in TgM83^{+/-} mice represents the only other human prion disease apart from that caused by PrP^{Sc} to induce a lethal phenotype in an animal model.

Attempts to transmit PD to TgM83^{+/-} mice were unsuccessful (Table 2). Notably, inoculation of brain fractions enriched for Lewy bodies from PD patients into WT mice and macaque monkeys induced limited Lewy body-like pathology, but neither species developed neurological disease (45). Using a similar approach, the insoluble protein fraction isolated from DLB patients induced phosphorylated α -synuclein pathology 15 mo after inoculation into WT mice, but the inoculations did not induce neurological deficits (46). Importantly, these human DLB transmission studies did demonstrate the spread of α -synuclein throughout the CNS and the phosphorylation of mouse α -synuclein. From our findings and those of others, we conclude that the putative α -synuclein prions causing PD represent one or more strains that differ from those causing MSA and from the one arising spontaneously in TgM83^{+/-} mice. Alternatively, post-translational chemical modifications might explain the difference between MSA and PD inocula. Ubiquitination, phosphorylation, nitrosylation, and sumoylation of α -synuclein have all been reported to play a role in α -synuclein toxicity (47–50).

Our hypothesis arguing that the MSA and PD prion strains are different is supported by the unique clinical presentations of these disorders and the distinct CNS locations of α -synuclein deposition: in MSA, within oligodendrocytes throughout the neuraxis, and in PD, within neuronal parikarya of the substantia nigra and the striatum, as well as surrounding axons. Our data and those of others contend that transmission of α -synuclein prions from MSA and PD patients to both an animal host and cultured cells requires different conditions to demonstrate infectivity.

Importantly, the transmission of MSA prions requires Tg mice expressing the A53T mutation found in familial PD, as mice expressing WT mouse or human α -synuclein were not capable of supporting MSA prion propagation (Table S4). Although the A53T mutation is likely to accelerate α -synuclein prion propagation by lowering the free energy barrier for replicating MSA prions, it is unclear if this amino acid substitution fundamentally altered the prions themselves. That said, single amino acid substitutions in PrP transgenes dramatically changed the susceptibility to PrP prion infection (51, 52).

Our discovery that MSA is caused by α -synuclein prions seems likely to force a revision in thinking about several important health care issues. First, deep brain stimulation (DBS) has become a widely used adjunct therapeutic intervention in PD, and many MSA cases may be initially misdiagnosed as PD. Our findings argue that the DBS electrodes, together with any associated equipment such as guide tubes and positioning micro-electrodes that come in contact with CNS tissue, should not be reused. The accidental transmission of CJD prions from depth electrodes that were reused demands similar precautions for DBS equipment (53, 54). Our findings also contend that the same increased vigilance used in brain biopsies on suspected CJD cases should be applied to all synucleinopathy patients. Increased biocontainment should be considered for PD, DLB, and MSA patients undergoing DBS electrode implantation or other neurosurgical procedures. Even after PrP^{Sc} prions bound to stainless steel wires were subjected to routine decontamination procedures, they retained their ability to infect mice on brain implantation, as well as in cultures of susceptible cells (55, 56). The resistance of MSA prions to standard decontamination and sterilization procedures remains to be determined.

In conclusion, all 14 human brain samples collected from people who died of MSA could be transmitted to both Tg mice expressing α -syn140*A53T and to cultured HEK cells expressing the fusion protein α -syn140*A53T-YFP. Notably, neither normal control brain nor PD brain samples transmitted prions to TgM83^{+/-} mice or HEK cells. Furthermore, we found that the brains from mice infected with MSA, but neither control brain nor PD, were also infectious in the TgM83^{+/-} mouse and HEK cell assay. Based on these findings, we conclude that MSA is a prion disorder and that α -synuclein is the first new bona fide prion to be discovered, to our knowledge, in the last 50 y. Moreover, establishing that MSA is an α -synuclein prion disorder sets the stage for a new therapeutic campaign; disappointingly, past efforts have failed to produce a single drug that slows or halts the ravages of the synucleinopathies since the introduction of levodopa that ameliorates PD but not MSA symptoms (57).

Materials and Methods

All animal procedures were approved by the University of California, San Francisco, Institutional Animal Care and Use Committee, and all procedures are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication, *Guide for the Care and Use of Laboratory Animals* (58).

Human Tissue Samples. Frozen tissue samples were obtained from the Parkinson's UK Brain Bank at Imperial College London, the Sydney Brain Bank, the neuropathology core of the Massachusetts Alzheimer's Disease Research Center (ADRC), and the San Francisco VA Medical Center. Clinical reports were provided and are summarized in Table 1 and Table S1.

Patient Neuropathology. MSA and PD patient samples obtained from the Parkinson's UK Brain Bank were bisected, with one hemisphere fixed in 10% (vol/vol) buffered formalin for diagnostic workup and the other coronally sliced, photographed on a grid, and then rapidly frozen. Blocks of tissue from 20 key anatomical areas were sampled from the fixed hemisphere. Sections from each area were stained with H&E and Luxol fast blue (LFB). For assessment and staging of neurodegenerative pathology, appropriate sections were stained with antibodies against α -synuclein, β -amyloid, tau, and p62. MSA was diagnosed based on the presence of oligodendroglial α -synuclein inclusions. PD cases were staged according to Braak criteria (59).

MSA patient samples obtained from the Massachusetts ADRC were bisected longitudinally. One half was coronally sectioned and rapidly frozen, and the other half was fixed in 10% (vol/vol) neutral buffered formalin and then sectioned. Histological evaluation was performed on a set of blocked regions representative for a variety of neurodegenerative diseases. All blocks were stained with LFB and H&E. On selected blocks, immunohistochemical analysis, including α -synuclein (mouse monoclonal antibody LB509; Life Technologies 18-0215), β -amyloid, and phosphorylated tau, was performed. The neuropathological diagnosis of MSA required the presence of GCIs (15).

Human brain tissue acquired from the Sydney Brain Bank was bisected: one hemisphere was randomly designated for fresh dissection and the other fixed for at least 2 wk in 15% (vol/vol) buffered formalin [39% (vol/vol) aqueous formaldehyde solution] and then sectioned. Standard neuropathological assessment was performed on H&E-stained sections, and a modified Bielschowsky silver stain was used to identify Alzheimer-type pathologies. Immunohistochemical detection for phosphorylated α -synuclein (BD Biosciences USA; 1:7,000), phosphorylated tau (AT8, Thermo Scientific USA; 1:1,000), and β -amyloid (Dako Denmark; 1:500) was also carried out.

Quantification of GCIs and Lewy Bodies. Brain samples from each of the patients were stained for α -synuclein with clone 42 (BD Biosciences; 1:300), counterstained with hematoxylin, and scanned using an AxioScan.Z1 microscope (Zeiss). Within the AxioScan image analysis software, a grid was placed over each image to define 500- μ m square fields, starting in a corner and moving in a serpentine fashion, lesions were counted in every 10th (substantia nigra) or 50th (pons and basal ganglia) field with at least 90% coverage and averaged for the whole section.

Quantification of Total α -Synuclein. Brain tissue was homogenized in calcium- and magnesium-free PBS to 10% (wt/vol) and processed with an ELISA kit (Anaspec #AS-55550) according to the manufacturer's instructions. Briefly, brain homogenates were diluted in the buffer provided and incubated in precoated wells overnight at 4 °C. Wells were washed seven times with wash

buffer provided. After a final wash, color was developed using the TMB-ELISA substrate provided. Plates were read at 450 nm optical density with a SpectraMax Plus microplate reader (Molecular Devices).

Sequencing of *SNCA* and *COQ2* Genes. DNA was extracted from the brain homogenates used for cell infection and mouse bioassay studies. The exons of the α -synuclein (*SNCA*) and *COQ2* genes from all 20 cases (14 MSA and 6 PD), as well as a single nondiseased control, were sequenced. Briefly, all five coding exons of *SNCA* and seven exons of *COQ2* were amplified by PCR using primers validated for specificity; primers used in this study are outlined in Table S2. The PCR products, ranging from 340 to 940 bp, were sequenced by Retrogen using standard Sanger sequencing and analyzed using Sequencher (www.genecodes.com) DNA sequence analysis software. Sequences from primer sets C (*SNCA* exon 4) and L (*COQ2* exon 1), which failed quality control, were resequenced using primer sets M and N, respectively (Table 2). A high-quality sequence was obtained in all samples for *SNCA* exons 2–5 and *COQ2* exons 3–5 and 7. For all other exons, a high-quality sequence was obtained for >75% of samples, except *COQ2* exon 1, in which only 8 of 21 sequences were reliable. Identified SNPs are shown in Table S3.

Mice. Homozygous TgM83^{+/+} mice (60) expressing human α -synuclein with the A53T mutation maintained on a B6;C3 background were purchased from the Jackson Laboratory. Hemizygous TgM83^{+/-} mice were generated either by backcrossing to FVB-Tg(*Gfap-luc*)^{+/+} mice, as previously described (13), or to B6C3F1 mice. Because strain background and the presence of the *Gfap-luc* transgene did not appear to affect the incubation period, they are not distinguished further.

To determine the role of endogenous α -synuclein on the effect of MSA transmission, *Snca*^{0/0} mice (61), a gift from Robert L. Nussbaum, University of California, San Francisco, were crossed with TgM83^{+/+} mice. The resulting TgM83^{+/-} *Snca*^{0/+} mice were then intercrossed to generate TgM83^{+/-} *Snca*^{+/+}, TgM83^{+/-} *Snca*^{0/+}, and TgM83^{+/-} *Snca*^{0/0} mice on a matched genetic background. Mice expressing WT human α -synuclein driven by a P1 artificial chromosome, Tg(*SNCA*) *Snca*^{0/0} (62), were a gift from Robert L. Nussbaum.

Inoculations. Human and mouse brain tissues were homogenized in calcium- and magnesium-free PBS to 10% (wt/vol) and were then diluted to 1% for inoculation using 5% (wt/vol) BSA in PBS. Approximately 2-mo-old mice were anesthetized with isoflurane and inoculated in the right parietal lobe with 30 μ L 1% homogenate, unless otherwise stated. Mice were assessed for signs of neurological illness, based on standard diagnostic criteria for prion disease (63), twice a week, and euthanized once signs of progressive neurological dysfunction were apparent. In preliminary studies, mice died within a few days of clinical onset; therefore, to ensure analysis of fresh tissue in the studies reported here, mice were euthanized within 2 d of showing progressive CNS dysfunction. Brains were bisected, and the left hemisphere was frozen for biochemical analysis, whereas the right was fixed in formalin for neuropathology.

Immunohistochemistry of Mouse Brains. Mouse brains fixed in 10% (vol/vol) formalin were embedded in paraffin, and sections were cut at 8 μ m. Sections were deparaffinized and treated with 3% (vol/vol) hydrogen peroxide in methanol for 30 min. Slides were then blocked with 10% (vol/vol) normal goat serum and incubated with primary antibody overnight. Sections were stained with anti-phosphorylated synuclein EP1536Y (Abcam #ab51253; 1:1,000 dilution), as well as anti-GFAP (Dako #Z0334; 1:500 dilution). Bound

antibody was detected using a Vectastain ABC peroxidase kit (Vector Laboratories) and visualized using 3,3'-diaminobenzidine. Slides were counterstained with hematoxylin and imaged using an AxioScan.Z1 microscope (Zeiss).

Biochemical Analysis of Synuclein Aggregates. Brains were homogenized in calcium- and magnesium-free PBS and then diluted to 10% (wt/vol). Total protein was quantified using the bicinchoninic acid assay kit (Pierce), and 5 μ g total mouse brain tissue homogenate or 25 μ g human brain tissue homogenate was run on a 4–12% gradient polyacrylamide gel (Invitrogen). Proteins were transferred to PVDF membranes, blocked with 5% (vol/vol) milk, and probed with a monoclonal α -synuclein antibody, Syn211 (ThermoScientific; 1:4,000 dilution), to assess for total synuclein. To assess the presence of phosphorylated synuclein aggregates, 250 μ g total protein was incubated with buffer (0.01 M Tris HCl, pH 8.0; 0.15 M NaCl; 0.5% Nonidet P-40; 0.5% deoxycholic acid) for 30 min at room temperature with shaking. Samples were then centrifuged at 100,000 \times g for 1 h at 4 $^{\circ}$ C. The pellet was resuspended in 1 \times NuPAGE loading buffer. Samples were separated and transferred to PVDF as described above, and blots were probed with the antiphosphorylated α -synuclein antibody EP1536Y (Abcam #ab51253; 1:4,000 dilution).

α -Synuclein Prion Cell Assay. The prion infectivity assay was carried out as previously described (14). Briefly, 10% (wt/vol) brain homogenate was combined with benzonase and sarkosyl to a final concentration of 2% (vol/vol) and incubated at 37 $^{\circ}$ C for 2 h on a shaking incubator. To this, PTA, pH 7.0, was added to a final concentration of 2% (vol/vol) and incubated overnight. The insoluble fraction was isolated by spinning at 16,000 \times g for 30 min; the pellet was resuspended in 2% (vol/vol) sarkosyl and PTA and incubated at 37 $^{\circ}$ C for 1 h. Samples were centrifuged for 30 min at 16,000 \times g, and the pellet was resuspended in PBS. Samples were diluted 1:4 and incubated with 1,000 HEK cells stably expressing the α -syn140**A53T*-YFP fusion protein in a 384-well black-walled plate (Greiner) for 4 d before imaging with an IN-Cell Analyzer 6000 (GE). Four images were collected from each well of the plate, and six replicate wells per sample were tested. Images were analyzed for aggregate formation using an algorithm we developed to detect YFP-positive aggregates among living cells, which was determined by nuclear stain. The four images from each well were averaged to determine a value for each well, and the six replicate wells were averaged to determine mean \pm SEM for each sample.

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Supporting Information

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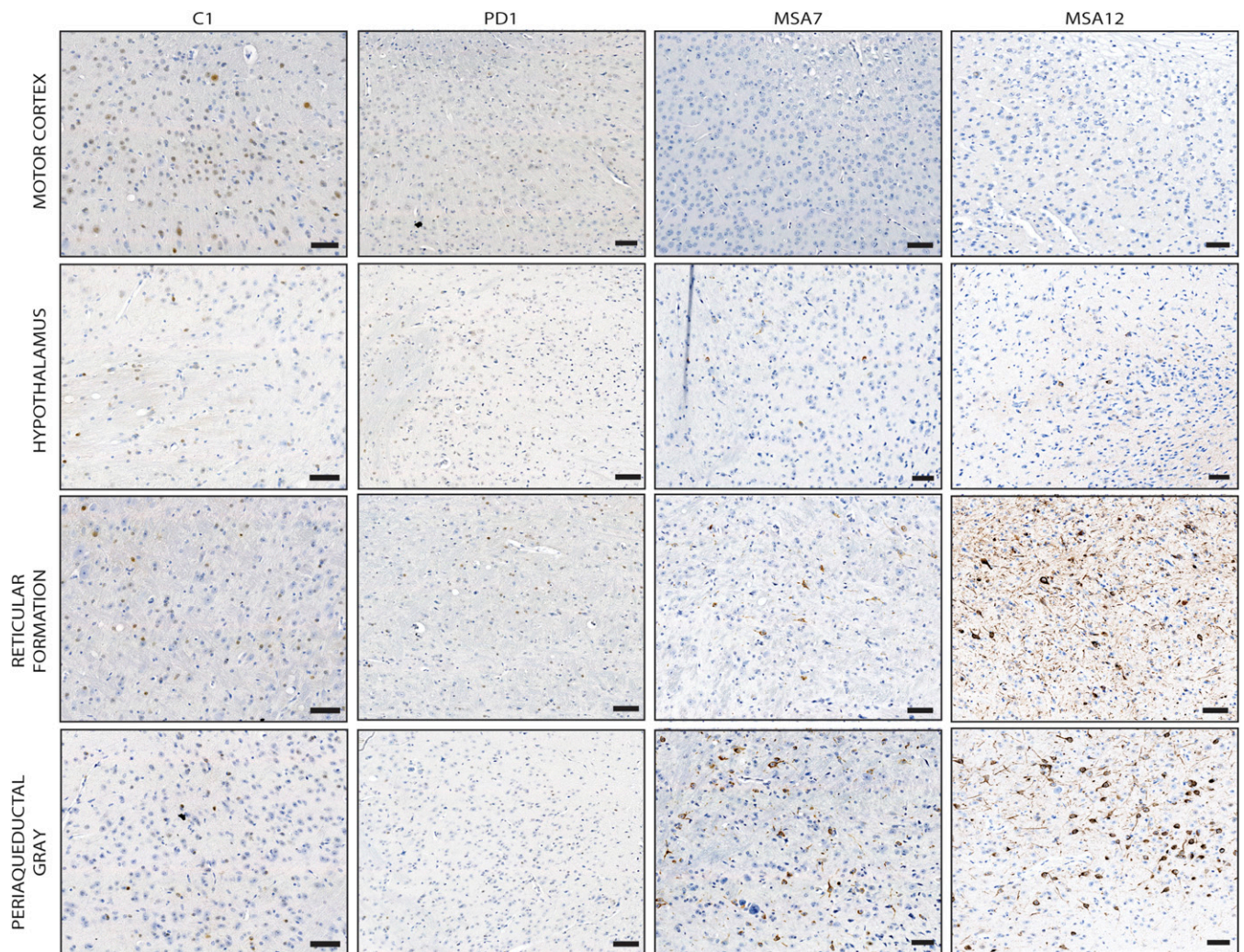


Fig. S1. Distribution of phosphorylated α -synuclein deposition in the brains of TgM83^{+/-} mice inoculated with control, PD, and MSA cases. Immunostaining for phosphorylated α -synuclein using antibody EP1536Y was performed in the brains of TgM83^{+/-} mice inoculated with homogenate prepared from a nondiseased control brain (360 d after inoculation), a PD case (360 d after inoculation), and two MSA cases (90 and 109 d after inoculation). Several brain regions were assessed for the deposition of phosphorylated α -synuclein, which was only found following inoculation with MSA homogenates. (Scale bars, 50 μ m.)

Table S1. Clinical and neuropathological characteristics of patient samples

Case	Autonomic symptoms	Cerebellar symptoms	Parkinsonism symptoms	Clinical interventions*	Gross brain changes	Microscopic brain changes [†]	
						Aβ plaques	Tau inclusions
PD1	CP, IC, OH, REM, SIA		TRE, DYS, DYA	Madopar, amantadine	Depigmentation of substantia nigra, cerebellar discoloration	+	+
PD2	CP, IC, OH		TRE, RGD	Ropinirole, Sinemet, Cabergoline	Depigmentation of substantia nigra	+	+
PD3 [‡]	SIA			Madopar	Depigmentation of substantia nigra	-	+
PD5			RGD, BK, DYS	Sinemet, Madopar, apomorphine	Depigmentation of brainstem	-	-
PD6					Depigmentation of brainstem	+	-
PD7	IC		TRE, RGD, BK	Kinson, Artane, Sifrol, Reminyl	Depigmentation of brainstem	+	+
MSA1	CP, IC	Action tremor	DYS, DYA	Sinemet, entacapone, Pergolide	Depigmentation of substantia nigra, cerebellar discoloration, small basal ganglia	+	-
MSA2	CP, IC, SIA		RGD, DYS, DYA	Madopar, Sinemet, entacapone, Ropinirole, amantidine	Depigmentation of substantia nigra, autolytic cerebellum, small pons	+	-
MSA3	CP, OH, IC, SIA		BK, MG, RGD, PI	Sinemet, Ropinirole, Madopar, entacapone, Pramipexole, amantidine	Depigmentation of substantia nigra, discoloration of white matter of cerebellum	-	+
MSA4	CP, OH, IC	Action tremor		Ropinirole, Sinemet, amantidine, Rasagiline, entacapone, Madopar	Depigmentation of substantia nigra, shrunken, brown, softened basal ganglia	+	-
MSA5	OH		TRE, RGD, BK, PI	Madopar, entacapone, Ropinirole, Selegiline	Softening/discoloration of the cerebellum, darkened frontal lobe, putamen, and globus pallidus	+	-
MSA6	CP, OH, UR, SIA		BK, RGD, DYS	Entacapone, Sinemet, Madopar, amantidine, Ropinirole, pramipexole	Depigmentation of substantia nigra, softening of cerebellum	+	-
MSA7	ED, UR, OH, REM	Unsteady gait, dysidiadochokinesis	BK, PI	Levodopa, amantidine	Depigmentation of substantia nigra, softening of cerebellum	-	-
MSA8	ED, UR, CP	Dysidiadochokinesis, ataxia	BK, PI, DYS, DYA	Sinemet	Depigmentation of brainstem, white matter loss of cerebellum	-	-
MSA9	CP, MG		RGD, BK	Sinemet, entacapone, apomorphine	Atrophy/discoloration of the putamen, depigmentation of the brainstem	-	-
MSA10	ED, IC, OH, CP		RGD, BK	Madopar, Cabergoline, amantidine	Atrophy of putamen and globus pallidus, depigmentation of the brainstem	-	-
MSA11	IC, ED, MG, REM, OH		BK	Madopar, Sinemet, Stalevo, apomorphine	Atrophy/discoloration of putamen and globus pallidus, depigmentation of the brainstem	-	-
MSA12		Ataxia	RGD, BK, PI	Sinemet	Depigmentation of substantia nigra, atrophy of striatum, degeneration of cerebellum, pons, and inferior olives	-	-
MSA13	IC, OH, CP		RGD, BK, DYS		Depigmentation of substantia nigra, degeneration of substantia nigra, and pons	-	-
MSA14	IC, ED, REM, OH	Cerebellar ataxia			Degeneration of cerebellum and pons inferior olives	-	-

BK, bradykinesia; CP, constipation; DYA, dysarthria; DYS, dysphagia; ED, erectile dysfunction; IC, urinary incontinence; MG, micrographia; OH, orthostatic hypotension; PI, postural instability; REM, REM sleep disorder; RGD, rigidity; SIA, sialorrhea; SIA, sialorrhea; TRE, tremor; UR, urinary retention.

*Madopar = benserazide/levodopa; Sinemet & Kinson = carbidopa/levodopa; Stalevo = carbidopa/levodopa/entacapone.

[†]Aβ and tau pathology are reported as present (+) or absent (-).

[‡]Clinical report for PD3 was incomplete.

Table S2. Primers used for sequencing each exon of the α -synuclein (*SNCA*) and coenzyme Q10 (*COQ2*) genes

Target amplicon	Set	Primer sequence	hg19 location	GC%
<i>SNCA</i> exon 6	A	ACTTAAGGAACCCAGTGCATACC	chr4:90646983–90647922	45
		ATTTTAGTGTAAAGTGGGGAGCC	chr4:90646983–90647922	45
<i>SNCA</i> exon 5	B	ACGTAATGAGCATGTAGAGAGC	chr4:90650230–90650908	45
		GATGGGGTTTCATTGTAACAGC	chr4:90650230–90650908	45
<i>SNCA</i> exon 4	C	GGGTAAATGCCTGAAACAAGC	chr4:90743223–90743870	48
		TAACTGCACCTGTAAGAATTGC	chr4:90743223–90743870	41
<i>SNCA</i> exon 3	D	CAAAGCCCTCATTATTCTTGGC	chr4:90749076–90749675	45
		AAATTATCTTCTCACTGGGCC	chr4:90749076–90749675	45
<i>SNCA</i> exon 2	E	CCATTTAGGGCCACAATAGG	chr4:90756215–90756984	45
		AAATGTCTGCTTTGTCCAATGG	chr4:90756215–90756984	41
<i>COQ2</i> exon 7	F	TGCTCTAAATCTTCATCTTCAGGTT	chr4:84185233–84185606	36
		GCTGTTTTCTCCTCCGTGTTA	chr4:84185233–84185606	48
<i>COQ2</i> exon 6	G	CTTCAGTCCAGGAGTTAGAGGTT	chr4:84188446–84189301	48
		AGCTATGGGAAGAATTAGAGCTG	chr4:84188446–84189301	43
<i>COQ2</i> exon 5	H	AGCCATGGAAGTGAATGACT	chr4:84190836–84191215	45
		CCACTCCTTCCCTTTAGGATTC	chr4:84190836–84191215	50
<i>COQ2</i> exon 4	I	TGATGATAACCCTAATAAATATGCAA	chr4:84193038–84193382	27
		TCGTGGTTTTCTAAGAGGTAGTGG	chr4:84193038–84193382	48
<i>COQ2</i> exon 3	J	ACAAAAGTTTCGTTTTGTCTTGTT	chr4:84194152–84194957	29
		GATGGGAATTTTTAGTCTTTTCA	chr4:84194152–84194957	29
<i>COQ2</i> exon 2	K	GGTCACTGAATGATCTTGTTC	chr4:84199994–84200433	45
		GTAAGGGTCCCTTGTGATTTG	chr4:84199994–84200433	45
<i>COQ2</i> exon 1	L	GACTCGGAGGCTGCTACTTG	chr4:84205304–84206120	60
		GAAATCCCTTTTTTCATTAAGTATTCCT	chr4:84205304–84206120	30
<i>SNCA</i> exon 4	M	TGAAAATGAACCGTAATCTCACC	chr4:90743337–90743716	39
		GGTTGAACCACACTCACTTGG	chr4:90743337–90743716	52
<i>COQ2</i> exon 1	N	CGACTCGGAGGCTGCTACT	chr4:84205610–84206114	63
		GAAATCCCTTTTTTCATTAAGTATTC	chr4:84205610–84206114	31

Table S3. Exon sequence SNPs for coenzyme Q10 (*COQ2*) gene

Patient	rs1129617 <i>COQ2</i> exon 6	rs6535454 <i>COQ2</i> exon 5	rs6818847 <i>COQ2</i> exon 1
C1	GG	GG	AA
PD1	GG	GG	AA
PD2	AA	AA	CC
PD3	00	GG	AA
PD5	00	GA	AC
PD6	00	GG	AA
PD7	GG	GG	AA
MSA1	GG	GG	00
MSA2	AA	AA	00
MSA3	AA	AA	00
MSA4	GG	GG	AA
MSA5	GG	GG	AA
MSA6	GG	GG	AA
MSA7	GA	GA	AA
MSA8	GG	GG	AA
MSA9	GA	GA	AA
MSA10	GG	GG	AA
MSA11	GA	GA	AA
MSA12	GG	GG	AA
MSA13	00	GA	AA
MSA14	00	GA	AC

00, insufficient data to call bases.

Table S4. Effect of M83 transgene dose and endogenous α -synuclein on incubation periods of MSA prions

Inoculum (brain region)	Host	Mean incubation time \pm SEM (d)	n/n_0
MSA2 (basal ganglia)	TgM83 ^{+/-} <i>Snca</i> ^{0/0}	112 \pm 10	8/8
	TgM83 ^{+/-} <i>Snca</i> ^{0/+}	113 \pm 9	9/9
	TgM83 ^{+/-} <i>Snca</i> ^{+/+}	110 \pm 10	6/6
	TgM83 ^{+/+}	79 \pm 2	8/8
MSA3 (substantia nigra)	TgM83 ^{+/+}	112 \pm 9	8/8
MSA8 (pons)	TgM83 ^{+/+}	86 \pm 5	6/6

n , number of ill mice; n_0 , number of inoculated mice.