





Somatic *SNCA* Copy Number Variants in Multiple System Atrophy Are Related to Pathology and Inclusions

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ABSTRACT: Background: Somatic α -synuclein (*SNCA*) copy number variants (CNVs, specifically gains) occur in multiple system atrophy (MSA) and Parkinson's disease brains.

Objective: The aim was to compare somatic *SNCA* CNVs in MSA subtypes (striatonigral degeneration [SND] and olivopontocerebellar atrophy [OPCA]) and correlate with inclusions.

Methods: We combined fluorescent in situ hybridization with immunofluorescence for α -synuclein and in some cases oligodendrocyte marker tubulin polymerization promoting protein (TPPP).

Results: We analyzed one to three brain regions from 24 MSA cases (13 SND, 11 OPCA). In a region preferentially affected in one subtype (putamen in SND, cerebellum in OPCA), mosaicism was higher in that subtype, and cells with CNVs were 4.2 times more likely to have inclusions. In the substantia nigra, non-pigmented cells with CNVs and TPPP were about six times more likely to have inclusions.

Conclusions: The correlation between *SNCA* CNVs and pathology (at a regional level) and inclusions (at a single-cell level) suggests a role for somatic *SNCA* CNVs in MSA pathogenesis. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: *snca*; alpha-synuclein; mosaicism; multiple system atrophy; somatic mutation

Background

Multiple system atrophy (MSA) can be classified pathologically into striatonigral degeneration (SND), olivopontocerebellar atrophy (OPCA), and mixed.¹ α -Synuclein, encoded by *SNCA*, forms aggregates, and glial cytoplasmic inclusions (GCIs) in oligodendrocytes are likely central to pathogenesis.² Rare inherited *SNCA* mutations lead to synucleinopathies, generally classified as Parkinson's disease (PD), often with dementia. These are most often copy number variants (CNVs), specifically gains, which increase mRNA,³ and disease severity depends on gene dosage.⁴ Patients with *SNCA* mutations frequently also have MSA features with GCIs,⁵⁻⁸ suggesting that *SNCA* mutations can also affect oligodendrocytes. As MSA is a sporadic disorder, with heritability less than 7%,⁹ a major role of mutations may appear unlikely. Mutations can, however, also be acquired post-zygotically. These are termed "somatic" and lead to mosaicism, the presence of cells with genetic differences in an organism. Somatic mutations, including CNVs, occur in brain,¹⁰ have a role in several neuropsychiatric and neurodevelopmental conditions,¹¹ and may also have a role in neurodegeneration,¹² and the asymmetry is often observed.¹³ Somatic *SNCA* mutations could contribute directly to the etiopathogenesis of sporadic synucleinopathies.¹⁴

We previously reported that somatic *SNCA* CNVs (gains) in the substantia nigra (SN) and cingulate cortex

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are more frequent in MSA and PD patients than in controls using fluorescent in situ hybridization (FISH).^{15,16} We also used single-cell whole genome amplification and low coverage sequencing to demonstrate somatic CNVs in about 30% of brain cells from two MSA cases, including a gain of *SNCA*.¹⁶ To further investigate the role of somatic *SNCA* CNVs in MSA, we have now compared their abundance (mosaic level) in three different regions between SND and OPCA and correlated their presence with inclusions in the same cells.

Methods

Samples were provided by the UCL Queen Square Brain Bank for Neurological Disorders (Table S1) with informed consent for use of tissue in research and ethics approval by the UK National Research Ethics Service (07/MRE09/72). We analyzed 10- μ m frozen sections from MSA patient brains from the SN at the level of the red nucleus or decussation of the superior cerebellar peduncle, putaminal striatopallidal fibers at the level of the anterior commissure (henceforth referred to as “putamen”), and cerebellar white matter. We performed FISH for *SNCA* copy numbers and a reference gene (unless otherwise stated) combined with immunofluorescence (IF) as described.¹⁷ For *SNCA* FISH, we used the same probe as before (Agilent SureFISH *SNCA* 4q.22.1, G110997R-8) and a chromosome 7 probe as reference (Agilent SureFISH G110902G-8) (Santa Clara, California, USA). IF staining for α -synuclein (mouse monoclonal 211, Santa Cruz, 1:200 dilution) (Dallas, Texas, USA) was performed in most experiments. In the SN, we divided cells based on neuromelanin (NM) presence into NM+ (dopaminergic neurons) and NM- (glia and other neurons). For oligodendrocyte detection, we used a rabbit monoclonal antibody to tubulin polymerization promoting protein (TPPP) (Abcam ab92305, 1:50 dilution) (Cambridge, UK), interpreting a nuclear signal as positive and omitting the reference FISH probe to simultaneously detect α -synuclein aggregates. Staining was visualized using Alexa 647 goat anti-mouse for α -synuclein and Alexa 488 goat anti-rabbit for TPPP (Life Technologies, 1:500 dilution) (Carlsbad, California, USA). All experiments were performed blinded to disease subtype. Images were obtained on a Leica epifluorescence microscope coupled to an ORCAII Digital CCD camera (Hamamatsu, Shizuoka, Japan) and controlled by Leica Application Suite X (Leica, Wetzlar, Germany) as z-stacks of 16 images (separated by 0.5 μ m) using appropriate fluorescence filters and brightfield in the SN for NM. Statistical analysis was performed using GraphPad Prism (v.9). Pairwise comparisons were performed using unpaired *t* test, two sided, as normal distribution was confirmed using the

Kolmogorov–Smirnov test. 2×2 and 2×3 tables were analyzed using χ^2 test.

Results

We first investigated whether mosaicism level in a given region differs based on the involvement of the region in each MSA subtype by studying putamen, mostly affected in SND; cerebellum, mostly affected in OPCA; and SN, equally affected in both. We analyzed 5967 cells, derived from at least one brain region from 24 MSA cases (13 SND, 11 OPCA), including all three from 14 (Tables S1 and S2). We calculated percentage *SNCA* mosaicism as the percentage of cells containing unique gains of *SNCA* (more than two *SNCA* copies, two copies of reference) divided by the total number of cells with no other detectable genomic aberration, that is, two copies of reference and more than two copies of *SNCA*, as before (Fig. 1A; all individual results are presented in detail in Table S3). In the cerebellum, mosaicism was significantly higher in OPCA (11.1% vs. 8.6%), whereas in the putamen, it was significantly higher in SND (9.9% vs. 8.5%). Furthermore, comparing these figures within each subtype revealed that mosaicism in SND was significantly higher in putamen than cerebellum ($P = 0.006$) and that mosaicism in OPCA was significantly higher in cerebellum than putamen ($P = 0.003$). Therefore, in a region preferentially affected in one MSA subtype, mosaicism is higher in that subtype, and in a given subtype of MSA, mosaicism is higher in a preferentially affected rather than a relatively spared region. In the SN it was similar in both subtypes, and this was also true when divided into dopaminergic neurons (NM+) and other cells (NM-), which are mostly oligodendrocytes¹⁸ (Fig. S1).

To avoid possible sectioning artifacts, and for consistency with our previous work,^{15,16} we did not include cells with gains of both probes, or losses of either probe, in the main analysis. Gains of both probes could represent true gains of both *SNCA* and the reference. They could also, however, be artifacts if multiple complete or partial nuclei were counted together, despite the care taken to avoid this. We noted only 18 cells with gains of both probes (0.29%): 14 of which were in the SN (0.59%), 3 in the cerebellum (0.16%), and 1 in the putamen (0.05%) ($P < 0.0001$). Half of the SN cells with gains of both probes had more *SNCA* than reference copies, consistent with the excess *SNCA* signals observed overall (six 4 *SNCA*/3 reference, one 5 *SNCA*/3 reference). We also reviewed gains of the reference probe alone. As in previous work with other reference probes,^{15,16} these were extremely rare (18 cells, 0.29%). Of these, 15 were in the SN (0.63%), whereas there were only

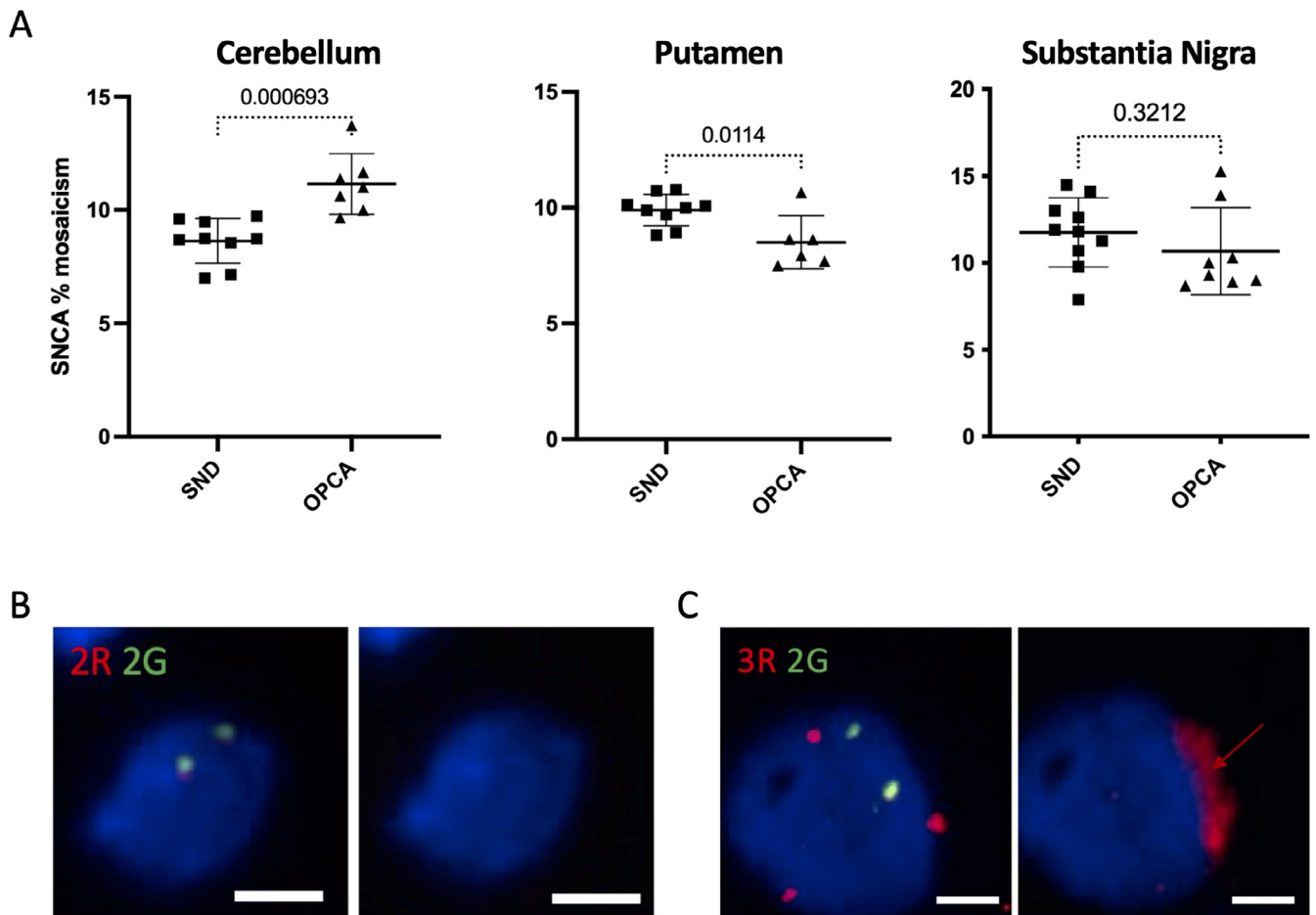


FIG. 1. SNCA CNV mosaicism in SND and OPCA. (A) Comparison of regional mosaicism between SND and OPCA in the three regions tested. Mean, SD, and P -value are shown. (B, C) Examples of FISH and α -synuclein IF. The left panel of each image shows the FISH (SNCA in red, reference in green), and the right panel shows the IF for α -synuclein. (B) A cell with no CNV and no inclusions and (C) a cell with a CNV and an inclusion (arrowed). Scale bars: 5 μ m. CNV, copy number variant; FISH, fluorescent in situ hybridization; IF, immunofluorescence; SD, standard deviation; SND, striatonigral degeneration. [Color figure can be viewed at wileyonlinelibrary.com]

2 in the cerebellum (0.1%) and 1 in the putamen (0.05%) ($P < 0.0001$).

We next performed further analysis of experiments where FISH had been combined with α -synuclein IF to compare the proportion of inclusions in different regions and subtypes and determine whether α -synuclein inclusions occur preferentially in cells with SNCA CNVs (Fig. 1B; 5007 cells). Inclusions were more frequent in the cerebellum and putamen in the subtype where each is preferentially affected (cerebellum: OPCA 14.3%, SND 6.9%, $P < 0.0001$; putamen: SND 10.3%, OPCA 5.5%, $P = 0.0002$). In the SN, inclusion prevalence was similar (SND 7.7%, OPCA 7.4%). As previous data had suggested increased inclusions in the SN in cells with CNVs, in a specific pattern (NM+ cells in Lewy body disorders and NM- cells in MSA-SND),¹⁶ we determined whether CNV presence is associated with a higher risk of inclusion in the same cell in each region and subtype (Table 1). Cells with CNVs were 4.2 times more likely to have inclusions in

the preferentially affected region in each subtype (cerebellum in OPCA, putamen in SND). In the relatively spared regions, a significant effect, but smaller (2.7 times), was observed only in SND. In the SN, NM- cells with CNVs were about six times more likely to have inclusions in both subtypes, but an association with inclusions in NM+ cells was observed only in OPCA. We conclude that CNVs are associated with inclusions in the same cells in affected regions.

As there is significant heterogeneity of oligodendrocytes,¹⁹ we hypothesized that the association of CNVs and inclusions may occur only in those with certain expression profiles. TPPP/P25 α is an oligodendrocyte protein that contributes to α -synuclein aggregation.²⁰⁻²³ We combined SNCA FISH with IF for TPPP and α -synuclein in SN from four additional MSA cases (two SND, two OPCA; 473 cells, of which 382 NM-; Table S4). We found robust nuclear TPPP staining in 53.4% of NM- cells, within which inclusions were present more often in TPPP+ cells (11.8%,

TABLE 1 Relative risk (RR) for α -synuclein inclusions in cells with and without somatic SNCA CNVs

Region	SND					OPCA				
	n	Percentage inclusion		RR	P	n	Percentage inclusion		RR	P
		CNV+	CNV-				CNV+	CNV-		
Cerebellum	7	16.2	6.0	2.7	0.004	5	43.3	10.4	4.2	<0.0001
Putamen	9	32.8	7.8	4.2	<0.0001	6	9.0	5.2	1.7	0.25
SN										
Overall	9	21.9	5.1	4.3	<0.0001	7	23.8	4.6	5.2	<0.0001
NM+		8.3	7.5	1.2	0.745		15.2	3.4	4.5	0.014
NM-		23.9	3.7	6.5	<0.0001		27.9	5.1	5.5	<0.0001

Note: Divided by MSA type, region, and NM status in SN. *n* is the number of cases. "Percentage inclusion" is the percentage of cells that had inclusions divided by CNV presence (+) or absence (-). Significant results are in bold font.

Abbreviations: CNV, copy number variant; MSA, multiple system atrophy; NM, neuromelanin; OPCA, olivopontocerebellar atrophy; SN, substantia nigra; SND, striatonigral degeneration.

vs. 4.5% TPPP-, $P = 0.015$). In these experiments, as no reference FISH probe was used, % SNCA mosaicism was calculated as the proportion of cells with more than two SNCA copies among all cells with two copies or more. The mosaicism level did not depend on TPPP presence, with CNVs in 10.1% of TPPP+ and 7.8% of TPPP- cells ($P = 0.5$), but the association between CNVs and inclusions was observed only in TPPP+ cells. These were six times more likely to have an inclusion if they had a CNV in SND and 5.7 times in OPCA ($P = 0.017$ and 0.0009 , respectively; Table S4). Among NM- cells in the SN, a cell with both a CNV and TPPP expression may therefore be most likely to develop inclusions.

Conclusions

We provide the first evidence in MSA, and to our knowledge in any neurodegenerative disorder, that the level of a potentially relevant somatic mutation in a brain region is higher in the disease subtype where this region is preferentially affected but similar in a region equally affected in both subtypes. The higher prevalence of cells with genomic instability beyond SNCA gains, that is, gains of the reference alone or of both probes, in the SN merits further investigation. We confirm a significant association between CNVs and inclusions in the same cells, in regions affected in each subtype, but reduced or absent in a region relatively spared. Presence of a CNV may lead to increased SNCA mRNA, which could be of particular importance in oligodendrocytes, where endogenous expression is low.^{22,24} This would lead to increased availability of α -synuclein to aggregate locally, and mosaicism levels in different regions could thus determine pathology subtype. We have not, however, demonstrated increased mRNA, which cannot presently be measured together with DNA FISH.

Furthermore, we cannot exclude the possibility that α -synuclein aggregation causes DNA damage²⁵ leading to somatic mutations such as SNCA CNVs, although even in this case a CNV may further potentiate aggregation.¹² Inclusion development in an oligodendrocyte with a CNV may be facilitated by TPPP expression, which leads to the highly toxic MSA α -synuclein strain.²³ Validation will require larger studies, including other regions, and detailed characterization of cells with CNVs to determine whether all (excluding dopaminergic neurons) are oligodendrocytes. It will also be crucial to determine the timing at which CNVs arise and whether enhancement of DNA repair is a valid target, for example, by augmenting nicotinamide adenine dinucleotide, as suggested in PD.²⁶ Overall, our results suggest a direct role of somatic SNCA CNVs in MSA etiopathogenesis and determination of pathological subtype. ■

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Data Availability Statement

Brain samples used in this study, with corresponding results, can be made available by formal application directly to the Queen Square Brain Bank.

References

- Miki Y, Foti SC, Asi YT, et al. Improving diagnostic accuracy of multiple system atrophy: a clinicopathological study. *Brain* 2019; 142:2813–2827.
- Monzio Compagnoni G, Di Fonzo A. Understanding the pathogenesis of multiple system atrophy: state of the art and future perspectives. *Acta Neuropathol Commun* 2019;7:113.
- Miller DW, Hague SM, Clarimon J, et al. Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology* 2004;62:1835–1838.

4. Book A, Guella I, Candido T, et al. A meta-analysis of α -Synuclein multiplication in familial Parkinsonism. *Front Neurol* 2018;9:1021.
5. Fujishiro H, Imamura AY, Lin W-L, et al. Diversity of pathological features other than Lewy bodies in familial Parkinson's disease due to SNCA mutations. *Am J Neurodegener Dis* 2013;2:266–275.
6. Kiely AP, Ling H, Asi YT, et al. Distinct clinical and neuropathological features of G51D SNCA mutation cases compared with SNCA duplication and H50Q mutation. *Mol Neurodegener* 2015;10:41.
7. Konno T, Ross OA, Puschmann A, Dickson DW, Wszolek ZK. Autosomal dominant Parkinson's disease caused by SNCA duplications. *Parkinsonism Relat Disord* 2016;22(Suppl 1):S1–S6.
8. Pasanen P, Myllykangas L, Siitonen M, et al. Novel α -synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. *Neurobiol Aging* 2014;35(2180):e1–e5.
9. Federoff M, Price TR, Sailer A, et al. Genome-wide estimate of the heritability of multiple system atrophy. *Parkinsonism Relat Disord* 2016;22:35–41.
10. Rohrbach S, Siddoway B, Liu CS, Chun J. Genomic mosaicism in the developing and adult brain. *Dev Neurobiol* 2018;78:1026–1048.
11. Bizzotto S, Walsh CA. Genetic mosaicism in the human brain: from lineage tracing to neuropsychiatric disorders. *Nat Rev Neurosci* 2022;23:275–286.
12. Proukakis C. Somatic mutations in neurodegeneration: an update. *Neurobiol Dis* 2020;144:105021.
13. Proukakis C. Somatic mutations may contribute to asymmetry in neurodegenerative disorders. *Brain Commun* 2022;4:fcac184.
14. Proukakis C, Houlden H, Schapira AH. Somatic alpha-synuclein mutations in Parkinson's disease: hypothesis and preliminary data. *Mov Disord* 2013;28:705–712.
15. Mokretar K, Pease D, Taanman J-W, et al. Somatic copy number gains of α -synuclein (SNCA) in Parkinson's disease and multiple system atrophy brains. *Brain* 2018;141:2419–2431.
16. Perez-Rodriguez D, Kalyva M, Leija-Salazar M, et al. Investigation of somatic CNVs in brains of synucleinopathy cases using targeted SNCA analysis and single cell sequencing. *Acta Neuropathol Commun* 2019;7:219.
17. Garcia-Segura ME, Perez-Rodriguez D, Proukakis C. Combined fluorescent In situ hybridization (FISH) and immunofluorescence for the targeted detection of somatic copy number variants in Synucleinopathies. In: Proukakis C, ed. *Genomic Structural Variants in Nervous System Disorders*. New York, NY: Springer; 2022.
18. Agarwal D, Sandor C, Volpato V, et al. A single-cell atlas of the human substantia nigra reveals cell-specific pathways associated with neurological disorders. *Nat Commun* 2020;11:4183.
19. Seeker LA, Williams A. Oligodendroglia heterogeneity in the human central nervous system. *Acta Neuropathol* 2021; published online Dec 3 143:143–157. <https://doi.org/10.1007/s00401-021-02390-4>
20. Lindersson E, Lundvig D, Petersen C, et al. p25alpha stimulates alpha-synuclein aggregation and is co-localized with aggregated alpha-synuclein in alpha-synucleinopathies. *J Biol Chem* 2005;280:5703–5715.
21. Kragh CL, Fillon G, Gysbers A, et al. FAS-dependent cell death in α -synuclein transgenic oligodendrocyte models of multiple system atrophy. *PLoS One* 2013;8:e55243.
22. Mavroei P, Arvanitaki F, Karakitsou A-K, et al. Endogenous oligodendroglial alpha-synuclein and TPPP/p25 α orchestrate alpha-synuclein pathology in experimental multiple system atrophy models. *Acta Neuropathol* 2019;138:415–441.
23. Ferreira N, Gram H, Sorrentino ZA, et al. Multiple system atrophy-associated oligodendroglial protein p25 α stimulates formation of novel α -synuclein strain with enhanced neurodegenerative potential. *Acta Neuropathol* 2021;142:87–115.
24. Asi YT, Simpson JE, Heath PR, et al. Alpha-synuclein mRNA expression in oligodendrocytes in MSA. *Glia* 2014;62:964–970.
25. El-Saadi MW, Tian X, Grames M, et al. Tracing brain genotoxic stress in Parkinson's disease with a novel single-cell genetic sensor. *Sci Adv* 2022;8:eabd1700.
26. Brakedal B, Dölle C, Riemer F, et al. The NADPARK study: a randomized phase I trial of nicotinamide riboside supplementation in Parkinson's disease. *Cell Metab* 2022;34:396–407.e6.

Supporting Data

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(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript: A. Writing of the first draft, B. Review and critique.

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D.C.: 1B, 1C, 3B

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