





## SHORT COMMUNICATION

# Human $\alpha$ -synuclein overexpression upregulates SKOR1 in a rat model of simulated nigrostriatal ageing

Noelia Morales-Prieto<sup>1</sup> | Rebekah Bevans<sup>1</sup> | Adam O'Mahony<sup>1</sup> | Aaron Barron<sup>1,2</sup> |  
Conor Giles Doran<sup>1</sup> | Erin McCarthy<sup>1</sup> | Ruth M. Concannon<sup>1</sup> | Susan R. Goulding<sup>1</sup> |  
Cathal M. McCarthy<sup>2</sup> | Louise M. Collins<sup>1,3,4</sup> | Aideen M. Sullivan<sup>1,4,5</sup>  |  
Gerard W. O'Keefe<sup>1,4,5</sup> 

<sup>1</sup>Department of Anatomy and Neuroscience, School of Medicine, University College, Cork, Ireland

<sup>2</sup>Department of Pharmacology and Therapeutics, School of Medicine, University College Cork, Cork, Ireland

<sup>3</sup>Department of Physiology, School of Medicine, University College Cork, Cork, Ireland

<sup>4</sup>Parkinson's Disease Research Cluster (PDRC), University College Cork, Cork, Ireland

<sup>5</sup>APC Microbiome Ireland, University College Cork, Cork, Ireland

## Correspondence

Gerard W. O'Keefe, Aideen M. Sullivan and Louise M. Collins, Department of Anatomy and Neuroscience, University College, Cork, Ireland.  
Email: [g.okeefe@ucc.ie](mailto:g.okeefe@ucc.ie), [a.sullivan@ucc.ie](mailto:a.sullivan@ucc.ie) and [l.collins@ucc.ie](mailto:l.collins@ucc.ie)

## Funding information

Science Foundation Ireland, Grant/Award Number: 19/FFP/6666; Cure Parkinson's Trust, Grant/Award Number: CP:GO01; HORIZON EUROPE Marie Skłodowska-Curie Actions, Grant/Award Number: 890290

## Abstract

Parkinson's disease (PD) is characterised by progressive loss of dopaminergic (DA) neurons from the *substantia nigra* (SN) and  $\alpha$ -synuclein ( $\alpha$ Syn) accumulation. Age is the biggest risk factor for PD and may create a vulnerable pre-parkinsonian state, but the drivers of this association are unclear. It is known that ageing increases  $\alpha$ Syn expression in DA neurons and that this may alter molecular processes that are central to maintaining nigrostriatal integrity. To model this, adult female Sprague–Dawley rats received a unilateral intranigral injection of adeno-associated viral (AAV) vector carrying wild-type human  $\alpha$ Syn (AAV- $\alpha$ Syn) or control vector (AAV-Null). AAV- $\alpha$ Syn induced no detrimental effects on motor behaviour, but there was expression of human wild-type  $\alpha$ Syn throughout the midbrain and ipsilateral striatum at 20 weeks post-surgery. Microarray analysis revealed that the gene most-upregulated in the ipsilateral SN of the AAV- $\alpha$ Syn group was the SKI Family Transcriptional Corepressor 1 (SKOR1). Bioenergetic state analysis of mitochondrial function found that SKOR1 overexpression reduced the maximum rate of cellular respiration in SH-SY5Y cells. Furthermore, experiments in SH-SY5Y cells revealed that SKOR1 overexpression impaired neurite growth to the same extent as  $\alpha$ Syn, and inhibited BMP-SMAD-dependent transcription, a pathway that promotes DA neuronal survival and growth. Given the normal influence of ageing on DA neuron loss in human SN, the extent of  $\alpha$ Syn-induced SKOR1 expression may influence whether an individual undergoes normal nigrostriatal ageing or reaches a threshold for prodromal PD. This provides new insight into mechanisms through which ageing-related increases in  $\alpha$ Syn may influence molecular mechanisms important for the maintenance of neuronal integrity.

**Abbreviations:** AAV, adeno-associated viral vector; BMP, bone morphogenetic protein; DA, dopaminergic; DAT, dopamine transporter; PD, Parkinson's disease; RLS, restless leg syndrome; SKOR1, SKI Family Transcriptional Corepressor 1; SN, *Substantia nigra*; TH, tyrosine hydroxylase;  $\alpha$ Syn,  $\alpha$ -synuclein.

Rebekah Bevans, Adam O'Mahony, Aaron Barron and Conor Giles Doran have contributed equally to this article.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Aging Cell* published by Anatomical Society and John Wiley & Sons Ltd.



## KEYWORDS

ageing, microarray, mitochondria, nigrostriatal, SKOR1,  $\alpha$ -Synuclein

## 1 | INTRODUCTION, RESULTS AND DISCUSSION

Parkinson's disease (PD) is the fastest-growing neurological disorder (Dorsey et al., 2018; Group, 2017). It is a neurodegenerative disease characterised by the progressive degeneration of midbrain dopaminergic (DA) neurons in the *substantia nigra* (SN), which form the nigrostriatal tract (Bloem et al., 2021; Kordower et al., 2013). Ageing is the biggest risk factor for PD, which affects 1% of people over the age of 60, rising to 5% of those over the age of 85. Despite the wealth of epidemiological evidence for ageing as the primary risk factor for PD, the biological drivers of this association are largely unknown. However, it is thought that SN neuronal loss occurs in humans as a result of ageing, at a rate of approximately 7% per decade (Stark & Pakkenberg, 2004). This is supported by imaging studies in humans showing that ageing of the nigrostriatal tract begins in the third decade and progresses throughout life until old age, when it exhibits significant degeneration (Seo & Koo, 2021). In agreement, a study in non-human primates has shown that ageing-related changes in the dopamine system actively create a vulnerable pre-parkinsonian state (Collier et al., 2017). The drivers of these changes are unknown, but a leading candidate is ageing-related change in  $\alpha$ -synuclein ( $\alpha$ Syn) expression.

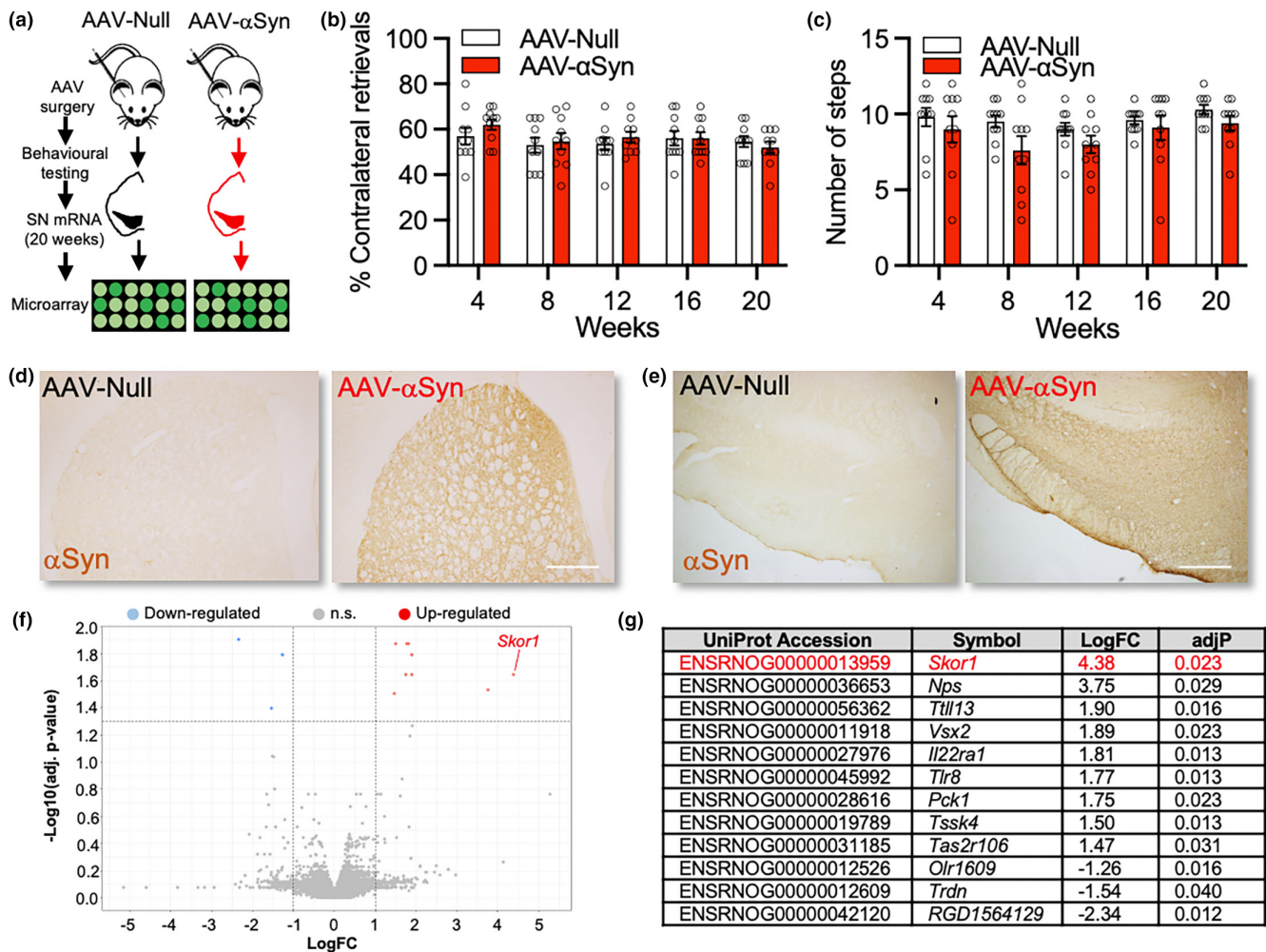
Misfolded  $\alpha$ Syn is a major component of Lewy bodies, the intracellular proteinaceous inclusions that characterise PD pathology (Braak et al., 2003, 1999; Spillantini et al., 1998, 1997). Moreover, mutations (Kruger et al., 1998; Polymeropoulos et al., 1997), or duplications and triplications (Nishioka et al., 2006; Singleton et al., 2003), in the  $\alpha$ Syn gene, SNCA, cause autosomal-dominant PD.  $\alpha$ Syn is a normal synaptic protein that displays a change in intraneuronal localisation as a result of ageing (Chu & Kordower, 2007; Collier et al., 2017). Ageing leads to a prominent increase in  $\alpha$ Syn staining within the soma of SN neurons, which is correlated with reduced expression of phenotypic markers of DA neurons (Chu & Kordower, 2007; Collier et al., 2017). This suggests that age-related increases in intraneuronal  $\alpha$ Syn expression may alter gene expression in the SN, thus affecting molecular processes that are central to maintaining nigrostriatal integrity.

To determine the effects of overexpression of wild-type human  $\alpha$ Syn on the transcriptome of a rat model of nigrostriatal ageing, adult female Sprague–Dawley rats received a unilateral intranigral injection of an adeno-associated viral (AAV) vector carrying the wild-type human  $\alpha$ Syn transgene (AAV- $\alpha$ Syn), or a null control vector (AAV-Null) (Figure 1a). This has been shown to lead to 36% reduction in TH-immunoreactive, and ~30% reduction in DAT-immunoreactive, neurons at 20 weeks post-surgery (Goulding et al., 2021). Although estimates vary, it is thought that the motor symptoms of PD do not manifest until ~60% of SN DA neurons

have been lost (Cheng et al., 2010). In agreement, we found no significant effect of  $\alpha$ Syn in the corridor ( $F_{(1,9)}=0.5606$ ,  $p=0.473$ ) (Figure 1b) or stepping ( $F_{(1,9)}=3.782$ ,  $p=0.084$ ) (Figure 1c) tests of sensorimotor function at 4, 8, 12, 16 and 20 weeks post-surgery. Immunohistochemistry for human wild-type  $\alpha$ Syn on sections of the midbrain and striatum showed that  $\alpha$ Syn was expressed throughout the ipsilateral striatum (Figure 1d) and SN (Figure 1e). To confirm if the increase in  $\alpha$ Syn resulted in reduced expression of phenotypic markers of DA neurons (Chu & Kordower, 2007; Collier et al., 2017), we examined the expression of 84 PD-relevant genes in the ipsilateral SN, using the PD RT<sup>2</sup> Profiler PCR Array. The AAV- $\alpha$ Syn group had significant reductions in the expression of transcripts for genes involved in the dopamine biosynthesis pathway, including *Th*, *Slc6a3* (*Dat1*) and *Ddc* (*dopa decarboxylase*), as well as reductions in *Park7* (Figure S1). This model recapitulates features of an aged nigrostriatal system in that there is increased expression of  $\alpha$ Syn and reduced expression of phenotypic markers of DA neurons, but not to such a degree that leads to motor impairments.

We next investigated global transcriptome changes in the ipsilateral SN. cRNA was prepared from the mRNA extracted from the ipsilateral SN and hybridised to oligonucleotide microarrays. Of the 39,956 sequences represented on the array, 2608 of these were found to be differentially expressed between the AAV-Null and the AAV- $\alpha$ Syn group ( $p < 0.05$ ), before a multiple testing correction was applied (data not shown). This included genes found in the PCR array, including *Th* ( $p=0.0043$ ), *Slc6a3* ( $p=0.0122$ ) and *Ddc* ( $p=0.0198$ ). Of the  $n=2608$  sequences,  $n=21$  remained statistically significant after a multiple testing correcting using the Benjamini–Hochberg method ( $adjp < 0.05$ ) (Benjamini & Hochberg, 1995). These 21 sequences mapped to 12 unique gene identifiers. The gene most upregulated in the  $\alpha$ Syn group was the SKI Family Transcriptional Corepressor 1 (*Skor1*) (Log FC = 4.3821;  $adjp=0.0225$ ) (Figure 1f,g). SKOR1 is highly expressed in the CNS of humans and mice. It plays a regulatory role in the transcription of genes that are involved in pathways related to restless legs syndrome (RLS) (Sarayloo et al., 2020), and variants in SKOR1 have been associated with RLS (Jimenez-Jimenez et al., 2018).

We next hypothesised that if SKOR1 is involved in  $\alpha$ Syn-mediated cellular changes, then SKOR1 overexpression should mimic the effects of  $\alpha$ Syn on cellular function. To test this hypothesis, we used two functional readouts that are known to be negatively affected by  $\alpha$ Syn: mitochondrial function (Di Maio et al., 2016; McCarthy et al., 2022) and neurite growth (Goulding et al., 2019; Mazzocchi et al., 2020). To investigate mitochondrial function in SH-SY5Y cells expressing FLAG or SKOR1, we analysed cellular bioenergetic state using the Agilent Seahorse XF platform. Like that seen in cells overexpressing  $\alpha$ Syn (Di Maio et al., 2016; McCarthy et al., 2022), a consistent reduction in OCR was observed in SKOR1-expressing



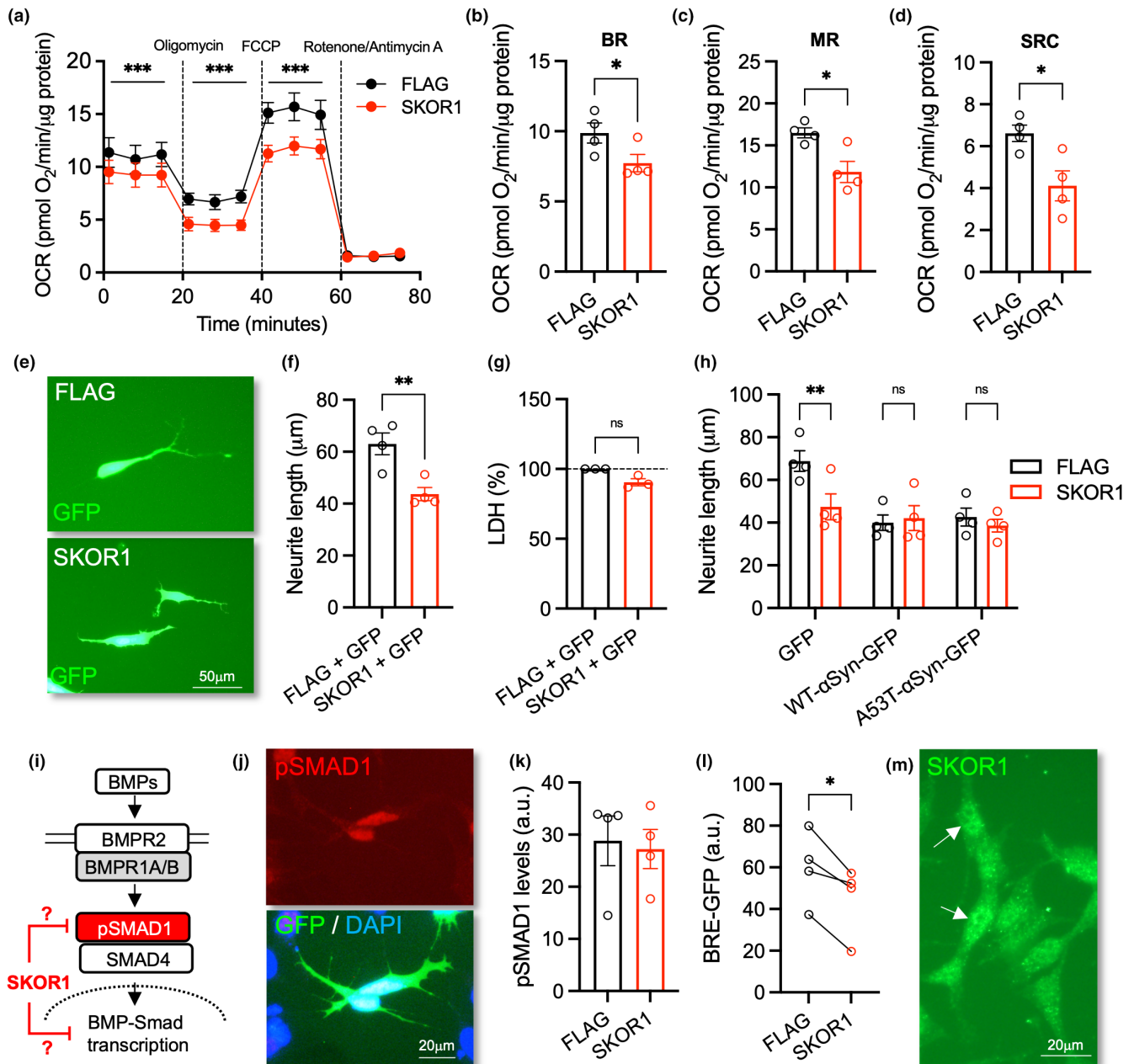
**FIGURE 1** Overexpression of wild-type human  $\alpha$ -synuclein in the rat *substantia nigra* upregulates *Skor1*. (a) Schema of the experimental design. (b, c) Graphs showing the results of (b) the corridor test and (c) the stepping test at 4–20 weeks post-surgery. (d, e) Representative images showing wild-type human  $\alpha$ -synuclein ( $\alpha$ Syn) immunostaining in (d) striatum and (e) SN at 20 weeks after unilateral stereotaxic injection of either an AAV-Null or AAV- $\alpha$ Syn vector into the SN. Scale bars = 40  $\mu$ m. (f) Volcano plot (adjusted  $p$ -value vs. log fold-change) and (g) list of the genes overexpressed and under-expressed (following a Benjamini–Hochberg multiple-testing correction) in the SN, in the AAV- $\alpha$ Syn compared to the AAV-Null group at 20 weeks after unilateral stereotaxic injection of either an AAV-Null or AAV- $\alpha$ Syn vector into the SN. Microarray analysis was performed on  $n=3$  per group. *Skor1* was the top-ranked overexpressed gene in the AAV- $\alpha$ Syn group (logFC = 4.38, Benjamini–Hochberg adjusted  $p=0.023$ ). n.s., not significant.

cells, relative to the FLAG controls (Figure 2a). We also examined individual parameters of respiration and found significant reductions in several parameters in the SKOR1 group, including maximal respiration (Figure 2b–d). Maximal respiration induced by the addition of FCCP mimics a physiological energy demand by stimulating mitochondria to operate at maximum capacity (Brand & Nicholls, 2011). These data show that SKOR1 overexpression reduces the maximum rate of respiration that a cell can achieve.

We next used neurite outgrowth as a second functional readout to determine if SKOR overexpression mimics the inhibitory effect of  $\alpha$ Syn on neurite growth, which has previously been shown in SH-SY5Y cells (Goulding et al., 2019; Mazzocchi et al., 2020), rodent neurons overexpressing  $\alpha$ Syn (Koch et al., 2015), and human iPSC-derived DA neurons carrying *SNCA* mutations, duplications or triplications (Kouroupi et al., 2017; Oliveira et al., 2015). We found

that SKOR1 overexpression impaired neurite growth in SH-SY5Y cells expressing FLAG or SKOR1 (Figure 2e,f), but did not adversely affect cell viability (Figure 2g). We repeated this experiment with or without co-transfection with plasmids expressing GFP-tagged WT or A53T- $\alpha$ Syn. SKOR1 had the same inhibitory effect on neurite growth in cells co-transfected with WT- $\alpha$ Syn or A53T- $\alpha$ Syn, and did not exacerbate reductions in neurite growth induced by either (Figure 2h). This is consistent with SKOR1 being a downstream mediator of the detrimental effects of  $\alpha$ Syn on neurite growth.

We next investigated the cellular mechanisms underpinning the effects of SKOR1 overexpression on neurite growth. Interestingly, SKOR1 has been reported to be a negative regulator of the BMP-Smad pathway in HEK-293 T cells (Fischer et al., 2012) (Figure 2i). Two members of the bone morphogenetic protein family, growth differentiation factor 5 (GDF5; also known as BMP14) (Goulding et al., 2021)



**FIGURE 2** Overexpression of SKOR1 impairs neurite growth, mitochondrial function and BMP-SMAD signalling in SH-SY5Y cells. (a) Oxygen consumption rate (OCR) on the Seahorse XF Mito Stress Test in SH-SY5Y cells at 72 h post-transfection with 500 ng of a plasmid expressing either FLAG or FLAG-tagged SKOR1. (b–d) The graphs of individual parameters of respiration showing (b) basal respiration (BR), (c) maximal respiration (MR) and (d) spare respiratory capacity (SRC). Data are mean ± SEM of OCR values normalised to protein content per well from *n* = 4 independent experiments. Mixed effects model with either (a) post-hoc Bonferroni test (\*\**p* < 0.001), or (b–d) Student's *t*-test (\**p* < 0.05, \*\*\**p* < 0.001). (e) Representative photomicrographs and (f) the graphs of neurite length and (g) LDH assay of SH-SY5Y cells at 72 h post-transfection with 500 ng of a plasmid expressing either FLAG or FLAG-tagged SKOR1 plus GFP to visualise transfected cells. (h) The graph of neurite length of SH-SY5Y cells at 72 h post-transfection with 500 ng of a plasmid expressing either FLAG or FLAG-tagged SKOR1 with either GFP or GFP-tagged wild-type α-synuclein (WT-αSyn) or GFP-tagged A53T-αSyn. (e–h) Data are mean ± SEM from *n* = 4 independent experiments. Student's *t*-test. (\*\**p* < 0.01). (i) Schema of the BMP-Smad signalling pathway. (j) Representative photomicrographs showing pSMAD1 staining, and graphs showing (k) pSMAD1 levels and (l) GFP fluorescence in SH-SY5Y cells at 72 h post-transfection with 500 ng of a plasmid expressing either FLAG or FLAG-tagged SKOR1 plus (k) GFP plasmid or (l) with a reporter plasmid expressing GFP under the control BMP-responsive element (BRE). (m) Immunocytochemistry showing the cellular localisation of SKOR1 (green) in SH-SY5Y cells. Scale bar = 20 μm. Data are mean ± SEM from *n* = 4 independent experiments. (k, l) Paired *t*-test (\**p* < 0.05).



and BMP5/7 (Vitic et al., 2021), have been shown to prevent  $\alpha$ Syn-induced DA degeneration in vivo. Moreover, activation of this pathway promotes neurite growth in SH-SY5Y cells (Hegarty et al., 2013) and rodent DA neurons (Hegarty et al., 2014). To investigate this further, we examined cellular levels of phospho-SMAD1 and found that, while SKOR1 overexpression did not affect these (Figure 2i,j), it significantly inhibited BMP-dependent transcription in SH-SY5Y cells transfected with a BMP-responsive element (BRE)-GFP reporter construct. The effect of SKOR1 on BMP-SMAD-dependent transcription is consistent with the predominantly-nuclear localisation of SKOR1 in SH-SY5Y cells (Figure 2m). Since BMP-Smad signalling has also been shown to enhance mitochondrial activity (Li et al., 2022), it is possible that SKOR1-induced impairments in the BMP-SMAD pathway (Figure 2m) may contribute to SKOR1-induced alterations in both mitochondrial function and neurite growth.

It is interesting to note in a wider context that SMAD1 was one of three transcription factors found to be downregulated in blood samples from PD patients (Tan et al., 2018), while there was increased expression of the BMP inhibitory SMAD6 in the PD SN (Vitic et al., 2021). Furthermore, a gene ontology analysis of transcriptome from human SN reported alterations in the BMP-SMAD pathway in Braak stage 1–2 PD subjects in comparison to controls (Dijkstra et al., 2015). It is worth noting that, while known genetic variants in SKOR1 are associated with RLS (Jimenez-Jimenez et al., 2018), these variants are not linked to PD risk, although one marker, rs12593813, in SKOR1 has been associated with a high frequency of tremor in PD patients (Gan-Or et al., 2015). This suggests a possible effect of SKOR1 on the motor system, but this requires further investigation.

In future work, it will be important to confirm these findings in males, as only female rats were used in the current study for logistic reasons. It will also be crucial to validate our findings at the protein level, and to determine the precise role, if any, of SKOR1 in DA degeneration in vivo. Additionally it is difficult to extrapolate the short-term overexpression of SKOR1 in vitro to the in vivo scenario; however, these data suggest a model in which age-related increases in  $\alpha$ Syn expression in DA neurons in the SN (Chu & Kordower, 2007; Collier et al., 2017) lead to increased cellular levels of SKOR1, which inhibit the BMP-SMAD signalling pathway during normal ageing. Given the natural variation in DA neuron number in healthy humans (von Linstow et al., 2020), and the age-related 7% per decade loss of these cells in the human SN (Stark & Pakkenberg, 2004), it is possible that the extent to which  $\alpha$ Syn upregulates SKOR1 expression may influence the process of normal nigrostriatal ageing, perhaps through the modulation of mitochondrial function and axonal integrity, thereby exacerbating an individual's risk of developing PD due to age.

#### AUTHOR CONTRIBUTIONS

NMP and CGD generated, analysed and curated the transcriptome data. RB, AOM performed the in vitro experiments. AB analysed mitochondrial function. NMP and EM performed the PCR array. RMC and SRG performed the surgery and behavioural testing. CMM,

LMC, AMS and GOK supervised the work. NMP, AMS and GOK wrote the manuscript. All authors contributed to reviewing and editing of the final manuscript.

#### ACKNOWLEDGEMENTS

We thank the staff of the Central Service for Research Support (SCAI) at the University of Cordoba for technical support with the microarrays analyses. Open access funding provided by IReL.

#### FUNDING INFORMATION

Work in the authors laboratories is supported by Science Foundation Ireland (Grant 19/FFP/6666), Cure Parkinson's (Grant CP:GO01) and the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie grant agreement No 890290.

#### CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

#### DATA AVAILABILITY STATEMENT

All data are either publicly available (GSE252918) or are available from the corresponding authors upon reasonable request.

#### ORCID

Aideen M. Sullivan  <https://orcid.org/0000-0001-9692-6438>

Gerard W. O'Keefe  <https://orcid.org/0000-0001-5149-0933>

#### REFERENCES

- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Statistical Methodology)*, 57, 289–300.
- Bloem, B. R., Okun, M. S., & Klein, C. (2021). Parkinson's disease. *Lancet*, 397, 2284–2303.
- Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, 24, 197–211.
- Braak, H., Sandmann-Keil, D., Gai, W., & Braak, E. (1999). Extensive axonal Lewy neurites in Parkinson's disease: A novel pathological feature revealed by alpha-synuclein immunocytochemistry. *Neuroscience Letters*, 265, 67–69.
- Brand, M. D., & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *The Biochemical Journal*, 435, 297–312.
- Cheng, H. C., Ulane, C. M., & Burke, R. E. (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Annals of Neurology*, 67, 715–725.
- Chu, Y., & Kordower, J. H. (2007). Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: Is this the target for Parkinson's disease? *Neurobiology of Disease*, 25, 134–149.
- Collier, T. J., Kanaan, N. M., & Kordower, J. H. (2017). Aging and Parkinson's disease: Different sides of the same coin? *Movement Disorders*, 32, 983–990.
- Di Maio, R., Barrett, P. J., Hoffman, E. K., Barrett, C. W., Zharikov, A., Borah, A., Hu, X., McCoy, J., Chu, C. T., Burton, E. A., Hastings, T. G., & Greenamyre, J. T. (2016).  $\alpha$ -Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. *Science Translational Medicine*, 8, 342ra378.



- Dijkstra, A. A., Ingrassia, A., de Menezes, R. X., van Kesteren, R. E., Rozemuller, A. J., Heutink, P., & van de Berg, W. D. (2015). Evidence for immune response, axonal dysfunction and reduced endocytosis in the substantia nigra in early stage Parkinson's disease. *PLoS One*, 10, e0128651.
- Dorsey, E. R., Sherer, T., Okun, M. S., & Bloem, B. R. (2018). The emerging evidence of the Parkinson pandemic. *Journal of Parkinson's Disease*, 8, S3-S8.
- Fischer, S., Bayersdorfer, F., Harant, E., Reng, R., Arndt, S., Bosserhoff, A. K., & Schneuwly, S. (2012). fussel (fuss)—a negative regulator of BMP signaling in *Drosophila melanogaster*. *PLoS One*, 7, e42349.
- Gan-Or, Z., Alcalay, R. N., Bar-Shira, A., Leblond, C. S., Postuma, R. B., Ben-Shachar, S., Waters, C., Johnson, A., Levy, O., Mirelman, A., Gana-Weisz, M., Dupre, N., Montplaisir, J., Giladi, N., Fahn, S., Xiong, L., Dion, P. A., Orr-Urtreger, A., & Rouleau, G. A. (2015). Genetic markers of restless legs syndrome in Parkinson disease. *Parkinsonism & Related Disorders*, 21, 582-585.
- GBD 2015 Neurological Disorders Collaborator Group. (2017). Global, regional, and national burden of neurological disorders during 1990-2015: A systematic analysis for the global burden of disease study 2015. *The Lancet. Neurology*, 16, 877-897.
- Goulding, S. R., Concannon, R. M., Morales-Prieto, N., Villalobos-Manriquez, F., Clarke, G., Collins, L. M., Levesque, M., Wyatt, S. L., Sullivan, A. M., & O'Keefe, G. W. (2021). Growth differentiation factor 5 exerts neuroprotection in an  $\alpha$ -synuclein rat model of Parkinson's disease. *Brain*, 144, e14.
- Goulding, S. R., Sullivan, A. M., O'Keefe, G. W., & Collins, L. M. (2019). Gene co-expression analysis of the human substantia nigra identifies BMP2 as a neurotrophic factor that can promote neurite growth in cells overexpressing wild-type or A53T  $\alpha$ -synuclein. *Parkinsonism & Related Disorders*, 64, 194-201.
- Hegarty, S. V., Collins, L. M., Gavin, A. M., Roche, S. L., Wyatt, S. L., Sullivan, A. M., & O'Keefe, G. W. (2014). Canonical BMP-Smad signalling promotes neurite growth in rat midbrain dopaminergic neurons. *Neuromolecular Medicine*, 16, 473-489.
- Hegarty, S. V., Sullivan, A. M., & O'Keefe, G. W. (2013). BMP2 and GDF5 induce neuronal differentiation through a Smad dependant pathway in a model of human midbrain dopaminergic neurons. *Molecular and Cellular Neurosciences*, 56, 263-271.
- Jimenez-Jimenez, F. J., Alonso-Navarro, H., Garcia-Martin, E., & Agundez, J. A. G. (2018). Genetics of restless legs syndrome: An update. *Sleep Medicine Reviews*, 39, 108-121.
- Koch, J. C., Bitow, F., Haack, J., d'Hedouville, Z., Zhang, J. N., Tonges, L., Michel, U., Oliveira, L. M., Jovin, T. M., Liman, J., Tatenhorst, L., Bahr, M., & Lingor, P. (2015). Alpha-synuclein affects neurite morphology, autophagy, vesicle transport and axonal degeneration in CNS neurons. *Cell Death & Disease*, 6, e1811.
- Kordower, J. H., Olanow, C. W., Dodiya, H. B., Chu, Y., Beach, T. G., Adler, C. H., Halliday, G. M., & Bartus, R. T. (2013). Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain*, 136, 2419-2431.
- Kouroupi, G., Taoufik, E., Vlachos, I. S., Tsiaras, K., Antoniou, N., Papastefanaki, F., Chroni-Tzartou, D., Wrasidlo, W., Bohl, D., Stellas, D., Politis, P. K., Vekrellis, K., Papadimitriou, D., Stefanis, L., Bregestovski, P., Hatzigeorgiou, A. G., Masliah, E., & Matsas, R. (2017). Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E3679-E3688.
- Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., & Riess, O. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics*, 18, 106-108.
- Li, Y., Fu, G., Gong, Y., Li, B., Li, W., Liu, D., & Yang, X. (2022). BMP-2 promotes osteogenic differentiation of mesenchymal stem cells by enhancing mitochondrial activity. *Journal of Musculoskeletal & Neuronal Interactions*, 22, 123-131.
- Mazzocchi, M., Collins, L. M., Sullivan, A. M., & O'Keefe, G. W. (2020). The class II histone deacetylases as therapeutic targets for Parkinson's disease. *Neuronal Signaling*, 4, NS20200001.
- McCarthy, E., Barron, A., Morales-Prieto, N., Mazzocchi, M., McCarthy, C. M., Collins, L. M., Sullivan, A. M., & O'Keefe, G. W. (2022). Gene Co-expression analysis of the human substantia nigra identifies ZNHIT1 as an SNCA Co-expressed gene that protects against alpha-Synuclein-induced impairments in neurite growth and mitochondrial dysfunction in SH-SY5Y cells. *Molecular Neurobiology*, 59, 2745-2757.
- Nishioka, K., Hayashi, S., Farrer, M. J., Singleton, A. B., Yoshino, H., Imai, H., Kitami, T., Sato, K., Kuroda, R., Tomiyama, H., Mizoguchi, K., Murata, M., Toda, T., Imoto, I., Inazawa, J., Mizuno, Y., & Hattori, N. (2006). Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. *Annals of Neurology*, 59, 298-309.
- Oliveira, L. M., Falomir-Lockhart, L. J., Botelho, M. G., Lin, K. H., Wales, P., Koch, J. C., Gerhardt, E., Taschenberger, H., Outeiro, T. F., Lingor, P., Schule, B., Arndt-Jovin, D. J., & Jovin, T. M. (2015). Elevated  $\alpha$ -synuclein caused by SNCA gene triplication impairs neuronal differentiation and maturation in Parkinson's patient-derived induced pluripotent stem cells. *Cell Death & Disease*, 6, e1994.
- Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*, 276, 2045-2047.
- Sarayloo, F., Spiegelman, D., Rochefort, D., Akcimen, F., De Barros Oliveira, R., Dion, P. A., & Rouleau, G. A. (2020). SKOR1 has a transcriptional regulatory role on genes involved in pathways related to restless legs syndrome. *European Journal of Human Genetics*, 28, 1520-1528.
- Seo, J. P., & Koo, D. K. (2021). Aging of the Nigrostriatal tract in the human brain: A diffusion tensor imaging study. *Medicina (Kaunas, Lithuania)*, 57, 994.
- Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muentner, M., Baptista, M., Miller, D., ... Gwinn-Hardy, K. (2003). alpha-Synuclein locus triplication causes Parkinson's disease. *Science*, 302, 841.
- Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., & Goedert, M. (1998). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 6469-6473.
- Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. *Nature*, 388, 839-840.
- Stark, A. K., & Pakkenberg, B. (2004). Histological changes of the dopaminergic nigrostriatal system in aging. *Cell and Tissue Research*, 318, 81-92.
- Tan, C., Liu, X., & Chen, J. (2018). Microarray analysis of the molecular mechanism involved in Parkinson's disease. *Parkinson's Disease*, 2018, 1590465.
- Vitic, Z., Safory, H., Jovanovic, V. M., Sarusi, Y., Stavsky, A., Kahn, J., Kuzmina, A., Toker, L., Gitler, D., Taube, R., Friedel, R. H., Engelder, S., & Brodski, C. (2021). BMP5/7 protect dopaminergic



neurons in an  $\alpha$ -synuclein mouse model of Parkinson's disease. *Brain*, 144, e15.

von Linstow, C. U., DeLano-Taylor, M., Kordower, J. H., & Brundin, P. (2020). Does developmental variability in the number of midbrain dopamine neurons affect individual risk for sporadic Parkinson's disease? *Journal of Parkinson's Disease*, 10, 405–411.

#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Morales-Prieto, N., Bevans, R., O'Mahony, A., Barron, A., Giles Doran, C., McCarthy, E., Concannon, R. M., Goulding, S. R., McCarthy, C. M., Collins, L. M., Sullivan, A. M., & O'Keefe, G. W. (2024). Human  $\alpha$ -synuclein overexpression upregulates SKOR1 in a rat model of simulated nigrostriatal ageing. *Aging Cell*, 23, e14155. <https://doi.org/10.1111/accel.14155>