

**A common polymorphism in *SNCA* is associated with accelerated motor decline in *GBA*-
Parkinson's disease**

**TB Stoker^{1,2}, M Camacho¹, S Winder-Rhodes¹, G Liu^{3,4,5}, CR Scherzer^{4,5}, T Foltynie⁶, RA
Barker^{1,2}, CH Williams-Gray¹**

1. John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Forvie Site, Robinson Way, Cambridge CB2 0PY, UK
2. Wellcome Trust - Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge, UK
3. School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China
4. Precision Neurology Program, Harvard Medical School, Brigham & Women's Hospital, Boston, USA
5. APDA Center for Advanced Parkinson Research, Harvard Medical School, Brigham & Women's Hospital, Boston, USA
6. Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, Queen Sq, London. WC1N 3BG.

***Author for correspondence**

Thomas Stoker

John van Geest Centre for Brain Repair, E.D. Adrian Building, Forvie Site, Robinson Way, Cambridge
CB2 0PY, UK

01223 331160

tbs26@cam.ac.uk

Word count: 1432

Key words: α -synuclein, *GBA*, glucocerebrosidase, Parkinson's disease, *SNCA*

A growing number of genetic susceptibility factors have been identified for Parkinson's disease (PD). The combination of inherited risk variants is likely to affect not only risk of developing PD but also its clinical course. Variants in the *GBA* gene are particularly common, being found in approximately 5 to 10% of patients, and they lead to more rapid disease progression¹. However, the effect of concomitant genetic risk factors on disease course in *GBA*-PD is not known.

The aggregation of α -synuclein, encoded by the *SNCA* gene, is central to the pathogenesis of PD. The *SNCA* rs356219 A/G polymorphism alters the risk of developing PD, with homozygotes for guanine (G/G) having an increased risk compared to carriers of an adenine (G/A or A/A) at this locus². The relationship between glucocerebrosidase (the enzyme encoded by the *GBA* gene) and α -synuclein is complex. These proteins have been shown to interact directly *in vitro*, as well as to influence the intracellular levels and processing of each other, potentially in a bidirectional feedback loop^{3 4}. Interestingly, a recent genome-wide association study found that the presence of this *SNCA* polymorphism was associated with an increased likelihood of developing PD in *GBA* mutation-carriers⁵. We therefore hypothesised that the presence of the *SNCA* rs356219 polymorphism would accelerate the clinical course of *GBA* variant-associated PD. Here we report on the effect of this *SNCA* polymorphism on clinical outcomes within the *GBA*-PD population.

Longitudinal data from *GBA*-variant carriers was analysed from the community-based "Cambridgeshire Incidence of Parkinson's disease from General Practice to Neurologist" (CamPaIGN) cohort (n=142)⁶. This study was approved by the local ethics committee and written informed consent was obtained from all subjects. Newly diagnosed patients were followed up with assessments every 2 years for up to 18 years. Time to development of dementia (defined as Mini-Mental State Examination (MMSE) score of 24 or less, with fulfilment of Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria), progression to postural instability (Hoehn and Yahr stage three (HY3)), and death were determined.

Sequencing of the *GBA* gene was carried out in 114 patients in the CamPaIGN cohort, as described here⁷. A further 16 patients underwent targeted genetic screening for common *GBA* variants using the Illumina Multi-Ethnic Genotyping Array (MEGA) chip. Genetic analysis of the *SNCA* rs356219

polymorphism had also been previously performed in 124 patients from the cohort². *GBA* variants were identified in 20 patients, which were included in survival analyses. Of these, 7 carried the high-risk (G/G) *SNCA* genotype, with 13 carrying low-risk (G/A or A/A) genotypes. There were no differences in age at diagnosis nor in baseline assessments including total and part III Unified Parkinson's Disease Rating Scale scores, Hoehn and Yahr scores, MMSE scores, or levodopa equivalent doses between these *SNCA* genotype groups. All carriers of the G/G genotype were male, compared to 53.8% in the low-risk group ($p=0.03$) (supplementary table 1).

Survival analyses for the aforementioned outcome measures were performed, with statistical significance determined using log-rank tests. P-values were adjusted for multiple comparisons using the Bonferroni method. Amongst carriers of *GBA* variants, there were no differences between the high- and low-risk *SNCA* groups for time to dementia ($p=0.29$, adjusted $p=0.86$) or death ($p=0.43$, adjusted $p=1.28$) (figure 1A and B). Progression to HY3 however, was significantly faster in the G/G *SNCA* group ($p=0.02$, adjusted $p=0.07$), with mean time to development of postural instability 2.0 years (95% confidence interval 1.3-2.7) compared to 4.9 years (95% confidence interval 2.5-7.3) in A carriers (figure 1C). All G/G carriers reached HY3 within three years of diagnosis.

To account for potential confounders, time to HY3 was also compared using a Cox regression model controlling for sex and age at diagnosis. The G/G *SNCA* genotype was associated with a hazard ratio for progression to HY3 of 3.8 (95% confidence interval 0.9-16.1) $p=0.07$, adjusted $p=0.21$) when controlling for these confounders. Because all of the *SNCA* G/G carriers were male, we also performed a survival analysis in which only male subjects were included, to further control for sex as a potential confounder. An increased risk of progression to HY3 was again observed in G/G carriers in comparison to A carriers ($p=0.036$, adjusted $p=0.11$; supplementary figure 1). *SNCA* genotype had no effect on progression to HY3 in non-carriers of *GBA* variants ($n=85$) in the CamPaIGN cohort ($p=0.88$, adjusted $p=2.65$; supplementary figure 2).

This cohort contained four patients with the non-coding c. 762 – 18 T>A variant (supplementary table 2). Whilst this has been reported to be a potential risk factor for PD⁸, its importance is not clear. We therefore also performed analysis after exclusion of these patients, and found that progression to HY3

was greater in the G/G carrier group, with a hazard ratio of 5.3 (95% confidence interval 1.1-26.2; $p=0.041$, adjusted $p=0.12$).

To our knowledge, no previous studies have investigated the influence of concomitant genetic risk factors on the progression of *GBA*-PD. Here we report that the *SNCA* rs356219 polymorphism significantly affects motor progression in *GBA*-PD, with the G/G genotype associated with a particularly aggressive disease course. This effect was not observed in PD patients without *GBA* variants, suggesting that it was relatively specific to *GBA*-PD.

This new study therefore raises the interesting possibility that *GBA* variants and the G/G *SNCA* rs356219 polymorphism act synergistically to accelerate pathology and clinical progression in PD. *GBA* mutations are thought to increase the risk of PD predominantly through perturbations in the lysosome-autophagy system – a system important in α -synuclein clearance. Furthermore, glucocerebrosidase and α -synuclein have been shown to interact directly *in vitro*, and to co-exist in Lewy bodies of PD patients, with a greater proportion of Lewy bodies containing glucocerebrosidase in patients with *GBA* mutations compared to those in PD patients not carrying a *GBA* variant^{4,9}. It has been speculated that mutant GCase potentiates the aggregation of α -synuclein, and it is therefore feasible that *SNCA* variants such as the rs356219 polymorphism alter the degree to which *GBA* variants predispose to PD pathology and disease progression. It has also been suggested that α -synuclein impedes the transit of glucocerebrosidase from the endoplasmic reticulum to the lysosome, further supporting the idea that the relationship between these two proteins is directly important in the pathogenesis of *GBA*-PD³. The proposed synergistic interaction between variants in the *GBA* gene and *SNCA* gene therefore could also potentially be explained by differences in the degree to which α -synuclein impairs normal GCase processing associated with specific *SNCA* variants.

We acknowledge that our sample size is small, but these preliminary observations raise the possibility that *GBA* variants and the G/G *SNCA* rs356219 polymorphism synergistically alter motor progression in PD. In this study we have considered the time from diagnosis to the development of important clinical milestones in PD. The time to diagnosis may vary between patients, so it should be recognised that this does not necessarily reflect disease duration. Time from disease onset to the development of clinical

milestones may be more representative of disease duration but onset is very difficult to ascertain accurately, given that many patients experience prodromal symptoms that are not initially attributed to PD.

There is a degree of genotype-phenotype correlation in *GBA*-PD, with severe mutations accelerating disease course to a greater extent than less severe variants¹⁰. However, because our sample size was small, it was not possible to stratify the *GBA*-PD patients into those with non-severe and severe *GBA* variants, so our *GBA*-PD population was genetically heterogeneous (supplementary table 2). Mean age at diagnosis was approximately five years later in G/G carriers which may potentially have contributed to their accelerated disease course. However, there were no differences in time to dementia or death, and when age at diagnosis was accounted for in the Cox regression model, the accelerated progression to HY3 persisted. Another limitation of our study is that all subjects in the G/G group were male, and though this was accounted for in the Cox regression model, it would be important to investigate the relationship between these two genetic risk factors in a large cohort, to reduce the effect of such confounders, and to allow for the stratification of G/G carriers into those with severe and those with mild *GBA* variants.

Competing interests

The authors declare no competing interests.

Funding statement

The CamPaIGN study has received financial support from the Wellcome Trust, the Medical Research Council, Parkinson's UK and the Patrick Berthoud Trust. CHWG is supported by an RCUK/UKRI Innovation Fellowship awarded by the Medical Research Council. RAB is supported by the Wellcome Trust Stem Cell Institute (Cambridge). TBS received financial support from the Cure Parkinson's Trust. The study is also supported by the National Institute for Health Research (NIHR) Cambridge

Biomedical Research Centre Dementia and Neurodegeneration Theme (reference number 146281).

The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. CRS' work is supported in part by NIH grants R01AG057331, U01NS100603, R01AG057331, and the American Parkinson Disease Association. Illumina MEGA Chip genotyping was made possible by a philanthropic investment from Dooley LLC (to Brigham & Women's Hospital and CRS).

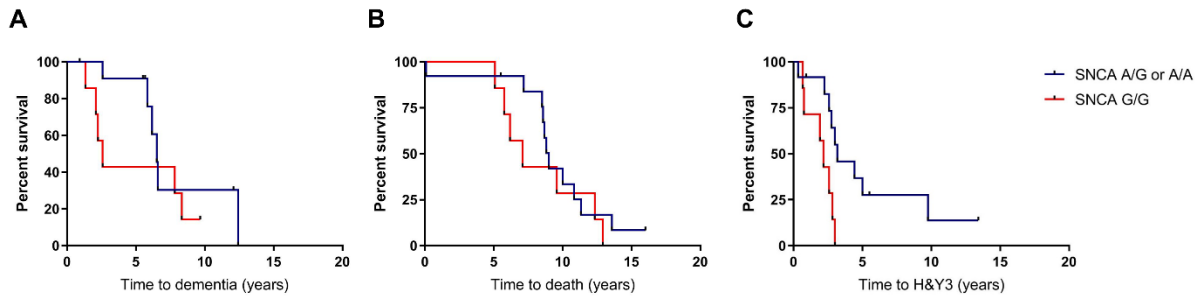


Figure 1 – Survival analyses in GBA1-PD patients comparing those with high- and low-risk variants in the SNCA rs356219 polymorphism. Kaplan-Meier curves for time to dementia (A), death (B) and postural instability (C). Abbreviations: H&Y3 = Hoehn and Yahr stage three

References

1. Cilia R, Tunesi S, Marotta G, et al. Survival and dementia in GBA-associated Parkinson's disease: The mutation matters. *Ann Neurol* 2016;80(5):662-73. doi: 10.1002/ana.24777
2. Goris A, Williams-Gray CH, Clark GR, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Annals of neurology* 2007;62(2):145-53.
3. Mazzulli JR, Xu YH, Sun Y, et al. Gaucher disease glucocerebrosidase and α -synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* 2011;146(1):37-52. doi: 10.1016/j.cell.2011.06.001
4. Yap TL, Velayati A, Sidransky E, et al. Membrane-bound α -synuclein interacts with glucocerebrosidase and inhibits enzyme activity. *Mol Genet Metab* 2013;108(1):56-64. doi: 10.1016/j.ymgme.2012.11.010
5. Blauwendraat C, Reed X, Krohn L, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. *Brain* 2019 doi: 10.1093/brain/awz350 [published Online First: 2019/11/22]
6. Williams-Gray CH, Mason SL, Evans JR, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *Journal of neurology, neurosurgery, and psychiatry* 2013;84(11):1258-64. doi: 10.1136/jnnp-2013-305277
7. Winder-Rhodes SE, Evans JR, Ban M, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. *Brain : a journal of neurology* 2013;136(Pt 2):392-9. doi: 10.1093/brain/aws318
8. Moors TE, Paciotti S, Ingrassia A, et al. Characterization of Brain Lysosomal Activities in GBA-Related and Sporadic Parkinson's Disease and Dementia with Lewy Bodies. *Mol Neurobiol* 2019 Feb;56(2):1344-1355.
9. Goker-Alpan O, Stubblefield BK, Giasson BI, et al. Glucocerebrosidase is present in α -synuclein inclusions in Lewy body disorders. *Acta Neuropathol* 2010;120(5):641-9. doi: 10.1007/s00401-010-0741-7
10. Liu G, Boot B, Locascio JJ, et al. Neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann Neurol* 2016 doi: 10.1002/ana.24781

Supplementary Information

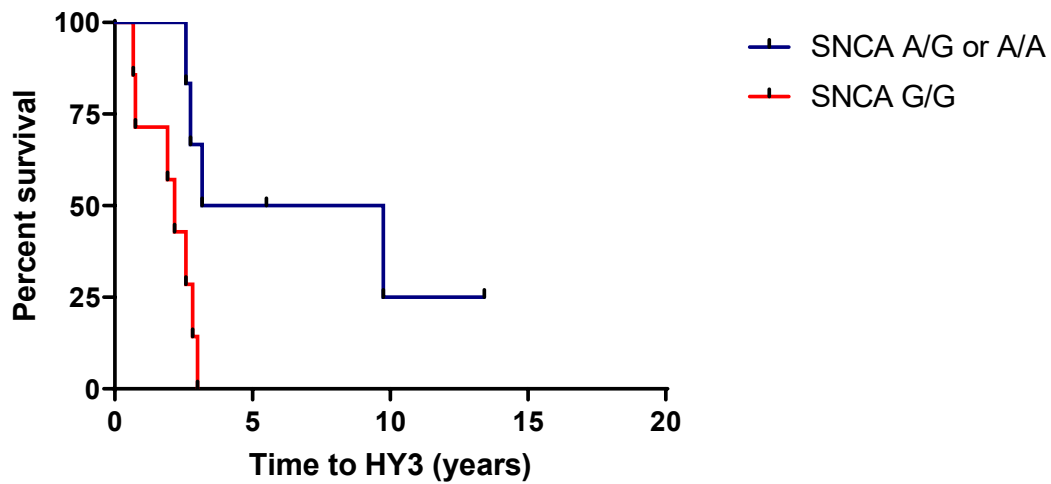
	G/G	G/A and A/A	P-value
Number	7	13	-
Gender M:F	7:0	7:6	0.032
Age at diagnosis (SD)	72.4 years (10.3)	75.5 years (9.3)	0.297
Years in education (SD)	9.7 years (1.1)	10.6 years (2.8)	0.428
Total UPDRS at baseline (SD)	48.8 (15.7)	44.1 (14.0)	0.5
Motor UPDRS at baseline (SD)	21.9 (13.0)	30.8 (9.9)	0.825
Hoehn & Yahr stage at baseline (SD)	2.1 (0.6)	2.3 (0.6)	0.445
MMSE at baseline (SD)	27.6 (1.5)	27.2 (1.9)	0.624
BDI at baseline (SD)	7.9 (5.5)	7.4 (5.6)	0.858
Baseline levodopa equivalent dose (SD)	188.6 mg (201.0)	185.4mg (213.3)	0.974

Supplementary Table 1 – Patient demographics.

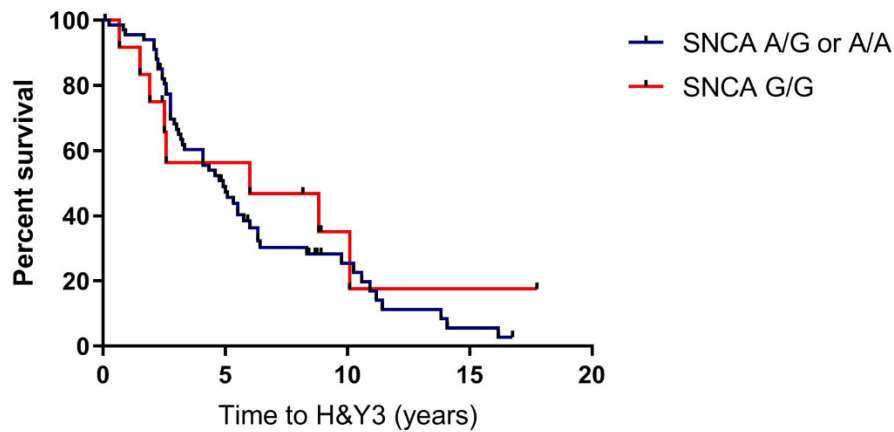
Patient Number	<i>GBA</i> Variant	<i>SNCA</i> Genotype
1	E326K	G/G
2	T369M	G/G
3	N370S*	G/G
4	c. 762 - 18 T>A	G/G
5	L444P*	G/G
6	T369M	G/G
7	c. 762 - 18 T>A	G/G
8	T369M	A/G
9	c. 762 - 18 T>A	A/G
10	E326K	A/G
11	T369M	A/G
12	E326K	A/G
13	T369M	A/G
14	R257Q*	A/G
15	E326K	A/A
16	R48W*	A/A
17	L119L	A/A
18	c. 762 - 18 T>A	A/A
19	E326K	A/A
20	N462K*	A/A

Supplementary Table 2 – *GBA* Variants and *SNCA* rs356219 carrier status in all subjects.

Asterixes indicate variants previously associated with Gaucher disease.



Supplementary figure 1 – Survival analyses in *GBA1*-PD patients comparing those with high- and low-risk variants in the *SNCA* rs356219 polymorphism. Kaplan-Meier curves for time to postural instability. To control for sex as a potential confounder, female patients were excluded, as all subjects in the *SNCA* G/G group were male. Abbreviations: HY3 = Hoehn and Yahr stage three.



Supplementary Figure 2 – Time to postural instability in PD patients without *GBA* variants, comparing those with high- and low-risk variants in the *SNCA* rs356219 polymorphism. Kaplan-Meier curve for time to postural instability in patients, comparing those carrying the G/G *SNCA* rs356219 variant to A carriers at this locus. Abbreviations: H&Y3 = Hoehn and Yahr stage three.