Serotonergic pathology and disease burden in the premotor and motor phase of A53T α-synuclein parkinsonism: a cross-sectional study

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Summary

Background Because of the highly penetrant gene mutation and clinical features consistent with idiopathic Parkinson's disease, carriers of the autosomal dominant Ala53Thr (A53T; 209G→A) point mutation in the α-synuclein (*SNCA*) gene are an ideal population to study the premotor phase and evolution of Parkinson's pathology. Given the known neurochemical changes in the serotonergic system and their association with symptoms of Parkinson's disease, we hypothesised that carriers of the A53T *SNCA* mutation might show abnormalities in the serotonergic neurotransmitter system before the diagnosis of Parkinson's disease, and that this pathology might be associated with measures of Parkinson's burden.

Methods In this cross-sectional study, we recruited carriers of the A53T *SNCA* mutation from specialist Movement Disorders clinics in Athens, Greece, and Salerno, Italy, and a cohort of healthy controls with no personal or family history of neurological or psychiatric disorders from London, UK (recruited via public advertisement) who were age matched to the A53T *SNCA* carriers. We also recruited one cohort of patients with idiopathic Parkinson's disease (cohort 1) from Movement Disorders clinics in London, UK, and retrieved data on a second cohort of such patients (cohort 2; n=40) who had been scanned with a different scanner. 7-day continuous recording of motor function was used to determine the Parkinson's disease status of the A53T carriers. To assess whether serotonergic abnormalities were present, we used [^{11}C]DASB PET non-displaceable binding to quantify serotonin transporter density. We constructed brain topographic maps reflecting Braak stages 1–6 and used these as seed maps to calculate [^{11}C]DASB non-displaceable binding potential in our cohort of A53T *SNCA* carriers. Additionally, all participants underwent a battery of clinical assessments to determine motor and non-motor symptoms and cognitive status, and [^{123}I]FP-CIT single-photon emission CT (SPECT) to assess striatal dopamine transporter binding and MRI for volumetric analyses to assess whether pathology is associated with measures of Parkinson's disease burden.

Findings Between Sept 1, 2016, and Sept 30, 2018, we recruited 14 A53T *SNCA* carriers, 25 healthy controls, and 25 patients with idiopathic Parkinson's disease. Seven (50%) of 14 A53T *SCNA* carriers were confirmed to have motor symptoms and confirmed to have Parkinson's disease, and the absence of motor symptoms was confirmed in seven (50%) A53T *SCNA* carriers (ie, premotor), in whom [^{123}I]FP-CIT SPECT confirmed the absence of striatal dopaminergic deficits. Compared with healthy controls, premotor A53T *SNCA* carriers showed loss of [^{11}C]DASB non-displaceable binding potential in the ventral (p<0·0001) and dorsal (p=0·0002) raphe nuclei, caudate (p=0·00015), putamen (p=0·036), thalamus (p=0·00074), hypothalamus (p<0·0001), amygdala (p=0·0041), and brainstem (p=0·046); and in A53T *SNCA* carriers with Parkinson's disease this loss was extended to the hippocampus (p=0·0051), anterior (p=0·022) and posterior cingulate (p=0·036), insula (p=0·0051), frontal (p=0·0016), parietal (p=0·019), temporal (p<0·0001), and occipital (p=0·0053) cortices. A53T *SNCA* carriers with Parkinson's disease showed a loss of striatal [^{123}I]FP-CIT-specific binding ratio compared with healthy controls (p<0·0001). Premotor A53T *SNCA* carriers had loss of [^{11}C]DASB non-displaceable binding potential in brain areas corresponding to Braak stages 1–3, whereas [^{11}C]DASB non-displaceable binding potential was largely preserved in areas corresponding to Braak stages 4–6. Except for one participant who was diagnosed with Parkinson's disease in the past year, all A53T *SNCA* carriers with Parkinson's disease had decreases in [^{11}C]DASB non-displaceable binding potential in brain areas corresponding to Braak stages 1–6. Decreases in [^{11}C]DASB non-displaceable binding potential in the brainstem were associated with increased Movement Disorder Score-Unified Parkinson's Disease Rating Scale total scores in all A53T *SNCA* carriers (r –0·66, 95% CI –0·88 to –0·20; p=0·0099), idiopathic Parkinson's disease cohort 1 (r –0·66, –0·84 to –0·36; p=0·00031), and idiopathic Parkinson's disease cohort 2 (r –0·71, –0·84 to –0·52; p<0·0001).

Interpretation The presence of serotonergic pathology in premotor A53T *SNCA* carriers preceded development of dopaminergic pathology and motor symptoms and was associated with disease burden, highlighting the potential early role of serotonergic pathology in the progression of Parkinson's disease. Our findings provide evidence that molecular imaging of serotonin transporters could be used to visualise premotor pathology of Parkinson's disease in

vivo. Future work might establish whether serotonin transporter imaging is suitable as an adjunctive tool for screening and monitoring progression for individuals at risk or patients with Parkinson's disease to complement dopaminergic imaging, or as a marker of Parkinson's burden in clinical trials.

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Introduction

The neuropathology of Parkinson's disease is characterised by the presence of α-synuclein (SNCA) aggregates, which form the main components of Lewy bodies and neurites.[1] According to Braak's histopathological staging of Parkinson's disease, Lewy body pathology spreads in a gradual ascending fashion, starting from the olfactory nucleus and medulla in premotor stages and spreading to subcortical and cortical areas at later stages of the disease,[2] affecting both dopaminergic and non-dopaminergic neurons—eg, the serotonergic neurons.[3] Neuropathological studies have shown involvement of serotonergic neurons in idiopathic Parkinson's disease[4] associated with the presence of Lewy body pathology in the raphe nuclei at early stages of disease,[2] suggesting that caudal serotonergic brainstem neurons might be affected before dopaminergic neurons in the midbrain as the disease progresses. However, to date, this concept has not been proven, particularly in an in-vivo context.

The PET radioligand [^{11}C]DASB, which is selective for the serotonin transporter, has been of use in the study of presynaptic serotonergic terminal integrity in idiopathic Parkinson's disease. Patients with idiopathic Parkinson's disease show early progressive loss of serotonergic function,[5] which has been associated with the development of motor and non-motor symptoms and complications such as tremor,[6] dyskinesias,[7] fatigue,[8] sleep problems,[9] and depression.[10] A PET study[11] in a cohort of asymptomatic carriers of a familial dominant *LRRK2* mutation showed increased expression of serotonin transporters, whereas expression of serotonin transporter was decreased in *LRRK2* mutation carriers with manifested Parkinson's disease. However, about half of *LRRK2* mutation carriers do not show classic Lewy body pathology,[12] and therefore

Research in context

Evidence before this study

We reviewed scientific literature on familial Parkinson's disease, A53T α-synuclein (*SNCA*), and related neuropathology by searching PubMed on Oct 2, 2018, with no start date, for publications in English, using the search terms "familial Parkinson's disease", "A53T α-synuclein", "p.A53T α-synuclein", "positron-emission tomography", "magnetic resonance imaging", "alpha-synuclein", "serotonin transporter, SERT, or "DASB", and "dopamine transporter, or DAT". We found most neuroimaging studies on familial Parkinson's disease have focused on the most common monogenic forms, such as *LRRK2*. Neuroimaging studies in A53T *SNCA* familial Parkinson's disease have focused on assessing striatal dopaminergic function in individual case reports and small cohorts of A53T *SNCA* carriers. Studies in idiopathic Parkinson's disease report early loss of serotonin transporter availability associated with motor and non-motor symptoms. In familial Parkinson's disease, serotonin transporter has only been investigated in vivo in *LRRK2* mutation carriers. The expression of serotonin transporters was increased in *LRRK2* mutation carriers without manifest Parkinson's disease, whereas serotonin transporter expression was decreased in *LRRK2* mutation carriers with Parkinson's disease.

Added value of this study

To our knowledge, this is the first study to assess serotonergic and dopaminergic pathology in A53T *SNCA* mutation carriers in vivo and to elucidate the pathophysiology underlying Parkinson's disease. Premotor A53T *SNCA* carriers presented with normal motor and striatal dopaminergic function; whereas, striatal dopaminergic dysfunction becomes exclusively prominent in A53T *SNCA* carriers with Parkinson's disease. All A53T *SNCA* carriers, whether premotor or with manifest Parkinson's disease, exhibited serotonergic pathology, with patterns consistent with Braak's histopathological staging showing caudal to rostral ascending progression. Furthermore, we showed brainstem serotonergic pathology, measured with [^{11}C]DASB PET, as an in-vivo marker of total disease burden.

Implications of all the available evidence

Serotonergic pathology is present in premotor A53T *SNCA* carriers, before striatal dopaminergic loss, highlighting the early role of serotonergic pathology in the progression of Parkinson's disease. Our findings suggest that measuring serotonergic integrity could be a useful in-vivo tool to identify individuals at risk of developing Parkinson's disease, before evidence of a dopaminergic deficit exists, preceding disease onset by many years. Thus, such a measurement could serve as a sensitive marker of Parkinson's disease burden. Differing patterns of serotonergic and dopaminergic pathology across familial forms of Parkinson's disease suggests that distinct pathologies underlie different phenotypes of Parkinson's disease.
The classification of Parkinson's disease based on different pathological phenotypes, assessed in vivo, could lead to a more targeted therapeutic approach.

association of changes in the serotonergic system detected in vivo with Parkinson's disease pathology in the absence of histopathological data is challenging.

One of the major challenges of Parkinson's disease research is the study of premotor pathology in vivo. Although Braak and colleagues[2] have suggested a large premotor period, which might be as lengthy as the symptomatic period, identification of this period in clinical practice has proven to be challenging. Autosomal dominant and highly penetrant familial forms of Parkinson's disease, which present with a similar phenotype to idiopathic forms of the disease, provide an ideal population for in-vivo study to understand premotor stages and the progression of Parkinson's disease. Of the several mutated genes associated with familial forms of Parkinson's disease, the point mutation A53T (209G→A) in the *SNCA* gene was identified in Italian and Greek families and was the first mutation to be associated with the autosomal dominant development of Parkinson's disease.[13] Carriers of the A53T *SNCA* mutation typically present with symptoms of Parkinson's disease that are indistinguishable from idiopathic patients;[14,15] however, motor symptoms commonly manifest early, have rapid progression, and are often associated with cognitive impairment.[16–19] Furthermore, histopathological studies have shown classic Lewy body pathology in carriers of the A53T *SNCA* mutation.[20]

In this study, we investigated serotonergic and dopaminergic pathology in carriers of the A53T *SNCA* mutation in vivo by using [^{11}C]DASB PET to detect serotonin transporters and [^{123}I]FP-CIT SPECT to detect presynaptic dopamine transporters. To increase our understanding, we compared data between cohorts of A53T *SNCA* carriers in premotor stages of Parkinson's disease, carriers with manifestation of the disease, patients with idiopathic Parkinson's disease, and age-matched healthy controls. We hypothesised that the serotonergic pathology might be evident at premotor stages of the disease and before dopaminergic deficits can be detected in vivo and might be associated with measures of Parkinson's disease burden.

Methods

Study design and participants

In this cross-sectional study, we recruited a cohort of A53T *SNCA* carriers (carrier cohort), comprising both individuals in the premotor stages of disease and those who had manifested Parkinson's disease, from specialist Movement Disorders clinics at the University of Athens, Athens, Greece, and the University of Salerno, Salerno, Italy, and patients with idiopathic Parkinson's disease (cohort 1) from specialist Movement Disorders clinics at King's College Hospital, London, UK. A control group of healthy individuals, age matched to the A53T *SNCA* carriers, was recruited through public advertisement in London, UK. Healthy controls and patients with idiopathic Parkinson's disease were eligible if they had no personal or family history of neurological or psychiatric disorders. Parkinson's disease diagnosis for both idiopathic patients and A53T *SNCA* mutation carriers was determined according to the UK Brain Bank diagnostic criteria. For all participants, use of medication with known action on the serotonergic system (eg, current or previous use of selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors) was an exclusion criteria. Participants were eligible if they were screened successfully to undertake PET and MRI scanning following safety criteria.

We retrieved data for a second cohort of 40 patients with idiopathic Parkinson's disease (cohort 2) from our electronic database (owned by Neurodegeneration Imaging Group at King's College London, London, UK), who had been scanned on a different PET scanner from those in the carrier cohort, cohort 1, and the control cohort to be use as a comparator. Cohort 2 was sex matched with cohort 1.

The study was approved by the London-Bloomsbury research ethics committee and the institutional review board at King's College London, London, UK. Permission to use radioactive substances was obtained from the Radioactive Substances Advisory Committee (ARSAC), Department of Health and Social Care, UK. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki.

Procedures

All recruited participants travelled to King's College London, London, UK, for clinical assessments, and to Invicro, Hammersmith Hospital (London, UK) for PET and MRI assessments; for each participant, all assessments were completed within 3 weeks.

The carrier cohort was assessed for the presence or absence of clinical features of Parkinson's disease as defined by Motor Disorder Society (MDS) Parkinson's disease Criteria.[22] The absence of motor symptoms (ie, premotor) was confirmed with a 24 h continuous recording of the participant's mobility for 7 days by use of an automated wrist-worn device (PKG, Globral Kinetics Corporation Ltd, Australia) on both arms. Whereas cardinal motor symptoms were indicative of manifested Parkinson's disease. For ease of analysis, all A53TThr *SCNA* carriers were given a numerical identifier.

All recruited participants underwent clinical assessments to determine motor and non-motor symptoms and cognitive status, including the University of Pennsylvania Smell Identification Test (UPSIT), the Non-Motor Symptom Scale (NMSS), the Beck Depression Inventory-II (BDI-II), the Montreal Cognitive Assessment (MoCA), the Mini Mental State Examination (MMSE), the Parkinson's Disease Questionnaire-39 (PDQ-39; not administered to controls), the Scales for Outcomes for Parkinson's disease–Autonomic function test (SCOPA-AUT), and the Movement Disorder Unified Parkinson's Disease Rating Scale (MDS-UPDRS; appendix p 2). All A53T *SNCA* carriers, idiopathic Parkinson's disease cohort 1, and

See Online for appendix

healthy controls underwent [11C]DASB PET, [123I]FP-CIT single photon emission CT (SPECT), and 3-Tesla MRI scans. PET imaging assessments were done on a Siemens Biograph Hi-Rez 6 PET-CT scanner (Erlangen, Germany), and MRIs were acquired by use of a 32-channel head coil on a Siemens Magnetom TrioTim syngo MR B17 (Erlangen, Germany), all at Invicro, Hammersmith Hospital, London, UK.

Clinical, PET, and MRI data for cohort 2 were retrieved from our electronic database including [11C]DASB PET and MRI imaging data and clinical data. [11C]DASB PET had been acquired with a GE Discovery RX PET-CT scanner (Paris, France) and MRIs had been acquired with a 3-Tesla Siemens Magnetom Avanto (Erlangen, Germany). Full acquisition parameters are outlined in the appendix (p 3).

All PET and SPECT imaging was done in an off-medication state and after an overnight withdrawal of their normal medications for all participants with idiopathic Parkinson's disease and all A53T *SNCA* carriers with Parkinson's disease.

[11C]DASB PET data processing and kinetic modelling was done by use of the Molecular Imaging and Kinetic Analysis Toolbox version 4.2.6 (MIAKAT; Invicro, London, UK), implemented in MATLAB version r2015a (The Mathworks, Natick, MA, USA). [123I]FP-CIT SPECT images were reconstructed by use of the HERMES Hybrid Recon-Neurology software, and BRASS was used for the semi-quantification of striatal specific binding ratio (full details are in the appendix pp 3–4). MRI volumetric analysis was done using all MRI scans from healthy controls and A53T *SNCA* carriers (appendix pp 14–16).

Regions of interest were defined by use of Multi-Atlas Propagation with Enhanced Registration (MAPER) to automatically segment individual participants' T1-weighted MRI into 95 anatomic regions.[21] Individual participants' MAPER atlas and manual regions of interest were overlaid on coregistered PET data and sampled in ANALYZE medical imaging software (version 12.0, Mayo Foundation AnalyzeDirect, Overland Park, KS, USA). First, we quantified [11C]DASB non-displaceable binding potential in regions of interest across the cohorts (carriers, idiopathic cohort 1, and controls); we then investigated the spread of pathology according to Braak's histopathological staging[2] for Lewy body pathology (appendix p 6). [11C]DASB non-displaceable binding potential values for each Braak stage were extracted from [11C]DASB parametric maps, taking region volume-weighted averages for individual A53T *SNCA* carriers and healthy controls. For each Braak stage, the presence of serotonergic pathology was graded in each anatomical region as one or two SDs from the control mean. Regions were further categorised into groups according to their anatomical location; grouping frontal, temporal, occipital, parietal, insula, and subcortical regions depending on the regions within each Braak stage (appendix pp 7–8). The number of groups, within each stage, with one or two SDs from the control mean, was considered when grading the severity of serotonin pathology (appendix p 8). Images showing dopaminergic and serotonergic function are presented by use of FMRIB Software Library MNI152 1 mm brain template.

We used FreeSurfer image analysis suite (version 5.3.0; Martinos Centre for Biomedical Imaging, Charlestown, MA, USA) to derive measures of cortical thickness and subcortical deep grey matter nuclei volumes. Additionally, we used voxel-based morphometry, implemented in SPM12 (Wellcome Department of Cognitive Neurology, London, UK), to assess intensity differences of subcortical grey matter as a measure of grey matter atrophy.

Outcomes

We used [11C]DASB PET imaging to assess whether A53T *SNCA* mutation carriers show abnormalities in the serotonergic system before a clinical diagnosis of Parkinson's disease by comparing A53T *SNCA* mutation carriers with and without motor symptoms with healthy controls and patients with idiopathic Parkinson's disease. We also looked at whether this pathology is associated with measures of Parkinson's disease burden by clinical assessments of global disease burden (assessed with MDS-UPDRS total scores), cognitive impairment (assessed with MoCA) and non-motor symptoms (assessed with SCOPA-AUT, UPSIT, and NMSS total scores), and whether the presence of serotonergic abnormalities preceded dopaminergic loss (as assessed by [123I]FP-CIT SPECT). To investigate whether the spread of serotonergic pathology follows patterns consistent with Braak's staging, showing caudal to rostal ascending pathology, we assessed topographic decreases of [11C]DASB non-displaceable binding potential in association with Braak's histopathological grading of Lewy bodies and neurites pathology.[2] To investigate whether serotonergic dysfunction, assessed by use of [11C]DASB PET, could be used a marker of disease burden, the same methods were to be used an a second population of patients with Parkinson's disease who had been scanned with a different PET scanner.

Statistical analysis

For all variables, variance homogeneity and Gaussianity were tested with Bartlett and Kolmogorov-Smirnov tests. We proceeded with parametric tests because our imaging and clinical data were normally distributed. We used multivariate ANOVA to assess group differences in clinical, PET, and MRI data. If the overall multivariate test was significant, we used two-tailed exact *t* tests for between-group comparisons in each imaging method in predefined regions of interest and calculated p values for each variable using Bonferroni's multiple comparisons test. We interrogated correlations between PET and clinical data using Pearson's *r* correlation coefficient and applied Benjamini-Hochberg correction to decrease the false discovery rate. The false discovery rate cut-off was set at 0·05. Age and sex were used as covariates in the multivariate ANOVA to assess group differences in PET

	Healthy controls (n=25)	Idiopathic Parkinson's disease cohort 1 (n=25)		Idiopathic Parkinson's disease cohort 2 (n=40)		Premotor A53T *SNCA* carriers (n=7)		A53T *SNCA* carriers with Parkinson's disease (n=7)	
		Value or estimate	p value	Value or estimate	p value	Value or estimate	p value	Value or estimate	p value
Sex									
Female	15 (60%)	14 (56%)	..	16 (40%)	..	6 (86%)	..	5 (71%)	..
Male	10 (40%)	11 (44%)	..	24 (60%)	..	1 (14%)	..	2 (29%)	..
Age, years	45·64 (7·54)	54·36 (7·78)	0·0002	60·01 (11·12)	0·0001	41·71 (11·16)	0·28	43·86 (8·53)	0·60
Disease duration, years	NA	9·59 (3·92)	..	11·41 (7·46)	..	NA	..	5·61 (2·41)	..
MDS-UPDRS Total score	1·04 (3·47)	46·28 (28·06)	<0·0001	81·72 (43·23)	<0·0001	7·00 (2·00)	0·0002	118·00 (41·05)	<0·0001
MDS-UPDRS Part-III off	0·26 (0·81)	41·61 (15·60)	<0·0001	48·92 (17·50)	<0·0001	0·86 (1·57)	0·18	51·14 (18·00)	<0·0001
UPSIT	32·18 (5·03)	22·65 (6·97)	0·0001	20·50 (5·89)	0·0001	31·86 (3·53)	0·88	16·71 (5·59)	0·0001
BDI-II	1·85 (1·87)	6·74 (9·06)	0·011	8·25 (10·87)	0·0051	3·29 (4·82)	0·23	18·57 (13·59)	0·0001
MoCA	28·53 (2·84)	27·38 (2·60)	0·14	27·50 (0·84)	0·035	27·51 (2·51)	0·40	25·57 (3·87)	0·049
MMSE	29·75 (0·77)	29·65 (0·71)	0·64	28·22 (3·01)	0·016	29·14 (0·69)	0·068	28·57 (2·15)	0·027
SCOPA-AUT	3·13 (4·75)	24·50 (7·23)	0·0001	26·52 (6·87)	0·0001	5·14 (3·39)	0·31	22·86 (11·23)	0·0001
NMSS	4·43 (6·11)	63·22 (31·00)	<0·0001	79·48 (35·12)	<0·0001	7·57 (5·09)	0·22	86·14 (38·57)	<0·0001
PDQ-39	..	21·83 (18·19)	..	38·77 (22·11)	..	1·27 (2·21)	..	57·29 (27·63)	..

Data are n (%) or mean (SD), and p values are compared with healthy controls. A53T=Ala53Thr. MDS-UPDRS=Movement Disorder Society-unified Parkinson's Disease Rating Scale. UPSIT=University of Pennsylvania Smell Identification Test. BDI-II=Beck depression inventory-II. MoCA=Montreal cognitive assessment. MMSE=mini mental state examination. SCOPA-AUT=scale for outcomes for Parkinson's disease–autonomic function. NMSS=non-motor symptom scale. PDQ-39=Parkinson's Disease Questionnaire-39.

Table 1: **Demographic and clinical characteristics of cohorts of A53T *SNCA* mutation carriers, idiopathic Parkinson's disease patients, and healthy controls**

and MRI data. All data are presented as mean (SD), and the α level was set to a p value of less than 0·05 for all comparisons.

We did all statistical analyses with Statistical Package for Social Science version 23.0 (SPSS, Chicago, IL, USA) and graph illustration with GraphPad Prism (version 7.0c).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author has full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 1, 2016, and Sept 30, 2018, we recruited 14 A53T *SNCA* carriers from Greece (n=10) and Italy (n=4), 25 patients with idiopathic Parkinson's disease from London, UK (cohort 1), and 25 healthy controls from London, UK.

In the carrier cohort, seven (50%) participants were determined to be in the premotor stage of the disease (appendix pp 17–18), and seven (50%) to have Parkinson's disease (appendix pp 19–20). Only two A53T *SCNA* carriers, one premotor and one with manifest Parkinson's disease, were related by blood. Baseline demographic and clinical characteristics for all cohorts are shown in table 1. No differences in age were seen between the cohorts of A53T *SNCA* carriers and healthy controls; whereas patients with idiopathic Parkinson's disease in cohort 1 and cohort 2 were significantly older than those in the carrier cohort and control group (table 1). UPDRS total scores were higher in A53T *SNCA* carriers and patients with idiopathic Parkinson's disease than in healthy controls. Non-motor symptoms, as measured by UPSIT, SCOPA-AUT, NMSS, and BDI-II, were increased in A53T *SNCA* carriers with Parkinson's disease compared with healthy controls; whereas premotor A53T *SNCA* carriers had no significant differences in non-motor symptoms compared with healthy controls (table 1). In the group of A53T *SNCA* carriers with Parkinson's disease, only three participants had high substantial self-reported depression (BDI-II scores of ≥17).[23] Whereas premotor A53T *SNCA* carriers did not show significant increases in total non-motor symptom burden compared with controls, three particiants in this group had NMSS total scores between 9 and 13, suggesting the development of early, mild non-motor symptoms. The A53T *SNCA* carriers with Parkinson's disease, but not the premotor A53T *SNCA* carriers, had lower scores in global measures of cognitive performance (ie, MoCA and MMSE) than healthy controls (table 1).

Figure 1 shows mean-summed images derived from 25 healthy controls (mean striatal [^{123}I]FP-CIT specific binding ratio 3·09 [SD 0·47]; [^{11}C]DASB non-displaceable binding potential 1·21 [0·2]); 25 patients in idiopathic Parkinson's disease in cohort 1, 12 of whom had early unilateral Parkinson's disease (mean striatal [^{123}I]FP-CIT specific binding ratio 1·51 [SD 0·31]; [^{11}C]DASB non-displaceable binding potential 0·97 [SD 0·2]), and 13 of whom had advanced bilateral Parkinson's disease (mean striatal [^{123}I]FP-CIT specific binding ratio 1·06 [SD 0·38]; [^{11}C]DASB non-displaceable binding potential 0·84 [SD 0·1]); seven premotor A53T *SNCA* carriers (mean

striatal [123I]FP-CIT specific binding ratio 3·29 [SD 0·96]; [11C]DASB non-displaceable binding potential 0·93 [SD 0·1]); and seven A53T *SNCA* carriers with Parkinson's disease (mean striatal [123I]FP-CIT specific binding ratio 0·91 [SD 0·40]; [11C]DASB non-displaceable binding potential 0·55 [SD 0·2]). Premotor A53T *SNCA* carriers had no differences in [123I]FP-CIT striatal specific binding ratio, whereas A53T *SNCA* carriers with Parkinson's disease showed significant loss of 71% in [123I]FP-CIT striatal specific binding ratio compared with healthy controls (table 2, figure 1). Compared with participants in idiopathic Parkinson's disease in cohort 1, A53T *SNCA* carriers with Parkinson's disease showed increased loss of [123I]FP-CIT caudate specific binding ratio (left caudate p=0·049; right caudate p=0·025) but no differences in [123I]FP-CIT putamen specific binding ratio (left putamen p=0·47; right putamen p=0·50; appendix pp 12–13).

Premotor A53T *SNCA* carriers showed significantly decreased [11C]DASB non-displaceable binding potential in the ventral and dorsal raphe nuclei, caudate, putamen, thalamus, hypothalamus, amygdala, and the brainstem compared with healthy controls (table 2; figure 1). A53T *SNCA* carriers with Parkinson's disease showed additional significant decreases in [11C]DASB non-displaceable binding potential in the hippocampus, anterior and posterior cingulate, insula, and in the frontal, temporal, and occipital cortex compared with healthy controls (table 2, figure 1; appendix p 11). Premotor A53T *SNCA* carriers showed an average loss in [11C]DASB non-displaceable binding potential of 34% in the raphe nuclei and 22% in the stritaum; whereas the A53T *SNCA* carriers with Parkinson's disease showed losses of 48% in the raphe nuclei and 57% in the striatum compared with healthy controls. The severity of serotonergic loss in premotor A53T *SNCA* carriers was in line with decreases in participants in idiopathic Parkinson's disease cohort 1 (ie, no differences in regions of interest, except in the occipital cortex, cuneus, lingual gyrus, and long and short insula gyrus; appendix p 11–12), whereas A53T *SNCA* carriers with Parkinson's disease showed significantly increased loss of [11C]DASB non-displaceable binding potential in the putamen, caudate, hypothalamus, and amygdala and in the insula posterior short gyrus and insula anterior long gyrus compared with participants in idiopathic Parkinson's disease cohort 1 (appendix pp 11–12).

Having shown the presence of serotonergic pathology in premotor and Parkinson's disease A53T *SNCA* carriers, we proceeded to investigate topographic decreases of [11C]DASB non-displaceable binding potential in association with Braak's histopathological grading of Lewy bodies and neurites pathology.[2] Premotor A53T *SNCA* carriers had loss of [11C]DASB non-displaceable binding potential in brain areas corresponding to Braak stages 1–3, whereas [11C]DASB non-displaceable binding potential was largely preserved in areas corresponding to Braak stages 4–6 (figure 2). All premotor A53T *SNCA* carriers showed mean decreases of more than two SDs in [11C]DASB

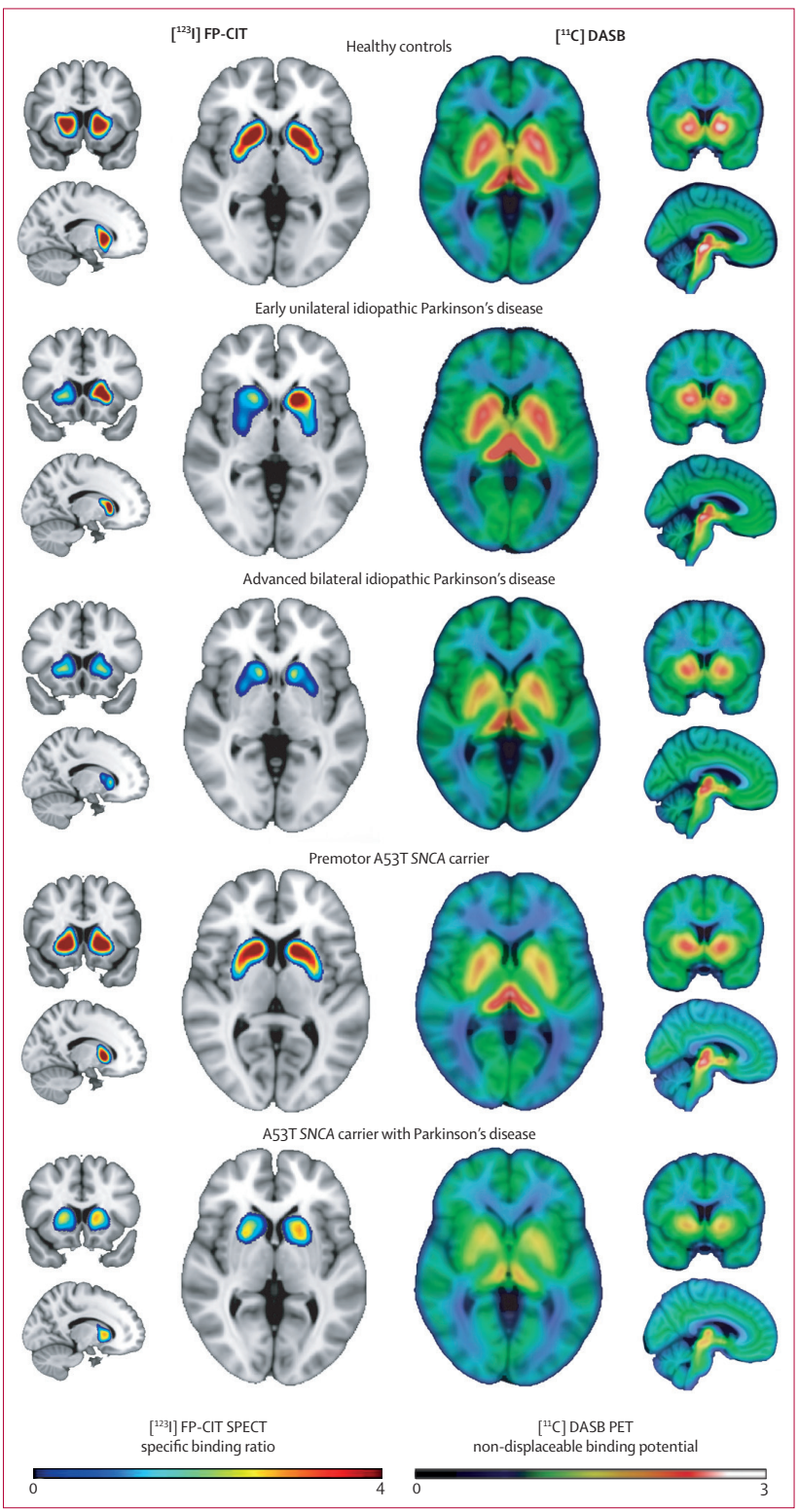

Figure 1: **Dopaminergic and serotonergic dysfunction in A53T *SNCA* carriers compared with healthy controls and patients with idiopathic Parkinson's disease (cohort 1)**
Axial, sagittal, and coronal mean summed images for [123I]FP-CIT SPECT for dopamine transporter (left), and [11C] DASB PET for serotonin transporter availability (right). [123I]FP-CIT SPECT MNI co-ordinates: X=75; Y=134; Z=77; [11C]DASB PET MNI coordinates: X=93; Y=134; Z=6.

	Healthy controls (n=25)	Idiopathic Parkinson's disease cohort 1 (n=25)	Premotor A53T *SNCA* carriers (n=7)		A53T *SNCA* carriers with Parkinson's disease (n=7)	
			Value or estimate	p value*	Value or estimate	p value*
[^{123}I]FP-CIT SPECT specific binding ratio						
Left putamen	2·70 (0·49)	0·91 (0·36)	2·72 (0·41)	0·85	0·78 (0·36)	<0·0001†
Right putamen	2·72 (0·46)	0·85 (0·35)	2·69 (0·31)	0·73	0·74 (0·28)	<0·0001†
Left caudate	3·60 (0·48)	1·49 (0·50)	3·34 (0·78)	0·33	1·12 (0·49)	<0·0001†
Right caudate	3·48 (0·42)	1·61 (0·70)	3·33 (0·80)	0·46	1·02 (0·39)	<0·0001†
Left striatum	3·15 (0·46)	1·28 (0·35)	3·08 (0·36)	0·95	0·95 (0·44)	<0·0001†
Right striatum	3·10 (0·40)	1·27 (0·45)	3·04 (0·39)	0·86	0·86 (0·37)	<0·0001†
[^{11}C]DASB PET non-displaceable binding potential						
Ventral raphe nuclei	1·53 (0·25)	0·97(0·21)	0·99 (0·15)	<0·0001†	0·78 (0·34)	<0·0001†
Dorsal raphe nuclei	1·76 (0·28)	1·20 (0·20)	1·19 (0·20)	0·00020†	0·94 (0·42)	<0·0001†
Brainstem	0·57 (0·10)	0·33 (0·17)	0·34 (0·10)	0·046†	0·26 (0·21)	<0·0001†
Putamen	1·22 (0·21)	0·90 (0·17)	1·02 (0·18)	0·036†	0·59 (0·24)	<0·0001†
Caudate	1·18 (0·20)	0·72 (0·21)	0·80 (0·10)	0·00015†	0·40 (0·24)	<0·0001†
Thalamus	1·31 (0·19)	0·98 (0·18)	0·99 (0·14)	0·00074†	0·77 (0·30)	0·00015†
Hypothalamus	1·14 (0·20)	0·67 (0·19)	0·40 (0·12)	<0·0001†	0·23 (0·13)	<0·0001†
Amygdala	0·81 (0·15)	0·60 (0·18)	0·59 (0·16)	0·0041†	0·35 (0·18)	<0·0001†
Hippocampus	0·32 (0·10)	0·28 (0·13)	0·32 (0·07)	0·85	0·16 (0·10)	0·0051†
Anterior cingulate	0·27 (0·09)	0·20 (0·16)	0·26 (0·06)	0·73	0·15 (0·11)	0·022†
Posterior cingulate	0·25 (0·10)	0·14 (0·13)	0·25 (0·03)	0·98	0·14 (0·09)	0·036†
Insula	0·44 (0·12)	0·32 (0·14)	0·44 (0·11)	0·94	0·23 (0·15)	0·0051†
Frontal cortex	0·22 (0·07)	0·14 (0·14)	0·19 (0·05)	0·20	0·093 (0·09)	0·0016†
Temporal cortex	0·20 (0·08)	0·085 (0·14)	0·17 (0·06)	0·34	0·05 (0·08)	<0·0001†
Occipital cortex	0·13 (0·09)	0·011 (0·09)	0·19 (0·07)	0·16	0·005 (0·08)	0·0053†

Data are mean (SD) unless otherwise stated. A53T=Ala53Thr. SPECT=Single Photon Emission Computed Tomography. *p values are compared with healthy controls. †p values show significant loss of dopamine transporter via [^{123}I]FP-CIT SPECT and serotonin transporter via [^{11}C]DASB in A53T *SNCA* carriers compared with healthy controls.

Table 2: **[^{123}I]FP-CIT SPECT and [^{11}C]DASB PET BP_{ND} in premotor A53T *SNCA* carriers and A53T *SNCA* carriers with Parkinson's disease compared with healthy controls and idiopathic Parkinson's disease patients by region of interest**

non-displaceable binding potential values compared with healthy controls in medulla oblongata (Braak stage 1) and pontine tegmentum (Braak stage 2). The youngest premotor A53T *SNCA* carriers (SNCA05 and SNCA06) had means decreases of more than one SD in [^{11}C]DASB non-displaceable binding potential values compared with healthy controls in midbrain and basolateral forebrain (Braak stage 3), whereas all other premotor A53T *SNCA* carriers were in the more than two SD range. Participants SNCA01 and SNCA14 had areas with a mean decrease of more than one SD in [^{11}C]DASB non-displaceable binding potential values compared with healthy controls within the temporal mesocortex and allocortex (Braak stage 4); and SNCA14 in the higher-order association areas of the neocortex (Braak stage 5). Premotor A53T *SCNA* carrier SNCA14 had an MoCA score of 28 and an MMSE score of 29 and no indication of subtle cognitive or behavioural changes. However, participant SNCA01 had an MoCA score of 23 and an MMSE score of 29, and mild changes in their visuospatial and executive cognitive function and working memory, as indicated by the MoCA subitem scores. With regards to A53T *SNCA* carriers with Parkinson's disease (figure 2B), all carriers showed mean decerases of more than two SDs in [^{11}C]DASB non-displaceable binding potential values compared with healthy controls in medulla oblongata, pontine tegmentum, and basolateral forebrain (Braak stages 1–3). Participants SNCA07, SNCA08, SNCA11, and SNCA12 had mean decreases of more than two SDs in [^{11}C]DASB non-displaceable binding potential values compared with healthy controls in the temporal mesocortex and allocortex (Braak stage 4); SNCA08 and SNCA11 in the higher-order association areas of the neocortex (Braak stage 5); and SNCA11 in higher cortical structures including primary sensory and motor areas (Braak stage 6). With the exception of SNCA09, who had been diagnosed with Parkinson's disease in the past year, all A53T *SNCA* carriers with Parkinson's disease showed at least some areas with mean decreases of more than one SD in [^{11}C]DASB non-displaceable binding potential values compared with healthy controls that corresponded to Braak stages 4–6. With the exception of one participant, SNCA09, who had been diagnosed with Parkinson's disease in the past year, all A53T *SNCA* carriers with Parkinson's disease

showed mean decerases of more than two SDs (Braak stage 1–3) and at least some areas with mean decreases of more than one SD (Braak stage 4–6) in [11C]DASB non-displaceable binding potential values compared healthy controls in brain areas corresponding to Braak stages as indicated (figure 2).

To assess whether serotonergic dysfunction could be a marker of disease burden, we looked for associations between [11C]DASB non-displaceable binding potential across the brain and MDS-UPDRS total scores. In the cohort of A53T *SNCA* carriers, decreased brainstem [11C]DASB non-displaceable binding potential correlated with high total UPDRS (n=14; *r* –0·66, 95% CI –0·88 to –0·20; p=0·0099; figure 3A). Decreased brainstem [11C]DASB non-displaceable binding potential correlated with high total UPDRS score also within the subgroups of premotor A53T *SNCA* carriers (n=7; *r* –0·75, 95% CI –0·96 to <–0·01; p=0·049) and A53T *SNCA* carriers with Parkinson's disease (n=7; *r* –0·76, –0·96 to –0·01; p=0·049; appendix p 21). Similarly, in the idiopathic Parkinson's disease cohort 1 (n=25), decreased brainstem [11C]DASB non-displaceable binding potential correlated with a high total UPDRS score (*r* –0·66, 95% CI –0·84 to –0·36; p=0·00031; figure 3B). We then wanted to test the applicability of these findings to a different cohort of patients with idiopathic Parkinson's disease (n=40; cohort 2) who were scanned previously with [11C]DASB PET in a different scanner. We found that decreased brainstem [11C]DASB non-displaceable binding potential also correlated with high total UPDRS score in this cohort (*r* –0·71, 95% CI –0·84 to –0·52; p<0·0001; figure 3C). We noted that as the sample size increased, the correlation coefficient became larger. Furthermore, decreased brainstem [11C]DASB non-displaceable binding potential was correlated with decreased [11C]DASB non-displaceable binding potential in regions that reflect Braak stage 1 (*r* 0·87, 95% CI 0·64 to 0·96; p<0·0001), Braak stage 2 (*r* 0·90, 0·71 to 0·97; p<0·0001), and Braak stage 3 (*r* 0·88, 0·66 to 0·96; p<0·0001; appendix p 22).

We investigated whether an association existed between [11C]DASB non-displaceable binding potential and cognitive impairment and non-motor symptoms. In the cohort of A53T *SNCA* carriers, low MoCA scores correlated with decreased [11C]DASB non-displaceable binding potential in Braak stage 4 (*r* 0·63, 95% CI 0·14 to 0·87; p=0·017; figure 4A) and with decreased [11C]DASB non-displaceable binding potential in Braak stage 5 (*r* 0·61, 0·11 to 0·86; p=0·022; figure 4B). No correlations were found between regional [11C]DASB non-displaceable binding potential and SCOPA-AUT or UPSIT scores. Decreased brainstem [11C]DASB non-displaceable binding potential correlated with high NMSS total scores in the cohort of A53T *SNCA* carriers (n=14; *r* –0·71, 95% CI –0·90 to –0·29; p=0·0042), and in subgroups of premotor A53T *SNCA* carriers (n=7; *r* –0·78, 95% CI –0·97 to –0·06; p=0·040) and A53T *SNCA* carriers with Parkinson's disease (n=7; *r* –0·76, –0·96 to –0·02; p=0·047; appendix p 23). FreeSurfer and voxel-based morphometry cortical thickness and subcortical volumetric analysis revealed no atrophy (appendix pp 14–16,24).

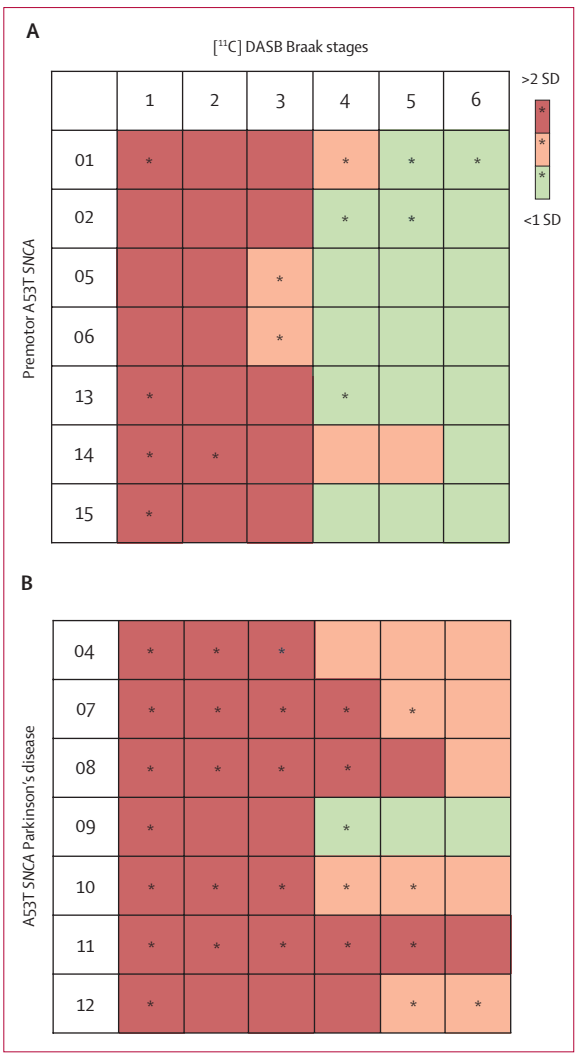

Figure 2: **In-vivo Braak staging of serotonergic pathology based on [11C]DASB PET in A53T *SNCA* mutation carriers by individual participant**
(A) Premotor A53T *SNCA* carriers and (B) A53T *SNCA* carriers with Parkinson's disease, listed by participant identifier. The colour scale represents a range of the number of regions with one to two SDs from the control mean to indicate the degree of serotonergic pathology for Braak stage across each A53T *SNCA* carrier with red* indicating the highest, followed by red, then orange*, orange, green*, and finally green indicating the lowest degree of serotonergic pathology. For full details and description please see expanded figure legend in appendix (pp 9–10). The regions included in each Braak stage are described in the appendix (p 6), the grading according to each anatomical region is on p 7–8, and the colour coding for staging is on p 8.

Discussion

In this cross-sectional study, we assessed molecular, structural, and clinical markers of pathology in a cohort of A53T *SNCA* gene mutation carriers and compared them with patients with idiopathic Parkinson's disease and healthy controls. Half of the cohort of A53T *SNCA*

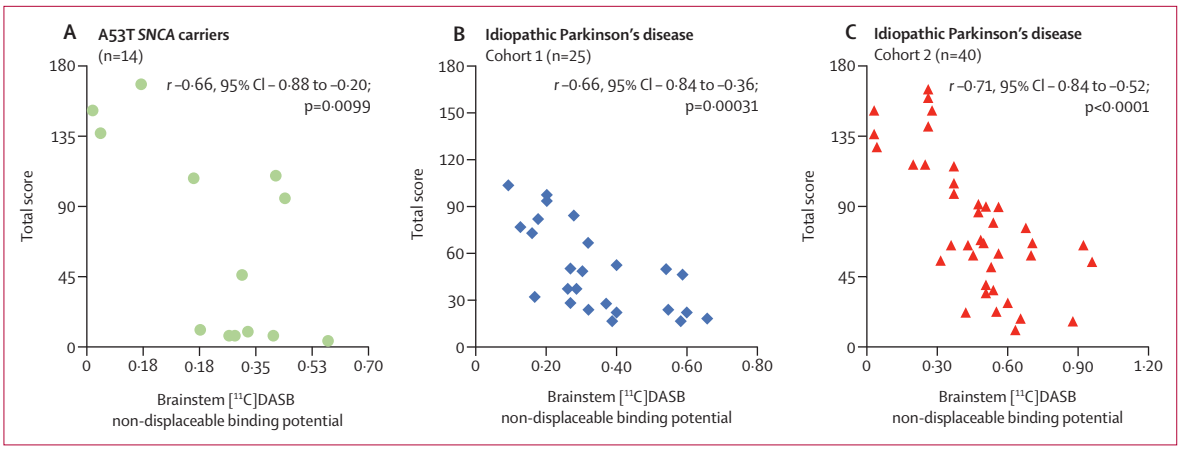

Figure 3: **Correlations between Unified Parkinson's disease Rating Scale total scores and brainstem [^{11}C]DASB non-displaceable binding for serotonin transporters for all A53T *SNCA* carriers (A) and patients with idiopathic Parkinson's disease from cohort 1 (B) and cohort 2 (C)**

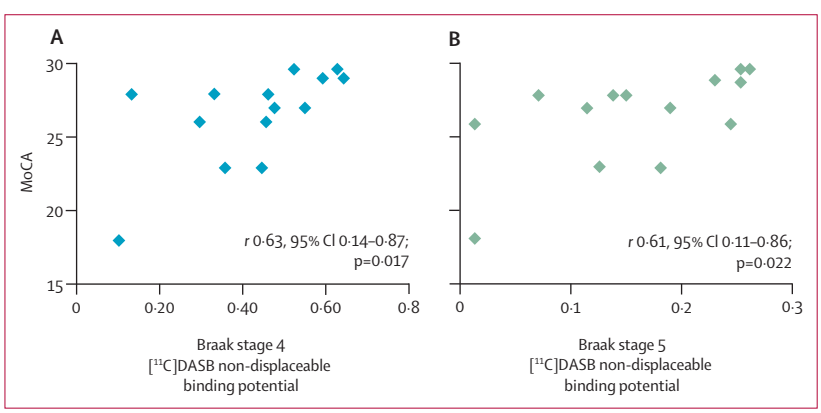

Figure 4: **Correlations between global cognitive status with Braak stage 4 [^{11}C]DASB non-displaceable binding (A) and Braak stage 5 [^{11}C]DASB non-displaceable binding (B) in all A53T *SNCA* mutation carriers (n=14)**
MoCA=Montreal cognitive assessment.

mutation carriers were at the premotor stage of Parkinson's disease, which was confirmed clinically and by use of digital continuous recordings of motor function. Our findings provide novel insights into the premotor pathology and evolution of Parkinson's disease, suggesting that serotonergic dysfunction, which can be detected by use of in-vivo molecular imaging in patients at risk of Parkinson's disease, precedes the development of motor symptoms and visualisation of dopaminergic pathology. Moreover, we found that the presence of serotonergic pathology in the brainstem is associated with the overall burden of Parkinson's disease.

Premotor A53T *SNCA* carriers had normal striatal dopamine transporter scans, but loss of serotonin transporters was noted in raphe nuclei, brainstem, striatum, thalamus, hypothalamus, and amygdala. A53T *SNCA* carriers with Parkinson's disease had loss of striatal dopamine transporters and loss of serotonin transporters extended to additional subcortical and cortical regions (eg, cingulate and insula), which were not seen in premotor A53T *SNCA* carriers. Our findings indicate that premotor A53T *SNCA* carriers with normal visualisation of dopamine transporters show an average of 34% loss of serotonin transporters in raphe nuclei and 22% loss in the striatum compared with healthy controls. In A53T *SNCA* carriers with Parkinson's disease, the loss of serotonin transporters is extended to 48% in raphe nuclei and 57% in striatum, whereas the loss of striatal dopamine transporters in this group is 71%. In line with previous studies,[18,19,24] A53T *SNCA* carriers with Parkinson's disease showed increased loss of dopamine transporters in the caudate and no differences in the putamen compared with patients with idiopathic Parkinson's disease. Furthermore, the severity of loss of serotonin transporter in premotor A53T *SNCA* carriers was in line with decreases observed in patients with idiopathic Parkinson's disease, whereas A53T *SNCA* carriers with Parkinson's disease showed greater losses of serotonin transporters than did idiopathic patients. Combined, these findings suggest similarities in the pathophysiology between patients with idiopathic Parkinson's disease and A53T *SNCA* carriers with Parkinson's disease, but with faster progression in those who are A53T *SNCA* mutation carriers.

In a previous [^{11}C]DASB PET study in patients with idiopathic Parkinson's disease,[5] we have hypothesised that serotonergic pathology could be an early phenomenon in the course of the disease, although it evolves at a slower pace than with dopaminergic pathology. Additional [^{11}C]DASB PET studies[6–10] in patients with idiopathic Parkinson's disease have shown an association between serotonergic pathology with non-motor symptoms such as fatigue,[8] depression,[10] and sleep problems,[9] and motor symptoms and complications such as tremor[6] and levodopa-induced dyskinesias.[7] By contrast, dopaminergic markers correlate well with the symptoms of rigidity and bradykinesia that also respond well to dopamine replacement therapy.[25]

The neurons of the raphe nuclei, which are located in the brainstem, are the main source of serotonergic neurotransmission in the human brain and innervate a large part of the brain through the rostral and caudal pathways

while modulating a large number of physiological functions.[26] Similarly, Braak and colleagues[2] have used histopathology to describe the distribution of Lewy body and neurite spread in brain tissue samples from people with Parkinson's disease, which closely follows the topographic distribution of serotonergic circuits in the brain. Moreover, SNCA is expressed in the perikarya and neuritic processes of serotonergic raphe nuclei neurons and has been shown to directly affect serotonin transporters by generating a negative modulation and decreasing the availability of its cell surface.[27] The influence of SNCA on serotonin transporters arises via direct binding between the two proteins, predominantly involving the non-amyloidogenic component domain of SNCA. This binding is particularly interesting because the A53T mutation, which has substantially increased aggregation kinetics, might hinder the ability of SNCA to form α-helices, thus promoting β-sheet configuration and SNCA aggregation, leading to the sequestration of serotonin transporter into aggregates, resulting in its depletion, as reflected by our results.

Our findings further support the potential association of loss of [11C]DASB binding potential—reflecting serotonergic pathology—with the distribution of Lewy body and neurite pathology. We constructed brain topographic maps reflecting Braak stages 1–6 and used these as seed maps to calculate [11C]DASB binding potential in the cohort of A53T *SNCA* carriers. In line with the Braak stages, premotor A53T *SNCA* carriers showed serotonergic pathology in brain areas corresponding to stages 1–3, whereas [11C]DASB binding potential was largely preserved in brain areas corresponding to stages 4–6. Interestingly, the youngest premotor A53T *SNCA* carriers (SNCA05 and SNCA06) had extensive loss of [11C]DASB binding potential in areas corresponding to stages 1 and 2 and only partial loss in areas corresponding to stage 3. Furthermore, A53T *SNCA* carriers with Parkinson's disease had serotonergic pathology in brain areas corresponding to stages 4–6. SNCA09, who had been diagnosed with Parkinson's disease in the past year, had minimal loss of [11C]DASB binding potential in areas corresponding to stage 4, whereas [11C]DASB binding potential was largely preserved in brain areas corresponding to stages 5 and 6.

If loss of [11C]DASB binding potential in the brain of a patient with Parkinson's disease—indicating serotonergic pathology detected in vivo—was to follow the progression and spread of Lewy body and neurite pathology, and if serotonergic pathology could provide an overall weighted capture of motor and non-motor symptomatology in line with the role of the serotonergic system in a high number of human physiological functions, then we hypothesised that an association should exist between loss of [11C]DASB binding potential and overall Parkinson's burden. Indeed, our findings indicate that serotonergic pathology in the brainstem, which was present in all A53T *SNCA* carriers, was associated with total UPDRS scores, which captures the overall burden of the disease including both motor and non-motor symptoms. This association was also present in both subgroups of premotor and manifest Parkinson's disease A53T *SNCA* carriers, suggesting that the correlation between brainstem serotonergic pathology and overall Parkinson's disease burden was driven by the presence of the mutation in both groups. To further test and generalise the applicability of this finding, we attempted similar correlations in two larger cohorts of patients with idiopathic Parkinson's disease, with one cohort having been scanned using a different scanner than all other participants herein. In both cohorts, the correlation remained true, and we noted that by increasing the sample size the correlation coefficient became larger. This correlation highlights the potential applicability of our findings from A53T *SNCA* carriers to patients with idiopathic Parkinson's disease and suggests the potential application of brainstem [11C]DASB PET as a marker of disease burden across different scanners and sites. This preliminary evidence could be useful for future multicentre studies and highlights the need for further studies to investigate brainstem [11C]DASB PET as a potentially robust biomarker to monitor disease progression. Larger cross-sectional and longitudinal studies are required to confirm these findings.

Non-motor symptoms typically present before the onset of cardinal motor symptoms in idiopathic Parkinson's disease, indicated by the accumulation of Lewy bodies in Braak stage 1–3.[2] We investigated the association of serotonergic pathology with non-motor symptoms in A53T *SNCA* carriers. In A53T *SNCA* carriers, loss of [11C]DASB in the brainstem was associated with higher global burden of non-motor symptoms; this correlation was also present in both subgroups of premotor and manifest Parkinson's disease A53T *SNCA* carriers. Therefore, these data suggest that brainstem serotonergic pathology might be preceding the gradual development of the non-motor symptom burden. Our findings are in line with previous studies in idiopathic Parkinson's disease that support a link between non-motor symptoms and serotonergic pathology.[8–10] Additionally, the serotonergic system plays a key role in the regulation of several important human functions including mood, sleep, appetite, cognition, and behaviour. As such, previous studies using serotonergic PET imaging in patients with idiopathic Parkinson's disease have shown correlations between serotonergic pathology and depression, apathy, sleep problems, fatigue, and weight loss. Unfortunately, we did not have enough power to explore for associations between serotonergic pathology in A53T *SNCA* mutation carriers and the specific symptoms of sleep, depression, behaviour, appetite, and others. We did not find any association between [11C]DASB binding and dysautonomic or olfactory symptoms, suggesting other neurotransmitter systems, such as the noradrenergic system, might play a more prominent role in their pathophysiology.

The presence of serotonergic pathology in Braak stage 4 and 5 was associated with global cognitive deficits. One

premotor A53T *SNCA* carrier (SNCA01) with serotonergic pathology in the temporal mesocortex and allocortex (Braak stage 4) presented with subtle cognitive deficits, in visuospatial and executive cognitive function and working memory. These observations suggest that the accumulation of serotonergic pathology in basal prosencephalon, mesocortical, and neocortical regions could play a role in the development of cognitive deficits, which are often prominent in A53T *SNCA* carriers.[16] Histopathological evidence suggests tau neurofibrillary tangles and amyloid-β plaques can coexist with SNCA accumulation.[28] In-vivo PET studies have shown the presence of amyloid-β and tau neurofibrillary tangles in patients with Parkinson's disease with cognitive impairment.[29,30] Therefore, the role of tau neurofibrillary tangles and amyloid-β plaques in the development of cognitive impairment in A53T *SNCA* carriers warrants further investigation in vivo.

The combined use of thorough clinical observation with molecular imaging, which encompasses nanomolar sensitivity, and the study of A53T *SNCA* carriers and how their gene mutation is directly linked with Lewy body pathology and Parkinson's disease susceptibility, allowed insight into the early role of serotonergic pathology in the progression of Parkinson's disease. To our knowledge, our findings provide the first in-vivo imaging data to potentially corroborate the Braak staging scheme, in terms of showing a neurotransmitter deficit corresponding to stage 2 preceding the dopaminergic deficit that occurs in stage 3. Although PET molecular imaging is expensive and A53T *SNCA* carriers are rare, our study highlights the potential to extend findings in A53T *SNCA* carriers to classic forms of idiopathic Parkinson's disease. However, further studies are required to fully elucidate the molecular pathology and disease mechanisms across familial forms of Parkinson's disease compared with idiopathic Parkinson's disease. Although the Parkinson's disease field is trying to identify reliable markers sensitive to disease progression and candidates at risk of novel neuroprotective treatments, we provide evidence that the detection of serotonergic pathology, which can be visualised in vivo in humans, could identify individuals at risk even before evidence of a dopaminergic deficit or occurrence of premotor symptoms, thus preceding disease onset by many years. Given the high signal-to-noise ratio of [^{123}I]FP-CIT SPECT, it could also be a useful tool to detect longitudinal changes in A53T *SNCA* carriers. Future studies are warranted to assess longitudinal changes in [^{123}I]FP-CIT SPECT and [^{11}C]DASB PET as potential markers to monitor disease progression. Provided that accurate serotonin transporter imaging can be labelled with longer lived ^{18}F isotopes for wider PET applicability or transferred to the less expensive SPECT platform, it has the potential to become a more affordable method for screening and monitoring disease progression than current methods. Future work could allow the development of serotonin transporter imaging into an adjunctive tool for screening and monitoring progression for individuals at risk of developing Parkinson's disease or for patients with Parkinson's disease, to complement existing molecular imaging tools such as dopaminergic imaging and serve as a sensitive marker of Parkinson's burden.

Contributors

MPo conceived the study, conceptualised the experimental design, and obtained funding for the study. LS and PB gave input to experimental design. MPo, HW, GD, GP, CK, MPi, SP, BG, MS, RNG, EAR, MTP, PB, and LS organised the study. GD, GP, CK, MPi, SP, AS, BG, MS, MTP, PB, and LS contributed to participant recruitment. GD, GP, and BG did the clinical assessments. HW, GD, GP, and TY collected the data. HW, GD, GP, TY, ZC, and BC analysed the data. MPo generated the figures. HW and MPo wrote the first draft of the manuscript. All authors contributed in data interpretation and reviewed and gave input to the manuscript.

Declaration of interests

HW reports support from CHDI Foundation outside of the submitted work. GD reports support from Edmond and Lily Safra Foundation and The Michael J Fox Foundation outside of the submitted work. GP reports support from Edmond and Lily Safra Foundation and Curium outside of the submitted work; and reports a patent for adrenoceptors antagonists for the prevention and treatment of neurodegenerative conditions issued to Cedars-Sinai Medical Center. CK and AS report personal fees from The Michael J Fox Foundation outside of the submitted work. TY reports support from the UK Medical Research Council outside of the submitted work. MPi and LS report support from The Michael J Fox Foundation outside of the submitted work. MS reports grants from The Michael J Fox Foundation, and other support from Biogen, AbbVie, UCB, Specifar Pharmaceuticals, The International Parkinson's disease and Movement Disorders Society, Oxford University Press, Cambridge University Press, and Elsevier outside of the submitted work. EAR reports grants from National Institute for Health Research Biomedical Research Centre at South London, Maudsley National Health Service Foundation Trust, and King's College London outside of the submitted work. PB reports grants from The Michael J Fox Foundation during the conduct of the study and personal fees from UCB, Chiesi, Zambon, and Bial outside of the submitted work. MPo reports grants from European Commission, The Michael J Fox Foundation, Edmond and Lily Safra Foundation, Glaxo Wellcome R&D, the CHDI Foundation, Alzheimer's Research UK, Curium, the UK Medical Research Council, and AVID Radiopharmaceuticals; grants and other support from Life Molecular Imaging; other support from UCB, Alliance Medical, Dementech, Road International, and Global Kinetics; and personal fees from United Neuroscience and Lundbeck outside of the submitted work. All other authors declare no competing interests.

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