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Serotonergic pathology linked with the premotor phase of A53T α-synuclein parkinsonism and with disease burden: cross-sectional studies

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Abstract

Background: Due to the highly penetrant gene mutation and the clinical features consistent with idiopathic Parkinson's disease, carriers of the autosomal dominant A53T (p.Ala53Thr, c.209G>A) point mutation in the α-synuclein gene *(SNCA)* represent 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 A53T *SNCA* mutation carriers might show abnormalities in the serotonergic neurotransmitter system before the diagnosis of Parkinson's disease, and that this pathology may be associated with measures of Parkinson's burden.

Methods: Between September 2016 and September 2018, we recruited 14 A53T *SNCA* mutation carriers (seven premotor without Parkinson's disease). We compared their data with two cohorts of 25 and 40 patients with idiopathic Parkinson's disease, and a cohort of 25 healthy controls. [^{11}C]DASB PET non-displaceable binding (BP_{ND}) was used 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 BP_{ND} in the cohort of A53T *SNCA* carriers. In addition, all participants underwent a battery of clinical assessments, [^{123}I]FP-CIT SPECT to assess striatal dopamine transporter binding and MRI for volumetric analyses.

Findings: Seven-day continuous recording of motor function confirmed the absence of motor symptoms and [^{123}I]FP-CIT SPECT the absence of striatal dopaminergic deficits in premotor A53T *SNCA* carriers (p>0·10). Premotor *A53T* SNCA carriers showed loss of [^{11}C]DASB BP_{ND} in the raphe nuclei (p<0·001), caudate (p<0·001), putamen (p=0·036), thalamus (p=0·001), hypothalamus (p<0·001), amygdala (p=0·004) and brainstem (p=0·046), which was extended to hippocampus (p=0·005), anterior (p=0·022) and posterior cingulate (p=0·036), insula (p=0·005), frontal (p=0·002), parietal (p=0·019), temporal (p=0·001) and occipital (p=0·005) cortices in A53T *SNCA* Parkinson's disease. A53T *SNCA* Parkinson's disease patients showed a loss of striatal [^{123}I]FP-CIT specific binding ratio (p<0·001). Premotor *A53T* SNCA had loss of [^{11}C]DASB BP_{ND} in brain areas corresponding to Braak stages 1-3, whereas [^{11}C]DASB BP_{ND} was largely preserved in areas corresponding to Braak stages 4-6. With the exception of a recently diagnosed subject with Parkinson's disease, A53T *SNCA* Parkinson's subjects had [^{11}C]DASB BP_{ND} decreases in brain areas corresponding to Braak stages 1-6. [^{11}C]DASB BP_{ND} decreases in brainstem were associated with increased MDS-UPDRS total scores in A53T *SNCA* carriers (r=-0·66; p=0·0003; 95% CI -0·84 to -0·36), idiopathic Parkinson's patients (r=-0·71; p<0·0001; 95% CI -0·84 to -0·52), and a second cohort of

idiopathic Parkinson's patients scanned on a different scanner (r=-0·71; p<0·0001; 95% CI -0·84 to -0·52).

Interpretation: Our findings indicate the presence of serotonergic pathology in premotor A53T *SNCA* mutation carriers, that precedes the development of dopaminergic pathology and motor symptoms. The presence of brainstem serotonergic pathology is associated with the overall burden of Parkinson's disease. Our findings provide evidence that molecular imaging of serotonin transporters may provide with an imaging tool to visualise *in vivo* premotor Parkinson's pathology. Future work may allow for the development of serotonin transporter imaging into an adjunctive tool for screening and monitoring progression for individuals at risk or patients with Parkinson's disease, to complement existing molecular imaging tools such as dopaminergic imaging, and could serve as a sensitive marker of Parkinson's burden in clinical trials.

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Research in context

Evidence before this study: We reviewed current literature on familial Parkinson's disease, A53T α-synuclein (*SNCA*) and related neuropathology by searching PubMed on 2nd October 2018, for published articles containing 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", "dopamine transporter, or DAT". To-date, the majority of neuroimaging studies on familial Parkinson's disease have focused on the most common monogenic forms, such as the Leucine Rich Repeat Kinase (*LRRK2*). Neuroimaging studies in A53T *SNCA* familial Parkinson's 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, while serotonin transporter expression was reduced 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* gene mutation carriers *in vivo* to elucidate the pathophysiology underlying Parkinson's disease. Premotor A53T *SNCA* carriers, presented with normal motor and striatal dopaminergic function; while striatal dopaminergic dysfunction becomes exclusively prominent in A53T *SNCA* carriers with Parkinson's disease. All A53T *SNCA* carriers, premotor and with a Parkinson's diagnosis, exhibited serotonergic pathology, with patterns consistent with Braak's histopathological staging showing caudal to rostral ascending progression. Furthermore, we demonstrate 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, prior to striatal dopaminergic loss; highlighting the very early role of serotonergic pathology in the progression of Parkinson's disease. Our findings highlight that measuring serotonergic integrity may serve as a useful *in vivo* tool to identify individuals at risk before there is evidence of a dopaminergic deficit, preceding disease onset by many years; thus, such a measurement could serve as a sensitive marker of Parkinson's 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 based on different pathological phenotypes, assessed *in vivo*, could lead to a more targeted therapeutic approach.

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, Lewy pathology spreads in a gradual ascending fashion, starting from the olfactory nucleus and the medulla in premotor stages and spreading to subcortical and cortical areas at later stages of the disease,(2) affecting both dopaminergic and non-dopaminergic containing neurons, such as the serotonergic neurons.(3) Neuropathological studies demonstrated involvement of serotonergic neurons in idiopathic Parkinson's disease,(4) associated with the presence of Lewy pathology within the raphe nuclei at early disease stages,(2) suggesting that caudal serotonergic brainstem neurons may be affected prior to dopaminergic neurons in the midbrain, as the disease evolves. However, to date, there has been no proof provided for this concept, in particular in an *in vivo* context.

The PET radioligand [^{11}C]DASB, which is selective for the serotonin transporter, has been employed to study presynaptic serotonergic terminal integrity in idiopathic Parkinson's disease. Idiopathic Parkinson's patients 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(9) and depression.(10) A recent PET study in a cohort of familial dominant *LRRK2* mutation carriers,(11) showed increased expression of serotonin transporters, while serotonin transporter expression was reduced in *LRRK2* mutation carriers with manifest Parkinson's. About half of *LRRK2* mutation carriers, however, do not show the classical Lewy body pathology,(12) and therefore, it is challenging to associate changes in the serotonergic system detected *in vivo* with Parkinson's pathology in the absence of histopathological data.

One of the major challenges of Parkinson's research is the ability to study premotor pathology *in vivo*. Although Braak and colleagues have suggested a large premotor period, which may be as lengthy as the symptomatic;(2) identification of this period in clinic has been proven challenging. Autosomal dominant and highly penetrant familial forms of Parkinson's disease, which present with a similar phenotype to idiopathic cases, provide an ideal population to study *in vivo* in order to understand premotor stages and the evolution of Parkinson's disease progression. Of the several mutated genes associated with familial forms of Parkinson's, the point mutation A53T (p.Ala53Thr, c.209G>A) in the *SNCA* gene was the first mutation identified in an autosomal dominant pedigree of Italian and Greek families and was associated with the development of Parkinson's disease. (13) Carriers of the A53T *SNCA* mutation

typically present with Parkinson's symptoms which are indistinguishable from idiopathic cases,(14, 15), however motor symptoms commonly manifest early, have rapid progression, and are often associated with cognitive impairment.(16-19) Furthermore, histopathological studies have demonstrated classical Lewy body pathology in A53T *SNCA* mutation carriers.(20)

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

Methods

Study design and participants

This is a cross-sectional study that included seven premotor A53T *SNCA* mutation carriers, seven A53T *SNCA* mutation carriers with a Parkinson's disease diagnosis, 25 healthy controls, and two cohorts of 25 and 40 idiopathic Parkinson's disease patients (table 1). Parkinson's disease diagnosis, for both idiopathic patients and A53T *SNCA* mutation carriers, was determined according to the UK Brain Bank diagnostic criteria. A53T *SNCA* carriers and idiopathic Parkinson's disease patients (cohort-1) were recruited between September 2016 and September 2018. Data from the second cohort of 40 idiopathic Parkinson's disease patients were retrieved from our electronic database and was added to investigate whether serotonergic dysfunction, assessed with [^{11}C]DASB PET, could be used a marker of disease burden across a second population of Parkinson's patients scanned on a different PET scanner. Healthy individuals, age matched for A53T *SNCA* carriers, served as the control group. Within the cohort of A53T *SNCA* mutation carriers only two, one premotor and one with manifest Parkinson's disease, were related by blood. The study was approved by the institutional review boards and the research ethics committee. Permission to use radioactive substances was obtained by the Radioactive Substances Advisory Committee (ARSAC), Department of Health

and Social Care, United Kingdom. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki.

Procedures

All participants underwent a battery of clinical assessment to assess motor and non-motor symptoms and cognitive status (supplemental materials). Fourteen A53T *SNCA* carriers, 25 idiopathic Parkinson's patients and 25 healthy controls underwent [^{11}C]DASB PET, [^{123}I]FP-CIT SPECT and a 3-Tesla MRI scan. PET imaging assessments were performed on a Siemens Biograph Hi-Rez 6 PET-CT scanner (Erlangen, Germany), and MR imaging was acquired with a 32-channel head coil on a Siemens Magnetom TrioTim syngo MR B17 (Erlangen, Germany), performed at Invicro LLC, UK. An additional second cohort of 40 idiopathic Parkinson's disease patients with [^{11}C]DASB PET were included; and these patients were scanned using a GE Discovery RX PET/CT scanner and MR imaging acquired using a 3-Tesla Siemens Magnetom Avanto. Full acquisition parameters are outlined in the supplemental material. For all idiopathic Parkinson's disease patients and A53T *SNCA* Parkinson's patients, all PET and SPECT imaging was performed in an "OFF" medication state and following an overnight withdraw of their normal medications.

[^{11}C]DASB PET data processing and kinetic modelling was carried out using the Molecular Imaging and Kinetic Analysis Toolbox version 4·2·6 (MIAKAT™, Invicro LLC, London), implemented in MATLAB® version r2015a (The Mathworks, Natick, MA, USA). [^{123}I]FP-CIT SPECT images were reconstructed using the HERMES Hybrid Recon™-Neurology software, and BRASS™ was used for the semi-quantification of striatal specific binding ratio (supplemental materials).

Regions-of-interest were defined using the multi-atlas propagation with enhanced registration (MAPER) to automatically segmented individual subjects' T1 MRI into 95 anatomic regions.(21) Individual subjects' MAPER atlas and manual regions-of-interest were overlaid on co-registered PET data and sampled in ANALYZE medical imaging software (version 12·0, Mayo Foundation AnalyzeDirect). First, we quantified [^{11}C]DASB BP_{ND} in regions-of-interest across cohorts; we then investigated the spread of pathology according to Braak's histopathological staging,(2) for SNCA pathology (table S1). [^{11}C]DASB BP_{ND} values for each Braak stage were extracted, from [^{11}C]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 standard deviations from the control mean. Regions where further categorized into groups according to their anatomical location, by grouping frontal, temporal, occipital, parietal, insula and subcortical regions depending on the regions within each Braak stage (table S2). The number of groups, within each stage, with one or two standard deviations from the control mean was considered for grading the severity of serotonin pathology (table S3).

FreeSurfer image analysis suite (version 5·3·0) was used to derive measures of cortical thickness and subcortical deep grey matter nuclei volumes. Additionally, voxel-based morphometry, implemented in SPM12 (Wellcome Department of Cognitive Neurology, London, UK), was used to assess subcortical grey matter intensity differences as a measure of grey matter atrophy.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Science version 23·0 (SPSS, Inc, Chicago, IL, USA) and graph illustration with GraphPad Prism (version 7·0c). For all variables, variance homogeneity and Gaussianity were tested with Bartlett and Kolmogorov-Smirnov tests. We proceeded with parametric tests as our imaging and clinical data were normally distributed. Multivariate analysis of covariance (MANOVA) was used to assess group differences in clinical, PET and MR imaging data. If the overall multivariate test was significant, two-tailed exact t-tests were used for between-group comparisons in each imaging modality in predefined regions-of-interest and p-values for each variable were calculated following 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 reduce false discovery rate. The false discovery rate cut-off was set at 0·05. Cohorts of idiopathic Parkinson's disease patients were older compared to healthy controls and A53T *SNCA* mutation carriers, and there were gender differences across the group; therefore, age and gender were used as covariates in the MANOVA to assess group differences in PET and MR imaging data. All data are presented as mean ±SD, and the level α was set for all comparisons at $p<0·05$.

Role of the funding source

The funder 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

Fourteen A53T *SNCA* carriers were recruited between September 2016 and September 2018. A53T *SNCA* carriers were recruited from specialist Movement Disorders clinics at the University of Athens, Greece, and the University of Salerno, Italy. Twenty-five idiopathic Parkinson's disease patients (cohort-1) were recruited from specialist Movement Disorders clinics at King's College Hospital, London, UK. Twenty-five healthy controls were recruited through public advertisement. All participants travelled to King's College London, UK, for clinical assessments and to Invicro, LLC, UK, for PET and MR imaging assessments; all assessments were performed within three weeks. Clinical, PET and MR imaging data of idiopathic Parkinson's disease (cohort-2) were retrieved from our electronic database.

A53T *SNCA* mutation carriers were subdivided into two subgroups according to the presence (A53T *SNCA* Parkinson's disease) or absence (premotor A53T *SNCA*) of a Parkinson's disease diagnosis, as defined by MDS PD Criteria.(22) The absence of motor symptoms in premotor A53T *SNCA* was confirmed with a 24-hour continuous recording of their mobility for seven days, using automated wrist-worn devices in both sides (figure S1). Whereas, measures obtained in A53T *SNCA* Parkinson's disease patients presented with cardinal motor symptoms of Parkinson's disease (figure S2).

There were no differences in age between the cohorts of A53T *SNCA* carriers compared to healthy controls; while the cohorts of idiopathic Parkinson's patients were significantly older compared to the healthy controls and cohorts of A53T *SNCA* carriers (table 1). UPDRS total scores were higher in the cohorts of A53T *SNCA* carriers and in the cohorts of idiopathic Parkinson's patients compared to the healthy controls. Non-motor symptoms, including UPSIT, SCOPA-AUT, NMSS, BDI-II were increased in A53T *SNCA* Parkinson's disease compared to healthy controls; while premotor A53T *SNCA* showed no significant differences compared to healthy controls (table 1). Within the group of A53T *SNCA* Parkinson's disease only three subjects had high depression levels (BDI-II scores $\geq$ 17),(23) which may be of clinical significance. While premotor A53T *SNCA* did not show significant increases in total non-motor symptom burden, three premotor A53T *SNCA* carriers had NMSS total scores between 9-13 suggesting the development of early mild non-motor symptoms. The cohort of A53T *SNCA* Parkinson's disease, but not premotor A53T *SNCA*, showed lower scores in global measures of cognitive performance, MoCA and MMSE, compared to healthy controls (table 1).

Premotor A53T *SNCA* exhibited no differences in [^{123}I]FP-CIT striatal specific binding ratio ($p>0{\cdot}10$), whilst A53T *SNCA* Parkinson's disease patients showed loss of [^{123}I]FP-CIT striatal specific binding ratio compared to healthy controls ($p<0{\cdot}001$; table 2, figure 1). Compared to idiopathic Parkinson's disease, A53T *SNCA* Parkinson's disease patients showed greater loss of [^{123}I]FP-CIT caudate specific binding ratio (left caudate: $p=0{\cdot}049$; right caudate $p=0{\cdot}025$) but no differences in [^{123}I]FP-CIT putamen specific binding ratio (left putamen: $p=0{\cdot}47$; right putamen: $p=0{\cdot}50$; table S5).

Premotor A53T *SNCA* showed decreased [^{11}C]DASB BP_{ND} in the ventral ($p<0{\cdot}001$) and dorsal raphe nuclei ($p<0{\cdot}001$), caudate ($p<0{\cdot}001$), putamen ($p=0{\cdot}036$), thalamus ($p=0{\cdot}001$), hypothalamus ($p<0{\cdot}001$), amygdala ($p=0{\cdot}004$) and the brainstem ($p=0{\cdot}046$) compared to healthy controls ($F(8,17)=17{\cdot}327$, $p<0{\cdot}001$; table 2; figure 1). A53T *SNCA* Parkinson's disease showed additional [^{11}C]DASB BP_{ND} decreases in the hippocampus ($p=0{\cdot}005$), anterior ($p=0{\cdot}022$) and posterior cingulate ($p=0{\cdot}036$), insula ($p=0{\cdot}005$) and in frontal ($p=0{\cdot}002$), temporal ($p=0{\cdot}001$) and occipital cortex ($p=0{\cdot}005$) compared to healthy controls ($F(8,17)=3{\cdot}073$, $p=0{\cdot}025$; table 2, table S4; figure 1). The severity of serotonergic loss in premotor A53T *SNCA* was in line with reductions in idiopathic Parkinson's patients, while A53T *SNCA* Parkinson's disease showed greater loss of [^{11}C]DASB BP_{ND} in the putamen ($p=0{\cdot}005$), caudate ($p=0{\cdot}004$), hypothalamus ($p<0{\cdot}001$) and amygdala ($p=0{\cdot}004$) compared to idiopathic Parkinson's disease patients (table S5).

Having demonstrated the presence of serotonergic pathology in premotor and Parkinson's disease A53T *SNCA*, we proceeded to investigate topographic reductions of [^{11}C]DASB BP_{ND} in relation to Braak's histopathological grading of Lewy bodies and neurites pathology,(2) by constructing [^{11}C]DASB BP_{ND} maps reflecting Braak stages one to six (table S1 and table S2). Premotor *A53T* SNCA had loss of [^{11}C]DASB BP_{ND} in brain areas corresponding to Braak stages 1-3, whereas [^{11}C]DASB BP_{ND} was largely preserved in areas corresponding to Braak stages 4-6. SNCA14 had a MoCA score of 28 and an MMSE score of 29 and there was no indication of subtle cognitive or behavioural changes. However, SNCA01 had a MoCA score of 23 and an MMSE score of 29, and there were mild changes in the visuospatial/executive cognitive function and working memory as indicated by the MoCA subitem scores. With the exception of a recently diagnosed subject with Parkinson's disease, A53T *SNCA* Parkinson's subjects had [^{11}C]DASB BP_{ND} decreases in brain areas corresponding to Braak stages 1-6 (figure 2).

To assess whether serotonergic dysfunction could be a marker of disease burden, we looked for associations between [^{11}C]DASB BP_{ND} across the brain and MDS-UPDRS total scores. In the cohort of A53T *SNCA* carriers, reduced brainstem [^{11}C]DASB BP_{ND} correlated with higher total UPDRS (n=14; r=-0·66; p=0·009; 95% CI -0·88 to -0·20; figure 3A). Reduced brainstem [^{11}C]DASB BP_{ND} correlated with higher total UPDRS also within the subgroups of premotor A53T *SNCA* (n=7; r=-0·75; p=0·049; 95% CI -0·96 to -0·004; figure S3A) and A53T *SNCA* Parkinson's disease (n=7; r=-0·76; p=0·049; 95% CI -0·96 to -0·005; figure S3B). Similarly, in the cohort of idiopathic Parkinson's disease patients (n=25), reduced brainstem [^{11}C]DASB BP_{ND} correlated with higher total UPDRS (r=-0·66; p=0·0003; 95% CI -0·84 to -0·36; figure 3B). We then wanted to test the applicability of these findings to a different cohort of idiopathic Parkinson's disease patients (n=40), who were scanned previously with [^{11}C]DASB PET in a different scanner. We found that also in this cohort, reduced brainstem [^{11}C]DASB BP_{ND} correlated with higher total UPDRS (r=-0·71; p<0·0001; 95% CI -0·84 to -0·52; figure 3C). We noted that as the sample size increased the correlation became stronger. Furthermore, reduced brainstem [^{11}C]DASB BP_{ND} correlated with lower [^{11}C]DASB BP_{ND} in regions reflecting Braak stage 1 (r=0·87; p<0·0001; 95% CI 0·64 to 0·96; figure S4A), Braak stage 2 (r=0·90; p<0·0001; 95% CI 0·71 to 0·97; figure S4B) and Braak stage 3 (r=0·88; p<0·0001; 95% CI 0·66 to 0·96; figure S4C).

We investigated whether there was a relationship between [^{11}C]DASB BP_{ND} with cognitive impairment and non-motor symptoms. In the cohort of A53T *SNCA*, lower MoCA scores correlated with reduced [^{11}C]DASB BP_{ND} in Braak stage 4 (r=0·63; p=0·017; 95% CI 0·14 to 0·87; figure 4A) and with reduced [^{11}C]DASB BP_{ND} in Braak stage 5 (r=0·61; p=0·022; 95% CI 0·11 to 0·86; figure 4B). No correlations were found between regional [^{11}C]DASB BP_{ND} and SCOPA-AUT or UPSIT scores. Reduced brainstem [^{11}C]DASB BP_{ND} correlated with higher NMSS total scores in the cohort of A53T *SNCA* (n=14; r=-0·77; p=0·0042; 95% CI -0·90 to -0·29; figure S5A), and in subgroups of premotor A53T *SNCA* (n=7; r=-0·78; p=0·040; 95% CI -0·97 to -0·055; figure S5B) and A53T *SNCA* Parkinson's disease (n=7; r=-0·76; p=0·047; 95% CI -0·96 to -0·016; figure S5C). FreeSurfer and voxel-based morphometry cortical thickness and subcortical volumetric analysis revealed no atrophy (supplemental results, tables S6-S8, figure S6).

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 with idiopathic Parkinson's disease patients and healthy controls. Half of the cohort of the A53T *SNCA* mutation carriers was at the premotor stage which was confirmed clinically and with the aid from 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 with *in vivo* molecular imaging in patients at risk for Parkinson's disease, precedes the development of motor symptoms and the visualisation of dopaminergic pathology. Moreover, 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 in raphe nuclei, brainstem, striatum, thalamus, hypothalamus and amygdala. A53T *SNCA* Parkinson's disease patients had loss of striatal dopamine transporters, and loss of serotonin transporters extended to further subcortical (e.g. cingulate, insula) and cortical regions. Our findings indicate that premotor A53T *SNCA* with normal visualisation of dopamine transporters show an average of 34% loss of serotonin transporters in raphe nuclei and 22% loss in the striatum. In A53T *SNCA* Parkinson's disease patients the serotonin transporters losses are 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* Parkinson's disease patients showed greater loss of dopamine transporters in the caudate, while there were no differences in the putaminal binding ratios, compared with idiopathic Parkinson's disease. Furthermore, the severity of serotonin transporter loss in premotor A53T *SNCA* carriers was in line with reductions observed in idiopathic Parkinson's patients, while A53T *SNCA* Parkinson's disease patients showed even greater loss of serotonin transporters. Combined these findings suggest similarities in the pathophysiology between idiopathic Parkinson's disease and A53T *SNCA* Parkinson's disease but with a faster progression in A53T *SNCA* mutation carriers.

In a previous [^{11}C]DASB PET study in idiopathic Parkinson's disease,(5) we have contemplated that serotonergic pathology could be an early phenomenon in the course of the disease, though it evolves at a slower pace compared to dopaminergic pathology. Additional [^{11}C]DASB PET studies in idiopathic Parkinson's disease have demonstrated an association of serotonergic pathology with non-motor symptoms such as fatigue,(8) depression,(10) and

sleep,(9) and motor symptoms and complications such as tremor,(6) and levodopa-induced dyskinesias.(7) On the contrary dopaminergic markers correlate well with the symptoms of rigidity and bradykinesia which are also responding 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 through the rostral and caudal pathways innervate a very large part of the brain, while modulating a large number of physiological functions.(26) Similarly, Braak and colleagues,(2) have described with histopathology the distribution of Lewy body and neurite spread, in tissue of Parkinson's brains, which follows closely 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 impact on serotonin transporters by generating a negative modulation and reducing its cell-surface availability.(27) The influence of SNCA on serotonin transporter arises through a direct binding between the two proteins, predominantly involving the non-amyloidogenic component domain of SNCA. This is particularly interesting as the A53T mutation, which has drastically increased aggregation kinetics, may hinder the ability of SNCA to form α-helices, thus promoting β-sheet configuration and SNCA aggregation. This could lead 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 [^{11}C]DASB binding potential loss, reflecting serotonergic pathology, with the distribution of Lewy body and neurite pathology. We went on to construct brain topographic maps reflecting Braak stages 1-6 and used these as seed maps to calculate [^{11}C]DASB binding potential in the cohort of A53T *SNCA* carriers. In line with Braak, premotor A53T SNCA carriers showed serotonergic pathology in brain areas corresponding to stages 1-3, whereas [^{11}C]DASB binding potential was largely preserved in brain areas corresponding to stages 4-6. Interestingly, the youngest premotor A53T *SNCA* carriers (SNCA05 and SNCA06), showed extensive loss of [^{11}C]DASB binding potential in areas corresponding to stages 1 and 2 and only partial loss in areas corresponding to stage 3. Furthermore, A53T *SNCA* Parkinson's disease patients showed serotonergic pathology in brain areas corresponding to stages 4-6. SNCA09 who had very recently been diagnosed with Parkinson's disease showed minimal loss of [^{11}C]DASB binding potential in areas corresponding to stage 4, whereas [^{11}C]DASB binding potential was largely preserved in brain areas corresponding to stages 5 and 6.

If loss of [^{11}C]DASB binding potential in the Parkinson's brain, reflecting 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 there should be an association between loss of [^{11}C]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 correlated with total UPDRS scores, which captures the overall burden of the disease including both motor and non-motor symptoms. This correlation was also present in both subgroups of premotor and manifest Parkinson's A53T *SNCA* suggesting that the correlation between brainstem serotonergic pathology and overall Parkinson's burden was driven by both premotor and manifest Parkinson's A53T *SNCA* carriers. In order 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, one of which scanned on a different scanner. In both occasions the correlation remained true, and we noted that by increasing the sample size the significance of correlation was becoming stronger. This highlights the potential applicability of our findings from A53T *SNCA* carriers into patients with idiopathic Parkinson's disease and suggests the potential application of brainstem [^{11}C]DASB PET as a marker of disease burden across different scanners and sites. This preliminary evidence could be useful for future multi-centre studies and highlights the need for further studies to investigate brainstem [^{11}C]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 symptom in idiopathic Parkinson's disease, marked 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 [^{11}C]DASB in the brainstem was associated with higher global burden of non-motor symptoms; this correlation was present also in both subgroups of premotor and manifest Parkinson's A53T *SNCA* carriers. Therefore, suggesting that brainstem serotonergic pathology may be preceding the gradual development of non-motor symptom burden. Our findings are in line with previous studies in idiopathic Parkinson's disease supporting a link between non-motor symptoms and serotonergic pathology.(8-10) We did not have enough power in the present study to investigate the relationship between [^{11}C]DASB binding with depression levels in A53T *SNCA* carriers. We

did not find any association between [^{11}C]DASB binding and dysautonomic or olfactory symptoms; suggesting other neurotransmitter systems, such as the noradrenergic system, may 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 with serotonergic pathology in the temporal mesocortex and allocortex (Braak stage 4) presented with subtle cognitive deficits, in visuospatial/executive cognitive function and working memory. Therefore, suggesting 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 demonstrated the presence of amyloid-β and tau neurofibrillary tangles in Parkinson's cases 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*.

In conclusion, the combined use of thorough clinical observation with molecular imaging, which encompasses nanomolar sensitivity, and the study of A53T *SNCA* carriers, related to a gene mutation 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. Our findings provide the first to our knowledge *in vivo* imaging data that corroborate the Braak staging scheme, in terms of showing a neurotransmitter deficit corresponding to stage 2 antedating the dopaminergic deficit that occurs in stage 3. Although PET molecular imaging is expensive and A53T *SNCA* carriers rare, our study highlights the potential to extend findings in A53T *SNCA* carriers to classic forms of idiopathic Parkinson's disease, which is the second most common neurodegenerative disorder. 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. While our community is in the pursuit to identify reliable markers sensitive to disease progression, and also to identify candidates at risk for 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 there is evidence of a dopaminergic deficit or 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 provide a useful tool to detect longitudinal changes in A53T

SNCA carriers. Future studies are warranted to evaluate 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 F-18 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. Future work could allow for the development of serotonin transporter imaging into an adjunctive tool for screening and monitoring progression for individuals at risk or patients with Parkinson's disease, to complement existing molecular imaging tools such as dopaminergic imaging, and could serve as a sensitive marker of Parkinson's burden.

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