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1 **Serotonergic pathology linked with the premotor phase of A53T  $\alpha$ -synuclein**  
2 **parkinsonism and with disease burden: cross-sectional studies**

3

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36

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39 MPi, SP, BG, MS, RNG, EAR, MTP, PB and LS organised the study. GD, GP, CK, MPi, SP,  
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41 the assessments. HW, GD, GP and TY collected the data. HW, GD, GP, TY, ZC, and BC  
42 analysed the data. MP generated the figures. HW and MP wrote the first draft of the manuscript.  
43 All authors contributed in data interpretation, reviewed and gave input to the manuscript.

44

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## 83 **Abstract**

84 **Background:** Due to the highly penetrant gene mutation and the clinical features consistent  
85 with idiopathic Parkinson's disease, carriers of the autosomal dominant A53T (p.Ala53Thr,  
86 c.209G>A) point mutation in the  $\alpha$ -synuclein gene (*SNCA*) represent an ideal population to  
87 study the premotor phase and evolution of Parkinson's pathology. Given the known  
88 neurochemical changes in the serotonergic system and their association with symptoms of  
89 Parkinson's disease, we hypothesised that A53T *SNCA* mutation carriers might show  
90 abnormalities in the serotonergic neurotransmitter system before the diagnosis of Parkinson's  
91 disease, and that this pathology may be associated with measures of Parkinson's burden.

92 **Methods:** Between September 2016 and September 2018, we recruited 14 A53T *SNCA*  
93 mutation carriers (seven premotor without Parkinson's disease). We compared their data with  
94 two cohorts of 25 and 40 patients with idiopathic Parkinson's disease, and a cohort of 25  
95 healthy controls. [ $^{11}\text{C}$ ]DASB PET non-displaceable binding ( $\text{BP}_{\text{ND}}$ ) was used to quantify  
96 serotonin transporter density. We constructed brain topographic maps reflecting Braak stages  
97 1-6 and used these as seed maps to calculate [ $^{11}\text{C}$ ]DASB  $\text{BP}_{\text{ND}}$  in the cohort of A53T *SNCA*  
98 carriers. In addition, all participants underwent a battery of clinical assessments, [ $^{123}\text{I}$ ]FP-CIT  
99 SPECT to assess striatal dopamine transporter binding and MRI for volumetric analyses.

100 **Findings:** Seven-day continuous recording of motor function confirmed the absence of motor  
101 symptoms and [ $^{123}\text{I}$ ]FP-CIT SPECT the absence of striatal dopaminergic deficits in premotor  
102 A53T *SNCA* carriers ( $p>0.10$ ). Premotor A53T *SNCA* carriers showed loss of [ $^{11}\text{C}$ ]DASB  
103  $\text{BP}_{\text{ND}}$  in the raphe nuclei ( $p<0.001$ ), caudate ( $p<0.001$ ), putamen ( $p=0.036$ ), thalamus  
104 ( $p=0.001$ ), hypothalamus ( $p<0.001$ ), amygdala ( $p=0.004$ ) and brainstem ( $p=0.046$ ), which was  
105 extended to hippocampus ( $p=0.005$ ), anterior ( $p=0.022$ ) and posterior cingulate ( $p=0.036$ ),  
106 insula ( $p=0.005$ ), frontal ( $p=0.002$ ), parietal ( $p=0.019$ ), temporal ( $p=0.001$ ) and occipital  
107 ( $p=0.005$ ) cortices in A53T *SNCA* Parkinson's disease. A53T *SNCA* Parkinson's disease  
108 patients showed a loss of striatal [ $^{123}\text{I}$ ]FP-CIT specific binding ratio ( $p<0.001$ ). Premotor A53T  
109 *SNCA* had loss of [ $^{11}\text{C}$ ]DASB  $\text{BP}_{\text{ND}}$  in brain areas corresponding to Braak stages 1-3, whereas  
110 [ $^{11}\text{C}$ ]DASB  $\text{BP}_{\text{ND}}$  was largely preserved in areas corresponding to Braak stages 4-6. With the  
111 exception of a recently diagnosed subject with Parkinson's disease, A53T *SNCA* Parkinson's  
112 subjects had [ $^{11}\text{C}$ ]DASB  $\text{BP}_{\text{ND}}$  decreases in brain areas corresponding to Braak stages 1-6.  
113 [ $^{11}\text{C}$ ]DASB  $\text{BP}_{\text{ND}}$  decreases in brainstem were associated with increased MDS-UPDRS total  
114 scores in A53T *SNCA* carriers ( $r=-0.66$ ;  $p=0.0003$ ; 95% CI -0.84 to -0.36), idiopathic  
115 Parkinson's patients ( $r=-0.71$ ;  $p<0.0001$ ; 95% CI -0.84 to -0.52), and a second cohort of

116 idiopathic Parkinson's patients scanned on a different scanner ( $r=-0.71$ ;  $p<0.0001$ ; 95% CI -  
117 0.84 to -0.52).

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

128 **Funding:** The study was funded by the Lily Safra Hope Foundation and by the National  
129 Institute for Healthy Research (NIHR) Biomedical Research Centre at King's College London.

## 130 **Research in context**

131 **Evidence before this study:** We reviewed current literature on familial Parkinson's disease,  
132 A53T  $\alpha$ -synuclein (*SNCA*) and related neuropathology by searching PubMed on 2<sup>nd</sup> October  
133 2018, for published articles containing the search terms "familial Parkinson's disease", "A53T  
134  $\alpha$ -synuclein", "p.A53T  $\alpha$ -synuclein", "positron-emission tomography", "magnetic resonance  
135 imaging", "alpha-synuclein", "serotonin transporter, SERT, or "DASB", "dopamine  
136 transporter, or DAT". To-date, the majority of neuroimaging studies on familial Parkinson's  
137 disease have focused on the most common monogenic forms, such as the Leucine Rich Repeat  
138 Kinase (*LRRK2*). Neuroimaging studies in A53T *SNCA* familial Parkinson's have focused on  
139 assessing striatal dopaminergic function in individual case reports and small cohorts of A53T  
140 *SNCA* carriers. Studies in idiopathic Parkinson's disease report early loss of serotonin  
141 transporter availability associated with motor and non-motor symptoms. In familial  
142 Parkinson's disease, serotonin transporter has only been investigated *in vivo* in *LRRK2*  
143 mutation carriers. The expression of serotonin transporters was increased in *LRRK2* mutation  
144 carriers without manifest Parkinson's disease, while serotonin transporter expression was  
145 reduced in *LRRK2* mutation carriers with Parkinson's disease.

146 **Added value of this study:** To our knowledge, this is the first study to assess serotonergic and  
147 dopaminergic pathology in A53T *SNCA* gene mutation carriers *in vivo* to elucidate the  
148 pathophysiology underlying Parkinson's disease. Premotor A53T *SNCA* carriers, presented  
149 with normal motor and striatal dopaminergic function; while striatal dopaminergic dysfunction  
150 becomes exclusively prominent in A53T *SNCA* carriers with Parkinson's disease. All A53T  
151 *SNCA* carriers, premotor and with a Parkinson's diagnosis, exhibited serotonergic pathology,  
152 with patterns consistent with Braak's histopathological staging showing caudal to rostral  
153 ascending progression. Furthermore, we demonstrate brainstem serotonergic pathology,  
154 measured with [<sup>11</sup>C]DASB PET, as an *in vivo* marker of total disease burden.

155 **Implications of all the available evidence:** Serotonergic pathology is present in premotor  
156 A53T *SNCA* carriers, prior to striatal dopaminergic loss; highlighting the very early role of  
157 serotonergic pathology in the progression of Parkinson's disease. Our findings highlight that  
158 measuring serotonergic integrity may serve as a useful *in vivo* tool to identify individuals at  
159 risk before there is evidence of a dopaminergic deficit, preceding disease onset by many years;  
160 thus, such a measurement could serve as a sensitive marker of Parkinson's burden. Differing  
161 patterns of serotonergic and dopaminergic pathology across familial forms of Parkinson's  
162 disease suggests that distinct pathologies underlie different phenotypes of Parkinson's disease.

163 The classification of Parkinson's based on different pathological phenotypes, assessed *in vivo*,  
164 could lead to a more targeted therapeutic approach.



## 165 **Introduction**

166 The neuropathology of Parkinson's disease is characterised by the presence of  $\alpha$ -synuclein  
167 (SNCA) aggregates, which form the main components of Lewy bodies and neurites.(1)  
168 According to Braak's histopathological staging, Lewy pathology spreads in a gradual  
169 ascending fashion, starting from the olfactory nucleus and the medulla in premotor stages and  
170 spreading to subcortical and cortical areas at later stages of the disease,(2) affecting both  
171 dopaminergic and non-dopaminergic containing neurons, such as the serotonergic neurons.(3)  
172 Neuropathological studies demonstrated involvement of serotonergic neurons in idiopathic  
173 Parkinson's disease,(4) associated with the presence of Lewy pathology within the raphe nuclei  
174 at early disease stages,(2) suggesting that caudal serotonergic brainstem neurons may be  
175 affected prior to dopaminergic neurons in the midbrain, as the disease evolves. However, to  
176 date, there has been no proof provided for this concept, in particular in an *in vivo* context.

177 The PET radioligand [<sup>11</sup>C]DASB, which is selective for the serotonin transporter, has been  
178 employed to study presynaptic serotonergic terminal integrity in idiopathic Parkinson's  
179 disease. Idiopathic Parkinson's patients show early progressive loss of serotonergic  
180 function,(5) which has been associated with the development of motor and non-motor  
181 symptoms and complications such as tremor,(6) dyskinesias,(7) fatigue,(8) sleep(9) and  
182 depression.(10) A recent PET study in a cohort of familial dominant *LRRK2* mutation  
183 carriers,(11) showed increased expression of serotonin transporters, while serotonin transporter  
184 expression was reduced in *LRRK2* mutation carriers with manifest Parkinson's. About half of  
185 *LRRK2* mutation carriers, however, do not show the classical Lewy body pathology,(12) and  
186 therefore, it is challenging to associate changes in the serotonergic system detected *in vivo* with  
187 Parkinson's pathology in the absence of histopathological data.

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

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

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

211

## 212 **Methods**

### 213 **Study design and participants**

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

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

## 231 **Procedures**

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

245 [<sup>11</sup>C]DASB PET data processing and kinetic modelling was carried out using the Molecular  
246 Imaging and Kinetic Analysis Toolbox version 4.2.6 (MIAKAT<sup>TM</sup>, Invicro LLC, London),  
247 implemented in MATLAB<sup>®</sup> version r2015a (The Mathworks, Natick, MA, USA). [<sup>123</sup>I]FP-CIT  
248 SPECT images were reconstructed using the HERMES Hybrid Recon<sup>TM</sup>-Neurology software,  
249 and BRASS<sup>TM</sup> was used for the semi-quantification of striatal specific binding ratio  
250 (supplemental materials).

251 Regions-of-interest were defined using the multi-atlas propagation with enhanced registration  
252 (MAPER) to automatically segmented individual subjects' T1 MRI into 95 anatomic  
253 regions.(21) Individual subjects' MAPER atlas and manual regions-of-interest were overlaid  
254 on co-registered PET data and sampled in ANALYZE medical imaging software (version 12.0,  
255 Mayo Foundation AnalyzeDirect). First, we quantified [<sup>11</sup>C]DASB BP<sub>ND</sub> in regions-of-interest  
256 across cohorts; we then investigated the spread of pathology according to Braak's  
257 histopathological staging,(2) for *SNCA* pathology (table S1). [<sup>11</sup>C]DASB BP<sub>ND</sub> values for each  
258 Braak stage were extracted, from [<sup>11</sup>C]DASB parametric maps, taking region-volume-  
259 weighted averages for individual A53T *SNCA* carriers and healthy controls. For each Braak  
260 stage, the presence of serotonergic pathology was graded in each anatomical region as one or

261 two standard deviations from the control mean. Regions were further categorized into groups  
262 according to their anatomical location, by grouping frontal, temporal, occipital, parietal, insula  
263 and subcortical regions depending on the regions within each Braak stage (table S2). The  
264 number of groups, within each stage, with one or two standard deviations from the control  
265 mean was considered for grading the severity of serotonin pathology (table S3).

266 FreeSurfer image analysis suite (version 5.3.0) was used to derive measures of cortical  
267 thickness and subcortical deep grey matter nuclei volumes. Additionally, voxel-based  
268 morphometry, implemented in SPM12 (Wellcome Department of Cognitive Neurology,  
269 London, UK), was used to assess subcortical grey matter intensity differences as a measure of  
270 grey matter atrophy.

### 271 **Statistical analysis**

272 Statistical analysis was performed with Statistical Package for Social Science version 23.0  
273 (SPSS, Inc, Chicago, IL, USA) and graph illustration with GraphPad Prism (version 7.0c). For  
274 all variables, variance homogeneity and Gaussianity were tested with Bartlett and  
275 Kolmogorov-Smirnov tests. We proceeded with parametric tests as our imaging and clinical  
276 data were normally distributed. Multivariate analysis of covariance (MANOVA) was used to  
277 assess group differences in clinical, PET and MR imaging data. If the overall multivariate test  
278 was significant, two-tailed exact t-tests were used for between-group comparisons in each  
279 imaging modality in predefined regions-of-interest and p-values for each variable were  
280 calculated following Bonferroni's multiple comparisons test. We interrogated correlations  
281 between PET and clinical data using Pearson's *r* correlation coefficient and applied Benjamini-  
282 Hochberg correction to reduce false discovery rate. The false discovery rate cut-off was set at  
283 0.05. Cohorts of idiopathic Parkinson's disease patients were older compared to healthy  
284 controls and A53T *SNCA* mutation carriers, and there were gender differences across the group;  
285 therefore, age and gender were used as covariates in the MANOVA to assess group differences  
286 in PET and MR imaging data. All data are presented as mean  $\pm$ SD, and the level  $\alpha$  was set for  
287 all comparisons at  $p < 0.05$ .

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

292

## 293 **Results**

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

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

310 There were no differences in age between the cohorts of A53T *SNCA* carriers compared to  
311 healthy controls; while the cohorts of idiopathic Parkinson's patients were significantly older  
312 compared to the healthy controls and cohorts of A53T *SNCA* carriers (table 1). UPDRS total  
313 scores were higher in the cohorts of A53T *SNCA* carriers and in the cohorts of idiopathic  
314 Parkinson's patients compared to the healthy controls. Non-motor symptoms, including  
315 UPSIT, SCOPA-AUT, NMSS, BDI-II were increased in A53T *SNCA* Parkinson's disease  
316 compared to healthy controls; while premotor A53T *SNCA* showed no significant differences  
317 compared to healthy controls (table 1). Within the group of A53T *SNCA* Parkinson's disease  
318 only three subjects had high depression levels (BDI-II scores  $\geq 17$ ),(23) which may be of  
319 clinical significance. While premotor A53T *SNCA* did not show significant increases in total  
320 non-motor symptom burden, three premotor A53T *SNCA* carriers had NMSS total scores  
321 between 9-13 suggesting the development of early mild non-motor symptoms. The cohort of  
322 A53T *SNCA* Parkinson's disease, but not premotor A53T *SNCA*, showed lower scores in global  
323 measures of cognitive performance, MoCA and MMSE, compared to healthy controls (table  
324 1).

325 Premotor A53T *SNCA* exhibited no differences in [<sup>123</sup>I]FP-CIT striatal specific binding ratio  
326 ( $p > 0.10$ ), whilst A53T *SNCA* Parkinson's disease patients showed loss of [<sup>123</sup>I]FP-CIT striatal  
327 specific binding ratio compared to healthy controls ( $p < 0.001$ ; table 2, figure 1). Compared to  
328 idiopathic Parkinson's disease, A53T *SNCA* Parkinson's disease patients showed greater loss  
329 of [<sup>123</sup>I]FP-CIT caudate specific binding ratio (left caudate:  $p = 0.049$ ; right caudate  $p = 0.025$ )  
330 but no differences in [<sup>123</sup>I]FP-CIT putamen specific binding ratio (left putamen:  $p = 0.47$ ; right  
331 putamen:  $p = 0.50$ ; table S5).

332 Premotor A53T *SNCA* showed decreased [<sup>11</sup>C]DASB BP<sub>ND</sub> in the ventral ( $p < 0.001$ ) and dorsal  
333 raphe nuclei ( $p < 0.001$ ), caudate ( $p < 0.001$ ), putamen ( $p = 0.036$ ), thalamus ( $p = 0.001$ ),  
334 hypothalamus ( $p < 0.001$ ), amygdala ( $p = 0.004$ ) and the brainstem ( $p = 0.046$ ) compared to  
335 healthy controls ( $F(8,17) = 17.327$ ,  $p < 0.001$ ; table 2; figure 1). A53T *SNCA* Parkinson's disease  
336 showed additional [<sup>11</sup>C]DASB BP<sub>ND</sub> decreases in the hippocampus ( $p = 0.005$ ), anterior  
337 ( $p = 0.022$ ) and posterior cingulate ( $p = 0.036$ ), insula ( $p = 0.005$ ) and in frontal ( $p = 0.002$ ),  
338 temporal ( $p = 0.001$ ) and occipital cortex ( $p = 0.005$ ) compared to healthy controls  
339 ( $F(8,17) = 3.073$ ,  $p = 0.025$ ; table 2, table S4; figure 1). The severity of serotonergic loss in  
340 premotor A53T *SNCA* was in line with reductions in idiopathic Parkinson's patients, while  
341 A53T *SNCA* Parkinson's disease showed greater loss of [<sup>11</sup>C]DASB BP<sub>ND</sub> in the putamen  
342 ( $p = 0.005$ ), caudate ( $p = 0.004$ ), hypothalamus ( $p < 0.001$ ) and amygdala ( $p = 0.004$ ) compared to  
343 idiopathic Parkinson's disease patients (table S5).

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

357 To assess whether serotonergic dysfunction could be a marker of disease burden, we looked  
358 for associations between [<sup>11</sup>C]DASB BP<sub>ND</sub> across the brain and MDS-UPDRS total scores. In  
359 the cohort of A53T *SNCA* carriers, reduced brainstem [<sup>11</sup>C]DASB BP<sub>ND</sub> correlated with higher  
360 total UPDRS (n=14; r=-0.66; p=0.009; 95% CI -0.88 to -0.20; figure 3A). Reduced brainstem  
361 [<sup>11</sup>C]DASB BP<sub>ND</sub> correlated with higher total UPDRS also within the subgroups of premotor  
362 A53T *SNCA* (n=7; r=-0.75; p=0.049; 95% CI -0.96 to -0.004; figure S3A) and A53T *SNCA*  
363 Parkinson's disease (n=7; r=-0.76; p=0.049; 95% CI -0.96 to -0.005; figure S3B). Similarly,  
364 in the cohort of idiopathic Parkinson's disease patients (n=25), reduced brainstem [<sup>11</sup>C]DASB  
365 BP<sub>ND</sub> correlated with higher total UPDRS (r=-0.66; p=0.0003; 95% CI -0.84 to -0.36; figure  
366 3B). We then wanted to test the applicability of these findings to a different cohort of idiopathic  
367 Parkinson's disease patients (n=40), who were scanned previously with [<sup>11</sup>C]DASB PET in a  
368 different scanner. We found that also in this cohort, reduced brainstem [<sup>11</sup>C]DASB BP<sub>ND</sub>  
369 correlated with higher total UPDRS (r=-0.71; p<0.0001; 95% CI -0.84 to -0.52; figure 3C).  
370 We noted that as the sample size increased the correlation became stronger. Furthermore,  
371 reduced brainstem [<sup>11</sup>C]DASB BP<sub>ND</sub> correlated with lower [<sup>11</sup>C]DASB BP<sub>ND</sub> in regions  
372 reflecting Braak stage 1 (r=0.87; p<0.0001; 95% CI 0.64 to 0.96; figure S4A), Braak stage 2  
373 (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;  
374 95% CI 0.66 to 0.96; figure S4C).

375 We investigated whether there was a relationship between [<sup>11</sup>C]DASB BP<sub>ND</sub> with cognitive  
376 impairment and non-motor symptoms. In the cohort of A53T *SNCA*, lower MoCA scores  
377 correlated with reduced [<sup>11</sup>C]DASB BP<sub>ND</sub> in Braak stage 4 (r=0.63; p=0.017; 95% CI 0.14 to  
378 0.87; figure 4A) and with reduced [<sup>11</sup>C]DASB BP<sub>ND</sub> in Braak stage 5 (r=0.61; p=0.022; 95%  
379 CI 0.11 to 0.86; figure 4B). No correlations were found between regional [<sup>11</sup>C]DASB BP<sub>ND</sub>  
380 and SCOPA-AUT or UPSIT scores. Reduced brainstem [<sup>11</sup>C]DASB BP<sub>ND</sub> correlated with  
381 higher NMSS total scores in the cohort of A53T *SNCA* (n=14; r=-0.77; p=0.0042; 95% CI -  
382 0.90 to -0.29; figure S5A), and in subgroups of premotor A53T *SNCA* (n=7; r=-0.78; p=0.040;  
383 95% CI -0.97 to -0.055; figure S5B) and A53T *SNCA* Parkinson's disease (n=7; r=-0.76;  
384 p=0.047; 95% CI -0.96 to -0.016; figure S5C). FreeSurfer and voxel-based morphometry  
385 cortical thickness and subcortical volumetric analysis revealed no atrophy (supplemental  
386 results, tables S6-S8, figure S6).

387

388

## 389 **Discussion**

390 In this cross-sectional study we assessed molecular, structural and clinical markers of  
391 pathology in a cohort of A53T *SNCA* gene mutation carriers and compared with idiopathic  
392 Parkinson's disease patients and healthy controls. Half of the cohort of the A53T *SNCA*  
393 mutation carriers was at the premotor stage which was confirmed clinically and with the aid  
394 from digital continuous recordings of motor function. Our findings provide novel insights into  
395 the premotor pathology and evolution of Parkinson's disease, suggesting that serotonergic  
396 dysfunction, which can be detected with *in vivo* molecular imaging in patients at risk for  
397 Parkinson's disease, precedes the development of motor symptoms and the visualisation of  
398 dopaminergic pathology. Moreover, the presence of serotonergic pathology in the brainstem is  
399 associated with the overall burden of Parkinson's disease.

400 Premotor A53T *SNCA* carriers had normal striatal dopamine transporter scans, but loss of  
401 serotonin transporters in raphe nuclei, brainstem, striatum, thalamus, hypothalamus and  
402 amygdala. A53T *SNCA* Parkinson's disease patients had loss of striatal dopamine transporters,  
403 and loss of serotonin transporters extended to further subcortical (e.g. cingulate, insula) and  
404 cortical regions. Our findings indicate that premotor A53T *SNCA* with normal visualisation of  
405 dopamine transporters show an average of 34% loss of serotonin transporters in raphe nuclei  
406 and 22% loss in the striatum. In A53T *SNCA* Parkinson's disease patients the serotonin  
407 transporters losses are extended to 48% in raphe nuclei and 57% in striatum, whereas the loss  
408 of striatal dopamine transporters in this group is 71%. In line with previous studies,(18, 19, 24)  
409 A53T *SNCA* Parkinson's disease patients showed greater loss of dopamine transporters in the  
410 caudate, while there were no differences in the putaminal binding ratios, compared with  
411 idiopathic Parkinson's disease. Furthermore, the severity of serotonin transporter loss in  
412 premotor A53T *SNCA* carriers was in line with reductions observed in idiopathic Parkinson's  
413 patients, while A53T *SNCA* Parkinson's disease patients showed even greater loss of serotonin  
414 transporters. Combined these findings suggest similarities in the pathophysiology between  
415 idiopathic Parkinson's disease and A53T *SNCA* Parkinson's disease but with a faster  
416 progression in A53T *SNCA* mutation carriers.

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



422 sleep,(9) and motor symptoms and complications such as tremor,(6) and levodopa-induced  
423 dyskinesias.(7) On the contrary dopaminergic markers correlate well with the symptoms of  
424 rigidity and bradykinesia which are also responding well to dopamine replacement therapy.(25)

425 The neurons of the raphe nuclei, which are located in the brainstem, are the main source of  
426 serotonergic neurotransmission in the human brain, and through the rostral and caudal  
427 pathways innervate a very large part of the brain, while modulating a large number of  
428 physiological functions.(26) Similarly, Braak and colleagues,(2) have described with  
429 histopathology the distribution of Lewy body and neurite spread, in tissue of Parkinson's  
430 brains, which follows closely the topographic distribution of serotonergic circuits in the brain.  
431 Moreover, SNCA is expressed in the perikarya and neuritic processes of serotonergic raphe  
432 nuclei neurons, and has been shown to directly impact on serotonin transporters by generating  
433 a negative modulation and reducing its cell-surface availability.(27) The influence of SNCA  
434 on serotonin transporter arises through a direct binding between the two proteins,  
435 predominantly involving the non-amyloidogenic component domain of SNCA. This is  
436 particularly interesting as the A53T mutation, which has drastically increased aggregation  
437 kinetics, may hinder the ability of SNCA to form  $\alpha$ -helices, thus promoting  $\beta$ -sheet  
438 configuration and SNCA aggregation. This could lead to the sequestration of serotonin  
439 transporter into aggregates, resulting in its depletion, as reflected by our results.

440 Our findings further support the potential association of [<sup>11</sup>C]DASB binding potential loss,  
441 reflecting serotonergic pathology, with the distribution of Lewy body and neurite pathology.  
442 We went on to construct brain topographic maps reflecting Braak stages 1-6 and used these as  
443 seed maps to calculate [<sup>11</sup>C]DASB binding potential in the cohort of A53T SNCA carriers. In  
444 line with Braak, premotor A53T SNCA carriers showed serotonergic pathology in brain areas  
445 corresponding to stages 1-3, whereas [<sup>11</sup>C]DASB binding potential was largely preserved in  
446 brain areas corresponding to stages 4-6. Interestingly, the youngest premotor A53T SNCA  
447 carriers (SNCA05 and SNCA06), showed extensive loss of [<sup>11</sup>C]DASB binding potential in  
448 areas corresponding to stages 1 and 2 and only partial loss in areas corresponding to stage 3.  
449 Furthermore, A53T SNCA Parkinson's disease patients showed serotonergic pathology in brain  
450 areas corresponding to stages 4-6. SNCA09 who had very recently been diagnosed with  
451 Parkinson's disease showed minimal loss of [<sup>11</sup>C]DASB binding potential in areas  
452 corresponding to stage 4, whereas [<sup>11</sup>C]DASB binding potential was largely preserved in brain  
453 areas corresponding to stages 5 and 6.

454 If loss of [<sup>11</sup>C]DASB binding potential in the Parkinson's brain, reflecting serotonergic  
455 pathology detected *in vivo*, was to follow the progression and spread of Lewy body and neurite  
456 pathology; and if serotonergic pathology could provide an overall weighted capture of motor  
457 and non-motor symptomatology in line with the role of the serotonergic system in a high  
458 number of human physiological functions; then we hypothesised that there should be an  
459 association between loss of [<sup>11</sup>C]DASB binding potential and overall Parkinson's burden.  
460 Indeed, our findings indicate that serotonergic pathology in the brainstem, which was present  
461 in all A53T *SNCA* carriers correlated with total UPDRS scores, which captures the overall  
462 burden of the disease including both motor and non-motor symptoms. This correlation was also  
463 present in both subgroups of premotor and manifest Parkinson's A53T *SNCA* suggesting that  
464 the correlation between brainstem serotonergic pathology and overall Parkinson's burden was  
465 driven by both premotor and manifest Parkinson's A53T *SNCA* carriers. In order to further test  
466 and generalise the applicability of this finding we attempted similar correlations in two larger  
467 cohorts of patients with idiopathic Parkinson's disease, one of which scanned on a different  
468 scanner. In both occasions the correlation remained true, and we noted that by increasing the  
469 sample size the significance of correlation was becoming stronger. This highlights the potential  
470 applicability of our findings from A53T *SNCA* carriers into patients with idiopathic Parkinson's  
471 disease and suggests the potential application of brainstem [<sup>11</sup>C]DASB PET as a marker of  
472 disease burden across different scanners and sites. This preliminary evidence could be useful  
473 for future multi-centre studies and highlights the need for further studies to investigate  
474 brainstem [<sup>11</sup>C]DASB PET as a potentially robust biomarker to monitor disease progression.  
475 Larger cross-sectional and longitudinal studies are required to confirm these findings.

476 Non-motor symptoms typically present before the onset of cardinal motor symptom in  
477 idiopathic Parkinson's disease, marked by the accumulation of Lewy bodies in Braak stage 1-  
478 3.(2) We investigated the association of serotonergic pathology with non-motor symptoms in  
479 A53T *SNCA* carriers. In A53T *SNCA* carriers, loss of [<sup>11</sup>C]DASB in the brainstem was  
480 associated with higher global burden of non-motor symptoms; this correlation was present also  
481 in both subgroups of premotor and manifest Parkinson's A53T *SNCA* carriers. Therefore,  
482 suggesting that brainstem serotonergic pathology may be preceding the gradual development  
483 of non-motor symptom burden. Our findings are in line with previous studies in idiopathic  
484 Parkinson's disease supporting a link between non-motor symptoms and serotonergic  
485 pathology.(8-10) We did not have enough power in the present study to investigate the  
486 relationship between [<sup>11</sup>C]DASB binding with depression levels in A53T *SNCA* carriers. We

487 did not find any association between [<sup>11</sup>C]DASB binding and dysautonomic or olfactory  
488 symptoms; suggesting other neurotransmitter systems, such as the noradrenergic system, may  
489 play a more prominent role in their pathophysiology.

490 The presence of serotonergic pathology in Braak stage 4 and 5 was associated with global  
491 cognitive deficits. One premotor A53T *SNCA* carrier with serotonergic pathology in the  
492 temporal mesocortex and allocortex (Braak stage 4) presented with subtle cognitive deficits, in  
493 visuospatial/executive cognitive function and working memory. Therefore, suggesting that the  
494 accumulation of serotonergic pathology in basal prosencephalon, mesocortical and neocortical  
495 regions could play a role in the development of cognitive deficits, which are often prominent  
496 in A53T *SNCA* carriers.(16) Histopathological evidence suggests tau neurofibrillary tangles  
497 and amyloid-β plaques can coexist with *SNCA* accumulation.(28) *In vivo* PET studies have  
498 demonstrated the presence of amyloid-β and tau neurofibrillary tangles in Parkinson's cases  
499 with cognitive impairment.(29, 30) Therefore, the role of tau neurofibrillary tangles and  
500 amyloid-β plaques in the development of cognitive impairment in A53T *SNCA* carriers  
501 warrants further investigation *in vivo*.

502 In conclusion, the combined use of thorough clinical observation with molecular imaging,  
503 which encompasses nanomolar sensitivity, and the study of A53T *SNCA* carriers, related to a  
504 gene mutation directly linked with Lewy body pathology and Parkinson's disease  
505 susceptibility; allowed insight into the early role of serotonergic pathology in the progression  
506 of Parkinson's disease. Our findings provide the first to our knowledge *in vivo* imaging data  
507 that corroborate the Braak staging scheme, in terms of showing a neurotransmitter deficit  
508 corresponding to stage 2 antedating the dopaminergic deficit that occurs in stage 3. Although  
509 PET molecular imaging is expensive and A53T *SNCA* carriers rare, our study highlights the  
510 potential to extend findings in A53T *SNCA* carriers to classic forms of idiopathic Parkinson's  
511 disease, which is the second most common neurodegenerative disorder. However, further  
512 studies are required to fully elucidate the molecular pathology and disease mechanisms across  
513 familial forms of Parkinson's disease compared with idiopathic Parkinson's disease. While our  
514 community is in the pursuit to identify reliable markers sensitive to disease progression, and  
515 also to identify candidates at risk for novel neuroprotective treatments, we provide evidence  
516 that the detection of serotonergic pathology, which can be visualised *in vivo* in humans, could  
517 identify individuals at risk even before there is evidence of a dopaminergic deficit or premotor  
518 symptoms, thus preceding disease onset by many years. Given the high signal-to-noise ratio of  
519 [<sup>123</sup>I]FP-CIT SPECT, it could also provide a useful tool to detect longitudinal changes in A53T

520 SNCA carriers. Future studies are warranted to evaluate longitudinal changes in [<sup>123</sup>I]FP-CIT  
521 SPECT and [<sup>11</sup>C]DASB PET as potential markers to monitor disease progression. Provided  
522 that accurate serotonin transporter imaging can be labelled with longer lived F-18 isotopes for  
523 wider PET applicability or transferred to the less expensive SPECT platform, it has the  
524 potential to become a more affordable method for screening and monitoring disease  
525 progression. Future work could allow for the development of serotonin transporter imaging  
526 into an adjunctive tool for screening and monitoring progression for individuals at risk or  
527 patients with Parkinson's disease, to complement existing molecular imaging tools such as  
528 dopaminergic imaging, and could serve as a sensitive marker of Parkinson's burden.

529

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