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Faster disease progression in Parkinson's disease with type 2 diabetes is not associated with increased α-synuclein, tau, amyloid-β or vascular pathology.

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ABSTRACT

Aims

Growing evidence suggests a shared pathogenesis between Parkinson's disease and diabetes although the underlying mechanisms remain unknown. The aim of this study is to evaluate the effect of type 2 diabetes on Parkinson's disease progression and to correlate neuropathological findings to elucidate pathogenic mechanisms.

Methods

In this cohort study, medical records were retrospectively reviewed of cases with pathologically-confirmed Parkinson's disease with and without pre-existing type 2 diabetes. Time to disability milestones (recurrent falls, wheelchair dependence, dementia, and care home placement) and survival were compared to assess disease progression and their risk estimated using Cox hazard regression models. Correlation with pathological data was performed, including quantification of α-synuclein in key brain regions and staging of vascular, Lewy and Alzheimer's pathologies.

Results

Patients with PD and diabetes (male 76%; age at death 78.6 ± 6.2 years) developed earlier falls ($P < 0.001$), wheelchair dependence ($P = 0.004$), dementia ($P < 0.001$), care home admission ($P < 0.001$) and had reduced survival ($P < 0.001$). Predating diabetes was independently associated with a two to three-fold increase in the risk of disability and death. Neuropathological assessment did not show any differences in global or regional vascular pathology, α-synuclein load in key brain areas, staging of Lewy pathology or Alzheimer's disease pathology.

Conclusions

Pre-existing type 2 diabetes contributes to faster disease progression and reduced survival in Parkinson's disease which is not driven by increased vascular, Lewy or Alzheimer's pathologies.

Additional non-specific neurodegeneration related to chronic brain insulin resistance may be involved.

ABBREVIATIONS

ANOVA, analysis of variance

CERAD, Consortium to Establish a Registry for Alzheimer's Disease

CI, confidence interval

CSF, cerebrospinal fluid

HR, hazard ratio

MRI, magnetic resonance imaging

NIA-AA, National Institute on Aging-Alzheimer's Association

PD, Parkinson's disease

TDP-43, transactivation response DNA-binding protein 43 kDa

T2DM, type 2 diabetes mellitus

VCING, Vascular Cognitive Impairment Neuropathology Guidelines

INTRODUCTION

A constellation of non-motor features are well recognised as part of the clinical spectrum of Parkinson's disease (PD) including metabolic and neuroendocrine disturbances.(1) Insulin signalling in the brain has a neuromodulatory role promoting neuronal homeostasis and survival,(2) in addition to its effects as a peripheral hormone regulating glucose metabolism. Evidence from different areas of research suggests a synergistic effect, overlap in risk and pathophysiological mechanisms between PD and type 2 diabetes mellitus (T2DM).(3) Higher prevalence of impaired glucose metabolism has been consistently reported in patients with PD,(4) and pre-existing T2DM has been shown to increase the risk of PD in large cohort studies.(5, 6) Furthermore, concomitant T2DM seems to influence the clinical presentation of PD contributing to faster motor progression,(7, 8) prominent gait difficulties,(9, 10) cognitive impairment (7, 11-13) and earlier motor complications.(14) These data suggest that T2DM may influence the progression of PD, although these studies are limited by short follow up periods and it is uncertain whether T2DM has a similar deleterious impact on survival.

The pathophysiological mechanisms underlying this association remain to be elucidated although T2DM and PD are likely to be synergistic conditions linked by dysregulated pathophysiological pathways, rather than parallel coincidental aging processes. Neuroinflammation, mitochondrial dysfunction, disruption of autophagy, protein synthesis, apoptosis, neuronal survival and synaptic plasticity have all been linked to brain insulin resistance.(3, 15) Little is known about how these dysregulated pathways ultimately promote neurodegeneration and contribute to the development and progression of PD. Increased cerebrovascular burden,(16) promotion of α-synuclein aggregation,(17) Alzheimer's disease pathological changes(18) and non-specific tau-related neuronal degeneration(7, 19) have all been proposed as potential mechanisms although neuropathological studies evaluating the association of T2DM and PD have not been conducted previously.

The aim of the present study is to evaluate the long-term effects of pre-existing T2DM on clinical progression to disability milestones and survival in a cohort of patients with detailed clinical data throughout their disease course and post-mortem confirmation of the diagnosis. We aim to elucidate pathogenic mechanisms linking PD and T2DM by evaluating the neuropathological

changes including quantitative and semiquantitative assessments of cerebrovascular pathology, Lewy pathology, and Alzheimer's disease pathological changes.

MATERIALS AND METHODS

This is a retrospective cohort study of patients with a neuropathologically-confirmed diagnosis of PD with and without concomitant predating T2DM identified from the Queen Square Brain Bank and the Parkinson's UK Brain Bank, London, United Kingdom. Written informed consent for brain donation and use of brain tissue for research was obtained from all donors. Brain donation program and research protocols have been approved by the relevant Research Ethics Committee.

Parkinson's disease with T2DM

All cases with neuropathology-confirmed PD and preceding T2DM (PD+T2DM) from the Queen Square and Parkinson's UK Brain Bank archives were included in the study. Cases with type 1 diabetes mellitus and those who developed T2DM after the clinical diagnosis of PD were excluded. T2DM was defined by documentation in the medical records of any of the following: (i) fasting plasma glucose ≥ 126 mg/dL or (ii) diagnosis of T2DM by treating primary care physician or (iii) regular treatment with hypoglycaemic medication.

Parkinson's disease without T2DM: Clinical control group

In order to compare the clinical progression of the disease, consecutive patients with pathology-proven PD without T2DM (Clinical PD-T2DM) were selected from the Queen Square Brain Bank archive between January 2009 and December 2017. Those with insufficient detailed clinical information throughout their disease course to evaluate disease progression were excluded. Patient selection and data collection methodologies for this cohort have been previously published.(20)

Parkinson's disease without T2DM: Neuropathological control group

A control group of patients with post-mortem confirmation of PD without T2DM was selected from the Queen Square Brain Bank archive (Neuropathology PD-T2DM) for histological comparison. These patients were matched by sex, age at death, PD duration and presence of hypertension in order to minimise bias of confounding factors that could account for potential differences in vascular or neurodegenerative pathologies.

Clinical assessment

Medical records comprising all documents from primary care, general practitioners, hospital specialists and Brain Bank evaluation forms, were systematically reviewed by a neurologist with expertise in PD (EdP-F). All cases had been evaluated by experienced hospital specialists (neurologists or geriatricians) in the United Kingdom throughout the course of their illness. Demographic data, information on T2DM (duration, treatment and end organ damage complications), vascular risk factors (hypertension, dyslipidaemia, body mass index, smoking status, symptomatic cerebrovascular disease) and PD (levodopa response, PD clinical motor subtype, levodopa equivalent dose(21)) were collated. To evaluate PD progression, time from diagnosis to specific disease milestones representing different domains of functioning impairment were selected:(20) regular falls and wheelchair dependence as markers of motor impairment; dementia, defined as cognitive impairment of enough severity to impair daily activities; and placement in residential or nursing home representing global disability. Time from diagnosis to death (survival) was also documented.

Neuropathological assessment

Brain tissue processing, sampling and staining was performed following Queen Square Brain Bank protocols. In summary, brain tissue was fixed in 10% formalin buffer and paraffin-embedded 8µm sections were obtained from representative brain regions. Tissue sections were stained with haematoxylin and eosin, and a panel of immunohistochemical stains for neurodegenerative

diseases using a standard avidin-biotin method with antibodies against α-synuclein (05-803; Millipore; 1:1500), amyloid-β (M0872; Dako; 1:100), phosphorylated tau (AT8 clone; MN1020; Thermo Scientific; 1:600) and transactivation response DNA-binding protein 43 kDa (TDP-43) (H00023435-M01; Abnova; 1:6000). Neuropathological evaluations for all cases were performed by an experienced neuropathologist (JLH) blinded to T2DM status.

Lewy pathology and Alzheimer's disease neuropathological changes

Severity and distribution of Lewy pathology was evaluated using Braak staging and McKeith criteria according to consensus recommendations.(22, 23) Alzheimer's disease neuropathological changes were evaluated using the ABC system proposed by the National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines, (24) based on scores for amyloid-β deposition Thal stages,(25) tau neurofibrillary tangle pathology staging proposed by Braak and Braak,(26) and neuritic plaque severity was based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol.(27)

Vascular pathology

An extensive assessment of vascular pathological changes was performed following the validated consensus recommendations from the Vascular Cognitive Impairment Neuropathology Guidelines (VCING).(28) In summary, tissue sections from representative areas stained with haematoxylin and eosin, Perls' stain (2% hydrochloric acid solution - VWR 2052.290 - and 2% potassium ferrocyanide – Alfa Aesar A15736) and immunohistochemical stains for amyloid-β (M0872; Dako; 1:100) and myelin basic protein (SMI94R; Covance; 1:500) were evaluated. A semiquantitative assessment of several cerebrovascular changes (arteriosclerosis and arteriolosclerosis; fibrinoid necrosis; microaneurysms; perivascular dilatation and haemosiderin leakage; atheroma of leptomeningeal and parenchymal vessels; myelin loss; microinfarcts, lacunar and large infarcts; microhaemorrhages and large haemorrhages; cortical, capillary and leptomeningeal cerebral amyloid angiopathy) was obtained for 13 representative brain regions.

Total scores and subscores for relevant brain areas were obtained for each patient to assess global and regional burden of cerebrovascular disease.

Quantitative image analysis of α-synuclein pathology

High-resolution digital images from α-synuclein immunohistochemistry slides were obtained using a high-resolution slide scanner (Leica SCN400F; Olympus VS120). The areas of interest containing the anterior cingulate, entorhinal and transentorhinal cortices were manually selected and the resulting image was processed with Image J software (https://imagej.nih.gov/ij/). These cortical areas were specifically evaluated as severity of Lewy pathology has been shown to accurately correlate with cognitive impairment in PD.(29) Using Bland-Altman plots to optimise sampling of the area of interest, five random squares of 1000 pixels were selected for image analysis. Using Image J software, the threshold setting was adjusted to correctly identify the area stained positive for α-synuclein immunoreactive pathological inclusions (including Lewy bodies and Lewy neurites) and an area fraction (defined as the percentage of pixels of α-synuclein immunoreactivity divided by the total pixels of the area of interest) was calculated for each random square. Mean values for the five random squares for each brain region (anterior cingulate, entorhinal and transentorhinal cortices) for each patient were determined and used for statistical comparisons. To account for learning curve effects, all samples were analysed twice and only the repeat area fraction was considered for statistical analysis. Furthermore, 10% of the samples were randomly selected for repetition of the analysis for each brain area and statistical analysis showed a very strong test-retest reliability (correlation coefficient > 0.85 for each brain area). All image analyses were performed blinded to the T2DM status.

Statistical analysis

Comparisons between groups were performed using non-parametric tests; analysis of variance (ANOVA) for continuous variables and Fisher exact test for categorical variables as appropriate. Ordinal neuropathology staging scores were analysed using the Kruskal-Wallis test.

Multivariable Cox proportional hazard regression models were used to estimate the risk of disease milestones and death for the presence of T2DM and other relevant variables. Potential confounders (age, sex, levodopa response, total levodopa equivalent dose, PD motor subtype) were included in the model as explanatory variables but only those with significant association in the multivariate analysis were retained in the final model. Kaplan-Meier curves for disease milestones and survival were plotted for PD with and without T2DM. Visual inspection of Kaplan-Meier curves and plots of scaled Schoenfeld residuals against time were used to assess the proportional hazards assumption of the models. Censoring was considered uninformative. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. To assess the association between these outcome variables and severity of T2DM Pearson correlation was used. Two-tailed tests were performed, statistical significance was set at $P < 0.05$, and Stata statistical software, version 12 (StataCorp), was used for statistical analysis.

RESULTS

Twenty-five patients with PD and preceding T2DM (19 men [76%]; mean ± SD age at diagnosis of PD of 70.4 ± 8.1 years) were included in the study and their demographic and clinical data are presented in table 1. T2DM preceded the diagnosis of PD by 6.9 ± 7.8 years, treatment with insulin was required by eight (32%) patients and end organ diabetic complications were present in six (12%) patients with nephropathy, six (12%) with retinopathy and nine (18%) with neuropathy.

Clinical progression and survival

PD+T2DM cases were compared with a group of 107 consecutive patients with PD without T2DM (64 men [60%]; 62.2 ± 11.5 years at diagnosis of PD). Patients with PD+T2DM were significantly older at the time of diagnosis of PD, although there were no significant differences in sex, age at death, levodopa response, maximum levodopa equivalent dose or PD motor subtype among groups (table 1).

Data on clinical progression and survival are presented in table 1. Patients with PD and concomitant T2DM had a reduced survival rate of 50% compared to those without diabetes (PD+T2DM 8.2 years vs PD-T2DM 16.2 years; $P < 0.001$). This could not be attributed to T2DM-related deaths as no differences between causes of death were found between those with and without diabetes. PD+T2DM had a significantly faster clinical progression developing all disability milestones earlier than those without concomitant T2DM. On average, PD+T2DM patients had regular falls or were dependent on a wheelchair to mobilise 6 years earlier, developed dementia 8 years earlier and were admitted to a care home 7 years earlier than those with PD without T2DM (table 1; figure 1).

Results of the multivariable Cox proportional hazard regression models showed that the presence of preceding T2DM was an independent factor increasing the risk of falls, wheelchair dependence, dementia, admission to care home and death by 2-3-fold after adjusting for potential confounders (table 2 for HR and 95% CI values; figure 1 for Kaplan-Meier curves). Additionally, older age at diagnosis, poor response to levodopa and postural-instability and gait-difficulties PD motor phenotype were the other variables independently associated with faster disability and reduced survival in the model.

To further explore potential pathophysiological mechanisms in the association between disability milestones and T2DM, sensitivity analyses were performed between these outcomes and T2DM duration, T2DM complications and treatment with insulin as a marker of T2DM severity in a regression analysis. Treatment with insulin was the only parameter showing a significant association with survival (coefficient 4.24; $R^2 = 0.248$; 95% CI = 1.06-7.42) although none of the other T2DM characteristics had an association with any of the disease milestones or survival.

Neuropathological findings

A group of 25 cases with PD without T2DM (16 men [64%] with mean ± SD age at diagnosis of PD of 71.4 ± 7.5 years) was selected as a control group for neuropathological comparison. Comparison of demographic and clinical data among PD+T2DM and the neuropathology PD-

T2DM group did not show any significant differences for demographic data and numerous vascular risk factors (table 1).

Lewy pathology and Alzheimer's disease neuropathological changes

Neuropathological assessment showed advanced Lewy pathology stages (80% Braak stage 6 and 80% neocortical subtype in PD+T2DM vs 88% Braak stage 6 and 88% neocortical subtype in PD-T2DM) in the majority of our cohort without significant differences between groups (table 3).

Alzheimer's disease neuropathological changes were predominantly low in most patients and no significant differences were found between groups using the NIA-AA staging (table 3). The analysis of tau neurofibrillary tangle pathology showed significantly higher stages in those patients without T2DM (Kruskal-Wallis; P = 0.04) although amyloid-β deposition were similar among groups (table 3).

Vascular pathology

No significant differences were found on global cerebrovascular pathology between patients with PD with and without T2DM. Similar results were found in the evaluation of different brain areas, with no significant differences in regional vascular load among groups by brain lobe, cortical areas and basal ganglia (table 3; Figure 2D-F). Only the hippocampus showed greater vascular changes in those with concomitant T2DM, although vascular pathology was generally mild in both groups (P = 0.04).

α-Synuclein pathology quantification

Due to tissue availability, α-synuclein immunoreactivity was quantified in 22 PD+T2DM cases and 18 PD-T2DM cases for the anterior cingulate cortex, and 23 PD+T2DM and 19 PD-T2DM for the entorhinal and transentorhinal cortices. Area fraction analysis of α-synuclein immunoreactivity of

these three brain cortical areas did not reveal any significant differences in Lewy pathology among PD with and without T2DM (table 3; Figure 2A-C).

DISCUSSION

The study shows that predating T2DM is an independent variable for faster disease progression in PD, with 2- to 3-fold increased risk of early motor, cognitive and global disability after adjusting for potential confounders. Pre-existing T2DM also confers a 3-fold increase in mortality risk, which cannot be explained by T2DM-associated complications. Our study provided a detailed neuropathological assessment and results revealed that these clinical differences in disease progression and survival were not associated with increased vascular, Lewy or Alzheimer's disease pathologies globally in the central nervous system or strategic brain areas. Taken together, these findings suggest that T2DM in patients with PD may increase neuronal vulnerability and induce neurodegeneration through other cellular mechanisms rather than disease-specific protein aggregation.

T2DM, Parkinson's disease progression and survival

Previous case-control studies have shown that T2DM may modify the presentation and progression in PD. Predating T2DM has been associated with poorer motor performance, particularly involving postural instability, and more severe cognitive impairment.(7-11, 13) However, only a few studies evaluated the effects longitudinally and data are limited by short follow up periods.(7, 13) Our results provide further evidence of the negative impact of T2DM on the progression of the disease, with mortality and clinical data throughout the entire disease course showing that concomitant diabetes is independently associated with early motor, cognitive and global disability, and with reduced survival in PD. PD+T2DM were older at diagnosis (as expected as both are age-related condition) although the presence of predating diabetes was a negative prognostic factor independent from age. Exploratory subgroup analyses did not show any correlation with indirect markers of diabetes severity such as diabetes duration, insulin requirement or presence of end organ complications.

T2DM, Parkinson's disease and cerebrovascular pathology

T2DM is a risk factor for cerebrovascular disease and systemic insulin resistance is associated with additional comorbidities (chronic hyperglycaemia, dyslipidaemia, hypertension, microvascular disease) that could potentially increase cerebrovascular burden responsible for the differences in phenotype. Pathological studies in T2DM individuals without neurological disease supported this hypothesis as they showed increased vascular pathology at post-mortem when compared with healthy controls.(16) The relationship between PD and comorbid cerebrovascular disease is more complex, although most pathological studies have not demonstrated any significant differences compared to controls.(30, 31) The results of our detailed comprehensive evaluation of vascular pathology covering numerous representative brain areas did not show any global or regional differences in vascular pathology between groups. Although there are no previous pathological studies, our findings are in agreement with a neuroimaging study that reported no differences on vascular leukoaraiosis on magnetic resonance imaging (MRI) in patients with PD with and without T2DM.(9) These data suggest that the association between PD and T2DM involve additional pathogenic mechanisms other than increased cerebrovascular disease.

T2DM, Parkinson's disease and neurodegeneration

The potential contribution of T2DM to neurodegeneration has been long hypothesised (32) and mounting evidence has become available over the last few decades suggesting that brain insulin resistance may play an important role in neurodegenerative diseases, including PD and Alzheimer's disease.(3, 33, 34) Although they remain largely unknown, multiple pathogenic mechanisms have been proposed including disease-specific pathological changes and/or increased neuronal vulnerability through non-specific pathways. Both are age-related diseases and PD+T2DM cases are likely to be older, as seen in our series. Although the negative impact of T2DM on PD was independent from age, these shared disrupted pathways may have a synergistic effect on a vulnerable ageing brain. Further evidence supporting T2DM as a contributing factor

for neuronal vulnerability in PD comes from several MRI studies. Using brain atrophy as a marker of neurodegeneration, they have reported variable patterns of cortical atrophy in the frontal and temporal lobes, and volume loss in the grey matter of individuals with PD and T2DM. (13, 35, 36)

Lewy pathology and α-synuclein load

Preclinical studies have shown that T2DM may contribute to α-synuclein aggregation in PD through abnormal synuclein expression,(37) autophagy disruption,(38) glycation secondary to increased neuroinflammation and oxidative stress,(39) or interaction with amyloidogenic pathogenic proteins such as amylin.(40, 41) Animal models of T2DM have also been shown to promote α-synuclein aggregation.(17) However, results of our pathological analysis did not show any significant differences in Lewy pathology between patients with PD with and without T2DM neither using global semiquantitative staging systems nor using quantification of α-synuclein immunoreactivity in key areas associated with cognitive function. No previous pathological studies in PD and T2DM are available although clinical studies using cerebrospinal fluid (CSF) α-synuclein as a biomarker reported normal results.

It remains unclear how α-synuclein elicits its neurodegenerative effects, although it is now well accepted that certain oligomeric species may be more neurotoxic.(42, 43) The α-synuclein immunohistochemistry used in our study recognises physiological and pathological α-synuclein species and it is considered the diagnostic gold standard for Lewy pathology detection.(44) However, it may not be a sensitive method of detection for the more toxic species (such as the oligomeric forms or post-translational modifications – glycation) that may require more specific techniques.(42, 45) It is therefore possible that the neurodegenerative effect of T2DM may be mediated by promotion of some of these more neurotoxic α-synuclein species that may have been missed in our assessment and this hypothesis should be evaluated in further studies.

Alzheimer's disease pathology

Additionally, growing evidence suggests that brain insulin resistance may play a pivotal role in the pathogenesis of Alzheimer's disease.(34) Although animal models of T2DM have shown an increased accumulation of Alzheimer's disease pathological changes including neurofibrillary tangles and amyloid deposition,(18) these findings have not been replicated in pathological studies of individuals with T2DM compared to healthy controls, and patients with Alzheimer's disease with and without diabetes.(46-49) Alzheimer's disease pathological changes are relatively common at post-mortem examination in PD individuals and it is now widely accepted that its severity contributes to the cognitive impairment.(50, 51) One could hypothesise that T2DM may induce changes in the presentation and progression of PD leading to prominent cognitive decline by promoting Alzheimer's disease pathology. However, our data do not support this hypothesis, as there were no significant differences in global Alzheimer's disease pathological changes using standard staging systems. When pathogenic protein aggregates involved in Alzheimer's disease neurodegeneration were analysed separately, a higher load of tau-related neurofibrillary tangles (but not Aβ-amyloid deposition or neuritic plaques) was seen in PD without T2DM. Although no previous pathological studies have been previously conducted, these results are in contrast with a clinical study using CSF biomarker data from the Parkinson's Progression Markers Initiative cohort showing an increase in CSF total tau in those with concomitant PD and T2DM.(7) Whilst phosphorylated tau and β-amyloid 42 in CSF are useful biomarkers in the antemortem diagnosis of Alzheimer's disease, increased CSF total tau is considered a rather non-specific marker of neuronal injury and it can be increased in other neurological conditions.(52) Analysis of CSF total tau levels in PD has shown variable results, with mostly normal or reduced values (53) and age is considered an important determinant of CSF levels.(54) Isolated increased CSF total tau (without abnormalities on CSF Aβ-amyloid levels) and frontoparietal atrophy on MRI (different to the mesial temporal lobe involvement seen in Alzheimer's disease) have been found in T2DM individuals.(19) Therefore, taken together these results suggest that T2DM may induced neuronal damage and promote neuronal vulnerability through non-specific mechanisms involving tau protein although these dysregulated pathways do not seem to ultimately lead to neurofibrillary tangle pathology seen in Alzheimer's disease.

Strengths and limitations

This study provides life-course clinical data that allows an evaluation of the long-term impact of T2DM on the progression of PD. Although the retrospective collection of clinical data from standard clinical practice performed by different clinicians may potentially account for some variability in documentation, disease milestones were selected on the basis that they reflect the different areas of disability associated with PD, require medical attention and are well documented in clinical records. No data on glycaemic control or anti-glycaemic drugs were available and we were unable to explore the influence of these factors on the association between PD and T2DM.(55) The systematic and detailed neuropathological ascertainment, including cerebrovascular and neurodegenerative pathologies using accepted staging criteria and quantitative methods, is the great strength of this study.

Conclusion

Our findings showed that pre-existing T2DM contributes to a faster disease progression with an independent increase in the risk of disability and survival in PD. The more aggressive course of the disease was not associated with increased cerebrovascular load, Lewy pathology or Alzheimer's disease neuropathological changes in the post-mortem assessment. T2DM may increase neuronal vulnerability and induce non-specific neurodegeneration through other cellular mechanisms. The exact molecular pathways shared between PD and T2DM remain largely unknown although they are likely to involve the additive deleterious effects of systemic and brain insulin resistance and may have a synergistic action with disease-specific neurodegenerative process such as those involved in PD and Alzheimer's disease. Further understanding of the shared mechanisms between PD and T2DM will provide novel therapeutic avenues for potential neuroprotective therapies.

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Authors' contributions:

Eduardo de Pablo-Fernández: conception and design; acquisition, analysis and interpretation of the data; drafting and review for important intellectual content.

Robert Courtney: acquisition of the data; review for important intellectual content.

Alice Rockliffe: acquisition of the data; review for important intellectual content.

Steve Gentleman: acquisition and analysis of the data; review for important intellectual content.

Janice L Holton: conception and design; acquisition and analysis of the data; review for important intellectual content.

Thomas T Warner: conception and design; review for important intellectual content.

ETHICAL APPROVAL

Queen Square Brain Bank and the Parkinson's UK Brain Bank brain donation programs and research protocols have been approved by the relevant Research Ethics Committee.

CONFLICT OF INTEREST

All co-authors report no conflict of interests.

DATA AVAILABILITY STATEMENT

All data were obtained from the archives of the Queen Square Brain Bank and Parkinson's UK Brain Bank, London, United Kingdom. General demographic, clinical and neuropathologic data are available through the Brain Bank websites after approval of the request by the local committee. Aggregated summary clinical data and detailed neuropathologic assessment may be available from the corresponding author where the data requested are considered appropriate and relevant to this study and comply with the ethical regulations approved for this research.

REFERENCES

1. De Pablo-Fernandez E, Breen DP, Bouloux PM, Barker RA, Foltynie T, Warner TT. Neuroendocrine abnormalities in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2017;88(2):176-85.

2. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the brain: sources, localization and functions. Mol Neurobiol. 2013;47(1):145-71.

3. Cheong JLY, de Pablo-Fernandez E, Foltynie T, Noyce AJ. The Association Between Type 2 Diabetes Mellitus and Parkinson's Disease. J Parkinsons Dis. 2020.

4. Marques A, Dutheil F, Durand E, Rieu I, Mulliez A, Fantini ML, et al. Glucose dysregulation in Parkinson's disease: Too much glucose or not enough insulin? Parkinsonism Relat Disord. 2018;55:122-7.

5. De Pablo-Fernandez E, Goldacre R, Pakpoor J, Noyce AJ, Warner TT. Association between diabetes and subsequent Parkinson disease: A record-linkage cohort study. Neurology. 2018;91(2):e139-e42.

6. Yue X, Li H, Yan H, Zhang P, Chang L, Li T. Risk of Parkinson Disease in Diabetes Mellitus: An Updated Meta-Analysis of Population-Based Cohort Studies. Medicine (Baltimore). 2016;95(18):e3549.

7. Pagano G, Polychronis S, Wilson H, Giordano B, Ferrara N, Niccolini F, et al. Diabetes mellitus and Parkinson disease. Neurology. 2018;90(19):e1654-e62.

8. Cereda E, Barichella M, Cassani E, Caccialanza R, Pezzoli G. Clinical features of Parkinson disease when onset of diabetes came first: A case-control study. Neurology. 2012;78(19):1507-11.

9. Kotagal V, Albin RL, Muller ML, Koeppe RA, Frey KA, Bohnen NI. Diabetes is associated with postural instability and gait difficulty in Parkinson disease. Parkinsonism Relat Disord. 2013;19(5):522-6.

10. Giuntini M, Baldacci F, Del Prete E, Bonuccelli U, Ceravolo R. Diabetes is associated with postural and cognitive domains in Parkinson's disease. Results from a single-center study. Parkinsonism Relat Disord. 2014;20(6):671-2.

11. Mollenhauer B, Zimmermann J, Sixel-Doring F, Focke NK, Wicke T, Ebentheuer J, et al. Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the De Novo Parkinson Cohort (DeNoPa). Mov Disord. 2018.

12. Bohnen NI, Kotagal V, Muller ML, Koeppe RA, Scott PJ, Albin RL, et al. Diabetes mellitus is independently associated with more severe cognitive impairment in Parkinson disease. Parkinsonism Relat Disord. 2014;20(12):1394-8.

13. Chung SJ, Jeon S, Yoo HS, Kim G, Oh JS, Kim JS, et al. Detrimental effect of type 2 diabetes mellitus in a large case series of Parkinson's disease. Parkinsonism Relat Disord. 2018.

14. Mohamed Ibrahim N, Ramli R, Koya Kutty S, Shah SA. Earlier onset of motor complications in Parkinson's patients with comorbid diabetes mellitus. Mov Disord. 2018;33(12):1967-8.

15. Santiago JA, Potashkin JA. Shared dysregulated pathways lead to Parkinson's disease and diabetes. Trends Mol Med. 2013;19(3):176-86.

16. Abner EL, Nelson PT, Kryscio RJ, Schmitt FA, Fardo DW, Woltjer RL, et al. Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology. Alzheimers Dement. 2016;12(8):882-9.

17. Rotermund C, Truckenmuller FM, Schell H, Kahle PJ. Diet-induced obesity accelerates the onset of terminal phenotypes in alpha-synuclein transgenic mice. J Neurochem. 2014;131(6):848-58.

18. Kimura N. Diabetes Mellitus Induces Alzheimer's Disease Pathology: Histopathological Evidence from Animal Models. Int J Mol Sci. 2016;17(4):503.

19. Moran C, Beare R, Phan TG, Bruce DG, Callisaya ML, Srikanth V. Type 2 diabetes mellitus and biomarkers of neurodegeneration. Neurology. 2015;85(13):1123-30.

20. De Pablo-Fernandez E, Lees AJ, Holton JL, Warner TT. Prognosis and Neuropathologic Correlation of Clinical Subtypes of Parkinson Disease. JAMA Neurol. 2019.

21. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord. 2010;25(15):2649-53.

22. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017;89(1):88-100.

23. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197-211.
24. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012;123(1):1-11.
25. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58(12):1791-800.
26. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112(4):389-404.
27. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology. 1991;41(4):479-86.
28. Skrobot OA, Attems J, Esiri M, Hortobagyi T, Ironside JW, Kalaria RN, et al. Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. Brain. 2016.
29. Kovari E, Gold G, Herrmann FR, Canuto A, Hof PR, Bouras C, et al. Lewy body densities in the entorhinal and anterior cingulate cortex predict cognitive deficits in Parkinson's disease. Acta Neuropathol. 2003;106(1):83-8.
30. Jellinger KA, Attems J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol. 2008;115(4):427-36.
31. Nanhoe-Mahabier W, de Laat KF, Visser JE, Zijlmans J, de Leeuw FE, Bloem BR. Parkinson disease and comorbid cerebrovascular disease. Nat Rev Neurol. 2009;5(10):533-41.
32. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol. 2004;3(3):169-78.
33. Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: A new target for disease modification? Prog Neurobiol. 2016;145-146:98-120.
34. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol. 2018;14(3):168-81.

35. Petrou M, Davatzikos C, Hsieh M, Foerster BR, Albin RL, Kotagal V, et al. Diabetes, Gray Matter Loss, and Cognition in the Setting of Parkinson Disease. Acad Radiol. 2016;23(5):577-81.
36. Ong M, Foo H, Chander RJ, Wen MC, Au WL, Sitoh YY, et al. Influence of diabetes mellitus on longitudinal atrophy and cognition in Parkinson's disease. J Neurol Sci. 2017;377:122-6.
37. Yuan YH, Yan WF, Sun JD, Huang JY, Mu Z, Chen NH. The molecular mechanism of rotenone-induced alpha-synuclein aggregation: emphasizing the role of the calcium/GSK3beta pathway. Toxicol Lett. 2015;233(2):163-71.
38. Heras-Sandoval D, Perez-Rojas JM, Hernandez-Damian J, Pedraza-Chaverri J. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal. 2014;26(12):2694-701.
39. Vicente Miranda H, Szego EM, Oliveira LMA, Breda C, Darendelioglu E, de Oliveira RM, et al. Glycation potentiates alpha-synuclein-associated neurodegeneration in synucleinopathies. Brain. 2017;140(5):1399-419.
40. Horvath I, Wittung-Stafshede P. Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease. Proc Natl Acad Sci U S A. 2016;113(44):12473-7.
41. Martinez-Valbuena I, Amat-Villegas I, Valenti-Azcarate R, Carmona-Abellan MDM, Marcilla I, Tunon MT, et al. Interaction of amyloidogenic proteins in pancreatic beta cells from subjects with synucleinopathies. Acta Neuropathol. 2018;135(6):877-86.
42. Sanderson JB, De S, Jiang H, Rovere M, Jin M, Zaccagnini L, et al. Analysis of α-synuclein species enriched from cerebral cortex of humans with sporadic dementia with Lewy bodies. Brain Commun. 2020;2(1):fcaa010.
43. Bengoa-Vergniory N, Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Alpha-synuclein oligomers: a new hope. Acta Neuropathol. 2017;134(6):819-38.
44. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol. 2009;8(12):1150-7.
45. Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. Brain. 2015;138(Pt 6):1642-57.

46. Sonnen JA, Larson EB, Brickell K, Crane PK, Woltjer R, Montine TJ, et al. Different patterns of cerebral injury in dementia with or without diabetes. Arch Neurol. 2009;66(3):315-22.
47. Ahtiluoto S, Polvikoski T, Peltonen M, Solomon A, Tuomilehto J, Winblad B, et al. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. Neurology. 2010;75(13):1195-202.
48. Beeri MS, Silverman JM, Davis KL, Marin D, Grossman HZ, Schmeidler J, et al. Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. J Gerontol A Biol Sci Med Sci. 2005;60(4):471-5.
49. Thambisetty M, Jeffrey Metter E, Yang A, Dolan H, Marano C, Zonderman AB, et al. Glucose intolerance, insulin resistance, and pathological features of Alzheimer disease in the Baltimore Longitudinal Study of Aging. JAMA neurology. 2013;70(9):1167-72.
50. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain. 2011;134(Pt 5):1493-505.
51. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol. 2012;72(4):587-98.
52. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673-84.
53. Parnetti L, Gaetani L, Eusebi P, Paciotti S, Hansson O, El-Agnaf O, et al. CSF and blood biomarkers for Parkinson's disease. Lancet Neurol. 2019;18(6):573-86.
54. Mollenhauer B, Locascio JJ, Schulz-Schaeffer W, Sixel-Doring F, Trenkwalder C, Schlossmacher MG. alpha-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. Lancet Neurol. 2011;10(3):230-40.
55. Brauer R, Wei L, Ma T, Athauda D, Girges C, Vijiaratnam N, et al. Diabetes medications and risk of Parkinson's disease: a cohort study of patients with diabetes. Brain. 2020;143(10):3067-76.

FIGURE LEGENDS

Figure 1. Disease milestones and Kaplan-Meier curves.

Kaplan-Meier curves of probability of survival (A) and cumulative risk of falls (B), wheelchair dependence (C), dementia (D) and admission to care home (E) in Parkinson's disease patients by type 2 diabetes status.

Figure 2. Box and whisker plots showing α-synuclein quantification and vascular pathology in key brain regions.

α-Synuclein quantification for anterior cingulate (A), entorhinal (B) and transentorhinal (C) cortices. Global (D), cortical (E) and basal ganglia (F) vascular pathology load.

Figure 3. Representative neuropathological findings in PD cases with type 2 diabetes mellitus.

Subacute macrophage-rich infarct in cerebral cortex with amyloid angiopathy evident in the centre (A; blue arrow). Immunostaining for amyloid-β accentuates widespread concentric cerebral amyloid angiopathy (B; blue arrow) and also shows diffuse parenchymal deposits in the neocortex. Marked hyaline arteriolosclerosis in subcortical white matter (C; red arrow). Diffuse parenchymal amyloid-β plaques are evident in the subiculum (D). Immunostaining for hyper-phosphorylated tau shows neuritic plaques and neurofibrillary tangle tau pathology in the subiculum (E). Immunostaining for α-synuclein in the anterior cingulate cortex (F) and representative appearance after application of threshold for identification and automated quantification of Lewy pathology (G). Scale bar: 250μm in A and B; 125μm in C and 350μm in D and E, 63μm in F and G.

TABLE LEGENDS

Table 1. Comparison of demographics, clinical data and disease progression of clinical and neuropathology cohorts of Parkinson's disease with and without type 2 diabetes.

Clinical cohort	**PD + T2DM (n=25)**	**PD - T2DM (n=107)**	**P value**
Sex – male	19 (76)	64 (60)	0.170
Age at diagnosis (years)	70.4 ± 8.1	62.2 ± 11.5	0.001
Age at death (years)	78.6 ± 6.2	78.4 ± 6.5	0.866
Motor phenotype			0.321
Tremor predominant	2 (8)	22 (21)	
Akinetic-rigid	16 (67)	67 (63)	
PIGD	6 (25)	18 (17)	
Levodopa response			0.110
Absent	0	6 (6)	
Moderate	3 (13)	6 (6)	
Good	9 (38)	22 (21)	
Excellent	12 (50)	71 (68)	
Levodopa equivalent dose (mg)	752.2 ± 443.0	918.4 ± 426.5	0.085
Cause of death			0.093
Advanced Parkinson's	15 (68)	41 (38)	
Infection	6 (27)	51 (48)	
Vascular	0	5 (5)	
Other	1 (5)	10 (9)	
Survival from diagnosis (years)	8.2 ± 4.1	16.2 ± 8.3	< 0.001
Falls	21 (84)	86 (80)	0.462
Time to falls (years)	5.4 ± 4.5	11.7 ± 7.0	<0.001
Wheelchair	15 (60)	58 (54)	0.660
Time to wheelchair (years)	6.9 ± 4.1	13.0 ± 7.6	0.004
Dementia	16 (64)	61 (57)	0.653
Time to dementia (years)	4.4 ± 3.0	12.6 ± 7.5	<0.001
Care home	13 (52)	49 (46)	0.658

Time to care home (years)	6.3 ± 2.9	14.0 ± 7.7	<0.001
Neuropathology cohort	**PD + T2DM (n=25)**	**PD - T2DM (n=25)**	**P value**
Sex – male	19 (76)	16 (64)	0.538
Age at diagnosis (years)	70.4 ± 8.1	71.4 ± 7.5	0.667
Age at death (years)	78.6 ± 6.2	78.1 ± 6.2	0.796
PD duration (years)	8.2 ± 4.1	6.8 ± 3.9	0.217
Clinical cerebrovascular disease	4 (17)	1 (4)	0.189
Hypertension	17 (68)	16 (64)	1.0
Dyslipidaemia	19 (76)	4 (16)	<0.001
Smoking			0.905
Never	16 (64)	14 (56)	
Ex-smoker	7 (28)	9 (36)	
Active	2 (8)	2 (8)	
BMI*	26.4 ± 4.2	24.6 ± 3.0	0.202

PIGD, postural instability and gait difficulty. *BMI available for PD+T2DM = 20 and PD-T2DM = 11.

Data presented as number (%) for categorical variables and median (standard deviation) for continuous variables. P value from comparisons using Fisher exact test for categorical variables, ANOVA for continuous variables and Kruskal-Wallis test for ordinal variables as appropriate.

Table 2. Cox proportional hazard regression models for disease disability milestones and survival.

Outcome variable	Predictors	Adjusted HR (95% CI)	P value
Survival			
	T2DM	2.96 (1.71-5.10)	<0.001
	Age at diagnosis	1.14 (1.11-1.17)	<0.001
	PIGD motor phenotype	3.57 (1.84-6.95)	<0.001
	Excellent levodopa response	0.23 (0.09-0.56)	<0.001
Falls			
	T2DM	2.39 (1.36-4.20)	0.002
	Age at diagnosis	1.11 (1.08-1.14)	<0.001
	PIGD motor phenotype	5.63 (2.59-12.26)	<0.001
	Excellent levodopa response	0.17 (0.06-0.48)	<0.001
Wheelchair			
	T2DM	2.60 (1.31-5.16)	0.006
	Age at diagnosis	1.09 (1.06-1.12)	<0.001
	PIGD motor phenotype	6.47 (2.24-18.72)	0.001
	Excellent levodopa response	0.10 (0.03-0.34)	<0.001
Dementia			
	T2DM	3.62 (1.73-7.58)	0.001
	Age at diagnosis	1.13 (1.09-1.17)	<0.001
	PIGD motor phenotype	5.24 (2.10-13.09)	<0.001
	Excellent levodopa response	0.03 (0.00-0.32)	0.004
Care home			
	T2DM	2.84 (1.19-6.77)	0.019
	Age at diagnosis	1.13 (1.09-1.18)	<0.001
	PIGD motor phenotype	4.63 (1.13-19.0)	0.033
	Excellent levodopa response	0.25 (0.07-0.85)	0.026

PIGD, postural instability and gait difficulty.

Table 3. Comparison of neuropathological data between neuropathological cohorts of Parkinson's disease with and without type 2 diabetes.

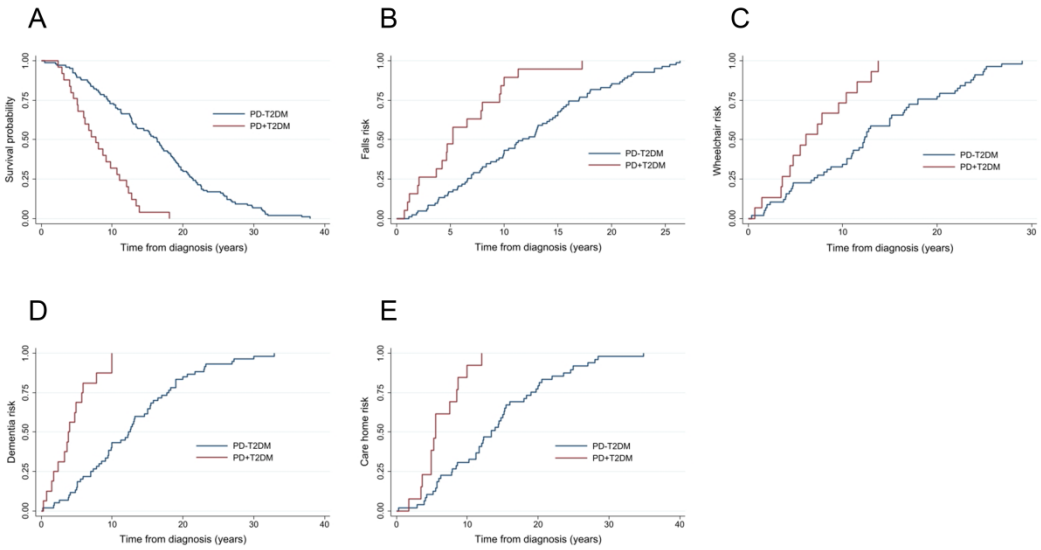
	PD + T2DM (n=25)	PD - T2DM (n=25)	P value
Anterior cingulate synuclein load (area fraction)*	0.61 ± 0.43	0.89 ± 0.89	0.201
Transentorhinal synuclein load (area fraction)**	0.72 ± 0.70	0.85 ± 0.88	0.607
Entorhinal synuclein load (area fraction)**	0.68 ± 0.48	0.78 ± 0.56	0.516
Cortical vascular load (VCING score)	13 ± 10	15 ± 12	0.561
Frontal	3 ± 2	4 ± 3	0.238
Temporal	3 ± 2	3 ± 3	0.511
Parietal	3 ± 3	3 ± 3	0.926
Occipital	4 ± 3	4 ± 4	0.953
Basal ganglia vascular load (VCING score)	9 ± 4	8 ± 4	0.465
Hippocampal vascular load (VCING score)	1 ± 1	0 ± 1	0.04
Global cerebral vascular load (VCING score)	27 ± 12	26 ± 15	0.959
Cerebral amyloid angiopathy load (VCING score)	4 ± 7	7 ± 9	0.339
Braak synuclein			0.493
Stage 4	0	1 (4)	
Stage 5	5 (20)	2 (8)	
Stage 6	20 (80)	22 (88)	
McKeith Lewy pathology			0.445
Limbic	5 (20)	3 (12)	
Neocortical	20 (80)	22 (88)	
Thal Aβ deposition			0.451
Phase 0	6 (24)	5 (20)	
Phase 1	7 (28)	7 (28)	
Phase 2	4 (16)	3 (12)	
Phase 3	5 (20)	3 (12)	
Phase 4	2 (8)	4 (16)	
Phase 5	1 (4)	3 (12)	
Braak and Braak neurofibrillary tangle			0.04
Stage 0	0	2 (8)	
Stage I	10 (40)	3 (12)	
Stage II	11 (44)	11 (44)	

Stage III	4 (16)	1 (4)	
Stage IV	0	6 (24)	
Stage V	0	1 (4)	
Stage VI	0	1 (4)	
CERAD neuritic plaque			0.152
Absent	12 (48)	10 (40)	
Sparse	10 (40)	5 (20)	
Moderate	3 (12)	9 (36)	
Frequent	0	1 (4)	
NIA Alzheimer's disease			0.143
Not	6 (24)	6 (24)	
Low	17 (68)	10 (40)	
Intermediate	2 (8)	7 (28)	
High	0	2 (8)	

VCING, Vascular Cognitive Impairment Neuropathology Guidelines.

Data presented as number (%) for categorical variables and median (standard deviation) for continuous variables. P value from comparisons using Fisher exact test for categorical variables, ANOVA for continuous variables and Kruskal-Wallis test for ordinal variables as appropriate.

*Cingulate available for PD+T2DM = 22 and PD-T2DM = 18. **Transentorhinal and entorhinal available for PD+T2DM = 23 and PD-T2DM = 19.



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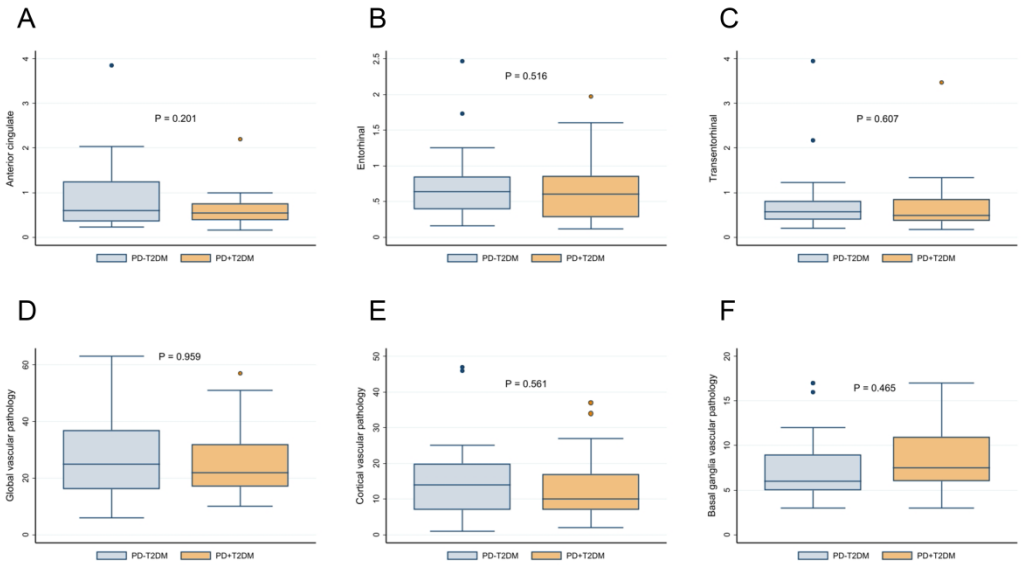

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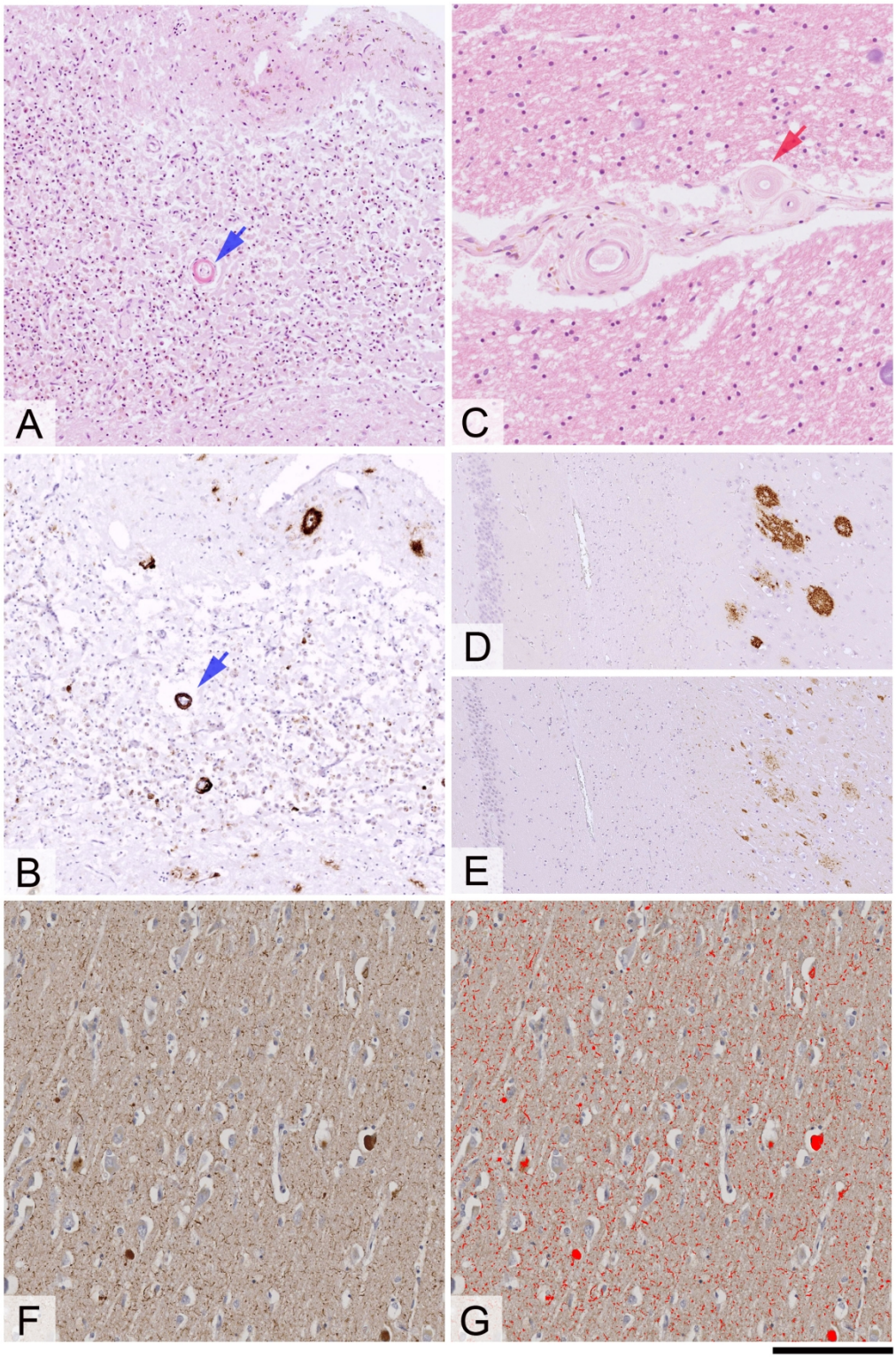

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