Brain Microglial Activation Increased in Glucocerebrosidase (GBA) Mutation Carriers without Parkinson’s disease

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ABSTRACT: Background: Glucocerebrosidase gene mutations are a common genetic risk factor for Parkinson’s disease. They exhibit incomplete penetrance. The objective of the present study was to measure microglial activation and dopamine integrity in glucocerebrosidase gene mutation carriers without Parkinson’s disease compared to controls.

Methods: We performed PET scans on 9 glucocerebrosidase gene mutation carriers without Parkinson’s disease and 29 age-matched controls. We measured microglial activation as \(^{11}\text{C}-(\text{R})-\text{PK11195}\) binding potentials, and dopamine terminal integrity with \(^{18}\text{F}\)-dopa influx constants.

Results: The \(^{11}\text{C}-(\text{R})-\text{PK11195}\) binding potential was increased in the substantia nigra of glucocerebrosidase gene carriers compared with controls (Student \(t\) test; right, \(t = -4.45, P = 0.0001\)). Statistical parametric mapping also localized significantly increased \(^{11}\text{C}-(\text{R})-\text{PK11195}\) binding potential in the occipital and temporal lobes, cerebellum, hippocampus, and mesencephalon. The degree of hyposmia correlated with nigral \(^{11}\text{C}-(\text{R})-\text{PK11195}\) regional binding potentials (Spearman’s rank, \(P = 0.0066\)). Mean striatal \(^{18}\text{F}\)-dopa uptake was similar to healthy controls.

Conclusions: In vivo \(^{11}\text{C}-(\text{R})-\text{PK11195}\) PET imaging detects neuroinflammation in brain regions susceptible to Lewy pathology in glucocerebrosidase gene mutation carriers without Parkinson’s. © 2020 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: Parkinson’s disease; microglia; substantia nigra; glucocerebrosidase; positron emission tomography

The glucocerebrosidase gene (GBA) encodes the lysosomal hydrolase glucocerebrosidase. In the biallelic (homozygous or compound heterozygous) state, GBA mutations may cause Gaucher disease (GD) which leads

to glucosylceramide accumulation in visceral organs and, in a minority of cases, the central nervous system (neuronopathic GD). GBA mutations are the most significant genetic risk factor for Parkinson’s disease (PD) and dementia with Lewy bodies (DLB)\(^1\)\(^{-3}\); however, penetration is only 10\%–30\%.\(^4\)\(^{-6}\) PD patients carrying a GBA mutation have an earlier disease onset and a higher risk of dementia.\(^7\)

At postmortem, \(\alpha\)-synuclein aggregations identical to those found in idiopathic PD\(^1\) and DLB\(^8\) are present in GBA-PD subjects. Asymmetrically reduced striatal \(^{18}\)F-dopa uptake,\(^9\)\(^{10}\) striatal dopamine transporter binding,\(^11\)\(^{12}\) and an altered striatal asymmetry index\(^13\) have been reported in PD patients with GBA mutations. Conversely \(^{123}\)I-isoflupane dopamine transporter uptake has been demonstrated to be upregulated in non-PD GBA carriers compared with controls and is higher in GBA PD compared to idiopathic PD cases.\(^14\)\(^{15}\) GBA mutation carriers without PD exhibit prodromal PD features,\(^16\)\(^{-19}\) which progress with time.\(^20\)

Glial activation has been demonstrated in postmortem PD brains.\(^21\)\(^{22}\) Nigral microglial activation along with reduced striatal \(^{18}\)F-Dopa uptake is present in idiopathic rapid eye movement sleep behavior disorder (RBD).\(^23\) It is also a feature of neuronopathic GD at postmortem\(^8\) and in GD mouse models.\(^24\) No studies have investigated in vivo the presence of brain microglial activation in GBA mutation carriers and related this to the presence of striatal dopaminergic dysfunction. We therefore measured \(^{11}\)C-(R)-PK11195 regional binding potentials (BPND) and \(^{18}\)F-dopa \(K_\text{D}\) in GBA mutation carriers without evidence of Parkinson’s disease.

**Methods**

**Recruitment and Clinical Assessments**

Between 2015 and 2016, 9 biallelic (homozygous or compound heterozygous) or heterozygous carriers of GBA mutations were recruited from University College London, UK (see Table 1 for characteristics). All subjects had exons 1–11 of the GBA gene sequenced (Table 1). Biallelic carriers had type 1 GD, whereas heterozygous carriers were drawn from GD kindreds. No subjects met PD (UK Brain Bank) diagnostic criteria, and none were genetically related. Two of 5 GD patients were receiving enzyme replacement therapy (ERT; velaglucerase 800 IU weekly and 4000 IU monthly) and 3 of 5 substrate reduction therapy (SRT; eliglustat 84 IU twice daily in 2 of 3, miglustat 300 mg once daily in 1 of 3). Both SRT and ERT were administered throughout the duration of the study. Ethical approval was obtained from London, UK (10/H0720/21), and Midtjylland, Denmark (M-2014-397-14), research ethics committees.

Each GBA carrier had \(^{11}\)C-(R)-PK11195 and \(^{18}\)F-dopa PET, an MRI, and neurological examination. Prodromal PD features were rated with the University of Pennsylvania Smell Identification Test (UPSIT), Montreal cognitive assessment, RBD questionnaire (RBDSQ), PD Non-Motor Symptoms Scale, the Movement Disorder Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) parts II and III, and Beck’s Depression Inventory.

All scans and examinations were performed at Aarhus University Hospital, Denmark. GBA carrier PET findings were compared with in-house PET data from 29 age-matched healthy controls (20 had \(^{11}\)C-(R)-PK11195 BPND PET, and 9 had \(^{18}\)F-dopa PET) recruited for a previously published study.\(^25\) Assessments of control prodromal PD features were not available.

**PET and MRI**

We performed prespecified region-of-interest (ROI) analyses comparing GBA mutation carriers with controls. Selected ROIs were the substantia nigra (SN), putamen, and caudate for \(^{11}\)C-(R)-PK11195 BPND and the putamen and caudate for \(^{18}\)F-dopa \(K_\text{D}\). We performed statistical parametric mapping (SPM) of \(^{11}\)C-(R)-PK11195 uptake across all brain voxels. Technical details of the PET and MRI scanning and analysis procedures are available in the supplementary materials.

**Statistics**

For the ROI analyses, statistical calculations and graphs were produced with Stata v14.2 software (StataCorp., College Station, TX). The \(^{18}\)F-dopa \(K_\text{D}\) and \(^{11}\)C-(R)-PK11195 BPND values from specified ROIs were compared in carrier and control groups using the Student \(t\) test (\(P < 0.05\)). When there was a significant difference in \(^{11}\)C-(R)-PK11195 BPND between the GBA and control groups, secondary analyses correlating PD prodromal features with \(^{11}\)C-(R)-PK11195BPND were undertaken (Spearman’s rank: all clinical scales were non normally distributed, \(P < 0.05\)). A Bonferroni correction was applied to all significant results.

**Results**

**Participants**

Participant characteristics are listed in Table 1. Nine GBA mutation carriers (5 biallelic and 4 heterozygous) were selected on the basis of their genotype and the absence of PD features. Two age-matched control groups (20 for \(^{11}\)C-(R)-PK11195 BPND PET and 9 for \(^{18}\)F-dopa PET) were included in the final GBA analysis. Some GD patients had musculoskeletal problems typical of GD reflected in raised MDS UPDRS III scores, but these were not specific for PD. This reflects the limitations of the MDS UPDRS when used in the context of non-PD
Substantia Nigra $^{11}$C-(R)-PK11195 BPND Is Increased in GBA+ Individuals Compared With Controls

ROI analysis localized a significant increase in mean nigral $^{11}$C-(R)-PK11195 BPND of the GBA carriers compared with controls (Student $t$ test, $t = -4.45$, $P = 0.0001$; Tables S1 and S2). Statistical significance was retained after correction for multiple comparisons (Table S2). For the GBA mutation carriers, mean SN $^{11}$C-(R)-PK11195 BPND was 0.15 ± 0.08 compared with −0.01 ± 0.09 for the control group (Table S1 and Fig. 1A). Interestingly, heterozygous carriers had disproportionately higher BP$_{ND}$ than biallelic (GD) patients (Table S1 and Fig. 1A).

$^{11}$C-(R)-PK11195 BPND Correlates With Olfactory Deficit in GBA+ Individuals

There was a negative correlation between nigral $^{11}$C-(R)-PK11195 BPND and UPSIT scores in GBA mutation carriers (Spearman’s rank, $P = 0.0066$; Table S2 and Fig. 1D), which did not survive correction for multiple comparisons (Table S2).

Upregulated Cortical, Hippocampal, and Mesencephalon $^{11}$C-(R)-PK11195 BP$_{ND}$ in GBA+ Group

SPM-localized clusters of voxels with significantly increased $^{11}$C-(R)-PK11195 BP$_{ND}$ in GBA carriers
bilateral in the occipital and temporal cortices, cerebellum, left hippocampus, and central and anterior mesencephalon (Table S3 and Fig. 1B,C). No brain regions showed reduced $^{11}$C-(R)-PK11195 BPND compared with controls.

**No Difference in Mean $^{18}$F-Dopa $K_i$ Between GBA+ and Control Participants**

The GBA carriers showed no significant decreases in mean $^{18}$F-dopa $K_i$ across striatal ROIs compared with controls (Tables S1 and S2, Fig. S1). Two participants had putamen and/or caudate $^{18}$F-dopa $K_i$ more than 2 SDs below the control mean (Table S4). Greater variance in $^{18}$F-dopa $K_i$ (see Table S1) was seen in the GBA group (SD of 0.002 in the putamen and caudate compared with SD of 0.001 in controls). Post hoc analysis (Student t test) comparing the anterior, medial, and posterior putamen did not show any significant mean differences between GBA mutation carriers and controls.

**No Correlation Between Nigral $^{11}$C-(R)-PK11195 BPND and $^{18}$F-Dopa $K_i$ in GBA+ Group**

There was no association between the SN $^{11}$C-(R)-PK11195 BPND and putamen or caudate (Table S2) $^{18}$F-dopa $K_i$ in the GBA group.

**Discussion**

Our data indicate that both heterozygous and biallelic GBA mutation carriers can have increased $^{11}$C-(R)-PK11195 BPND in brain regions susceptible to Lewy body formation.26 It is unclear whether this is a cytotoxic or neuroprotective process. Only 10%–30% of GBA mutation carriers will develop PD. It is therefore unlikely that all the participants in this study will convert. Which GBA carriers are likely to progress to PD and the mechanisms underlying this conversion are of particular interest.

$^{11}$C-(R)-PK11195 BPND values in the SN correlated with UPSIT scores, suggesting that those GBA carriers who have reduced olfactory function have higher nigral inflammation. Correlation of striatal $^{11}$C-(R)-PK11195
BPND with age and MDS UPDRS III score has also been shown in early PD cases.\[^{27}\]

Despite mean nigral \(^{11}\)C-(R)-PK11195 BPND being increased in the GBA group, no significant reduction in mean putamen \(^{18}\)F-dopa uptake was seen. It is known that \(^{18}\)F-dopa lacks the sensitivity to detect early dopaminergic dysfunction because of compensatory upregulation of dopa decarboxylase in the remaining terminals. Early reductions may be better detected with dopamine transporter markers.\[^{28,29}\] Our finding of normal striatal F-dopa uptake in GBA carriers may not necessarily equate to normal dopamine terminal function, although no GBA carrier exhibited clinical features of PD.

Interestingly \(^{18}\)F-dopa Ki was more variable in the GBA group compared with controls. Recently, 184 non-manifesting GBA carriers were reported to have increased dopamine transporter binding across striatal regions.\[^{15}\] This is in line with an increase in striatal \(^{18}\)F-dopa K\(_i\) found in a portion of our GBA+ cases. It has been reported that \(^{11}\)C-(R)-PK11195 binding to microglia “burns out” as amyloidosis in early Alzheimer’s disease advances\[^{30}\] but increases again as tau tangles form.\[^{31,32}\] A biphasic trajectory could explain the lack of correlation between \(^{18}\)F-dopa K\(_i\) and \(^{11}\)C-(R)-PK11195 BPND in our data set.

**Limitations**

The relatively small sample size, its cross-sectional design, and the unknown future disease status of GBA mutation carriers are limitations. We acknowledge that GBA mutations exhibit a variable penetrance and phenotype, in terms of both PD and GD. Reproducing these results in larger (ideally prospective) and more genotypically and phenotypically homogenous cohorts is needed. Nevertheless, we believe these are important and highly relevant pilot data that will inform the design of future studies.

The \(^{11}\)C-PK11195 BPND has high nonspecific binding, which provides a lower specific-to-background PET signal ratio than newer markers of activated microglia; therefore, our results may underestimate glial activation. This study used \(^{11}\)C-(R)-PK11195 BPND as a marker of the translocator protein (TSPO) expressed by the mitochondria of activated microglia, and, in contrast to newer TSPO tracers available, the binding is not influenced by the polymorphism of the TSPO expressed by individuals. The limitations of supervised cluster analysis in conditions with possible widespread microglial activation should also be acknowledged, as it could lead to an underestimation of \(^{11}\)C-(R)-PK11195 BPND, particularly in small ROIs.

Three of 5 and 2 of 5 subjects were taking substrate reduction therapy or enzyme replacement therapy (ERT), respectively. The former is under evaluation as a PD neuroprotective agent (clinicaltrials.gov, NCT02906020), ERT is not thought to cross the blood–brain barrier, although 1 report suggests a portion may.\[^{33}\] We cannot exclude the possibility that the reduced nigral and putamen \(^{11}\)C-(R)-PK11195 BPND in biallelic compared with heterozygous cases could represent suppression of glial activation by these drugs.

**Conclusions**

Our findings indicate that GBA mutations are associated with microglial activation in Lewy-susceptible brain regions in subjects without either a prodromal or clinical diagnosis of PD. Further studies are required to assess whether \(^{11}\)C-(R)-PK11195 BPND, (with or without additional biomarkers) can predict GBA carrier conversion to PD and striatal dopamine loss. ■

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**Data and Materials Availability**

Study data are available on reasonable request. ■

**References**


Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.