***GBA* variants in Parkinson’s disease: clinical, metabolomic and multimodal neuroimaging phenotypes**

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**Abstract:**

**Background:**

Alterations in the *GBA* gene (NM\_000157.3) are the most important genetic risk factor for Parkinson’s disease (PD). Biallelic *GBA* mutations cause the lysosomal storage disorder Gaucher´s disease. The *GBA* variants p.E365K and p.T408M are associated with Parkinson’s but not with Gaucher´s disease. The pathophysiological role of these variants needs to be further explored.

**Objective:**

This study analyzed clinical, neuropsychological, metabolic and neuroimaging phenotypes of PD patients carrying the *GBA* variants p.E365K and p.T408M.

**Methods:**

*GBA* was sequenced in 56 mid-stage PD patients. Carriers of *GBA* variants were compared to non-carriers regarding clinical history and symptoms, neuropsychological features, metabolomics and multimodal neuroimaging. Blood plasma gas chromatography coupled to mass spectrometry, 6-[18F]fluoro-L-Dopa PET, [18F]fluorodeoxyglucose PET, and resting-state functional magnetic resonance imaging were performed.

**Results:**

Sequence analysis detected 13 heterozygous *GBA* variant carriers (seven with p.E365K, six with p.T408M). One patient carried a *GBA* mutation (p.N409S) and was excluded. Clinical history and symptoms were not significantly different between groups. Global cognitive performance was lower in variant carriers. Metabolomic group differences were suggestive of more severe PD-related alterations in carriers versus non-carriers. Both PET scans showed signs of a more advanced disease; [18F]fluorodeoxyglucose PET and functional magnetic resonance imaging showed similarities with Lewy body dementia and PD dementia in carriers.

**Conclusions:**

This is the first study to comprehensively assess (neuro-)biological phenotypes of *GBA* variants in PD. Metabolomics and neuroimaging detected more significant group differences than clinical and behavioral evaluation. These alterations could be promising to monitor effects of disease-modifying treatments targeting glucocerebrosidase metabolism.

**Introduction:**

Alterations in the *GBA* gene (NM\_000157.3) represent the most common genetic risk factor for Parkinson’s disease recognized to date.[1] *GBA* encodes glucocerebrosidase, a lysosomal enzyme involved in sphingolipid metabolism.[2] A number of known *GBA* mutations cause the autosomal-recessive lysosomal storage disorder Gaucher’s disease in biallelic carriers.[3] It has been hypothesized that glucocerebrosidase plays a role in alpha-synuclein degradation and therefore aggregate formation may be facilitated when glucocerebrosidase function is impaired,[4,5] thereby increasing the risk to develop Parkinson’s disease and dementia with Lewy bodies (DLB) in mono- and biallelic carriers.[4,6] Clinical presentation and long-term clinical course of Parkinson’s disease patients with *GBA* mutations is not overtly different from patients without known genetic risk factors, however, many studies have presented evidence for an earlier age at onset,[7,8] faster progression of motor symptoms and cognitive decline,[1,4,8–10] and more visual hallucinations or psychotic symptoms.[8,11–13]

Recently, more frequently occurring alterations in *GBA* that do not cause Gaucher’s disease, termed *GBA* variants (rather than mutations), have also been recognized as genetic risk factors for Parkinson’s disease: large multicenter studies and a meta-analysis have established significant associations between Parkinson’s disease and the single nucleotide polymorphisms p.E365K and p.T408M (traditional nomenclature: p.E326K and p.T369M).[9,14,15] The 1000 Genomes Project reports p.E365K in 1% and p.T408M in about 0.4% of the world population, whereas they were found in up to 5% and 3.9% of Parkinson’s disease patients, respectively.[9,16] It has repeatedly been shown that carriers of p.E365K, like mutation carriers, suffer from a faster cognitive decline than non-carriers, which has not (yet) been demonstrated for p.T408M.[10,16,17] A meta-analysis of 13 Parkinson’s disease cohorts suggested a faster disease progression in both variants.[16]

Metabolic consequences of glucocerebrosidase dysfunction in affected Parkinson’s disease patients have rarely been explored *in vivo*.[18] Mass spectrometry-based analysis of dried blood spots showed reduced glucocerebrosidase enzymatic activity,[19] as well as increased levels of the lysosphingolipid hexosylsphingosine not only in Gaucher’s disease-related mutations but also in p.E365K and p.T408M.[18]

Neuroimaging studies comparing Parkinson’s disease patients with and without *GBA* mutations reported reduced cerebral blood flow in the parieto-occipital cortex, which resembled the pattern typically seen in DLB.[8,20,21] To the best of our knowledge, no neuroimaging studies to date have focused on *GBA* variants.

In summary, current evidence suggests that the clinical and neurobiological phenotype of Parkinson’s disease patients carrying *GBA* alterations (mutations or variants) is not fundamentally different from *GBA* non-carrier Parkinson’s disease patients, but may be more severe and/or progress slightly faster, especially with severe mutations,[8,9] whereas variants may have milder clinical effects.[9]

The current study aimed to comprehensively assess the phenotypes of Parkinson’s disease patients carrying the *GBA* variants p.E365K or p.T408M, compared to patients with wildtype *GBA*. Carriers and non-carriers were compared regarding clinical and family history, motor and non-motor symptom severity, cognitive function, metabolomics and multimodal neuroimaging using PET and functional MRI.

**Methods:**

*Subjects*

From a larger, well-characterized cohort (KFO 219), DNA samples were available for 56 Parkinson’s disease patients. Inclusion criteria were Hoehn and Yahr stage[22] ≤ 3, age ≥ 40 years, and absence of dementia,[23] deep brain stimulation or cerebral pathologies other than Parkinson’s disease. All patients were recruited at the University Hospital of Cologne and diagnosed by a movement disorder specialist according to the UK brain bank criteria.[24] The study was approved by the local medical ethics committee (EK12-265) and registered with the German Clinical Trials Register (DRKS00005388); informed consent was obtained from each participant per the declaration of Helsinki.

Patients in this cohort underwent an extensive study protocol with assessment of motor, cognitive, neuropsychiatric and other non-motor symptoms, multimodal neuroimaging, metabolomic and finally genetic analysis. Several of these procedures have been described in previous publications.[25–27]

*Clinical and behavioral data*

The motor part of the unified Parkinson’s disease rating scale (UPDRS-III, including subscores for tremor and akinesia-rigidity to determine motor subtypes[28]), collection of blood samples and functional neuroimaging were performed after antiparkinsonian medication was discontinued for a minimum of 12 hours (levodopa) and up to three days (dopamine agonists). Clinical history, neuropsychological and other non-motor data were obtained on dopaminergic medication. A cognitive test battery covered the domains executive function, memory, attention, language and visual-spatial abilities, from which a global cognition z-score was computed using age- and education-adjusted standard norms. Self-rated scales were applied to measure apathy, depression, (hypo-)mania, impulsivity and other non-motor symptoms.

Groups were compared using IBM SPSS statistics version 25. Categorical data were analyzed with Fisher’s exact test, Mann-Whitney U-test was used for ordinal variables. For continuous variables, normal distribution was tested by Shapiro-Wilk test; groups were compared by t-test or U-test as appropriate. Cognitive scores were compared by ANCOVA to adjust for points on the Beck depression inventory, version II (BDI-II), as depression is known to heavily influence test performance.[29]

*Biospecimen collection and processing*

Blood samples for metabolomic and genetic analyses were drawn after overnight fasting. A gene panel analysis was performed comprising 29 genes previously linked to parkinsonism or dystonia. Rare variants in Parkinson’s disease genes (*GBA*, *LRRK2*, *PARK7*, *PRKN*, *PINK1*, *SNCA*, *VPS35*) were validated by Sanger sequencing and confirmed alterations were registered.

Details of metabolomics processing and analysis in the KFO 219 cohort were previously published.[25] Plasma samples of 54 patients could be analyzed (Table 1). Gas chromatography coupled to mass spectrometry (GC-MS) was applied to measure polar and non-polar metabolite extracts. Metabolomic profiles were analyzed in an untargeted approach, comparing 71 metabolites detected by GC-MS between wildtype patients and *GBA* variant carriers using Welch’s t-test. In this exploratory analysis, suggestive group differences with uncorrected p < 0.05 are reported.

*Neuroimaging data acquisition and analysis*

Image acquisition and preprocessing have previously been described in detail.[27] 6-[18F]fluoro-L-Dopa ([18F]FDopa) PET to estimate dopaminergic denervation and [18F]fluorodeoxyglucose ([18F]FDG) PET to quantify cerebral metabolic activity were performed under standard conditions with an average dose of 185 MBq on a high resolution research tomograph (ECAT HRRT, Siemens) and processed in SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12). [18F]FDopa scans were aligned with the more affected body side according to UPDRS-III. PET images were normalized to MNI space (voxel size 1.22 mm) and spatially smoothed with a 3D Gaussian filter of 6 mm full width at half maximum (FWHM). Resting-state functional MRI (rs-fMRI) time series were acquired on a 3T Siemens Magnetom Prisma (TR 776 ms, TE 37.4 ms, 617 time points, 72 slices, voxel size 2x2x2 mm) and preprocessed using the SPM toolbox Conn[30] with default parameters: realignment, outlier detection for correction of motion artifacts, normalization to MNI space, 5 mm FWHM spatial smoothing, temporal band-pass filtering (0.01 – 0.1), linear detrending and anatomical component-based noise correction. [18F]FDopa PET was available for 39, [18F]FDG for 47 and fMRI for 54 of the 56 patients who underwent genetic testing (Table 1).

[18F]FDopa uptake was compared between patient groups in a voxel-wise two-sample t-test, restricting the search volume to the bilateral striatum. An uncorrected threshold of p < 0.05 was defined as a suggestive group difference. [18F]FDG PET scans were processed using the topographic rating algorithm implemented in ScAnVP (http://feinsteinneuroscience.org) to measure expression of two distinct Parkinson’s disease-related covariance patterns: the Parkinson’s disease related pattern (PDRP), associated with disease progression and motor symptoms,[31,32] and the Parkinson’s disease cognitive pattern (PDCP), associated with cognitive dysfunction.[33,34] A control sample of 11 healthy subjects with wildtype *GBA* and an identical [18F]FDG PET scan was used to z-transform raw subject scores. Regional metabolic changes were analyzed in a whole-brain voxel-wise two-sample t-test of normalized [18F]FDG uptake, thresholded at p < 0.05 family-wise error (FWE)-corrected at the cluster level. Proportional scaling with default settings was applied in PET analyses ([18F]FDopa and [18F]FDG global signals were similar between groups).

Rs-fMRI data were analyzed by seed-to-voxel functional connectivity (FC) analysis of each left and right putamen, caudate and nucleus accumbens; second-level group comparisons were thresholded at p < 0.05, cluster-level FWE-corrected. In all neuroimaging analyses, anatomical regions were defined with the Harvard-Oxford cortical and subcortical atlas as implemented in Conn. For each modality, mean cluster values ([18F]FDopa or [18F]FDG uptake for PET, FC beta values for rs-fMRI) were extracted from significant clusters found in the voxel-wise analyses.

**Results:**

*Genotyping results*

Gene panel analysis identified seven patients (12.5%) who were heterozygous for the c.1093G>A (p.E365K (p.E326K)) variant and six patients (10.7%) heterozygous for c.1223C>T (p.T408M (p.T369M)), thus 23.2% of the cohort carried a *GBA* variant. One patient carried the Gaucher’s-associated *GBA* mutation c.1226A>G (p.N409S (p.N370S)) and was excluded from further analysis. The remaining 42 patients had no variant in *GBA*.

Additionally, one *GBA* wildtype patient was heterozygous for the likely benign variant c.1000C>T (p.R334C) in *PRKN*; one carrier of *GBA*:p.E365K was also heterozygous for the likely benign variant c.587C>T (p.P196L) in *PINK1* and the *PRKN* mutation c.823C>T (p.R275W). While the *PRKN* mutation p.R275W in biallelic carriers is associated with autosomal-recessive Parkinson’s disease,[35] only a heterozygous carrier was detected here. Group comparisons were repeated without the *PRKN* mutation carrier, and since results were largely unaltered, the subject was not excluded from final analyses.

Since carriers of the two variants p.E365K and p.T408M were similar concerning all measures of interest in this study (see Fig. 1, Tables 2, 3, S1), variant carriers were combined into one group for all comparisons with non-carriers.

*Demographic data*

The 42 patients with wildtype *GBA* were 65.0 ± 10.2 years old and 27 (64.3%) of them were male. The 13 variant carriers were 66.7 ± 8.9 years old and 11 (84.6%) were male. In each group, three patients had a first-degree relative with Parkinson’s disease, corresponding to 7.1% of wildtype and 23.1% of variant carriers. These differences were not statistically significant; details are presented in Table 2.

*Clinical history and symptoms*

Clinical history, symptom severity and motor subtypes were not significantly different between genotypes and are detailed in Table 2. Wildtype patients had an average age at onset of 60.1 ± 10.2 years compared to 61.2 ± 8.1 years in the variant group, disease duration was 4.9 ± 4.0 (wildtype) versus 5.5 ± 3.9 (variant carriers) years. Median Hoehn and Yahr stages were similar between groups with 2.5 (range: 1-3) for patients with the *GBA* wildtype sequence and 2 (2-3) for variant carriers. Total UPDRS-III scores were 23.7 ± 9.1 in patients with wildtype *GBA* and 16% higher (27.5 ± 10.9) in variant carriers; levodopa equivalent daily dose (LEDD)[36] was 455.8 ± 286.1 mg in non-carriers and 26% higher (577.1 ± 253.9 mg) in carriers; these group differences were not statistically significant. Likewise, no significant differences were detected with the non-motor symptom scale.

Variant carriers scored lower in all five cognitive domains. The global cognition z-score was significantly lower when BDI-II was included as a covariate (p = 0.039). Among the cognitive domains, executive and visual-spatial functions were the most affected, although group differences were narrowly not significant. BDI-II scores were significantly lower in carriers (5.8 ± 6.4) than in non-carriers (11.6 ± 7.8; p = 0.012), whereas groups were similar concerning apathy, (hypo-)mania and impulsivity. Clinical and behavioral data are summarized in Table 2.

*Metabolomics*

Abundance of 1,5-anhydro-D-glucitol, asparagine, ornithine, glutamine and glycine, as well as the unknown metabolites with retention indices (RI) RI1169 and RI1568, was increased in *GBA* variant carriers compared to non-carriers, while an unknown metabolite RI1120 showed decreased levels in carriers. All variant carriers were included in the metabolomics analysis. Results are presented in Fig. 1 and Table S1.

*Neuroimaging*

Genotyping results in imaging subsamples can be found in Table 3. [18F]FDopa uptake was reduced in the bilateral caudate nuclei, the antero-medial putamen ipsilateral and nucleus accumbens contralateral to the more affected body side in variant carriers compared to non-carriers. PDRP expression was significantly higher in patients with *GBA* variants (3.07 ± 1.67) than in patients with wildtype *GBA* (0.99 ± 1.71, p = 0.0007), with similar scores in both variants (see Fig. 1C). PDCP expression was higher in carriers (0.64 ± 1.18) than non-carriers (0.11 ± 1.41) but not significantly different between groups (p = 0.250). *GBA* variant carriers showed significantly reduced [18F]FDG PET activity in the bilateral medial and lateral parietal lobe. Functional connectivity was significantly reduced between the left and right caudate nuclei and the bilateral occipital cortex in carriers; the right nucleus accumbens showed reduced connectivity with the left superior parietal and right occipital fusiform cortex. More precisely, FC values were near zero in wildtype and negative in variant carriers, showing anticorrelations between activity fluctuations of the seed regions and the occipital/parietal cortex. FC of the left nucleus accumbens and left and right putamen was not different between groups. Results of voxel-wise group comparisons are detailed in Table 3 and depicted in Fig. 2.

*Further analyses*

Group characteristics in the subsamples for each analysis were similar to the whole sample (see Tables S2a–d). In patients with [18F]FDG PET, the higher LEDD in carriers reached statistical significance (p = 0.017, Table S2b). To control for a potential influence of disease duration or antiparkinsonian medication, clinical, metabolomic and imaging data were additionally compared with correction for disease duration and LEDD, which had only minimal effects on group differences; PDRP results remained significant with UPDRS-III as covariate (p = 0.0015). The difference in BDI-II scores could not be explained by antidepressive medication or dopamine agonists, which were similar between groups (Tables 2, S2a-d). When apathy evaluation scale (AES) scores were corrected for BDI-II, a trend for more apathy in variant carriers was observed (p = 0.083).

**Discussion:**

This is the first study to date that provides detailed phenotypical data about the two main *GBA* variants that do not cause Gaucher’s disease but are associated with Parkinson’s disease, p.E365K and p.T408M. Results point to similarities with Gaucher’s disease-related *GBA* mutations and are suggestive of a more severe course of the disease.

The present cohort had a relatively high proportion of *GBA* variant carriers (23.2%) compared to less than 10% reported in previous studies, while the rate of *GBA* mutations (1/56) was comparably low.[9,16] The total frequency of *GBA* alterations was not significantly higher than in the Dutch PROPARK cohort,[9] and considering the sample size is probably coincidental. Mutations are much rarer than variants, most of them occurring far less frequently than p.N409S,[9] so they may be missed in a small cohort. The high proportion of variant carriers makes it extremely unlikely that exclusion criteria (e.g. dementia) were biased against them. Carriers had a trend for more first-degree relatives with Parkinson’s disease than non-carriers (23.1% vs. 7.1%); similar rates of a positive family history are regularly reported in *GBA* mutations.[1,7] A meta-analysis has not found higher UPDRS-III scores in variant carriers,[16] whereas LEDD may be increased[11] and should be investigated longitudinally in larger cohorts. Surprisingly, *GBA* carriers showed less symptoms of depression than wildtype patients. More depression in mutation carriers has been reported,[37] but often no effect was found.[11,12,38] In non-Gaucher’s-related variants, no change in depression has been described.[11,16,39] When BDI-II was taken into account, significantly reduced global cognition was detected. However, cognition z-scores in both groups indicated function within the normal range (-0.13 in wildtypes vs. -0.34 in carriers). An increased risk for cognitive deficits has previously only been demonstrated for p.E365K. In this study, both variants scored similarly in global cognition and BDI-II, thus contributing equally to the group difference.

While carriers and non-carriers presented only minor clinical differences, metabolomics and neuroimaging highlighted interesting effects of *GBA* variants. Levels of eight metabolites were suggestively altered in *GBA* variant carriers compared to non-carriers, i.e. 1,5-anhydro-D-glucitol, asparagine, ornithine, glutamine, glycine and two unidentified metabolites (RI1169 and RI1568) being increased, and the unknown metabolite RI1120 being decreased. Further elucidation is required for the identification and validation of the unknown metabolites. These preliminary results provide new hints to which pathways may be altered in *GBA*-related Parkinson’s disease.

Increases of anhydroglucitol and other polyol pathway metabolites have been shown in brain tissue of a *DJ-1* knockout Parkinson’s disease mouse model, together with decreased levels of glycolysis intermediates.[40] Thereby, the polyol pathway might be dysregulated in *GBA* variant carriers. Ornithine has been reported to be higher in Parkinson’s disease patients’ than healthy controls’ cerebrospinal fluid;[41] while blood plasma levels were increased in a rotenone-lesioned rat model.[42] The authors suspected a link to impairments in mitochondrial transport and the urea cycle. Asparagine, glutamine and glycine have all been found to be significantly increased in blood plasma of Parkinson’s disease patients compared to healthy controls.[43] Higher levels in *GBA* variant carriers may indicate that changes observed in Parkinson’s disease are more severe in patients with genetically determined glucocerebrosidase dysfunction. The results of this untargeted, hypothesis-generating approach are preliminary, but can help focus future investigations on certain pathways or metabolites.

Neuroimaging studies of *GBA*-related Parkinson’s disease so far have almost exclusively examined Gaucher’s disease causing mutations, while no publications could be found that applied imaging techniques specifically in the p.E365K and p.T408M variants. Recently, metabolic networks have been investigated in Parkinson’s disease patients with Gaucher’s-related *GBA* mutations, showing similar results of significantly increased PDRP scores and slightly, not significantly increased PDCP expression values.[44] Increased PDRP-scores are in line with a more severe course of the disease and suggest that subclinical neurobiological changes are present in *GBA* variant carriers even when clinical differences are not (yet) significant. The PDRP is already expressed in very early stages of the disease, even before the onset of motor symptoms in hemiparkinsonism[32] and prodromal Parkinson’s disease.[45] Conversely, increased PDCP expression values may only reach significance with more advanced impairment: they steadily increase with disease progression and cognitive decline, but a significant group difference was described only between patients with multi-domain cognitive impairment and healthy controls.[34]

Integrity of the dopaminergic system has not previously been examined in carriers of *GBA* variants. In mutation carriers, results were variable: [18F]FDopa uptake was reduced in Gaucher’s disease patients with and without Parkinson’s disease compared to healthy controls[20]; [123I]FP-CIT binding was lower in more advanced stage Parkinson’s disease patients with severe but not mild mutations compared to non-carriers,[8] while a recent study reported higher dopamine transporter density in carriers’ more affected striatum at an early stage.[46] Discrepancies between these and our results may be due to different methodologies (i.e. higher statistical threshold, region-of-interest based approach, radiotracer and target), and group differences may evolve with progression. Here, reduced uptake was detected at a low threshold, possibly due to the small number of variant carriers with [18F]FDopa PET (n = 7). Striatal dopaminergic loss progresses from posterior to anterior in Parkinson’s disease,[47] and the caudate nucleus is involved in cognition, particularly executive functions.[48]

Interestingly, variant carriers displayed FC anticorrelations between the caudate nuclei and the occipital cortex, while non-carriers’ FC values were near zero. An almost identical pattern of caudate-occipital anticorrelations was previously reported in Parkinson’s disease dementia (PDD), compared to positive correlations in healthy controls.[49] The striking similarity between these findings strongly suggests a link to more cognitive deficits in Parkinson’s disease patients with *GBA* alterations. Additionally, altered connectivity patterns detected here could relate to *GBA* mutation carrier’s susceptibility to psychotic symptoms: in Parkinson’s disease patients with hallucinations, occipital and striatal regions show reduced connectivity,[50] and visual areas are deactivated during the hallucinatory experience.[51] In schizophrenia with visual hallucinations, abnormal FC was described between the nucleus accumbens and higher visual areas.[52] Functional MRI has, to our knowledge, never before been applied in *GBA*-related Parkinson’s disease.

Parieto-occipital cortex hypoactivity in our cohort was very similar to previous findings in Parkinson’s disease with *GBA* mutations[20] and in DLB,[8] as well as in Parkinson’s disease with visual hallucinations.[53] In line with this, cortical Lewy body load has been shown to be higher in Parkinson’s patients carrying *GBA* mutations.[54] In our cross-sectional study of mid- and early-stage patients, hallucinations or psychotic symptoms were not observed, but imaging findings indicate an increased susceptibility to these symptoms in variant carriers, which has repeatedly been described in *GBA* mutation carriers.[1] It has been shown that hallucinations and temporo-parieto-occipital hypometabolism precede PDD,[53] but to date no imaging predictors of hallucinations in Parkinson’s disease have been described.

Despite a high degree of similarity between the two variants investigated here (tables, Fig. 1), future studies with more participants should address potential differences between them. To date, most neuroimaging studies are performed without genetic testing. The unexpectedly high number of carriers found here is a reminder that, especially in smaller cohorts, the rate at which certain genetic risk variants occur is somewhat random. Depending on the methods used, results could potentially be confounded by the genetic risk profile of included patients. The results of previous studies in the same cohort investigated here are unrelated to the differences detected between carriers and non-carriers of *GBA* variants.[25–27]

One reason why *GBA* has lately been drawing attention among researchers and clinicians is the hope for an – at least partially – causative therapy targeting glucocerebrosidase metabolism. Ambroxol, e.g., enhances glucocerebrosidase activity in Parkinson’s disease patients’ cerebrospinal fluid,[55] and a randomized controlled trial in PDD patients with and without *GBA* mutations or variants is currently recruiting.[56]

In conclusion, metabolomic and neuroimaging findings are suggestive of a more severe Parkinson’s disease pathology and demonstrate similarities with PDD and DLB even in carriers with only minimal cognitive decline. Group differences were apparent at the (neuro-)biological level but not significant at the clinical level. The lack of clinical differences is congruent with the hypothesis that more severe mutations have a more profound effect on the clinical course,[8] and evidence suggesting that patients with non-Gaucher’s-related variants fall in between carriers of mild mutations and non-carriers.[9,16] Similarities with DLB are thought to be most apparent with severe mutations,[1] but here were also seen in patterns of cortical hypoactivity. We demonstrate for the first time that even in the absence of a significantly more severe clinical syndrome, subclinical findings are present in “mild” *GBA* variants. To the best of our knowledge, this is the most in-depth description to date of clinical and biological phenotypes of Parkinson’s disease patients carrying the *GBA* variants p.E365K and p.T408M. Metabolomic changes have to be validated, and longitudinal studies are needed to investigate whether the observed neuroimaging changes progress, or if they are followed by significantly more severe motor symptoms, clinical dementia and psychotic symptoms. Similar approaches could be applied to other genetic variants associated with Parkinson’s disease, and the observed alterations could be promising to monitor effects of targeted disease-modifying treatments.

**Author Contributions:**

A.G., K.L., D.E., L.T., K.H., M.T., A.D., N.D., C.E.: conception and design of the study. A.G., J.-P.T., E.G., M.C.R., F.M., C.J., Z.H., K.L., Y.M.: acquisition and analysis of data. A.G., J.-P.T., N.D.: drafting of the manuscript. All authors: review of the manuscript.

**References:**

1 Blandini F, Cilia R, Cerri S, et al. Glucocerebrosidase mutations and synucleinopathies: Toward a model of precision medicine. *Movement Disorders* 2019; **34**: 9-21.

2 Kolter T, Sandhoff K. Sphingolipids—Their Metabolic Pathways and the Pathobiochemistry of Neurodegenerative Diseases. *Angewandte Chemie International Edition* 1999; **38**: 1532-1568.

3 Stirnemann J, Belmatoug N, Camou F, et al. A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. *International Journal of Molecular Sciences* 2017; **18**: 441.

4 Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol* 2012; **11**: 986-998.

5 Migdalska‐Richards A, Schapira AHV. The relationship between glucocerebrosidase mutations and Parkinson disease. *Journal of Neurochemistry* 2016; **139**: 77-90.

6 Nalls MA, Duran R, Lopez G, et al. A Multicenter Study of Glucocerebrosidase Mutations in Dementia With Lewy Bodies. *JAMA Neurol* 2013; **70**: 727-735.

7 Sidransky E, Nalls MA, Aasly JO, et al. Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson’s Disease. *New England Journal of Medicine* 2009; **361**: 1651-1661.

8 Cilia R, Tunesi S, Marotta G, et al. Survival and dementia in GBA-associated Parkinson’s disease: The mutation matters. *Annals of Neurology* 2016; **80**: 662-673.

9 Liu G, Boot B, Locascio JJ, et al. Specifically neuropathic Gaucher’s mutations accelerate cognitive decline in Parkinson’s. *Ann Neurol* 2016; **80**: 674-685.

10 Mata IF, Leverenz JB, Weintraub D, et al. GBA Variants are associated with a distinct pattern of cognitive deficits in Parkinson’s disease. *Mov Disord.* 2016; **31**: 95-102.

11 Jesús S, Huertas I, Bernal-Bernal I, et al. GBA Variants Influence Motor and Non-Motor Features of Parkinson’s Disease. *PLOS ONE* 2016; **11**: e0167749.

12 Thaler A, Gurevich T, Bar Shira A, et al. A ‘dose’ effect of mutations in the GBA gene on Parkinson’s disease phenotype. *Parkinsonism Relat. Disord.* 2017; **36**: 47-51.

13 Creese B, Bell E, Johar I, Francis P, Ballard C, Aarsland D. Glucocerebrosidase mutations and neuropsychiatric phenotypes in Parkinson’s disease and Lewy body dementias: Review and meta-analyses. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 2018; **177**: 232-241.

14 Mallett V, Ross JP, Alcalay RN, et al. GBA p.T369M substitution in Parkinson disease: Polymorphism or association? A meta-analysis. *Neurol Genet* 2016; **2**: e104.

15 Huang Y, Deng L, Zhong Y, Yi M. The Association between E326K of GBA and the Risk of Parkinson’s Disease. *Parkinsons Dis* 2018; DOI:10.1155/2018/1048084.

16 Iwaki H, Blauwendraat C, Leonard HL, et al. Genetic risk of Parkinson disease and progression: *Neurol Genet* 2019; **5**: e348.

17 Davis MY, Johnson CO, Leverenz JB, et al. Association of GBA Mutations and the E326K Polymorphism With Motor and Cognitive Progression in Parkinson Disease. *JAMA Neurol* 2016; **73**: 1217-1224.

18 Pchelina S, Baydakova G, Nikolaev M, et al. Blood lysosphingolipids accumulation in patients with parkinson’s disease with glucocerebrosidase 1 mutations. *Movement Disorders* 2018; **33**: 1325-1330.

19 Alcalay RN, Levy OA, Waters CC, et al. Glucocerebrosidase activity in Parkinson’s disease with and without GBA mutations. *Brain* 2015; **138**: 2648-2658.

20 Goker-Alpan O, Masdeu JC, Kohn PD, et al. The neurobiology of glucocerebrosidase-associated parkinsonism: a positron emission tomography study of dopamine synthesis and regional cerebral blood flow. *Brain* 2012; **135**: 2440-2448.

21 Oeda T, Umemura A, Mori Y, et al. Impact of glucocerebrosidase mutations on motor and nonmotor complications in Parkinson’s disease. *Neurobiology of Aging* 2015; **36**: 3306-3313.

22 Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967; **17**: 427-442.

23 Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson’s disease. *Movement Disorders* 2007; **22**: 1689-1707.

24 Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson’s disease. *Journal of Neurology, Neurosurgery & Psychiatry* 1988; **51**: 745-752.

25 Glaab E, Trezzi J-P, Greuel A, et al. Integrative analysis of blood metabolomics and PET brain neuroimaging data for Parkinson’s disease. *Neurobiology of Disease* 2019; **124**: 555-562.

26 Hammes J, Theis H, Giehl K, et al. Dopamine metabolism of the nucleus accumbens and fronto-striatal connectivity modulate impulse control. *Brain* 2019; **142**: 733-743.

27 Ruppert MC, Greuel A, Tahmasian M, et al. Network degeneration in Parkinson’s disease: multimodal imaging of nigro-striato-cortical dysfunction. *Brain*. DOI:10.1093/brain/awaa019.

28 Eggers C, Kahraman D, Fink GR, Schmidt M, Timmermann L. Akinetic-rigid and tremor-dominant Parkinson’s disease patients show different patterns of FP-CIT Single photon emission computed tomography. *Movement Disorders* 2011; **26**: 416-423.

29 Semkovska M, Quinlivan L, O’Grady T, et al. Cognitive function following a major depressive episode: a systematic review and meta-analysis. *Lancet Psychiatry* 2019; **6**: 851-861.

30 Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* 2012; **2**: 125-141.

31 Huang C, Tang C, Feigin A, et al. Changes in network activity with the progression of Parkinson’s disease. *Brain* 2007; **130**: 1834-1846.

32 Tang CC, Poston KL, Dhawan V, Eidelberg D. Abnormalities in Metabolic Network Activity Precede the Onset of Motor Symptoms in Parkinson’s Disease. *J. Neurosci.* 2010; **30**: 1049-1056.

33 Huang C, Mattis P, Tang C, Perrine K, Carbon M, Eidelberg D. Metabolic brain networks associated with cognitive function in Parkinson’s disease. *Neuroimage* 2007; **34**: 714-723.

34 Meles SK, Tang CC, Teune LK, et al. Abnormal metabolic pattern associated with cognitive impairment in Parkinson’s disease: a validation study. *J. Cereb. Blood Flow Metab.* 2015; **35**: 1478-1484.

35 Huttenlocher J, Stefansson H, Steinberg S, et al. Heterozygote carriers for CNVs in PARK2 are at increased risk of Parkinson’s disease. *Hum Mol Genet* 2015; **24**: 5637-5643.

36 Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson’s disease. *Movement Disorders* 2010; **25**: 2649-2653.

37 Brockmann K, Srulijes K, Hauser A-K, et al. GBA-associated PD presents with nonmotor characteristics. *Neurology* 2011; **77**: 276-280.

38 Alcalay RN, Caccappolo E, Mejia-Santana H, et al. Cognitive performance of GBA mutation carriers with early-onset PD: the CORE-PD study. *Neurology* 2012; **78**: 1434-1440.

39 Winder-Rhodes SE, Evans JR, Ban M, et al. Glucocerebrosidase mutations influence the natural history of Parkinson’s disease in a community-based incident cohort. *Brain* 2013; **136**: 392-399.

40 Hauser DN, Mamais A, Conti MM, et al. Hexokinases link DJ-1 to the PINK1/parkin pathway. *Molecular Neurodegeneration* 2017; **12**: 70.

41 Wuolikainen A, Jonsson P, Ahnlund M, et al. Multi-platform mass spectrometry analysis of the CSF and plasma metabolomes of rigorously matched amyotrophic lateral sclerosis, Parkinson’s disease and control subjects. *Mol. BioSyst.* 2016; **12**: 1287-1298.

42 Fagotti J, Targa ADS, Rodrigues LS, et al. Chronic sleep restriction in the rotenone Parkinson’s disease model in rats reveals peripheral early-phase biomarkers. *Sci Rep* 2019; **9**: 1-16.

43 Jiménez-Jiménez FJ, Molina J, Vargas C, et al. Neurotransmitter amino acids in cerebrospinal fluid of patients with Parkinson’s disease. *Journal of the Neurological Sciences* 1996; **141**: 39-44.

44 Schindlbeck KA, Vo A, Nguyen N, et al. LRRK2 and GBA Variants Exert Distinct Influences on Parkinson’s Disease-Specific Metabolic Networks. *Cereb Cortex* 2019; DOI:10.1093/cercor/bhz280.

45 Holtbernd F, Gagnon J-F, Postuma RB, et al. Abnormal metabolic network activity in REM sleep behavior disorder. *Neurology* 2014; **82**: 620-627.

46 Simuni T, Brumm MC, Uribe L, et al. Clinical and Dopamine Transporter Imaging Characteristics of Leucine- Rich Repeat Kinase 2 (LRRK2) and Glucosylceramidase Beta (GBA) Parkinson’s Disease Participants in the Parkinson’s Progression Markers Initiative: A Cross-Sectional Study. *Movement Disorders* 2020; DOI:10.1002/mds.27989.

47 Mishina M, Ishii K, Suzuki M, et al. Striatal Distribution of Dopamine Transporters and Dopamine D2 Receptors at Different Stages of Parkinson’s Disease: A CFT and RAC PET Study. *Neuroradiol J* 2011; **24**: 235-241.

48 Grahn JA, Parkinson JA, Owen AM. The cognitive functions of the caudate nucleus. *Progress in Neurobiology* 2008; **86**: 141-155.

49 Rektorova I, Krajcovicova L, Marecek R, Mikl M. Default Mode Network and Extrastriate Visual Resting State Network in Patients with Parkinson’s Disease Dementia. *NDD* 2012; **10**: 232-237.

50 Hepp DH, Foncke EMJ, Olde Dubbelink KTE, van de Berg WDJ, Berendse HW, Schoonheim MM. Loss of Functional Connectivity in Patients with Parkinson Disease and Visual Hallucinations. *Radiology* 2017; **285**: 896-903.

51 Goetz CG, Vaughan CL, Goldman JG, Stebbins GT. I finally see what you see: Parkinson’s disease visual hallucinations captured with functional neuroimaging. *Movement Disorders* 2014; **29**: 115-117.

52 Rolland B, Amad A, Poulet E, et al. Resting-State Functional Connectivity of the Nucleus Accumbens in Auditory and Visual Hallucinations in Schizophrenia. *Schizophr Bull* 2015; **41**: 291-299.

53 Gasca‐Salas C, Clavero P, García‐García D, Obeso JA, Rodríguez‐Oroz MC. Significance of visual hallucinations and cerebral hypometabolism in the risk of dementia in Parkinson’s disease patients with mild cognitive impairment. *Human Brain Mapping* 2016; **37**: 968-977.

54 Neumann J, Bras J, Deas E, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson’s disease. *Brain* 2009; **132**: 1783-1794.

55 Mullin S, Smith L, Lee K, et al. Ambroxol for the Treatment of Patients With Parkinson Disease With and Without Glucocerebrosidase Gene Mutations. *JAMA Neurol* 2020; DOI: 10.1001/jamaneurol.2019.4611.

56 Silveira CRA, MacKinley J, Coleman K, et al. Ambroxol as a novel disease-modifying treatment for Parkinson’s disease dementia: protocol for a single-centre, randomized, double-blind, placebo-controlled trial. *BMC Neurology* 2019; **19**: 20.

**Figure legends:**

**Figure 1:** Group comparison of metabolomics and [18F]FDG-PET covariance patterns

Metabolites with significantly A) increased and B) decreased levels in *GBA* variant carriers. C) PDRP and PDCP expression. The two variants are indicated by color and shape of dots, but were combined for group comparisons. Significance levels: \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

**Figure 2:** Reduced metabolism and functional connectivity in *GBA* variant carriers

Clusters found in voxel-wise group comparisons of A) [18F]FDopa uptake (3D view, template cut at y = 8); B) [18F]FDG uptake; C) functional connectivity analysis (red: seed in left caudate; dark blue: seed in right caudate; purple: overlap; light blue: seed in right accumbens). IL, ipsilateral; CL, contralateral; L, left; R, right; P, posterior; S, superior.

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