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Transcranial sonography and functional imaging in glucocerebrosidase mutation Parkinson disease

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Abstract

Background—Heterozygous glucocerebrosidase (*GBA*) mutations are the leading genetic risk factor for Parkinson disease, yet imaging correlates, particularly transcranial sonography, have not been extensively described.

Methods—To determine whether *GBA* mutation heterozygotes with Parkinson disease demonstrate hyperechogenicity of the substantia nigra, transcranial sonography was performed in Ashkenazi Jewish Parkinson disease subjects, tested for the eight most common Gaucher disease mutations and the *LRRK2* G2019S mutation, and in controls. [¹⁸F]-fluorodeoxyglucose or [¹⁸F]-fluorodopa positron emission tomography is also reported from a subset of Parkinson disease subjects with heterozygous *GBA* mutations.

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Results—Parkinson disease subjects with heterozygous *GBA* mutations (n=23) had a greater median maximal area of substantia nigral echogenicity compared to controls (n=34, aSNmax=0.30 vs. 0.18, p=0.007). There was no difference in median maximal area of nigral echogenicity between Parkinson disease groups defined by *GBA* and *LRRK2* genotype: *GBA* heterozygotes; *GBA* homozygotes/compound heterozygotes (n=4, aSNmax=0.27); subjects without *LRRK2* or *GBA* mutations (n=32, aSNmax=0.27); *LRRK2* heterozygotes/homozygotes without *GBA* mutations (n=27, aSNmax=0.28); and *GBA* heterozygotes/*LRRK2* heterozygotes (n=4, aSNmax=0.32, overall p=0.63). In secondary analyses among Parkinson disease subjects with *GBA* mutations, maximal area of nigral echogenicity did not differ based on *GBA* mutation severity or mutation number. [¹⁸F]-fluorodeoxyglucose (n=3) and [¹⁸F]-fluorodopa (n=2) positron emission tomography in Parkinson disease subjects with heterozygous *GBA* mutations was consistent with findings in idiopathic Parkinson disease.

Conclusions—Both transcranial sonography and positron emission tomography are abnormal in *GBA* mutation associated Parkinson disease, similar to other Parkinson disease subjects.

Keywords

Parkinson disease; parkinsonism; diffuse Lewy body disease; Transcranial sonography; PET; Glucocerebrosidase; *GBA*; Gaucher disease; *LRRK2*

Introduction

Heterozygous glucocerebrosidase (*GBA*) mutations have emerged as the leading genetic risk factor for Parkinson disease (PD) [1–3]. In Asian [4, 5] and non-Jewish European populations [6–8], 4–9% of PD patients have heterozygous *GBA* mutations, while in the Ashkenazi Jewish population, up to 20% of PD patients are *GBA* heterozygotes [8]. Although many individual PD patients with heterozygous *GBA* mutations clinically resemble PD without identified mutations, carriers overall have an earlier age of onset and greater cognitive impairment [8–11]. Neuroimaging modalities have the potential to further clarify the phenotype and pathophysiology of PD associated with *GBA* mutations. Transcranial sonography (TCS) detects substantia nigral hyperechogenicity in 90% of patients with idiopathic PD [12]. The nigral hyperechogenicity likely reflects increased local iron content and microglial activation [13,14]. With the exception of parkinsonism associated with *ATP13A2* mutations [15], TCS investigations in *PINK1* [16], *PRKN* [17], *SNCA* [18], and *LRRK2* associated PD [19], have revealed hyperechogenic substantia nigra. TCS in 3 Gaucher disease type 1 (GD1) patients with parkinsonism and 20 PD patients with heterozygous *GBA* mutations revealed hyperechogenic substantia nigra, suggesting that nigral hyperechogenicity is also a feature of *GBA*-associated PD [20,21]. The pathophysiological similarity of *GBA*-associated PD with idiopathic PD is also supported by functional imaging studies showing a presynaptic dopaminergic deficit in a small number of *GBA* homozygotes and heterozygotes [20,22]. Herein we evaluated TCS in a cohort with *GBA*-associated PD, including *GBA* heterozygotes with mild and severe mutations, *GBA* compound heterozygotes and homozygotes, and *GBA*/*LRRK2* G2019S mutation carriers, and compared these with *LRRK2* mutation carriers. We assessed whether *GBA* mutation severity and the number of mutant alleles is associated with the degree of hyperechogenicity in those with PD. We also extend the literature by reporting functional imaging with [¹⁸F]-fluorodeoxyglucose (FDG) and [¹⁸F]-fluorodopa (FDOPA) positron emission tomography (PET) in a subset.

Methods

Ashkenazi Jewish patients who met diagnostic criteria for PD were evaluated at the Movement Disorder Center at Beth Israel Medical Center and invited to participate in a

genetic study of PD, including mutation screening and transcranial sonography [23,24]. Blood or saliva samples were obtained, DNA was extracted, and samples were screened for the *LRRK2* G2019S mutation [24] and the eight most common Ashkenazi Jewish *GBA* mutations (N370S, L444P, 84GG, IVS2+1G→A, V394L, del55bp, D409H, and R496H) as previously reported [20]. PD subjects were characterized as *GBA* heterozygotes without *LRRK2* mutations (PD-*GBA* single), *GBA* homozygotes or compound heterozygotes (PD-*GBA* double), *GBA* heterozygotes with *LRRK2* mutations (PD-*GBA/LRRK2*), *LRRK2* heterozygotes (PD-*LRRK2* single), *LRRK2* homozygotes (PD-*LRRK2* double), and subjects without *LRRK2* or *GBA* mutations (PD-no mutation). Spouses and healthy laboratory controls (14 Ashkenazi Jewish, 16 non-Jewish) without neurological disease or a family history of PD were also recruited. The study procedures were approved by the institutional review board at Beth Israel Medical Center, and all subjects provided informed consent.

Transcranial Sonography

Experienced sonographers, blinded to the genetic status of the subjects, performed TCS on PD subjects and controls using the SONOS 5500 ultrasound system (Phillips) equipped with a 2.0–2.5 MHz sector transducer (S3 probe). The examination was performed bilaterally at the pre-auricular temporal bone window with a penetration depth of 14–15 cm. The images were adjusted for gain power, compression and time-gain compensation depending on the quality of the individual bone window. Images of the mesencephalic brainstem were digitally stored for later analysis. The area of hyperechogenicity in the ipsilateral SN was manually encircled by an independent investigator blinded to PD and gene status and measured using computer-based analysis (Scion Image Beta 4.02 Win software package). For statistical analysis, the larger aSN of each individual was selected (aSNmax), or in cases of an insufficient bone window on one side, the ipsilateral aSN of the analyzable side [25]. Analysis of TCS images included data from 30 non-PD controls, 52 new PD subjects and 35 PD subjects who were previously reported (22 PD-*LRRK2*, 6 PD-no mutation, 3 PD-*GBA/LRRK2*, 1 PD-*LRRK2* double [20], and 3 PD-*GBA* double subjects [19]). Representative TCS images are included in Figure 1.

Positron Emission Tomography

FDG PET had been performed in 3 subjects with heterozygous *GBA* mutations using previously described methods [26]: 35 to 45 minutes after injection with 5–6.4 mCi of FDG, 35 PET slices parallel to the orbitomeatal line were acquired in 3D over 10 minutes. A measured attenuation correction and corrections for scatter and randoms were applied. For each subject, expression of the Parkinson disease-related metabolic covariance pattern (PDRP), as determined by network analysis of FDG PET in 20 PD patients and 20 age-matched healthy controls [27,28], was quantified using a fully automated voxel-based algorithm [27,29]. The results were Z-transformed and the mean control PDRP score was set to zero.

In addition to FDG PET, Fluorodopa (FDOPA) PET had been performed in 2 different subjects with heterozygous *GBA* mutations using previously described methods [30]: 35 to 100 minutes after injection with 4.0 and 6.3 mCi of FDOPA, 35 PET slices parallel to the orbitomeatal line were acquired in 3D over 10 minutes. The striato-occipital ratio (SOR) was assessed by placing the regions of interest for the caudate, putamen, and the occipital cortex (0.6, 1.6, and 3.9 cm², respectively) on composite FDOPA PET slices. SOR values were obtained by dividing the difference between striatal and occipital activity by occipital activity. A dopaminergic deficit was defined as putamen SOR value 2 SD below the mean for 20 healthy controls (age: 51 ± 13 years) [31]. Additionally, putaminal FDOPA uptake

ratios were compared to the uptake for 20 early PD patients (mean age: 53 ± 9 years; disease duration: 2.6 ± 2.4 years) [31].

Statistical Analysis

Mann-Whitney and Fisher's exact tests were used for bivariate comparisons of continuous and categorical variables, respectively. For comparison of all parkinsonian groups, Kruskal-Wallis and Fisher's exact tests were used. (Stata 11, 2009. College Station, TX: StataCorp LP.).

Results

TCS was performed in 90 PD subjects: 23 PD-*GBA* single, 4 PD-*GBA* double, 32 PD-no mutation, 25 PD-*LRRK2* single, 2 PD-*LRRK2* double, and 4 PD-*GBA/LRRK2*. TCS was also performed in 34 non-PD controls. *GBA* mutations included 17 N370S, 2 R496H, 1 V394L, 2 L444P, and 1 84GG in the PD-*GBA* single group; 1N370S/N370S, 2N370S/R496H, and 1 N370S/84GG in the PD-*GBA* double group; and 3 N370S and 1 84GG in the PD-*GBA/LRRK2* group. The mutations IVS2+1G→A, del55bp, and D409H were not encountered in our sample. Demographic characteristics and distribution of mutations are presented in Table 1. Because PD in *LRRK2* homozygotes and *LRRK2* heterozygotes does not differ [32], these groups were combined for analysis (PD-*LRRK2*). All other groups, including PD-*GBA/LRRK2*, were considered separately. There was no difference in the gender distribution ($p=0.09$) or the age at exam ($p=0.22$) between PD-*GBA* single and controls. Among the five parkinsonian groups, there were no differences in gender ($p=0.71$), age at symptom onset ($p=0.49$), or duration of disease at exam ($p=0.45$). The UPDRS III score at exam was lower in the *LRRK2* mutation group compared to PD-*GBA* single ($p=0.006$), PD-*GBA* double ($p=0.03$), and PD-no mutation ($p=0.005$).

Seventy-six subjects had bilaterally adequate bone windows, and 10 had only one window (3 PD-*GBA* single, 2 PD-no mutation, 5 PD-*LRRK2*). One of the PD-*GBA* single subjects (N370S) did not have an adequate bone window and was not included in the analysis.

PD-*GBA* single were more echogenic than non-PD controls ($p=0.007$). There was no difference in aSNmax between the five parkinsonian groups ($p=0.63$) (Figure 2). The PD-*GBA* double subjects did not have a different aSNmax compared to the 23 PD-*GBA* single subjects ($p=0.68$). Nor did PD-*GBA/LRRK2* differ from the PD-*GBA* single group ($p=0.20$). Further, when PD-*GBA/LRRK2* and PD-*GBA* double were combined, echogenicity still did not differ from the heterozygous group ($p=0.57$).

A secondary analysis was performed to determine whether mutation severity, as previously defined, [33] was associated with the area of substantia nigra echogenicity. Among those with heterozygous *GBA* mutations, there was no difference in aSNmax between the 5 subjects with severe mutations (L444P, 84GG, or V394L) and the 22 subjects with mild mutations ($p=0.21$).

The characteristics of the PD-*GBA* single subjects who underwent FDG and FDOPA PET scans are summarized in Table 2. In the three subjects who had FDG PET, findings were consistent with idiopathic PD. PDRP z-scores were above the range for healthy controls (-1.09 to 0.88) and fell within 2 SD of the mean for PD controls (mean $\pm$ SD= 1.55 ± 0.74). In the two subjects with FDOPA PET, there were bilateral reductions in FDOPA uptake. Both putaminal SOR values were below the range for healthy controls (1.30 to 1.97) and within 2 SD of the mean for PD controls (mean $\pm$ SD= 0.67 ± 0.15) (Supplementary Figure 1). TCS aSNmax was enlarged in both subjects with FDOPA PET (aSNmax= 0.22 , 0.33) and in two of the three subjects with FDG PET (aSNmax= 0.33 , 0.23 , 0.05).

Discussion

Our data support that *GBA* mutation carriers with PD have greater substantia nigra echogenicity than non-PD controls and do not differ from PD subjects without *GBA* mutations [20,21]. This further strengthens the idea that Lewy body disorders, including *GBA*-associated PD, idiopathic PD, and *LRRK2*-associated PD, demonstrate nigral hyperechogenicity. While cases with non-G2019S *LRRK2* mutations less consistently show midbrain Lewy body pathology, the majority of neuropathology studies in *LRRK2* G2019S mutation-associated PD demonstrated prominent dopaminergic cell loss in the substantia nigra pars compacta and locus coeruleus and midbrain Lewy body deposition typical for PD. In these cases cortical Lewy bodies were variably present. (reviewed by [34]) Almost all reported autopsy cases of *GBA*-associated PD show nigral cell loss and Lewy body pathology, although the distribution of Lewy body pathology may be more likely to be cortical. While the more frequent neocortical pathology in those with PD and *GBA* mutations compared to non-mutation PD controls is concordant with a clinical phenotype more similar to dementia with Lewy bodies, [10,35,36] the shared Lewy body pathology and nigral hyperechogenicity among *GBA*-associated PD, *LRRK2* G2019S-associated PD, and idiopathic PD suggest a shared pathophysiological mechanism. Therefore, agents specifically targeting the mechanism of *LRRK2*- or *GBA*-associated PD may provide benefit for those with idiopathic PD.

The pathogenic etiology of *GBA*-associated PD is not yet defined. Proposed mechanisms include reduced α -synuclein degradation secondary to impairment of the ubiquitin-proteasome system by mutant protein; lysosomal dysfunction leading to either or both reduced α -synuclein degradation and impaired autophagy and mitophagy; and alteration in lipid metabolism and lipid membrane composition ultimately resulting in α -synuclein aggregation (reviewed by [37]). These mechanisms, however, may not be exclusive and other factors likely also contribute to the development of parkinsonism in *GBA* mutation carriers. The finding of nigral hyperechogenicity among our PD subjects with *GBA* mutations is consistent with the proposed mechanisms, as they may be associated with oxidative stress and apoptosis and, thus, iron deposition.

TCS in PD and non-PD subjects with *PRKN* mutations and in non-PD subjects with *PINK1* mutations suggests a possible gene dosage effect with homozygotes having larger areas of echogenicity than heterozygotes [16,17,38]. The loss of function mechanisms postulated for both *PRKN* and *PINK1* mutations support this difference. *GBA* mutations have been hypothesized to contribute to PD via both loss of function and gain-of-toxic-function mechanisms [37]. Among *GBA* mutation carriers with PD in our study, nigral hyperechogenicity does not appear to be dependent on the number of *GBA* mutations. This is not consistent with a gene dosage effect, and is a finding similar to *LRRK2* PD [19].

GBA mutations can be divided into mild and severe mutations based on their propensity to cause neuronopathic Gaucher disease phenotypes (Type 2 and 3) [33]. Compared to mild *GBA* mutations, severe mutations are associated with an increased risk of developing PD and an earlier age of PD onset [39]. In a secondary analysis we did not detect a difference in nigral echogenicity between PD subjects with severe mutations compared to those with mild mutations, although the small sample size may have limited our ability to detect a difference between these two subgroups.

One limitation to our study is that the *GBA* genotype of most of our controls was unknown. As the carrier frequency in the Ashkenazim is approximately 6% and is less than 1% in the non-Jewish population [40], we expect that very few, if any, of our controls were carriers. However, if they were, this would bias our results toward finding no difference between

parkinsonian patients and non-PD controls. Similar to *PRKN* and *PINK1* mutation carriers without PD, *LRRK2* mutation carriers without parkinsonism have also been shown to have hyperechogenic SN compared to controls, suggesting that TCS may be a risk marker in these genetic etiologies of parkinsonism [16,17,19]. Finally, whereas Brockmann et al. noted interrupted raphe in PD-*GBA* heterozygotes [21], we did not systematically evaluate the raphe in our scans, and cannot comment in our population.

Because there are few reports of functional imaging studies in PD subjects with *GBA* mutations we also reported PET imaging in a subset of those who had TCS. Overall our findings are consistent with those in idiopathic PD. In a study of five heterozygous *GBA* mutation carriers with parkinsonism and three asymptomatic carriers, FDG PET demonstrated supplemental motor area hypometabolism in all, while only *GBA* heterozygotes with parkinsonism showed parieto-occipital hypometabolism [22]. The latter finding is consistent with the greater cognitive impairment reported in parkinsonian *GBA* heterozygotes. Further, [^{11}C] CFT and [^{11}C] raclopride PET did not reveal dopaminergic dysfunction in the asymptomatic carriers [22]. In our prior study of GD1 subjects with parkinsonism, FDG PET revealed hypermetabolic lentiform nuclei and areas of cortical hypometabolism (parieto-occipital, temporal, and anteromedial frontal) [20]. FDOPA PET showed reduced striatal uptake bilaterally [20]. Here we extend this data and demonstrate that PD patients with heterozygous *GBA* mutations have hypermetabolic lentiform nuclei on FDG PET and reduced striatal FDOPA uptake bilaterally. While our primary analysis was the comparison of PDRP, heterozygous *GBA* mutation carriers also had regions of cortical hypometabolism on FDG-PET, specifically in the parietal and temporal, parietal and anteromedial frontal, and parieto-occipital and temporal regions.

Two of three subjects with FDG PET consistent with PD and both subjects with reduced FDOPA uptake showed nigral hyperechogenicity. This is similar to a previous study among *PRKN* mutation carriers that found that those with abnormal FDOPA PET, regardless of PD status, also demonstrated nigral hyperechogenicity on TCS [38]. In order to address whether TCS nigral hyperechogenicity and functional imaging abnormalities are associated with *GBA* mutations independent of PD, further imaging studies of asymptomatic *GBA* carriers are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative transcranial sonography images for control and PD subjects
Transcranial sonography images of midbrain through the preauricular bone window. The ipsilateral echogenic substantia nigral area is encircled with a yellow line for a control subject (A), a PD subject with a heterozygous *GBA* mutation without a *LRRK2* mutation (B), a PD subject with a *LRRK2* mutation without a *GBA* mutation (C), and a PD subject without a *GBA* or *LRRK2* mutation (D).

Figure 2. aSNmax of PD subjects by *GBA/LRRK2* status and controls

Bracket limits represent the 25th and 75th percentiles of the range of aSNmax values for each group. Abbreviations: aSNmax = area of maximal substantia nigral echogenicity; PD-GBA single = *GBA* heterozygotes without *LRRK2* mutations; PD-no mutation = subjects without *LRRK2* or *GBA* mutations; PD-LRRK2 = *LRRK2* heterozygotes and homozygotes without *GBA* mutations.

Table 1
TCS of Parkinson's disease patients by *GBA* and *LRRK2* mutation status and non-PD controls

	PD- <i>GBA</i> single (n=23)	PD- <i>GBA</i> double (n=4)**	PD-no mutation (n=32)*	PD- <i>LRRK2</i> single (n=25)*	PD- <i>LRRK2</i> double (n=2)*	PD- <i>GBA/LRRK2</i> PD (n=4)*	Controls (n=30)
Women, %	39.1	25.0	43.8	40.0	50.0	75	62.8
Age at onset	54 (48, 62)	51 (44.5, 59)	58.5 (52, 64)	60 (48, 70)	53.5 (48, 59)	55.5 (43, 63.5)	-
Duration of disease, y	9.0 (4.0, 13.9)	7.7 (5.0, 9.2)	6.9 (3.1, 9.0)	8.5 (4.5, 10.5)	10.9 (7.2, 14.7)	8.9 (3.8, 21.9)	-
UDRS III, score	19 (14, 25)	32 (22, 35)	19 (13, 24)	12 (6, 20)	13 (12, 14)	18 (16, 19)	-
Age at exam, y	65.0 (59.0, 68.2)	60.2 (50.2, 67.6)	64.8 (60.5, 73.8)	68.2 (60.6, 74.5)	64.4 (62.7, 66.2)	65.3 (64.3, 68.0)	60 (51, 68)
asfmax, cm ²	0.3 (.20, .32)	0.27 (0.17, 0.31)	0.27 (0.24, 0.33)	0.28 (0.24, 0.33)	0.23 (0.14, 0.32)	0.32 (0.29, 0.49)	0.18 (0.11, 0.27)

Data are presented as medians with interquartile ranges.

* PD-*LRRK2* single subjects; 6 PD-no mutation subjects; 1 PD-*LRRK2* double; and 3 PD-*GBA/LRRK2* subjects were previously reported.(19)

** Three PD-*GBA* double subjects were previously reported.(20)

Table 2

Characteristics of PD-*GBA* single subjects with brain PET imaging.

Subject	1	2	3	4	5
Age at PET, y	39	59	69	65	64
Gender	Female	Female	Male	Male	Female
Duration of PD, y	2	1	3	2	5
UPDRS Part I Mentation Score	1	0	1	2	0
<i>GBA</i> mutation	N370S	L444P	N370S	N370S	V394L
TCS (ΔSNmax)	.22	.33	.23	0.05	0.34
FDOPA PET putamen	71% below control	56% below control	-	-	-
FDG PET PDRP <i>z</i> -score	-	-	1.98	2.59	1.61
FDG PET cortical hypometabolism	-	-	Parietal, temporal	Parietal, anteromedial frontal	Parieto-occipital, temporal