Parkinsonism and Related Disorders 19 (2013) 186–191

Contents lists available at SciVerse ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Transcranial sonography and functional imaging in glucocerebrosidase mutation Parkinson disease

M.J. Barrett [a,*], J. Hagenah [b], V. Dhawan [c], S. Peng [c], K. Stanley [a], D. Raymond [a], A. Deik [a], S.J. Gross [d,e], N. Schreiber-Agus [e], A. Mirelman [f,g], K. Marder [h], L.J. Ozelius [i], D. Eidelberg [c], S.B. Bressman [a,j], R. Saunders-Pullman [a,j] The LRRK2 Ashkenazi Jewish Consortium

[a] Department of Neurology, Beth Israel Medical Center, NY 10003, USA
[b] Department of Neurology, University of Lübeck, Lübeck, Germany
[c] Center for Neurosciences, The Feinstein Institute for Medical Research, Manhasset, NY 11030, USA
[d] Department of Obstetrics and Gynecology, North Bronx Healthcare Network, Albert Einstein College of Medicine, Bronx, NY, USA
[e] Human Genetics Laboratory at Jacobi Medical Center, North Bronx Healthcare Network, Bronx, NY, USA
[f] Movement Disorders Unit, Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel
[g] Ben Gurion University, Beer Sheba, Israel
[h] Columbia University, Columbia University Medical Center, NY, USA
[i] Department of Genetics, Mount Sinai School of Medicine, NY 10029, USA
[j] Department of Neurology, Albert Einstein College of Medicine, NY 10461, USA

ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised form
9 August 2012
Accepted 15 September 2012

Keywords:
Parkinson disease
Parkinsonism
Diffuse Lewy body disease
Transcranial sonography
PET
Glucocerebrosidase
GBA
Gaucher disease
LRRK2

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. [^{18}F]-fluorodeoxyglucose or [^{18}F]-fluorodopa positron emission tomography is also reported from a subset of Parkinson disease subjects with heterozygous *GBA* mutations.

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. [^{18}F]-fluorodeoxyglucose ($n = 3$) and [^{18}F]-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.

1. 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

* Corresponding author. Department of Neurology, Beth Israel Medical Center, 10 Union Square East, Suite 5K, NY 10003, USA. Tel.: +1 212 844 8719; fax: +1 212 844 8461.
E-mail address: mjbarrett79@gmail.com (M.J. Barrett).

1353-8020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.parkreldis.2012.09.007

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 [^{18}F]-fluorodeoxyglucose (FDG) and [^{18}F]-fluorodopa (FDOPA) positron emission tomography (PET) in a subset.

2. 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 (18 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.

2.1. 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 34 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 Fig. 1.

2.2. Positron emission tomography

FDG PET had been performed in 3 subjects with heterozygous *GBA* mutations using previously described methods [26]: 35–45 min after injection with 5–6.4 mCi of FDG, 35 PET slices parallel to the orbitomeatal line were acquired in 3D over 10 min. 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]: 35–100 min 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 min. 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 cm^2, 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].

2.3. 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.).

3. 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 ($p = 0.63$) (Fig. 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 ($p = 0.21$).