ORIGINAL ARTICLE

Prodromal substantia nigra sonography undermines suggested association between substrate accumulation and the risk for *GBA*-related Parkinson's disease

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Background and purpose: Individuals with *GBA* (glucocerebrosidase) mutations are at increased risk of Parkinson's disease (PD). It is still debated, however, whether this increased risk results from impaired glucocerebrosidase activity leading to substrate accumulation. Comparing the presence of prodromal PD marker in *GBA* mutation carriers and patients with Gaucher disease (GD) (in which substrate accumulation is extensive) can assist in clarifying this issue.

Methods: In this cross-sectional study, we compared the hyperechogenic area of the substantia nigra, a prodromal PD marker, in large cohorts of *GBA* mutation carriers (n = 71) and patients with GD (n = 145). Our control populations were healthy, non-carriers (n = 49) and patients with *GBA*-related PD (n = 11). Substrate accumulation was assessed from dry blood spot levels of glucosylsphingosine.

Results: Our findings indicate no contribution of substrate accumulation, as the area of hyperechogenicity is similarly enlarged relative to healthy controls in both *GBA* mutation carriers and patients with GD. Moreover, this similarity between *GBA* carriers and patients with GD persists when comparing only carriers of the N370S (c.1226A>G) mutation (n = 38) with untreated patients with GD who were homozygotes for the same mutation (n = 47). In addition, measurements of hyperechogenic area did not correlate with levels of glucosylsphingosine in the untreated patients with GD.

Conclusion: The presence of a marker of prodromal PD (substantia nigra hyperechogenicity) is independent of substrate accumulation in a population with mutated *GBA*. Although further longitudinal studies are needed to determine the precise predictive value of this marker for *GBA*-related PD, our findings raise doubts regarding the contribution of substance reduction strategies to PD prevention.

Introduction

GBA mutations have been associated with increased risk for Parkinson's disease (PD) in both heterozygote carriers [1,2] and individuals with Gaucher disease (GD) (homozygote or compound heterozygotes) [3]. However, the molecular pathways that link between *GBA* mutations and PD remain far from clear and consequently there is a lack of information on effective treatment and prevention strategies [4]. Reduced glucocerebrosidase activity resulting in substrate accumulation is considered by some as one of the causes of increased PD risk among individuals with *GBA* mutations [5,6], supporting substrate reduction

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therapy as a strategy for PD prevention [7]. Other researchers have argued that the underlying mechanism is an endoplasmic reticulum stress response, induced by misfolded glucocerebrosidase proteins [8], and recommend using a pharmacological chaperone in prevention efforts [9].

The incidence of PD among patients with GD compared with carriers of the *GBA* mutation can shed light on the contribution of substrate accumulation in risk for PD. Provided that substrate accumulation contributes to the risk for PD, the incidence of the disease among patients with GD (who accumulate substrate) would be much higher than among carriers. A major limitation in studies comparing the incidence of PD in these two populations is, however, the overall low incidence of GD and the fact that only a minority of these would eventually develop PD [10]. To overcome this limitation, we employed a marker for the risk of PD, i.e. sonographic measurement of the substantia nigra (SN) hyperechogenic area [11], with a large cohort of patients from the two at-risk populations.

Methods

We recruited patients with GD and their blood relatives with a known *GBA* mutation established by Sanger sequencing (Centogene AG, Rostock, Germany) through the Shaare Zedek Medical Center Gaucher clinic (Jerusalem, Israel). Spouses of patients and age-matched hospital employees without a known *GBA* mutation were recruited as healthy controls. The study was approved by the ethical committee of Shaare Zedek Medical Center (SZMC-0168-16) and all participants signed informed consent.

Standard brainstem sonography of all study participants was conducted by a single trained technician (M.T.) through the pre-auricular acoustic bone window using a 2.5-MHz S4-2 FAST transducer in two-dimensional mode connected to a Sparq ultrasound system (Philips Ultrasound, Bothell, WA, USA). Following identification of the butterfly-shaped mesencephalic brainstem, the maximal area of the hyperechogenic ipsilateral SN was identified in an axial plane, the image was captured, the border of the hyperechogenic area was manually marked and planimetry was performed [12]. For each participant, we used the larger of the two brain sides for analysis. In case of a unilateral temporal bone window, the available SN area was used. After area measurement, a print of the marked area was produced. Because the sonographer was not blinded to the status of some participants, one of the authors (D.A.) blindly reviewed these prints to identify any inconsistencies between the marked area and the actual hyperechogenic area at the end of the study.

Blood glucosylsphingosine (Lyso-Gb1) levels were measured using mass spectrometry of a sample from dry blood spot (Centogene) as previously described [13].

We analyzed data from four groups of participants: (i) patients with GD (homozygotes or compound heterozygotes for pathogenic mutation in the *GBA* gene) but without PD (GD-NoPD); (ii) carriers of a single known pathogenic mutation in the *GBA* gene without PD (*GBA*-NoPD); (iii) healthy controls without known *GBA* mutation and without PD (No*GBA*-NoPD); and (iv) patients with PD as well as one or two pathogenic *GBA* mutations (*GBA*-Related-PD) (including nine patients with GD and two *GBA* mutation carriers). Group sizes and participant characteristics are presented in Table 1.

As our data did not show a normal distribution we used the non-parametric Mann–Whitney *U*-test to test for differences between participant groups. For the same reason, median values and ranges are used to present the data.

Results

Figure 1 compares the area of midbrain hyperechogenicity between the different study groups. The hyperechogenic area was significantly lower in healthy controls [range 0–0.31 (median 0.11) cm^2] than in the *GBA*-Related-PD group [>0.2 cm^2 in 10/11; range 0.15–0.35 (median 0.27) cm^2) (Mann–Whitney *U*-test, $P < 0.001$], as expected. Our data also showed, however, that the hyperechogenic area in healthy controls was significantly lower than in the two groups of participants who were at risk of developing PD [GD-NoPD: range 0–0.43 (median 0.14) cm^2; *GBA*-NoPD: range 0–0.45 (median) 0.14 cm^2] ($P < 0.05$ and $P < 0.01$, respectively). The area of hyperechogenicity was not correlated with the age of participants ($P = 0.57$) or with their sex ($P = 0.8$).

The hyperechogenic area of the two groups at risk (GD-NoPD and *GBA*-NoPD) was nearly identical ($P = 0.96$), although the number of *GBA* mutations per individual in the two groups was different (homozygotes or compound heterozygotes vs. heterozygotes, respectively). Because the risk for PD varies with different severities of *GBA* mutations [14,15], we further compared data between the patients with GD-NoPD who were homozygotes for the non-neuronopathic N370S (c.1226A>G) *GBA* mutation and individuals with *GBA*-NoPD who were N370S carriers (Fig. 2a). This comparison allowed testing of the effect of the number of *GBA* mutations on hyperechogenic area, showing that it did not significantly differ between our

Table 1 Group size and participant characteristics

	GD-NoPD	*GBA*-NoPD	No*GBA*-NoPD	*GBA*-Related-PD
Analyzed	145	71	49	11
Bilateral bone window	130	68	43	11
Unilateral bone window	15	3	2	0
Excluded (no bone window)	16 (1.0%)	5 (0.7%)	3 (0.6%)	0 (0%)
Age (years)	51 (40–88)	51 (40–77)	51 (40–73)	58 (49–74)
Females	74/145 (51%)	41/71 (58%)	23/49 (47%)	5/11 (46%)
Receiving ERT	95/145 (66%)	NA	NA	6/11 (55%)

ERT, enzyme-replacement therapy; *GBA*-NoPD, *GBA* mutation carriers without PD; *GBA*-Related-PD, known *GBA* mutations [carriers or Gaucher disease (GD)] with Parkinson's disease (PD); GD-NoPD, GD without PD; NA, not applicable; No*GBA*-NoPD, no *GBA* mutations without PD. Data are given as *n* (%) and median (range).

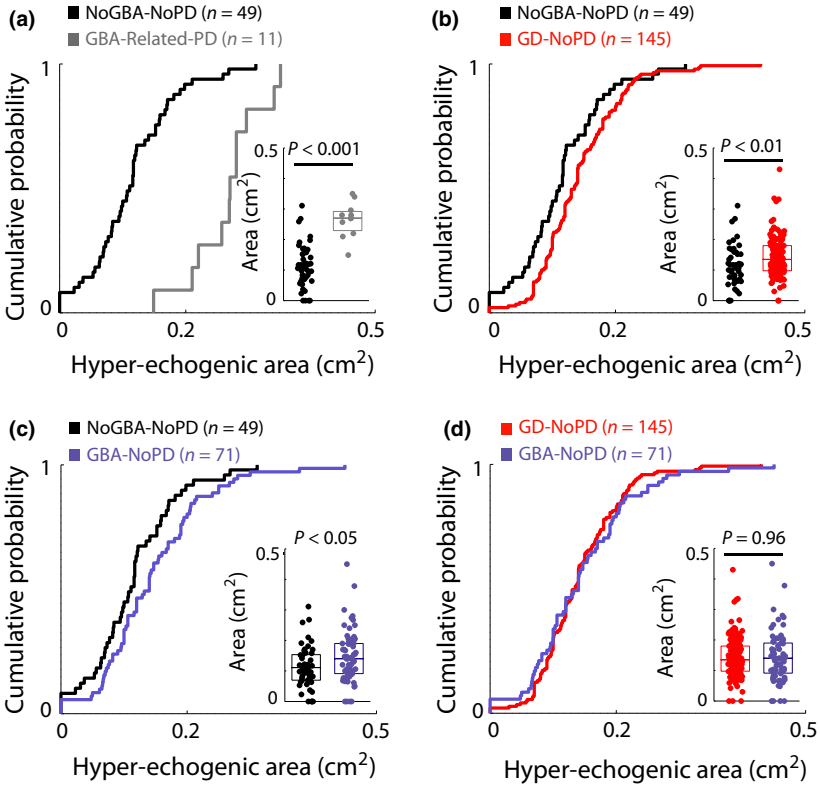

Figure 1 Maximal measured substantia nigra (SN) hyperechogenicity area. Plots of cumulative probabilities for the different groups (main axes). Right-shifted plots represent higher measured values. Data are also presented in scatter plots (inset) with boxes marking 25th, 50th and 75th percentiles. (a) Healthy non-carriers (No*GBA*-NoPD) compared with the results from known *GBA* mutation carriers and patients with Gaucher disease (GD) with Parkinson's disease (PD) (*GBA*-Related-PD). (b) No*GBA*-NoPD and patients with GD without PD (GD-NoPD). (c) No*GBA*-NoPD and *GBA* mutation carriers without PD (*GBA*-NoPD). (d) Similarity of SN area in the GD-NoPD and *GBA*-NoPD groups. [Colour figure can be viewed at wileyonlinelibrary.com]

two groups [$P = 0.25$; GD-NoPD N370S homozygotes: range 0–0.34 (median 0.13) cm^2; *GBA*-NoPD N370S carriers: range 0–0.45 (median) 0.14 cm^2]. To control for the possible contribution of enzyme-replacement therapy (ERT) itself or normalized substrate levels in treated patients with GD [16], we compared with this last result (similarity of echogenicity between carriers and patients with GD) only the group of patients with GD-NoPD who were N370S homozygotes but did not receive ERT [0–0.34 (median) 0.13 cm^2] with *GBA*-NoPD N370S carriers (Fig. 2b). The hyperechogenic area also did not differ significantly between these two groups ($P = 0.29$).

We then analyzed data on blood levels of Lyso-Gb1, the deacylated form of glucosylceramide, which were available for individuals from the group of untreated patients with GD-NoPD who were N370S homozygotes (34/38) and from *GBA*-NoPD N370S carriers (13/47). These two groups, with similarly enlarged hyperechogenic area, were clearly distinguished by their blood Lyso-Gb1 levels ($P < 10^{-6}$) (Fig. 3a). Whereas blood levels in the GD-NoPD group demonstrated accumulation of Lyso-Gb1 [18–398 (median 137.5) ng/mL], blood levels in the *GBA*-NoPD group were within the normal range [3.4–9.2 (median 5.6) ng/mL] (normal <10 ng/mL). Finally, we tested for possible correlation

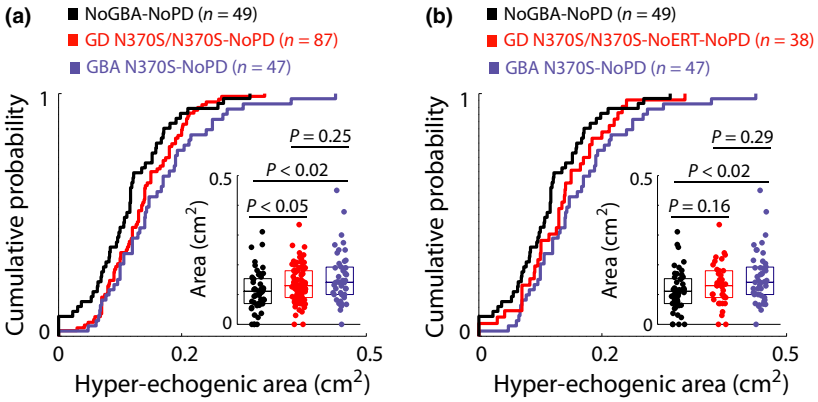

Figure 2 Maximal measured substantia nigra hyperechogenicity area. Plots of cumulative probabilities for the different groups (main axes). Right-shifted plots represent higher measured values. For clarity of interpretation, data are also presented in scatter plots (inset) with boxes marking 25th, 50th and 75th percentiles. (a) No *GBA* mutations without Parkinson's disease (PD) (No*GBA*-NoPD) compared with individuals in the Gaucher disease (GD) without PD (GD-NoPD) group who are homozygotes for the N370S mutation and individuals from the *GBA* mutation carriers without PD (*GBA*-NoPD) group who are heterozygote carriers of the N370S mutation. (b) Patients who received enzyme-replacement therapy (ERT) were excluded from the analysis (GD-NoPD group). [Colour figure can be viewed at wileyonlinelibrary.com]

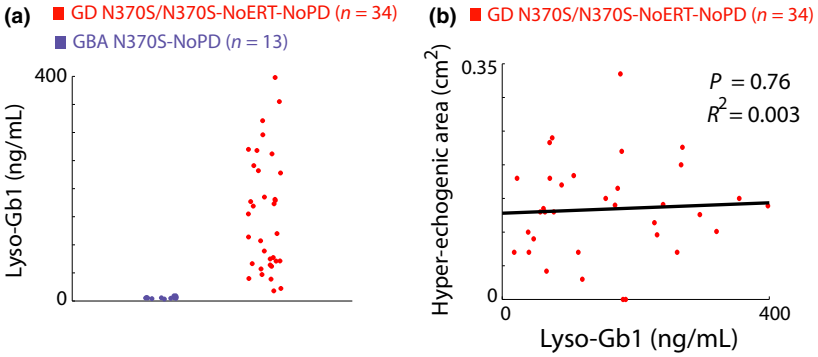

Figure 3 Glycolipid accumulation is not a risk factor for increased hyperechogenic substantia nigra (SN) area. (a) Blood glucosylsphingosine (Lyso-Gb1) levels in untreated patients with Gaucher disease (GD) who are homozygotes for the N370S mutation and in carriers of the same mutation. (b) SN hyperechogenicity area plotted against blood Lyso-Gb1 level in the untreated GD without Parkinson's disease (PD) (GD-NoPD) group who are homozygotes for the N370S mutation. ERT, enzyme-replacement therapy. [Colour figure can be viewed at wileyonlinelibrary.com]

between blood Lyso-Gb1 and the hyperechogenic SN area among the N370S homozygote untreated patients with GD-NoPD, showing a lack of correlation (Fig. 3b) (Pearson's $R^2 = 0.003$, $P = 0.76$).

Discussion

In agreement with previous studies [17–19], we show that an enlarged SN hyperechogenicity area occurs in the majority of patients with *GBA*-related PD (>0.2 cm^2 in >90% of the individuals). However, this study reveals that the mean area of hyperechogenicity is also enlarged in both *GBA* mutation carriers and patients with GD who do not have PD. Even after correcting for the type of *GBA* mutation and exposure to ERT, the hyperechogenic area is similarly enlarged in these two groups. In contrast, Lyso-Gb1 accumulation was seen only in patients with GD and did not correlate with the degree of substrate accumulation within the GD group.

Parkinson's disease is more common in both *GBA* single mutation carriers [1,2] and in patients with GD [3]. Other than their risk for PD, these two populations are clinically distinguished; *GBA* mutation carriers are healthy individuals, whereas non-neuronopathic patients with GD have spleen and liver enlargement, bone disease and thrombocytopenia [20]. Metabolically, patients with GD and not carriers accumulate glucocerebroside (as well as other glycolipids). Our results, pointing to similar susceptibility to PD among

GBA mutation carriers and patients with GD, counter the possibility that accumulation of glycolipids, secondary to impaired activity of the glucocerebrosidase enzyme, significantly influences the risk for PD. Our results are compatible with the retrospective observation that the incidence of PD is similar in these two populations [10] and stand against the logic of treating *GBA*-related PD with substrate reduction therapy [7].

We used the area of SN hyperechogenicity as a proxy for the risk for PD in our study populations. The low positive predictive value of this biomarker as a predictor for conversion of healthy individuals to PD limits routine use of this method in the clinical setting [11] and therefore it is used here to study the 'average' risk of a population and not of individual participants. Combining the presence of hyperechogenic area, which probably reflects iron accumulation [21], with the presence of other prodromal symptoms improves its predictive value to some degree [22]. In patients with PD, it has been suggested that sonography-based SN volumetry would improve the sensitivity and specificity of this biomarker [23], but the usefulness of this method in prodromal PD has not yet been established. These issues motivate the search for more innovative imaging techniques, such as neuromelanin imaging [24], that could be used to predict PD.

Although it is widely understood that a hyperechogenic area larger than 0.2 cm^2 predicts a risk for PD [25,26], the positive predictive value of areas below this value still need to be established through longitudinal studies. Nonetheless, our results show that the distribution of measured areas among the at-risk populations is shifted toward larger values. Similarly, enlarged areas of SN hyperechogenicity were demonstrated in asymptomatic carriers of other mutations of monogenic forms of PD, such as *LRRK2* [27,28], *PRKN* [29], *PINK1* [30] and *DJ1* [31], as well as in other at-risk populations, such as individuals with rapid eye movement sleep behavioral disorders [32], hyposmia [22], depression [33] or soft parkinsonian motor signs [34].

Finally, a prospective study will be needed to establish the precise risk level for PD among *GBA* mutation carriers and patients with GD. Addressing the latter issue will provide a way to determine the cellular pathways linking *GBA* mutations and PD with crucial epidemiological support.

Disclosure of conflicts of interest

D. Arkadir, T. Dinur, M. Tiomkin and M. Becker Cohen declare no financial or other conflicts of interest. N. Brüggemann was funded by the collaborative center for X-linked Dystonia-Parkinsonism, German Research Foundation (BR 4328/2-1) and Else Kroner-Fresenius Foundation. He received travel grants from Merz and St Jude Medical. C. Cozma and A. Rolfs are employees of Centogene AG. A. Zimran and S. Revel-Vilk receive honoraria from Shire, Pfizer and Sanofi/Genzyme. The Gaucher Clinic receives research grants from Shire and Pfizer, and support from Shire, Pfizer and Sanofi/Genzyme for participation in their respective registries (GOS, TALIAS and ICGG).

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