J Neurol (2013) 260:2523–2531
DOI 10.1007/s00415-013-7011-2

ORIGINAL COMMUNICATION

# Clinical, genetic, and brain sonographic features related to Parkinson's disease in Gaucher disease

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Received: 30 April 2013 / Revised: 13 June 2013 / Accepted: 14 June 2013 / Published online: 29 June 2013


**Abstract** Homozygous or compound heterozygous mutations in the glucocerebrosidase gene cause Gaucher disease. Moreover, heterozygous glucocerebrosidase gene mutations represent the most common genetic risk factor for Parkinson's disease (PD) known so far. *Substantia nigra* (SN) hyperechogenicity, a sonographic feature thought to reflect iron accumulation, has been described in both PD and Gaucher disease patients. Here we studied how clinical, genetic, and brain sonographic findings relate to the occurrence of PD in Gaucher disease. Sixteen Gaucher disease patients, 12 PD patients, and 32 control subjects were enrolled. The glucocerebrosidase genotypes were identified by DNA sequencing. All subjects underwent transcranial ultrasound, and eight Gaucher disease patients additionally MRI for comparison with SN ultrasound findings. SN hyperechogenicity and reduced echogenicity of brainstem raphe were more frequent in Gaucher disease patients (62, 37 %) than in controls (12, 12 %; $p < 0.001$, $p < 0.05$). SN hyperechogenicity in Gaucher disease patients was unrelated to type or severity of glucocerebrosidase gene mutation, but correlated with iron-sensitive MRI-T2 hypointensity of SN *pars compacta*, and with age at start of enzyme replacement therapy. While none of the five Gaucher disease patients with signs of PD (definite PD, $n = 4$; early PD, $n = 1$) had severe glucocerebrosidase gene mutations known to cause neuronopathic Gaucher disease, all carried a N370S allele, previously reported to predict non-neuronopathic Gaucher disease. Hyposmia, higher non-motor symptoms score (constipation, depression, executive dysfunction), and SN hyperechogenicity were characteristic features of Gaucher disease-related PD. We conclude that the combined clinical, genetic, and transcranial sonographic assessment may improve the PD risk evaluation in Gaucher disease.

**Keywords** Parkinson's disease · Gaucher disease · Glucocerebrosidase · GBA mutation · *Substantia nigra* hyperechogenicity

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## Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, clinically characterized by bradykinesia, rigidity, resting tremor, postural instability, and a variety of other motor and non-motor symptoms [1]. An increased prevalence of PD has been reported in patients with Gaucher disease (GD), an autosomal recessive lysosomal glycolipid storage disease caused by homozygous or compound heterozygous mutations in the glucocerebrosidase gene (*GBA*)

[2–4]. GD patients developing PD often present with an earlier onset of PD symptoms and a varying response to levodopa medication [2, 5, 6], and they show a high grade of heterogeneity of the underlying genotype [5]. It has been shown recently in cultured neurons derived from induced pluripotent stem cells from GD patients that functional loss of glucocerebrosidase compromises lysosomal protein degradation, causes accumulation of α-synuclein, and results in neurotoxicity through aggregation-dependent mechanisms [7]. On the other hand, the risk of PD was also found to be increased in carriers of heterozygous *GBA* mutations who do not develop GD. Results of large studies have confirmed that *GBA* mutations are the most common genetic risk factor for PD known so far [8, 9]. Severe (*84GG*, *IVS2(+1)*, *V394L*, *D409H*, *L444P*, *RecTL*) and mild (*N370S*, *R496H*) *GBA* mutations increased the risk of developing PD differentially, by 13.6- and 2.2-fold, and differentially affected the average age at PD onset [10]. Other well-established risk factors for PD in general population are the following: older age [1], male gender [11], and first-degree relatedness to a PD patient [12]. An about 20-fold increased risk of developing PD within 5 years has been found for subjects at ages over 50 years with *substantia nigra* (SN) hyperechogenicity on transcranial sonography (TCS) [13]. SN hyperechogenicity is a characteristic finding in PD, allowing a correct diagnosis in more than 90 % of individuals even in very early disease stages [14]. Animal and human post-mortem studies have shown that SN hyperechogenicity correlates with increased cellular iron content [15]. SN hyperechogenicity was also frequently found in PD patients carrying *GBA* mutations [16–19].

Here, we studied the relationship between demographic, sonographic, and genetic features and the presence of non-motor and motor symptoms of PD in GD patients.

## Methods

### Subjects

We investigated 16 Caucasian, non-Ashkenazi GD patients who were seen in our outpatient clinic for metabolic brain disorders. Four of the GD patients fulfilled the British Brain bank criteria for definite PD [20], and another one had early signs of PD (see "Results"). To establish a control group, we aimed to recruit at least two (non-GD) healthy subjects per GD patient without PD, and at least two (non-GD) PD patients per GD patient with signs of PD. Eventually, the non-GD control group comprised 32 healthy individuals, and another 12 patients with PD. In the non-GD PD patients, the two most common *GBA* mutations (*N370S*, *L444P*) and mutations in the *leucine-rich repeat kinase 2* gene had been excluded. The study was approved and registered by the ethics review board at Rostock University. All subjects gave written informed consent. Demographic and clinical data of the study cohort are displayed in Tables 1 and 2.

### Clinical investigations

A detailed medical history of GD- and PD-related symptoms including history of premotor PD symptoms (depression, hyposmia, constipation) and family history of PD was obtained from all study subjects using a structured interview. Bone disease severity in GD patients was graded as reported previously (Table 1) [4]. All subjects underwent a thorough neurological examination including the motor part of the Unified PD Rating Scale (UPDRS-III) [21]. Special attention was paid to the presence of mild motor symptoms of PD, especially lateralized motor slowing or rigidity [22]. History of depression was regarded positive if an individual reported depressive symptoms within the past 5 years that had impaired activities of daily life and/or resulted in antidepressant therapy, or if they scored ≥13 on the Beck Depression Inventory at the time of this study [23]. Diagnosis of idiopathic hyposmia was based on a patient's history and >3 failures on the 12-item smell identification test from Sniffin' Sticks [24]. The presence of constipation was assessed using established criteria [25]. Dementia was excluded using the Mini-Mental State Examination (score ≥25) [26]. Executive dysfunction was diagnosed if the test results on the Trail-Making Test (TMT-B) were below the age-related standardized normal ranges [27], albeit a normal result on the TMT-B may not entirely exclude the presence of mild executive dysfunction. An individual was assessed to exhibit signs of PD if she/he had a diagnosis of PD, or mild signs suggestive of early PD, with at least one lateralized motor sign scored ≥2 on the UPDRS-III in combination with idiopathic hyposmia and SN hyperechogenicity [22, 28]. For each individual, a score of the identified non-motor symptoms (depression, executive dysfunction, hyposmia, and constipation) was calculated, ranging from 0 to 4.

To search for cognitive deficits, the GD patients without PD underwent a neuropsychological test battery to determine intellectual functioning (the German adaptation of Cattel's Culture Fair Intelligence Test 20) [29], executive functions and mental flexibility (Wechsler Digit Symbol Test, TMT-A, TMT-B) [27, 30], focused attention (d2-Letter Cancellation Test [LCT]) [31], figural memory (Benton Visual Retention Test [BVRT]) [32], numerical memory span (Digit Memory Span Test) [30], and verbal memory (Wechsler Verbal Paired Associates [WVPA]) [30]. These tests were chosen because they allow measurements of a wide range of cognitive functions, afford limited amount of time (about 75 min), and therefore do usually not cause motivational problems of the participants.

**Table 1** Demographic and clinical characteristics of patients with Gaucher disease

| Patient no./ sex | Genotype | Age (years) | Age at onset of | | | Clinical manifestations (non-neuronopathic)[a] | ERT (years) | UPDRS (score) | SN–h |
|---|---|---|---|---|---|---|---|---|---|
| | | | GD (years) | DD (years) | PD (years) | | | | |
| 1/F[b] | *A88P/N370S* | 49 | 17 | 49 | 45 | HSM, splenectomy at age of 19, mild bone disease | 3 | 15 | + |
| 2/M[b] | *N370S/D409H* | 62 | 17 | 49 | 53 | HSM, mild bone disease | 12 | 53 | + |
| 3/M | *N370S/G202R* | 55 | 51 | 52 | 52 | HSM, thrombocytopenia, mild bone disease | 2 | 49 | + |
| 4/M | *N370S/RecNcil* | 63 | 43 | 60 | 61 | HSM, thrombocytopenia, moderate bone disease | 3 | 28 | − |
| 5/M[b] | *V398L/N370S* | 51 | 33 | 35 | NA | HSM, mild bone disease | 16 | 1 | + |
| 6/M[b] | *N370S/RecNcil* | 66 | 55 | NA | NA | HSM, thrombocytopenia, severe bone disease | 9 | 0 | + |
| 7/F[b] | *N370S/V398L* | 47 | 14 | 42 | NA | HSM, thrombocytopenia, moderate bone disease | 15 | 1 | + |
| 8/F[b] | *I93F/R359Q* | 21 | 3 | NA | NA | HSM, thrombocytopenia, mild bone disease | 17 | 0 | − |
| 9/F | *N370S/L444P* | 49 | 34 | NA | NA | HSM, thrombocytopenia | 12 | 0 | + |
| 10/F[b] | *T231R/N370S* | 39 | 3 | 35 | NA | HSM, thrombocytopenia, moderate bone disease | 11 | 0 | − |
| 11/F | *G202R/N370S* | 41 | 1 | NA | NA | HSM, anemia, moderate bone disease | 13 | 2 | + |
| 12/F | *S13L/P178S* | 55 | 43 | NA | NA | HSM, thrombocytopenia | NA | 1 | + |
| 13/F | *N370S/R285H* | 42 | 5 | NA | NA | HSM, thrombocytopenia, moderate bone disease | 7 | 0 | − |
| 14/M[b] | *L444P/L444P* | 41 | 1 | NA | NA | HSM, splenectomy at age of 1, moderate bone disease | 13 | 0 | − |
| 15/M | *N370S/IVS2(+1)* | 45 | 27 | NA | NA | HSM, thrombocytopenia, mild bone disease | 9 | 0 | − |
| 16/M | *F216Y/ RecTL + c1263Δ* | 54 | 36 | 46 | NA | HSM, severe bone disease | 13 | 0 | + |

*DD* Depressive disorder, *ERT* enzyme replacement therapy, *HSM* hepatosplenomegaly, *GD* Gaucher disease, *NA* not applicable, *PD* Parkinson's disease, *SN–h* marked hyperechogenicity of *substantia nigra* on transcranial sonography (+, present; −, absent), *UPDRS* Unified PD Rating Scale, motor part

[a] Bone disease classifications: mild, radiologic abnormalities or occasional mild pain; moderate, fractures (including avascular necrosis) or chronic pain; and severe, surgery or long-term disability due to pain [4]

[b] Cerebral MRI was performed at the time of this study

## Transcranial sonography

TCS was performed using a phased-array ultrasound system with a 2.5-MHz transducer (Acuson Antares; Siemens). The brainstem, basal ganglia, and ventricles were assessed at standardized axial scanning planes [14]. SN hyperechogenicity was defined as a planimetrically measured larger area of increased echogenicity at the anatomic site of the SN than found in 90 % of the healthy population (here: $\geq$0.24 cm$^2$). Brainstem raphe echogenicity was rated as reduced if its echosignals were interrupted or missing on the scanning from both sides despite visibility of the red nucleus. Echogenicity of thalami, lenticular nuclei, and heads of caudate nuclei was classified as hyperechogenic when it was more intense than the surrounding white matter. The width of the third ventricle was measured at the location with minimum distance of its lateral borders between the thalami. The width of contralateral frontal horn was measured at the most frontal position at which the bilateral frontal horns are in junction. The examiner was blinded to the mutational status and clinical data of the subjects. All images were made anonymous and independently re-assessed by a second reader; a brain structure was only regarded abnormal if both readings agreed.

## Magnet resonance imaging

Eight GD patients agreed to undergo 1.5-T MRI at the time of TCS (Table 1). Turbo spin-echo T2-weighted MR images (repetition time/echo time/number of signal averages = 4,620/98/1; slice thickness, 5 mm; slice gap, 1 mm; echo train length, 34) were obtained in the axial plane on a 1.5-T scanner (Magnetom Avanto, Siemens). The in-plane spatial resolution was 0.6 × 0.4 mm. Images were transferred to a Syngo acquisition workstation (Siemens) and analyzed quantitatively by a trained radiologist

**Table 2** Findings in subjects with and without GD

| | GD patients[a] ($n = 16$) | PD patients ($n = 12$) | Controls ($n = 32$) | $P^b$ | $P^c$ |
|---|---|---|---|---|---|
| Clinical data | | | | | |
| Gender, F/M (*n*) | 8/8 | 6/6 | 16/16 | 1.0[d] | 1.0[d] |
| Age, mean ± SD (years) | 48.8 ± 11.1 | 60.9 ± 4.1 | 48.2 ± 11.7 | **0.001**[e] | 0.87[e] |
| Age at onset of GD (years) | 23.9 ± 18.7 | NA | NA | | |
| Age at onset of DD (years)[f] | 46.0 ± 8.5 | 56.0 ± 4.9 | 42.8 ± 15.4 | **0.016**[e] | 0.72[e] |
| Age at onset of PD (years) | 52.8 ± 6.6 | 56.0 ± 4.1 | NA | 0.41[e] | |
| Family history of PD (%) (*n*) | 1 (6) | 1 (8) | 2 (6) | 1.0[d] | 1.0[d] |
| Idiopathic hyposmia (%) (*n*) | 5 (31) | 8 (67) | 4 (12) | 0.063[d] | 0.12[d] |
| Constipation (%) | 4 (25) | 7 (58) | 3 (9) | 0.074[d] | 0.15[d] |
| Executive dysfunction (%) (*n*) | 7 (44) | 10 (83) | 1 (3) | **0.034**[d] | **<0.001**[d] |
| DD within past 5 years (%) (*n*) | 8 (50) | 7 (58) | 4 (12) | 0.66[d] | **0.005**[d] |
| Motor signs of PD present (*n*) | 5 (31) | 12 (100) | 1 (3) | **<0.001**[d] | **0.005**[d] |
| Non-motor symptom score | 1.5 ± 1.5 | 2.7 ± 0.8 | 0.4 ± 0.7 | **0.014**[e] | **0.011**[e] |
| UPDRS-III score | 9.4 ± 17.9 | 19.9 ± 8.5 | 0.5 ± 1.1 | 0.052[e] | 0.064[e] |
| BDI score | 11.2 ± 10.1 | 11.3 ± 7.8 | 2.9 ± 3.9 | 0.98[e] | **0.012**[e] |
| Laboratory findings | | | | | |
| ChT activity (nmol mU/h/ml plasma) | 311 ± 326 | NA | NA | | |
| CCL18 concentration (ng/ml plasma) | 290 ± 228 | NA | NA | | |
| Transcranial sonography findings | | | | | |
| Bilateral SN echogenic area ($cm^2$) | 0.50 ± 0.13 | 0.60 ± 0.16 | 0.31 ± 0.10 | 0.099[e] | **<0.001**[d] |
| SN hyperechogenicity U/B/all (%) (*n*) | 3/7/10 (62) | 3/7/10 (83) | 3/1/4 (12) | 0.23[d] | **<0.001**[d] |
| Brainstem raphe reduced (%) (*n*) | 6 (37) | 4 (33) | 4 (12) | 0.82[d] | **0.044**[d] |
| Third-ventricle width (mm) | 3.8 ± 2.6 | 6.5 ± 2.2 | 3.3 ± 2.0 | **0.006**[d] | 0.56[d] |
| Frontal-horn width (mm) | 13.9 ± 3.3 | 13.1 ± 2.1 | 12.6 ± 1.9 | 0.44[d] | 0.20[d] |

Significant values $p < 0.05$ are in bold

*B* bilateral, *BDI* Beck Depression Inventory, *CCL18* CC chemokine ligand 18, *ChT* chitotriosidase, *DD* depressive disorder, *GD* Gaucher disease, *LN* lenticular nucleus, *NA* not applicable, *PD* Parkinson's disease, *SN substantia nigra*, *U* unilateral, *UPDRS-III* Unified PD Rating Scale, motor part (off-dopaminergic medication)

[a] PD was diagnosed in four GD patients

[b] Comparison of GD patients and PD patients

[c] Comparison of GD patients and control subjects

[d] $\chi^2$ test

[e] *t* test

[f] Eight GD patients, seven PD patients, and four controls affected

who was blind to clinical and TCS data. Based on a localization technique [33], circular ROIs not exceeding 2 mm in diameter were placed in the SN *pars compacta* and *pars reticulata*, and in the cerebrospinal fluid of the right lateral ventricular body. Separate measurements were taken from each hemisphere and averaged by calculating the mean of each ROI. The ROI of the cerebrospinal fluid was placed in the ventricle without including the adjacent parenchyma or choroid plexus. One axial slice was used for each measurement (the slice showing the largest part of structure). To correct for potential interscan variations in system scaling and gain, mean signal intensity in each ROI was divided by the mean signal intensity of lateral ventricular cerebrospinal fluid.

## Genotyping

*GBA* gene analysis was performed as previously described by polymerase chain reaction (PCR) and DNA sequencing [34]. The presence of a 55-base pair deletion spanning the *N370S* mutation was ascertained by PCR to detect a potential contamination with genomic DNA from the pseudogene and to avoid the misclassification of an *N370S*/55-base pair deletion as *N370S/N370S*.

### Statistical analysis

The Spearman correlation test was used for comparison of SN echogenic size (bilateral sum of planimetrically measured SN echogenic areas) and ventricle widths with demographic data and clinical scores, and of unilateral SN echogenicity measures with referring MRI T2-hypointensity measures. Normally distributed data were analyzed using the $t$ test, and categorical data using $\chi^2$ test and Fisher's exact test.

## Results

### Clinical findings

Apart from the four GD patients with definite PD, another one GD patient was found to show features suggestive of early PD, with reduced finger tapping and hand-movement frequency on the left side in combination with hyposmia and SN hyperechogenicity [22, 28]. Executive dysfunction and depression were more frequent in GD patients compared to healthy controls (Table 2). Excluding GD patients with definite PD, still neuropsychological findings (depression, executive dysfunction) were more frequent in GD patients (54 %) than in controls (12 %; $\chi^2$ test, $p = 0.004$). While the overall performance of the 11 GD patients without PD was normal and comparable to an age-related normal population, clinically significant deficits were detected in several cases on the TMT-B (executive function; $n = 4$), LCT (divided attention; $n = 4$), BVRT (figural memory; $n = 7$), and WVPA (verbal memory; $n = 4$). The five GD patients with definite ($n = 4$) or early ($n = 1$) PD signs differed from those without PD signs by higher frequency of hyposmia, and higher non-motor symptom score (Table 3). There was no correlation between presence of PD signs and bone disease severity.

### Relationship between clinical and TCS findings

SN hyperechogenicity (Fig. 1) was more frequent in GD patients compared to controls (Table 2), but did not discriminate GD patients with clinical PD signs from those without (Table 3). Bilateral sum of planimetrically measured SN echogenic areas correlated with age at start of enzyme replacement therapy (ERT) in the GD patients (Spearman test, $p = 0.044$; Fig. 2). This correlation persisted if the three patients with very early or late start of ERT (at ages <10 or >55 years) were removed from analysis. Also, the classification of GD patients into three groups with respect to SN echogenicity (1, normal; 2, unilaterally hyperechogenic; 3, bilaterally hyperechogenic) correlated with increasing age at start of ERT ($r = 0.54$, $p = 0.30$). SN echogenicity was unrelated to age at GD onset, GD duration, ERT duration, or any clinical score (each, $p > 0.05$). Other TCS findings did not correlate with clinical findings in neither group.

### Relationship between laboratory and clinical or TCS findings

Clinical scores and TCS findings were unrelated to chitotriosidase activity or CC-chemokine ligand 18 concentration in blood plasma of GD patients.

### Relationship between genetic and clinical findings

None of the five GD patients with definite ($n = 4$) or early ($n = 1$) PD signs had severe *GBA* mutations typically related to neuronopathic GD, i.e., a severe *GBA* mutation on both alleles or a null mutation on one allele [35, 36]. However, presence of a *N370S* allele, previously reported to predict non-neuronopathic GD irrespective of the other allele [37], was found in all GD patients with PD signs (Table 3). Neuropsychological performance and bone disease severity were unrelated to presence of severe *GBA* mutation, or a *N370S* allele.

### Relationship between genetic and TCS findings

The GD patients carrying severe *GBA* mutations on both alleles ($n = 1$; *L444P/L444P*) or a null mutation on one allele ($n = 2$; *IVS2(+1)*, *RecTL + c1263Δ*), often related to neuronopathic GD [35, 36], did not differ from GD patients with less severe *GBA* mutations with respect to echogenicity of SN, brainstem raphe or lenticular nucleus, or width of third ventricle or frontal horns (each, $p > 0.3$). The GD patients carrying an *N370S* allele did not differ from those without an *N370S* allele with respect to any TCS finding (each, $p > 0.2$).

### Relationship between TCS and MRI findings

One GD patient with definite PD (with SN hyperechogenicity on TCS) had smudging of the SN on T2-weighted MRI (Fig. 1). The other GD patients (definite PD, $n = 1$; no PD, $n = 6$) had normal appearance of SN on MRI irrespective of SN TCS findings. SN echogenic size (unilateral measures, $n = 16$) correlated negatively with T2-hypointensity measures of SN *pars compacta* ($r = -0.55$, $p = 0.027$) but not *pars reticulata* ($p = 0.47$). None of the patients with normal SN echogenicity on TCS had pronounced MRI-T2 hypointensity of SN *pars compacta* (i.e., a measure <0.30).

**Table 3** Findings in GD patients with and without signs of PD

| | GD and PD signs present[a] ($n = 5$) | GD without PD signs ($n = 11$) | $P$ |
|---|---|---|---|
| Clinical data | | | |
| Gender, F/M (*n*) | 2/3 | 6/5 | 1.0[b] |
| Age, mean ± SD (range) (years) | 52.6 ± 8.0 (41–63) | 46.4 ± 11.4 (21–66) | 0.19[c] |
| Age at onset of GD (years) | 25.8 ± 20.6 (1–51) | 23.1 ± 18.8 (1–55) | 0.81[c] |
| Age at onset of DD (years) | 52.5 ± 5.2 (49–60) | 39.5 ± 5.4 (35–46) | **0.014**[c] |
| Family history of PD (%) (*n*) | 0 | 1 (9) | 1.0[b] |
| Idiopathic hyposmia (%) (*n*) | 5 (100) | 0 | <**0.001**[b] |
| Constipation (%) (*n*) | 3 (60) | 1 (9) | 0.63[b] |
| Executive dysfunction (%) (*n*) | 4 (80) | 3 (27) | 0.11[b] |
| DD within past 5 years (%) (*n*) | 4 (80) | 4 (36) | 0.28[b] |
| Non-motor symptom score | 3.2 ± 1.3 (1–4) | 0.7 ± 0.8 (0–2) | **0.010**[c] |
| UPDRS-III score | 29.6 ± 21.5 (3–53) | 0.3 ± 0.5 (0–1) | **0.038**[c] |
| BDI score | 10.3 ± 5.1 (5–16) | 11.6 ± 11.9 (0–35) | 0.79[b] |
| Laboratory findings | | | |
| Presence of N370S allele (%)[d] (*n*) | 5 (100) | 7 (64) | 0.24[b] |
| Severe *GBA* mutation (%)[e] (*n*) | 0 | 3 (27) | 0.51[b] |
| ChT activity (nmol mU/h/ml plasma) | 126 ± 34 | 404 ± 370 | 0.071[c] |
| CCL18 concentration (ng/ml plasma) | 212 ± 145 | 325 ± 256 | 0.34[b] |
| Transcranial sonography findings | | | |
| Bilateral SN echogenic area ($cm^2$) | 0.57 ± 0.07 | 0.47 ± 0.15 | 0.11[c] |
| SN hyperechogenicity U/B/all (%) (*n*) | 1/3/4 (80) | 2/4/6 (54) | 0.59[b] |
| Brainstem raphe reduced (%) (*n*) | 4 (80) | 6 (54) | 0.59[b] |
| Third-ventricle width (mm) | 5.0 ± 3.7 | 3.2 ± 1.9 | 0.35[c] |
| Frontal-horn width (mm) | 15.4 ± 4.3 | 13.0 ± 2.5 | 0.30 |

Significant values $p < 0.05$ are in bold

*B* Bilateral, *BDI* Beck Depression Inventory, *CCL18* CC chemokine ligand 18, *ChT* chitotriosidase, *DD* depressive disorder, *GD* Gaucher disease, *LN* lenticular nucleus, *NA* not applicable, *PD* Parkinson's disease, *SN substantia nigra*, *U* unilateral, *UPDRS-III* Unified PD Rating Scale, motor part

[a] PD signs: diagnosis of PD or signs suggestive of early PD (for details, see text)

[b] Fisher's exact test

[c] *t* test

[d] Compound heterozygous *GBA* mutation

[e] For details, see text

### Findings associated with PD in GD patients

Of the previously reported risk factors of PD in general population (age, male gender, first-degree relative with PD, SN hyperechogenicity) [1, 11–13], none was related to PD or early PD signs in our cohort of GD patients (Table 3). While a single *N370S* allele was present in all GD patients with PD signs but only in 64 % of non-PD GD patients, this difference was not significant due to low number of subjects. On the other hand, all GD patients with PD or early motor PD signs exhibited idiopathic hyposmia but none of the non-PD GD patients (Fisher's exact test, $p < 0.001$). A non-motor symptom score >2 was only found in GD patients with PD motor signs (80 %) but not in those without ($p = 0.026$).

## Discussion

Of the 16 GD patients studied here, four had clinically definite PD, and another one was found to show the combination of mild asymmetric motor signs, hyposmia and SN hyperechogenicity suggestive of early PD. Present data show that SN hyperechogenicity, a TCS finding thought to reflect abnormal iron accumulation in the SN, is a frequent finding in GD patients irrespective of whether they had developed PD or not. SN hyperechogenicity was more frequent in GD patients compared to controls, was unrelated to type or severity of *GBA* mutation, correlated with MRI-T2 hypointensity of SN *pars compacta*, and was correlated with age at start of ERT.

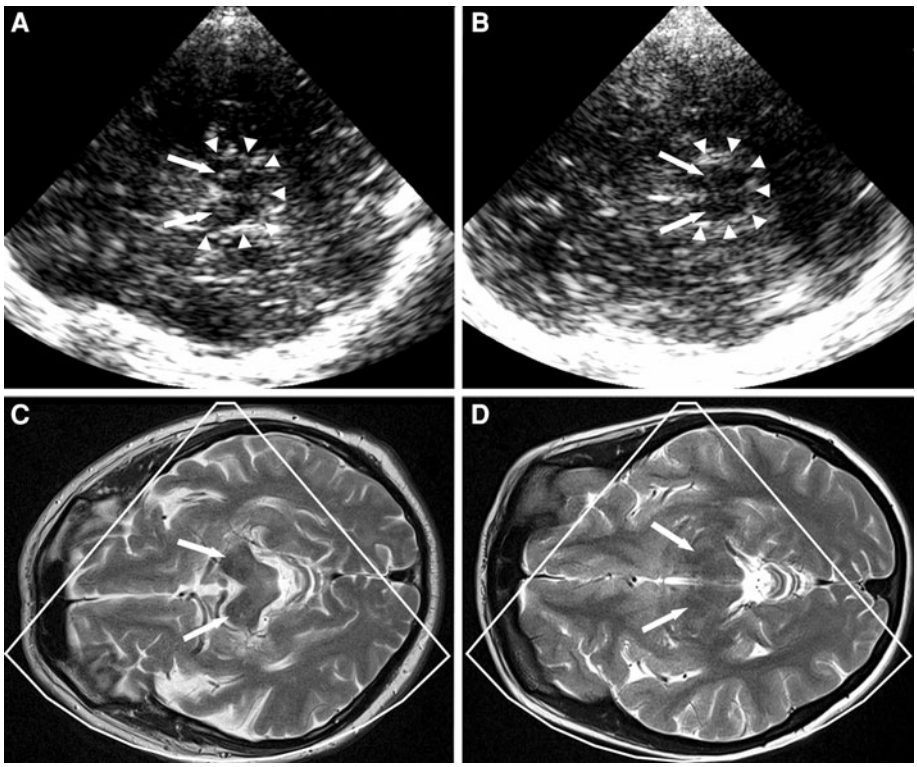


**Fig. 1** Transcranial sonography (TCS) and MRI of midbrain in two patients with Gaucher disease. **a** TCS image of a Gaucher patient with Parkinson's disease showing bilateral hyperechogenicity of *substantia nigra* (*arrows*, *arrowheads* highly echogenic basal cisterns surrounding the midbrain). **b** TCS image of a Gaucher patient without clinical signs of Parkinson's disease showing bilaterally normal (low) echogenicity of *substantia nigra* (*arrows*). **c** T2-weighted MRI in the same patient as shown in (**a**) revealing smudging of bilateral *substantia nigra* (*arrows*). **d** T2-weighted MRI in the same patient as shown in (**b**) with normal, clearly demarcated aspect of bilateral *substantia nigra* (*arrows*)

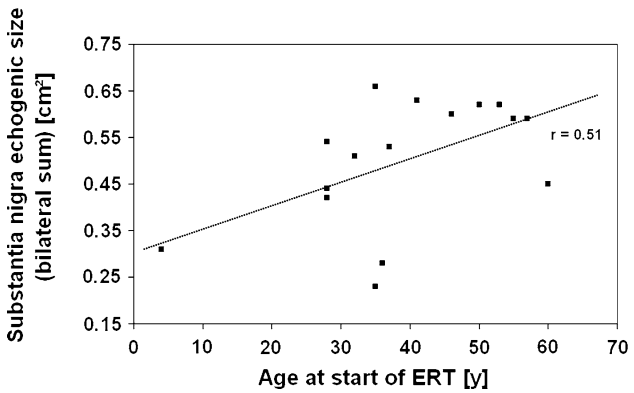


**Fig. 2** Diagram showing the correlation between *substantia nigra* echogenicity on transcranial sonography (sum of bilateral measures), a feature thought to reflect iron accumulation, and age at start of enzyme replacement therapy (ERT) in patients with Gaucher disease (Spearman test, $p = 0.044$)

The clinical features of the GD-related PD patients studied here are similar to idiopathic PD patients with respect to the motor phenotype and levodopa responsivity but also the early and frequent occurrence of non-motor symptoms such as hyposmia, constipation, and executive dysfunction. This is in line with previous reports [4, 16, 38]. Especially the finding of idiopathic hyposmia might be an early predictor of PD in GD according to the results of the present and earlier studies [16, 38]. While MRI did not show any gross brain pathology, TCS detected SN hyperechogenicity, known to be characteristic for idiopathic PD [14], in four (80 %) of five GD patients with signs of PD in the present study. In two previous TCS studies, six (86 %) of seven investigated GD patients with PD were reported to exhibit SN hyperechogenicity [16, 19]. It has been shown in post-mortem studies that SN hyperechogenicity, present in about 9 % of healthy adults but 90 % of PD patients [14], is related to increased iron content in the SN [15]. In non-GD subjects, the extent of SN hyperechogenicity correlated with MRI measures indicating increased SN iron content such as T2 relaxation times [39], or T2 hypointensity [33, 40]. We found the same correlation in our GD patients, suggesting that SN hyperechogenicity in GD is also caused by local iron accumulation. Hyperferritinemia and systemic iron accumulation have been frequently reported in GD, and may even lead to the misdiagnosis of hemochromatosis, and it has been demonstrated that ERT markedly reduces hyperferritinemia in GD patients [41, 42]. The surprisingly high frequency of SN hyperechogenicity found here also in non-PD GD patients suggests that disturbance of iron metabolism involves deep brain structures in GD patients, even in early disease stages. Interestingly in our cohort of GD patients SN hyperechogenicity was the lower the earlier ERT was started. While this finding needs to be interpreted with caution in view of the low number of subjects studied and missing TCS studies before and after start of ERT in the same subjects, it might imply that an earlier start of ERT could possibly reduce iron accumulation in the SN, and thereby the risk of PD. Even though the recombinant enzyme does not cross

efficiently the blood–brain barrier, ERT might influence via systemic effects, especially on iron metabolism, local inflammatory processes or iron accumulation within the SN and other brain structures. Whether SN hyperechogenicity can be altered by therapeutic measures is unclear so far. Recently reported life-long increase of SN echogenicity in general population at least suggests that this finding may change over time in an individual [43]. The mechanisms leading to disturbance of iron metabolism in GD are not yet fully understood. It has been speculated that increased serum levels of hepcidin, a peptide that is produced in the liver and binds to membrane ferroportin, a cellular iron exporter, could play a role [41]. Of note, SN hyperechogenicity was unrelated to type or severity of *GBA* mutation in our GD patients, which conforms to previous findings in PD patients with heterozygous *GBA* mutations and GD patients [17, 18].

Only 38 % of the mutant alleles in our GD patients were *N370S*, which compares to a previously reported 44 % fraction in non-Ashkenazi patients that was lower than in Ashkenazi-Jewish (77 %) patients [35]. Of our GD patients neither carried a homozygous *N370S* mutation. Compound-heterozygous *N370S* mutations were found in all five GD patients with PD signs but only in seven of 11 patients without PD. In earlier studies, 14 out of 17 [5], seven out of seven [4], and ten out of 11 GD-related PD patients harbored at least one *N370S* allele [44], thus further supporting the relevance of this allele for the development of PD in GD patients. This may be surprising since the *N370S* mutation is regarded as mild and thought to protect from neuronopathic GD irrespective of the severity of *GBA* mutation on the other allele [36]. This also contrasts to the previously reported positive correlation between the severity of single heterozygous *GBA* mutations and the risk of PD [10], and recently found greater risk of parkinsonism associated with non-N370S mutations in heterozygous *GBA* mutation carriers [45]. These differences might indicate diverse effects of distinct *GBA* mutations in GD patients and heterozygous *GBA* mutation carriers. The enzyme glucocerebrosidase co-localizes with α-synuclein in Lewy bodies in the brain of subjects with PD related to GD or to heterozygous *GBA* mutations but not in PD patients without *GBA* mutations [46]. As a consequence of lowered glucocerebrosidase enzymatic activity, the accumulation of its substrate, glucosylceramide, was found to affect α-synuclein aggregation by stabilizing the soluble high-molecular-weight (oligomeric) intermediates [7]. *GBA* point mutations have been demonstrated to measurably upregulate α-synuclein concentration in neural cells under both cellular and in vivo (Gaucher mice) conditions, thereby likely increasing the susceptibility to a late onset synucleinopathy disorder [47]. Interestingly, this effect was unrelated to the level of lowered glucocerebrosidase enzymatic activity. Even more, there was also a dissociation found between early occurring axonal neuronal pathology and still normal α-synuclein concentrations in the brain [47]. These findings underpin the idea of different pathogenetic mechanisms underlying the classical neuronopathic phenotype of GD and GD-related PD. It has been proposed that other genetic and environmental co-factors contribute to clinical heterogeneity observed in GD patients [35, 36, 41]. One of these co-factors predisposing to PD in GD patients may be an altered iron metabolism leading to iron accumulation in the SN. The finding of the present study that SN hyperechogenicity, thought to reflect such an iron accumulation [15, 39, 40], was frequent in the GD patients irrespective of associated PD fits in this hypothesis.

Currently, investigations are under way to explore whether TCS of SN might be useful for monitoring possible therapeutic effects of ERT on iron accumulation in the SN of GD patients. It remains to be elucidated whether the risk of PD can be lowered in GD patients with *N370S* mutation by an early start of ERT prior to development of GD-related symptoms.

**Acknowledgments** The authors wish to thank the patients for their participation in this study. Part of the data has been presented on the 9th International Meeting of the European Working Group on GD in Cologne, Germany, in June of 2010.

**Conflicts of interest** On behalf of all authors, the corresponding author states that there are no conflicts of interest.

**Ethical standard** This study has been approved by the appropriate ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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