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Transcranial sonography in patients with Parkinson's disease with *glucocerebrosidase* mutations

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ABSTRACT

Objectives: The aim of this study was to search for possible differences in the findings of transcranial sonography (TCS) between groups of patients with *glucocerebrosidase* (GBA)-associated Parkinson's disease (PD) (4 patients with Gaucher disease type 1 and parkinsonism [GD+PD+] and 18 PD patients with heterozygous *GBA* mutations; [GBA+PD+]) and groups of 12 patients with Gaucher disease type 1 and no signs of parkinsonism (GD+PD−), 9 asymptomatic carriers of heterozygous *GBA* mutations (GBA+PD−), 32 sporadic PD patients (sPD), and 43 healthy controls.

Results: In all groups of patients, except asymptomatic carriers of heterozygous GBA mutations (mean ± SD: 0.16 ± 0.03 cm^2), the maximal areas of substantia nigra hyperechogenicity (aSN-max) was higher (GD+PD+: 0.28 ± 0.15 cm^2; GD+PD−: 0.18 ± 0.06 cm^2; GBA+PD+: 0.27 ± 0.06 cm^2; sPD: 0.28 ± 0.10 cm^2) when compared to controls (0.12 ± 0.08 cm^2) ($p = 0.001$). In *GBA*-associated PD (GD+PD+ and GBA+PD+) and sPD, aSNmax values were very similar. Moderate or marked SN hyperechogenicity was present in 87.5% of sPD patients and in 83% of PD patients with heterozygous *GBA* mutations, but in only 11.6% of controls, and in 22.2% and 33.3% of patients from GBA+PD− and GD+PD− groups, respectively ($p < 0.001$). The prevalence of interrupted or missing echogenicity of the brainstem raphe differed between the groups ($p = 0.046$), while no difference was observed in the diameter of the third ventricle.

Conclusions: TCS findings in *GBA*-associated PD were consistent to those of patients with sporadic PD.

Mutations in both copies (homozygous or compound heterozygous) of the glucocerebrosidase gene (*GBA*; OMIM #606463) cause Gaucher disease (GD), the most common lysosomal storage disorder [1]. A subset of patients with GD develops parkinsonism [2], with brainstem or diffuse Lewy body (LB)-related pathology [3]. Further studies provided evidence that even heterozygous mutations of the *GBA* represented a strong genetic risk factor for Parkinson's disease (PD) in different populations (4–9% of PD patients in non-Jewish European cohorts had *GBA* mutations) [4–6].

The clinical features of PD patients with (*GBA*-associated PD) or without *GBA* mutations were similar, although several studies of *GBA*-associated PD revealed earlier age at onset, higher prevalence of a variety of non-motor symptoms, such as greater cognitive decline, neuropsychiatric disturbances, the presence of hallucinations, and autonomic dysfunction [5–9].

From the first report of an abnormality on transcranial brain sonography (TCS) that was specific for PD (i.e. hyperechogenicity of the substantia nigra [SN] in up to 90% of patients with PD, but also in approximately 10% of the healthy subjects) [10,11], this feature has been detected not only in sporadic, but also monogenic cases of PD [11] (except *ATP13A2* mutations) [12]. The SN hyperechogenicity was already present in the prediagnostic stages of PD and most studies were not able to show correlation between its extent and the disease progression (assessed both clinically, or as visualized with presynaptic striatal functional imaging), suggesting that hyperechogenicity of this region may be primarily a risk marker for nigral injury [11].

The aim of this study was to further evaluate TCS in *GBA*-associated PD patients in comparison to asymptomatic carriers of

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heterozygous *GBA* mutations, GD patients without PD, sporadic PD patients (sPD) and healthy controls.

1. Patients and methods

This study has been approved by the Ethical Committee of the School of Medicine, University of Belgrade (Serbia). Before entering the study all subjects provided the informed consent.

1.1. Patients

The study comprised 5 groups of patients: (1) patients with GD type 1 and parkinsonism (GD+PD+); (2) patients with GD type 1 and no signs of parkinsonism (GD+PD−); (3) PD patients with heterozygous *GBA* mutations (GBA+PD+); (4) asymptomatic carriers of heterozygous *GBA* mutations (GBA+PD−); (5) sPD patients without mutation in *GBA*, *LRRK2* and *parkin* gene; and a group of healthy controls (HC). All the patients were of Serbian ancestry. Patients with GD were recruited from the Center for GD, Clinic for Endocrinology, Belgrade. Patients from the GBA+PD− group were recruited through testing of the parents and siblings of patients with GD. Patients included in the GBA+PD+ and sPD groups, as well as HC, were identified through the preliminary genetic analysis of 415 outpatients recruited at the Department for Movement Disorders, Clinic of Neurology CCS (Belgrade) and 369 healthy subjects, whose only goal was to estimate the prevalence of *GBA* mutations in our population (for a larger part of this analysis see Ref. [13]). *GBA* mutations in GD+PD+ group included three N370S/D409H and one N370S/N370S, in GD+PD− group three N370S/D409H, three N370S/R463H, two N370S/Rec Ncil, two N370S/c.1265_1319del, one N370S/R120W, and one N370S/N370S mutations. In the GBA+PD− group 6 N370S, two R409H, and one L444P mutations were found. Mutation D409H was the most frequent in GBA+PD+ group (8 patients), followed with N370S (7 patients), while the L444P, D380V, and E388K mutations were found in only one individual each. Only after we obtained genetic data, patients with identified mutations were asked to participate in the TCS study.

Twenty-two PD patients (5.3%) were identified as *GBA* mutation carriers, but only 18 of them had a temporal bone window sufficient for an adequate sonographic analysis.

At the study entry all patients were personally examined by two specialists in movement disorders (VSK, MS) when detailed demographic and clinical history was taken. Disease stage was scored using the Hoehn and Yahr stage score [14], and disease severity using the Unified Parkinson's Disease Rating Scale III (UPDRS III) [15]. Within 48 h from TCS, global cognitive function, depression and anxiety were assessed using the Mini-Mental State Examination (MMSE) [16], Hamilton Depression (HDRS) [17] and Anxiety (HARS) [18] rating scales, respectively.

1.2. Transcranial sonography

For TCS we used a color-coded phased-array ultrasound system, equipped with a 2.5 MHz transducer (ProSound Alpha 10, Aloca, Japan). The specific normative values were obtained on 148 healthy controls (78 males; mean age: 55.2 ± 9.8 years; range: 17–89 years) (according to methods discussed in extensive review by Berg et al. [11]). The ultrasound parameters chosen were penetration depth of 14–16 cm and a dynamic range of 45–50 dB. Image brightness, contrast and time-gain compensation were adjusted to get the best image. The examination was performed through a preauricular acoustic bone window scanning supra and infratentorial brain areas in axial planes by tilting the probe. Substantia nigra (SN) echogenic size measurements were performed on axial TCS scans automatically after manually encircling the outer circumference of the SN's echogenic area. Echogenic sizes of <0.19 cm^2 were classified as normal, sizes of ≥ 0.25 cm^2 as markedly hyperechogenic, and sizes in-between as moderately hyperechogenic, with the ultrasound system used in this study. For classification of patients with respect to their SN echogenicity, the greater value of bilateral SN echogenic sizes of each patient was used. In addition to the classification of the SN echogenic size, we conducted comparisons between the maximal areas of SN hyperechogenicity (aSN-max) obtained through the measurement of the larger aSN of each subject, or the ipsilateral aSN if we had an insufficient bone window. Echogenicity of the brainstem raphe was considered abnormal if the signals were missing or were interrupted on the scanning of both sides despite hyperechogenicity of the red nucleus. The width of the third ventricle was measured on a standardized diencephalic axial scanning plane, and was determined by the minimum transverse diameter on axial TCS scan.

All TCS examinations were performed by an examiner (M.M.) who was blinded to the genetic and clinical data.

1.3. Statistical analysis

(Advanced Statistics, version 17.0, SPSS Inc., Chicago, USA). Normality of data was tested by the Kolmogorov–Smirnov test. Following tests were used for comparison between groups: chi-square test for nominal variables, Kruskal–Wallis test for continuous nonparametric variables and one way ANOVA for continuous and parametric variables. Post hoc analysis with Bonferroni correction was applied because of multiple comparisons. Results were considered statistically significant if $p < 0.05$.

2. Results

The characteristics of patients (divided in 5 groups) and HC, who underwent TCS, are summarized in Table 1 (total number of 118 out of 131 subjects with a temporal bone window sufficient for an adequate sonographic analysis at least on one side; 90.1%). Unfortunately, we were able to recruit only 4 patients (all males) in the GD+PD+ group. Although these patients developed parkinsonian signs almost 8 years before the age at onset in two other groups accompanied with PD (GBA+PD+ and sPD), this difference was not statistically significant ($p = 0.311$). Patients from the GD+PD+ group showed significantly more anxiety than those in GBA+PD+ and sPD groups ($p = 0.025$). Similar trend for depression did not prove to be significant ($p = 0.117$) (Table 1).

In all groups of patients, except GBA+PD− group, aSNmax was higher when compared to controls (Table 2 and Fig. 1). In *GBA*-associated PD (GD+PD+ and GBA+PD+), and sPD, aSNmax values were very similar. Finally, there was no difference in aSNmax between the GD+PD− and GBA+PD− groups (Table 2).

Table 1
Demographic and clinical characteristics of patients from different study groups.

	GD+PD+	GD+PD−	GBA+PD+	GBA+PD−	sPD	Controls	*p*
Number of patients	4	12	18	9	32	43	–
Gender (f:m)	0:4	1:5	1:1.6	2:1	1:2.6	1:2.9	0.085[a]
Age (years)[d]	49.0 ± 12.1	44.7 ± 19.0	62.6 ± 8.6	56.7 ± 11.7	61.5 ± 9.3	54.9 ± 14.9	0.014[b,*]
Age at onset of parkinsonian signs (years)[d]	45.6 ± 9.5	–	53.0 ± 8.2	–	53.3 ± 10.0	–	0.311[c]
Duration of parkinsonism (years)[d]	3.6 ± 3.7	–	9.6 ± 6.7	–	8.2 ± 5.8	–	0.201[b]
Hoehn and Yahr stage[d]	2.5 ± 1.2	–	2.7 ± 1.1	–	2.4 ± 0.8	–	0.648[b]
UPDRS III[d]	47.2 ± 27.7	–	38.6 ± 21.4	–	35.9 ± 15.5	–	0.433[b]
MMSE[d]	28.5 ± 1.3	–	27.9 ± 2.6	–	28.5 ± 2.4	–	0.838[c]
HDRS[d]	19.2 ± 6.3	–	9.4 ± 5.7	–	11.2 ± 9.0	–	0.117[b]
HARS[d]	20.7 ± 4.6	–	10.7 ± 7.1	–	9.1 ± 7.1	–	0.025[b,**]

GD+PD+: patients with Gaucher disease (GD) type 1 and parkinsonism; GD+PD−: patients with GD type 1 and no signs of parkinsonism; GBA+PD+: PD patients with heterozygous *GBA* mutations; GBA+PD−: asymptomatic carriers of heterozygous *GBA* mutations; sPD: sporadic PD patients (excluded mutations in *GBA, LRRK2* and *parkin* gene); UPDRS: Unified Parkinson's Disease Rating Scale; MMSE: Mini Mental State Examination; HDRS: Hamilton Depression Rating Scale; HARS: Hamilton Anxiety Rating Scale.

*: GD+PD− ($p = 0.36$), GBA+PD+ ($p = 0.018$) and sPD ($p = 0.032$) groups differ from Controls, and GD+PD− group differs from GBA+PD+ ($p = 0.000$), GBA+PD− ($p = 0.039$) and sPD ($p = 0.000$) groups; **: GD+PD+ group differs from sPD and GBA+PD+ groups.

[a] Chi-Square Test.
[b] Kruskal Wallis Test.
[c] One way ANOVA.
[d] Values presented as means ± SDs.