Blood Glucocerebrosidase Activity and α-Synuclein Levels in Patients with GBA1-Associated Parkinson's Disease and Asymptomatic *GBA1* Mutation Carriers

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Abstract

Introduction. *Mutations in a GBA1 gene, which encodes a lysosomal enzyme called glucocerebrosidase (GCase), are the most common genetic risk factor for Parkinson's disease (PD). The pathogenesis of PD results from the death of dopaminergic neurons in the substantia nigra of the brain, which is associated with the aggregation of α-synuclein protein. However, not all GBA1 mutation carriers develop PD during their lifetime.*

*The **aim** of this study was to evaluate GCase activity and α-synuclein levels in CD45⁺ blood cells of patients with PD associated with GBA1 mutations (GBA1-PD), asymptomatic carriers of GBA1 mutations (GBA1-carriers), and patients with sporadic PD (sPD), as well as correlation between the study parameters in the study groups.*

Materials and methods. *The study included patients with GBA1-PD (n = 25) and sPD (n = 147), and GBA1-carriers (n = 16). A control group included healthy volunteers (n = 154). The level of α-synuclein in CD45⁺ cells was measured by enzyme-linked immunosorbent assay, and GCase activity in dried blood spots was detected by high-performance liquid chromatography with tandem mass spectrometry.*

Results. *Increased level of α-synuclein protein was detected in CD45⁺ blood cells of patients with GBA1-PD, sPD, and GBA1-carriers compared to controls ($p = 0.0043$; $p = 0.0002$; $p = 0.032$, respectively). Decreased GCase activity was reported in GBA1-PD patients and GBA1-carriers compared to sPD patients ($p = 0.0003$; $p = 0.003$, respectively) and controls ($p < 0.0001$; $p < 0.0001$, respectively). However, negative correlation between α-synuclein levels and GCase activity was observed only in GBA1-PD patients, but not in GBA1-carriers.*

Conclusion. *Our data suggest a possible functional relationship between the activity of GCase and the metabolism of α-synuclein in PD associated with GBA1 mutations.*

Keywords: *Parkinson's disease; GBA1 gene; α-synuclein; glucocerebrosidase; glucocerebrosidase activity; blood*

Ethics approval. All procedures performed in human studies comply with the ethical standards of the National Committee on Research Ethics and the Helsinki Declaration or comparable standards of ethics. The research protocol was approved by the Ethics Committee of the N.P. Bekhtereva Institute of the Human Brain of the Russian Academy of Sciences (LEK Protocol No. 1, dated November 26, 2020). Informed voluntary consent was obtained from each of the participants included in the study.

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Активность глюкоцереброзидазы и уровень α-синуклеина в крови у пациентов с GBA1-ассоциированной болезнью Паркинсона и бессимптомных носителей мутаций в гене *GBA1*

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Аннотация

Введение. *Мутации в гене GBA1, кодирующем лизосомный фермент глюкоцереброзидазу (GCase), являются наиболее распространённым генетическим фактором риска развития болезни Паркинсона (БП), в основе патогенеза которой лежит гибель дофаминергических нейронов чёрной субстанции головного мозга, ассоциированная с агрегацией белка α-синуклеина. Однако не у всех носителей мутаций в гене GBA1 развивается БП в течение жизни.*

Целью *настоящего исследования являлась оценка активности GCase и уровня α-синуклеина в CD45⁺-клетках в крови пациентов с БП, ассоциированной с мутациями в гене GBA1 (GBA-БП), бессимптомных носителей мутаций в гене GBA1 (GBA-носители) и пациентов со спорадической формой БП (сБП), а также корреляции между изучаемыми параметрами в исследуемых группах.*

Материалы и методы. *В исследование включены пациенты с GBA-БП (n = 25) и сБП (n = 147), GBA-носители (n = 16). Контрольную группу составили здоровые лица (n = 154). Уровень α-синуклеина в CD45⁺-клетках определяли путём иммуноферментного анализа, активность GCase в сухом пятне крови — высокоэффективной жидкостной хроматографии в сочетании с тандем-масс-спектрометрией.*

Результаты. *Выявлен повышенный уровень белка α-синуклеина в CD45⁺-клетках крови в группе пациентов с GBA-БП, сБП, а также GBA-носителей по сравнению с контролем (p = 0,0043; p = 0,0002; p = 0,032 соответственно). Активность GCase была снижена у пациентов с GBA-БП и GBA-носителей по сравнению с пациентами с сБП (p = 0,0003; p = 0,003 соответственно) и контролем (p < 0,0001; p < 0,0001 соответственно). Однако обратная корреляция уровня α-синуклеина и активности GCase наблюдалась только у пациентов с GBA-БП, но не у GBA-носителей.*

Заключение. *Полученные данные свидетельствуют о возможной функциональной взаимосвязи между активностью GCase и метаболизмом белка α-синуклеина при БП, ассоциированной с мутациями в гене GBA1.*

Ключевые слова: *болезнь Паркинсона; ген GBA1; α-синуклеин; глюкоцереброзидаза; активность глюкоцереброзидазы; кровь*

Этическое утверждение. Все процедуры, выполненные в исследованиях с участием людей, соответствуют этическим стандартам Национального комитета по исследовательской этике и Хельсинкской декларации или сопоставимым нормам этики. От каждого участника исследования было получено информированное добровольное согласие. Протокол исследования одобрен Этическим комитетом Института мозга человека им. Н.П. Бехтеревой РАН (протокол № 1 от 26.11.2020).

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Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the death of dopaminergic neurons in the brain and associated with aggregation of a α-synuclein protein inside them. PD is generally a sporadic disease. However, in 10% of cases, there is a positive family history. A number of genes have been described with mutations leading to the development of inherited forms of PD [1, 2]. *GBA1* mutations are a high PD risk factor leading to the development of GBA-associated PD (GBA-PD). Depending on the population, the prevalence of GBA-associated PD is up to 10% in patients with PD [3–5].

The GBA1 gene encodes a lysosomal enzyme called glucocerebrosidase (GCase). GCase is involved in cleaving the lysosphingolipid glucosylceramide into glucose and ceramide. Biallelic mutations in the *GBA1* gene result in a rare autosomal recessive disorder, Gaucher disease, associated with decrease in the GCase activity (5% to 30% depending on the mutation) as well as accumulation of its substrate [3, 6, 7]. In heterozygous carriers of *GBA1* mutations, both in GBA-PD patients and asymptomatic carriers of *GBA1* mutations (GBA carriers), decreased enzymatic activity of GCase and increased concentration of the lysosphingolipid glucosylceramide are also reported but to a lesser extent compared to patients with Gaucher disease [8–10]. It should be noted that not all *GBA1* carriers develop PD during their lifetime, and the mechanism of pathogenesis of the disease remains unclear.

Currently, the bidirectional effect of GCase dysfunction on α-synuclein levels via a feedforward/feedback mechanism is suggested [11, 12]. *In vitro* experiments have shown that α-synuclein can directly interact with GCase, leading to decrease in GCase activity [13].

Several other studies have shown that GCase dysfunction can cause α-synuclein to accumulate in neurons derived from induced pluripotent stem cells [11]. Increased α-synuclein levels have been reported in animal models of GCase dysfunction [14], as well as in dopaminergic neurons from induced pluripotent stem cells, and peripheral blood mononuclear cells obtained from GBA-PD [15, 16] and Gaucher patients [15, 17].

The **aim** of the study is to evaluate levels of α-synuclein in $CD45^+$ blood cells and GCase activity in GBA-PD patients, GBA carriers, sPD patients, and controls, as well as the correlation between these parameters in the study groups.

Materials and methods

Study groups

The study included patients with GBA-PD (heterozygous carriers of *GBA1* mutations; $n = 25$), patients with sPD ($n = 147$), GBA carriers ($n = 16$), and controls ($n = 154$). Diagnosis was based on the criteria of the Parkinson's UK Brain Bank [18] and the International Parkinson and Movement Disorder Society [19]. PD patients were examined at the N.P. Bekhtereva Institute of the Human Brain of the Russian Academy of Sciences and Pavlov First Saint Petersburg State Medical University. The study included L-DOPA-naïve patients with sPD. Patients with GBA-PD received L-DOPA therapy.

The group of GBA carriers ($n = 16$, neurologically healthy individuals with heterozygous *GBA1* mutations) included relatives of patients with Gaucher disease. Direct Sanger sequencing was used to confirm the presence of mutations. To rule out neurodegenerative diseases, all study participants underwent a clinical neurological examination. The controls ($n = 154$) were examined at the Pavlov First Saint Petersburg State Medical University. In all patients with sPD and controls, polymerase chain reaction and restriction analysis were used to confirm the absence of the common *GBA1* mutations (*L444P*, *N370S*, *E326K*) [5]. The control and experimental groups did not differ in age or gender.

All study procedures involving human subjects complied with the ethical standards of the National Research Ethics Committee and the Declaration of Helsinki or equivalent ethical standards. All subjects provided their voluntary informed consent to participate in the study. The study protocol was approved by the Local Ethics Committee of N.P. Bechtereva Institute of the Human Brain of the Russian Academy of Science (Protocol #1 dated 26 November 2020).

Determination of α-synuclein levels in $CD45^+$ cells

$CD45^+$ cells were isolated from 8 mL of peripheral blood by Ficoll density gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare) followed by magnetic sorting using microparticles conjugated with antibodies to $CD45^+$ receptors and

miniMACS MS columns (Miltenyi Biotec). The cell suspension was aliquoted and frozen at –70°C.

Cells were lysed using the Chemicon Total Protein Extraction Kit (Millipore). Total protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific). The level of α-synuclein in $CD45^+$ cells was measured using Human alpha Synuclein ELISA kit (Thermo Fisher Scientific). All samples were adjusted for total protein (6 μg) and assayed in triplicate. Absorbance was measured using an XMark microplate spectrophotometer (Bio-Rad).

Measurement of blood GCase activity

GCase activity was measured by high performance liquid chromatography with tandem mass spectrometry in dried blood spots [10]. Enzymatic activity was evaluated by measuring the concentration of the product obtained as a result of the reaction of the enzyme with the following substrate: enzyme (E) + substrate (S) + (ES complex) E + product.

Mass spectrometry was performed using an API 3200 QTrap tandem mass spectrometer (ABSciex) in the multiple reaction monitoring mode. Activity was calculated by assuming that the amount of product obtained is directly proportional to the activity of lysosomal enzymes in the dried blood spot. As a control, samples with known levels of enzyme activity were added to each plate. Enzymes were provided by U.S. Centers for Disease Control and Prevention.

Statistical analysis

Statistical analysis was performed using R 3.6.2 software. The Shapiro–Wilk test was used to test the normality of the data. The Mann–Whitney U test was used for pairwise comparisons of variation series. $P < 0.05$ was considered statistically significant. The Spearman's rank correlation coefficient was used to assess the correlation between study groups. Clinical and experimental data are presented as mean ± standard deviation (M ± SD) and median (minimum-maximum), respectively.

Results

The clinical characteristics of the patients and controls enrolled in the study are shown in the table. Since the risk of PD is 6–7 times higher in carriers of the *N370S* and *L444P* mutations of the *GBA1* gene and 2 times higher in carriers of the *E326K* mutation, the levels of α-synuclein and GCase activity were evaluated both in the group of patients with the *N370S*, *L444P* mutations (GBA-PD – *N370S*, *L444P*) and in the general group including

Clinical characteristics and study parameters of participants in study groups

Parameter	Control	sPD	GBA carriers	GBA-PD, mutations	
				N370S, L444P, E326K	*N370S, L444P*
N	154[a] 68[g]	147[a] 40[6]	16[a] 15[g]	25	15
Gender (M/F)	75/79[a] 32/36[g]	61/86[a] 16/24[g]	5/11[a] 5/10[g]	15/10	9/6
Age, years (*M* ± *SD*)	62,02 ± 9,06[a] 59,68 ± 8,76[g]	63,36 ± 9,26[a] 61,57 ± 8,56[g]	53,93 ± 8,19[a] 53,26 ± 8,31[g]	61,74 ± 9,91	62,71 ± 11,19
Age of onset, years (*M* ± *SD*)	N/A	59,32 ± 10,00[a] 57,17 ± 8,91[g]	N/A	57,32 ± 9,91	57,00 ± 11,20
GBA1 mutations	N/A	N/A	5 L444P/N[a, g], 4 N370S/N[a, g], 1 L326P/N[a, g], 1 N227S/N[a, g], 1 R159W/N[a, g], 4 E326K/N[a]/3 E326K/N[g]	8 *L444P/N*, 7 *N370S/N*, 10 *E326K/N*	8 *L444P/N*, 7 *N370S/N*
Levels of α-synuclein, ng/mL	6,56 (0,46–45,70)	9,28 (0,63–65,60) p^* = 0,0002	12,80 (1,22–41,30) p^* = 0,032	10,80 (0,68–51,40) p^* = 0,0043	12,90 (2,92–37,50) p^* = 0,0014
Gcase activity, mM/L/h	8,14 (1,55–32,10)	7,60 (3,33–14,70)	4,67 (2,33–10,40) p^* = 3,9e-05 p^{**} = 0,003	4,28 (1,51–13,20) p^* = 5,1e-06 p^{**} = 0,00027	4,40 (1,51–6,13) p^* = 1,5e-06 p^{**} = 9,9e-05

Note. [a]Assay of α-synuclein levels; [g]Assay of GCase activity. N/A, not assessed. **p* compared to the control group; ***p* compared to sPD patients.

PD patients with the *N370S*, *L444P* and *E326K* mutations (all_GBA-PD).

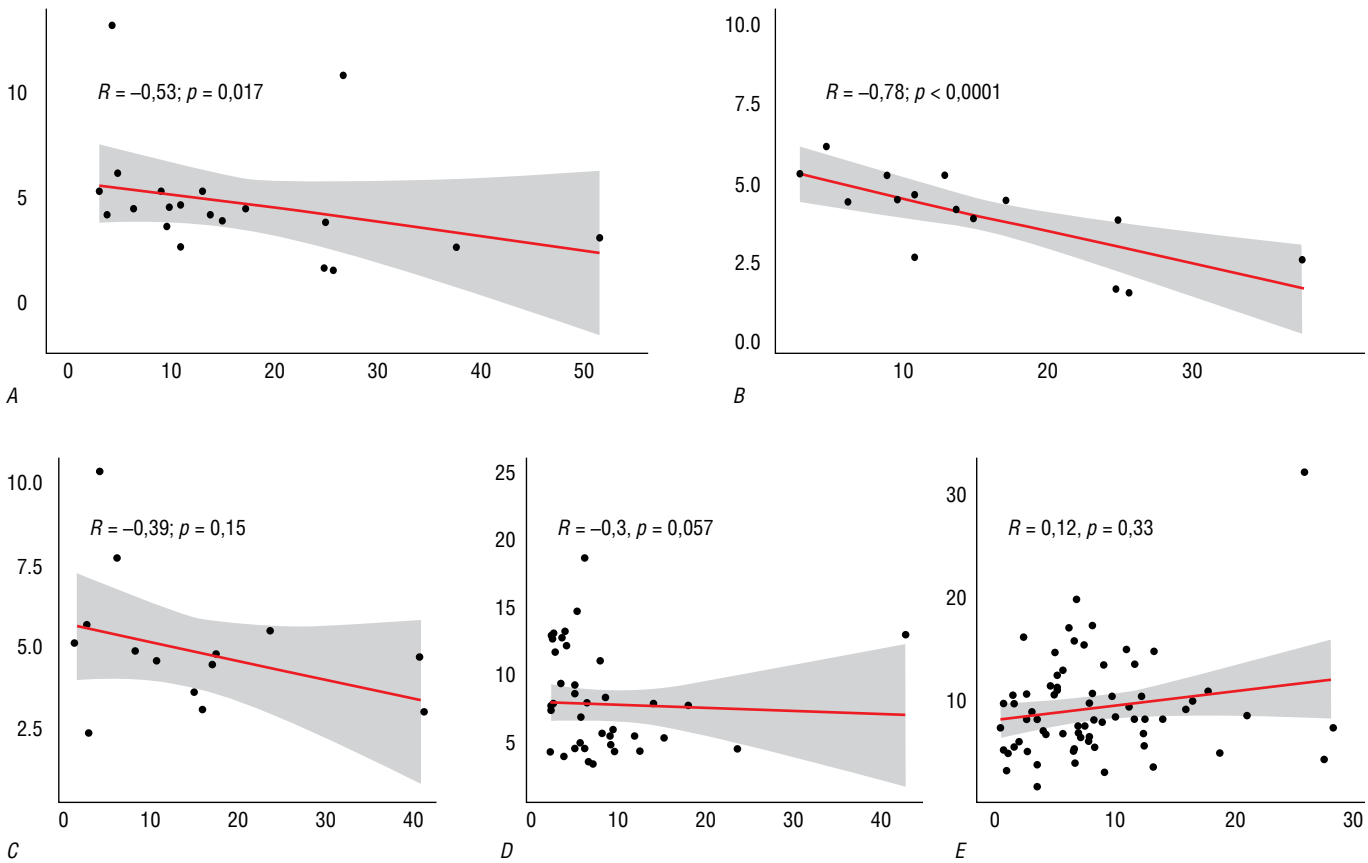
The study showed that the level of α-synuclein in CD45⁺ cells in all_GBA-PD and GBA-PD groups as well as in GBA carriers was increased compared to the control group ($p = 0.0043$; $p = 0.0014$; $p = 0.032$, respectively; Table). Levels of α-synuclein were also increased in patients with sPD compared to the control group ($p = 0.0002$). Decrease in GCase activity was observed in patients with GBA-PD and GBA carriers compared to patients with sPD ($p = 0.0003$; $p = 0.003$) and the control group ($p < 0.0001$; $p < 0.0001$) (Table), which is consistent with our previous results [2].

A negative correlation was found between GCase activity and α-synuclein levels in blood CD45⁺ cells in the all_GBA-PD group ($R = –0.53$; $p = 0.017$), the GBA-PD group ($R = –0.78$; $p < 0.0001$), but not in GBA carriers ($R = –0.39$; $p = 0.15$; Figure). In the sPD group, a negative correlation between GCase activity and α-synuclein levels in CD45⁺ cells were found at the threshold of statistical significance ($R = –0.3$, $p = 0.057$; Figure). At the same time, no correlation was found between the study parameters in the control group.

Discussion

The molecular mechanism underlying GBA-PD remains unclear. However, GCase dysfunction and intracellular α-synuclein oligomerization appear to be interrelated. However, the following questions are poorly understood. Whether a decrease in GCase activity and accumulation of lysosphingolipids, as well as changes in peripheral blood α-synuclein levels, precede disease development in *GBA1* mutation carriers, or is this a consequence of disease development, remains unknown. At the same time, it is crucial to study PD with a known etiology, as well as factors that precede and/or influence disease development in *GBA1* mutation carriers. This would allow identifying disease biomarkers and PD risk groups for *GBA1* mutation carriers in order to include this cohort in clinical trials of targeted therapies that increase the GCase activity [3].

For the first time, we showed that with increased α-synuclein levels and decreased blood GCase activity in patients with GBA-PD and GBA carriers, a negative correlation between α-synuclein levels and GCase activity was typical only for patients with GBA-PD, but not for GBA carriers.



Correlation between the level of α-synuclein in CD45⁺ blood cells and GCase activity in the all-GBA-PD group (*A*; *n* = 25), the GBA-PD group (*B*; *n* = 15), GBA carriers (*C*; *n* = 15), sPD patients (*D*; *n* = 40) and controls (*E*; *n* = 68).Abscissa: level of α-synuclein, ng/mL; ordinata: GCase activity, mM/L/h.

Increase in α-synuclein levels and decrease in peripheral blood GCase activity have previously been reported in PD patients with *GBA1* mutations [8, 10, 15]. For example, M. Avenali *et al.* found increased levels of α-synuclein in peripheral blood lymphocytes in the GBA-PD group compared to patients with sPD and controls [15]. Previously, we found increased plasma levels of oligomeric α-synuclein in patients with Gaucher disease and in PD patients with GBA1 mutations and polymorphic *GBA1* mutations compared to controls [9].

A link between GCase dysfunction and α-synuclein accumulation is being discussed [11]. *In vitro* studies demonstrated a direct effect of lysosphingolipids on α-synuclein aggregation [12, 20]. So, using α-synuclein isolated from dopaminergic neurons derived from induced pluripotent stem cells, it was shown that the GCase substrate called glucosylceramide induces aggregation of α-synuclein and promotes the conversion of its oligomeric forms into toxic aggregates of a specific conformation [21]. It is noteworthy that cyclic amplification of proteins with disrupted conformation has been used increasingly to evaluate such α-synuclein conformers in biological fluids of patients with synucleinopathies [22]. For example, M. Shahnawaz *et al.* used this technique to detect specific conformers of α-synuclein in cerebrospinal fluid samples obtained from patients with synucleinopathies compared to control samples without these conformers [23]. Therefore, the presence of abnormal α-synuclein forms, sensitive to decrease in GCase activity, in biological samples of PD patients may explain the observed negative correlation between GCase activity and the level of α-synuclein in blood $CD45^+$ cells in patients with GBA-PD and its absence in the group of GBA carriers. This assumption may also be supported by the negative correlation we found between the GCase activity and blood α-synuclein levels at the threshold of statistical significance in patients with sPD, but not in the control group.

Data on blood GCase activity in sPD are inconsistent [8, 24]. We found no differences in GCase activity in sPD patients compared to controls.

However, increased levels of α-synuclein were found in $CD45^+$ cells of sPD patients compared to controls, which is consistent with our previous findings [16]. In recent decades, the role of peripheral tissue levels of α-synuclein as a potential biomarker for PD has been discussed [25]. However, numerous studies had conflicting results, which may be explained by the differences in the methods and antibodies used, and other experimental factors. Despite the increased levels of α-synuclein in $CD45^+$ cells of patients with sPD shown in our study, the use of this marker for the differential diagnosis of PD does not seem possible because of the overlapping values obtained across the study groups. In healthy carriers of *GBA1* mutations, an even higher level of α-synuclein was detected in $CD45^+$ cells than in patients with sPD. Previous studies evaluating α-synuclein levels in peripheral blood mononuclear cells found no differences in patients with sPD compared to the control group [15, 26, 27]. In this context, further studies are required to assess the impact of α-synuclein levels in peripheral blood mononuclear cells on the development and progression of PD.

Our study had some strengths and weaknesses. The main strength of our study is the inclusion of asymptomatic *GBA1* mutation carriers, which allowed us to perform a comparative analysis of study parameters in the group of GBA carriers with and without PD. A homogeneous fraction of peripheral blood $CD45^+$ cells of study participants was used to assess α-synuclein levels, so it was possible to neutralize the effect of erythrocyte hemolysis on α-synuclein. It has been previously shown that peripheral blood mononuclear cells obtained by Ficoll gradient centrifugation may contain a mixture of red blood cells that include over 99% of the total α-synuclein in all blood cells [28]. Furthermore, our study included L-DOPA-naïve patients with sPD, which allowed us to exclude the potential influence of these agents on α-synuclein gene expression [29]. Most of the previous studies evaluating α-synuclein levels in mononuclear blood cells in PD patients have not considered effects of erythrocyte α-synuclein and use of L-DOPA-containing agents by PD patients.

The small size of the GBA-BP and GBA carrier groups is the major limitation of our study. Although there was no difference in the mean age between subjects with PD and subjects without PD, we cannot rule out the possibility that some GBA carriers may develop clinical symptoms of PD later in the lifetime.

Conclusion

Our data on the negative correlation between blood α-synuclein levels and GCase activity in GBA1 mutation carriers with PD, but not in asymptomatic GBA carriers, suggest that changes in blood α-synuclein levels and GCase activity in GBA1 mutation carriers may be observed with clinical manifestations of developing PD.

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