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Increased plasma oligomeric alpha-synuclein in patients with lysosomal storage diseases

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HIGHLIGHTS

- Alpha-synuclein oligomers can be detected in human plasma.
- Increase in oligomeric alpha-synuclein in Gaucher disease (GD) patients vs. controls.
- Increase in oligomeric alpha-synuclein in other lysosomal storage diseases vs. controls.
- Duration of an enzyme-replacement therapy for GD may influence plasma alpha-synuclein.

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ABSTRACT

A link between lysosomal storage diseases (LSDs) and neurodegenerative disorders associated with accumulation of presynaptic protein alpha-synuclein has been shown. Particularly, Gaucher disease (GD) patients with a deficiency of the lysosomal enzyme glucocerebrosidase (GBA) and carriers of GBA mutations are at increased risk of Parkinson's disease (PD). It remains unclear whether this link is due to increased alpha-synuclein oligomerization. Here we show that level of oligomeric alpha-synuclein form, associated with PD development, is increased in plasma of GD patients ($n = 41$, median = 22.9 pg/mL, range1.57–444.58 pg/mL; controls ($n = 40$, median = 6.02 pg/mL, range 1.05–103.14 pg/mL, $p < 0.0001$). This difference is absent in GD patients receiving enzyme replacement therapy (ERT) for more than 5 years. Moreover, the levels of alpha-synuclein oligomers in plasma are also higher in patients with other LSDs (Niemann-Pick type C, Krabbe disease, Wolman disease) compared to the median value in controls. Therefore, we suggest that mutations in the *GBA* gene and at least in several other LSDs genes may be associated with an increase in oligomeric alpha-synuclein in plasma. ERT applied for recovering of GBA functions in GD treatment might decrease formation of plasma oligomeric alpha-synuclein.

1. Introduction

Lysosomal storage diseases (LSDs) are the class of inherited metabolic human disorders caused by mutations in proteins critical for lysosomal function. The most common form of LSDs is Gaucher disease (GD) caused by mutations in the glucocerebrosidase (GBA) gene. Mutations in the *GBA* gene are well validated risk factors of Parkinson's disease (PD) in many populations including Russia [8,26]. Subjects with GBA mutations who develop PD demonstrate on autopsy typical neuropathological hallmarks with post mortem alpha-synuclein-positive inclusions and alpha-synuclein aggregates in neuronal cells [4]. Recently, mutations in the causative gene for Niemann-Pick disease (*SMPD1*), encoding another lysosomal enzyme, have been also associated with an increased risk of PD development supporting the link of PD and LSDs [12].

Based on genetic, neuropathological and experimental data alpha-synuclein is considered now as the main key stone in PD pathogenesis. Alpha-synuclein oligomers are believed to be the major neurotoxic agents in neurodegenerative process in PD and other synucleinopathies [5]. It was hypothesized that lysosomal

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dysfunctions observed in LSDs could affect the levels of alpha-synuclein and facilitate its oligomerization in patients bearing mutations in LSDs causative genes, as half of cellular protein is degraded via chaperone-mediated autophagy [6]. Brain accumulation of alpha-synuclein has been described in several LSDs [25].

However, it remains unknown whether alpha-synuclein levels are actually elevated in LSDs patients and whether alpha-synuclein provides the link between PD and LSDs. Here we examined the levels of oligomeric forms of alpha-synuclein in blood plasma of patients with GD and other LSDs (Niemann-Pick type C, Krabbe disease, Wolman disease), as well as in asymptomatic carriers of GBA mutations.

2. Materials and methods

41 GD patients were included in the study. Diagnosis of GD were put by demonstration of deficient GBA activity in leucocytes. In most cases the diagnosis was confirmed by genotyping. In 9 cases plasma samples were collected before therapy. 32 patients received ERT with Cerezyme (Imiglucerase), Genzyme. A group of neurologically healthy carriers with mutations in the *GBA* gene was formed from the first-degree relatives of GD patients. Additionally, patients with other LSDs (Niemann-Pick type C1 and C2 diseases, Krabbe disease and Wolman disease) were included. All diagnoses were confirmed by genotyping.

Three control groups were formed: 40 subjects as Controls-1 for GD patients, 19 subjects as Controls-2 for healthy heterozygous carriers of GBA mutations and 21 children as Controls-3 for other LSDs (male 9, median 15, range 3–16, years old). Patients and controls did not differ by age and sex. The study was approved by the local ethics committee. All participants provided informed consent for the use of stored plasma samples. Clinical characteristics of studied subjects are summarized in Table 1.

Plasma samples were collected from all patients using centrifugation at 3000 rpm at 4 °C for 20 min and stored at −70 °C. Samples were thawed on ice immediately before analysis.

Levels of oligomeric alpha-synuclein were estimated by means of enzyme-linked immunosorbent assay (ELISA) with Human Synuclein OLIGO kit (AJ Roboscreen, Germany) designated for detection of alpha-synuclein oligomers in human fluid samples. Undiluted plasma samples were examined. Each sample was measured in triplicate. Pathological related forms of alpha-synuclein were captured using mab 5G4 antibodies [16]. Synthetic aggregated alpha-synuclein standard was used as positive control during ELISA procedure. OD of negative controls, including monomeric recombinant alpha-synuclein (rPeptide, USA) in dilutions 1 ng/mL and 5 ng/mL was less than 0.2.

Study of conformity findings to normal distribution was tested using the Shapiro–Wilk test. To assess differences between groups, the Mann – Whitney test was used, the correlations were evaluated by linear regression analysis. Sex variables were analyzed with Chi-square test. The level of significance was set at $p < 0.05$. Statistical analysis was carried out using SPSS 12.0. Data are expressed as medians with a range.

3. Results

3.1. Oligomeric alpha-synuclein level decreases with age in the control and GD patients groups

The correlation analysis was performed to assess whether plasma levels of oligomeric alpha-synuclein is changed with age. We observed a statistically significant negative correlation of alpha-synuclein levels with age both in controls and in GD patients groups ($p = 0.007$ and $p = 0.03$, respectively (Fig. 1). This negative correlation persisted when all samples (GD, other LSDs patients, controls) were evaluated together ($N = 81$, $R^2 = 0.062$, $p = 0.0001$).

3.2. Oligomeric alpha-synuclein level is higher in GD patients compared to controls

Alpha-synuclein level was significantly higher in the GD compared to the control group (Table 2 and Fig. 2). According to age at the moment of investigation we divided patients into two groups: children with GD (age before 18 years old) and the adults (18 years old and elder) (Table 1). These groups were differed also in age of GD onset. However, there were no statistically significant differences in the disease duration. Levels of oligomeric alpha-synuclein were significantly higher in the group of GD children compared to the group of GD adults ($p = 0.003$) (Table 2). As the correlation between plasma oligomeric alpha-synuclein with age was shown in GD patients and controls we could not be aware if revealed increase in oligomeric alpha-synuclein in GD children is due to earlier disease onset or due to age at the examination. The statistically significant increase in the levels of alpha-synuclein as compared to the corresponding control group were shown both in children with GD and in adult patients (Table 2). At the same time we observed no statistically significant differences in oligomeric alpha-synuclein levels in healthy heterozygous carriers of GBA mutations compared to the control group (Fig. 2). Besides, alpha-synuclein levels did not differ between carriers of the most severe L444P mutation ($n = 7$) and the group of GD patients bearing mild mutations ($n = 18$) ($p = 0.18$).

3.3. Oligomeric alpha-synuclein levels in patients with other LSDs

We assessed plasma oligomeric alpha-synuclin in several patients with different LSDs (Fig. 2). As all of these diseases are rare (opposite to GD, which is more frequent) we combined all patients with rare LSDs in one group. The level of oligomeric alpha-synuclein in group with rare LSDs was significantly higher compared to controls (patients: median 126.35 pg/mL, range 5.42–378.50 pg/mL; controls: median 8.95 pg/mL, range 2.29–103.14 pg/mL, $p = 0.021$). The highest level was observed in patient P5 with Wolman disease. However, patient P4 with Krabbe disease bearing mutation IVS10del30 kb in one allele and unidentified mutation in another revealed plasma alpha-synuclein level less than median value in controls.

3.4. Oligomeric alpha-synuclein level in GD patients depends on the duration of substrate replacement therapy (ERT)

32 of 41 GD patient included in our study received ERT for different periods (from half a year to 14 years) and 9 patients were newly diagnosed and were not treated. GD patients were divided into three groups: patients who did not receive an enzyme-replacement therapy, those who receive this therapy for less than 5 years and those who received this therapy for 5 years and more. There were no statistically significant differences in age and sex between each patient group and the controls. The highest alpha-synuclein levels were shown in the group of untreated patients and the lowest levels in patients receiving an enzyme-replacement therapy for a longer time. The statistical analysis showed that that compared to the control group the alpha-synuclein levels were significantly higher in the untreated GD and GD patients receiving such therapy less than for 5 years but not in GD patients treated for more than 5 years (Table 2).

4. Discussion

The genetic link between GD and PD is now widely accepted. Following several case reports, clinical, epidemiological and