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Research paper

α-Synuclein dimerization in erythrocytes of Gaucher disease patients: correlation with lipid abnormalities and oxidative stress

Marina Moraitou[a], Georgia Dermentzaki[b], Evangelia Dimitriou[a], Ioannis Monopolis[a], Nick Dekker[c], Hans Aerts[c], Leonidas Stefanis[b,d], Helen Michelakakis[a,*]

[a] Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece
[b] Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[c] Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
[d] Second Department of Neurology, National and Kapodistrian University of Athens Medical School, Athens, Greece

HIGHLIGHTS

- Increased alpha-synuclein (a-Syn) dimer/monomer ratio in Gaucher disease (GD) erythrocyte membranes *vs*. controls.
- Positive correlation of a-Syn status with glucosylceramide levels and glucosylceramide/ceramide ratio observed in GD.
- Negative correlation of a-Syn status with plasmalogen levels and oxidative stress observed in GD.

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ABSTRACT

Several observations suggest that disturbed homeostasis of α-Synuclein (α-Syn) may provide a link between Gaucher disease (GD) and Parkinson's disease (PD). We recently reported increased dimerization of α-Syn in the red blood cell (RBC) membrane of patients with GD. Several studies indicate a crucial relationship between lipids, oxidative stress and α-Syn status. Here we investigated the relationship between the observed increased dimerization of α-Syn in the cell membranes of RBCs, cells devoid of lysosomes and lacking lysosomal enzyme synthesis, and the lipid abnormalities and oxidative stress already described in GD. Correlation studies showed that in GD the α-Syn dimer/monomer ratio is positively correlated with the levels of glucosylceramide (GlcCer) and the glucosylceramide/ceramide (GlcCer/Cer) ratio and negatively with the levels of malonyldialdehyde (MDA) and plasmalogens. In conclusion, we have shown that the increased tendency of α-Syn to form dimers in the RBC membrane of patients with GD, is correlated with both the level of lipids, including GlcCer, the primary lipid abnormality in GD, and the increased oxidative stress observed in this disorder. The study of other tissues, and in particular brain, will be important in order to elucidate the significance of these findings regarding the link between GD and PD.

1. Introduction

Gaucher disease (GD), a rare autosomal recessive metabolic disorder (MIM 230800, 230900 and 231000), is associated with deficient activity of β-glucocerebrosidase (Glcase; EC 3.2.1.45), a lysosomal enzyme encoded by the β-glucocerebrosidase gene (*GBA;* MIM #606463, GenBank accession no. J03059.1) [1].

GBA mutations are one of the most commonly reported risk factors for Parkinson's disease (PD) [2–4].

α-Synuclein (α-Syn), an abundant neuronal protein, has been linked to PD by genetic, neuropathological and experimental studies. It is generally accepted that α-Syn aggregation is involved in the neurodegenerative process in PD and other synucleinopathies [5] and there are suggestions that disturbed α-Syn homeostasis may provide a link between GD and PD. α-Syn aggregation has been observed in brain samples of individuals with *GBA* mutations [6]. Accumulation of toxic, soluble oligomeric intermediates of α-Syn

Abbreviations: α-Syn, α-Synuclein; $C_{16:0}$ DMA/$C_{16:0}$, palmitate dimethylacetal/palmitate; Cer, ceramide; GBA, glucocerebrosidase gene; GD, Gaucher disease; Glcase, β-glucocerebrosidase; GlcCer, glucosylceramide; iPS, induced pluripotent stem (cells); MDA, malonyldialdehyde; PD, Parkinson's disease; RB, Cred blood cell.

* Corresponding author. Fax: +30 2107700111.
E-mail address: inchildh@otenet.gr (H. Michelakakis).

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Table 1
Genotypes of the patients with Gaucher disease studied, their age and the corresponding α-Syn dimer/monomer ratios.

Gaucher diseasetype	Genotype	Number of patients	Age on diagnosis (years)	α-Syn dimer/monomer ratio
Type I	N370S/N370S	8	24	0.15
			25	1.70
			35	2.92
			40	1.00
			43	2.51
			55	1.00
			74	0.34
			77	1.14
			Mean: 41.5	Mean: 1.07
	N370S/D409H:H255Q	5	32	1.61
			64	0.15
			67	0.22
			67	0.86
			72	0.88
			Mean: 67	Mean: 0.86
	N370S/L444P	5	4.5	0.31
			27	0.81
			29	8.02
			60	5.59
			66	0.29
			Mean: 29	Mean: 0.81
	N370S/A309V	2	17	1.70
			20	0.84
			Mean: 18.5	Mean: 1.27
	N370S/H311R	1	62	2.94
	N370S/R496C	1	30	2.07
	N370S/T231I	1	14	1.45
	N370S/?	1	71	0.85
Type II	D409H:H255Q/D409H:H255Q	2	0.041	2.26
			0.958	1.36
			Mean: 0.5	Mean: 1.81
	RecNciI/IVS10-1G>A	1	0.019	2.81

has been demonstrated in primary neuronal cultures with deficient Glcase activity and in human iPS neurons derived from a *GBA* mutation carrier [7]. The pathogenic mechanism linking these entities is still a topic of debate. Loss of Glcase activity, direct interaction between Glcase and α-Syn molecules, triggering of the unfolded protein response by mutant Glcase protein and impaired lysosomal function have all been proposed as possible underlying mechanisms linking the entities [7–9].

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akai et al. [10] have shown that α-Syn is abundantly expressed in erythroid cells, including erythroblasts, reticulocytes and erythrocytes, in both the bone marrow and the peripheral blood, where α-Syn is localized in both the cytoplasm and the plasma membrane of circulating erythrocytes.

We found recently that the ratio of dimeric to monomeric α-Syn is significantly increased in the cell membranes of the red blood cells (RBC) of patients with GD compared to that in control subjects [11]. An increase in plasma oligomeric α-Syn has also been reported in patients with GD [12].

Several studies have indicated a crucial relationship between lipids, oxidative stress and α-Syn status [13–15]. Mazzulli et al. [7] showed that *in vitro* glucosylceramide (GlcCer) influences the aggregation of α-Syn by stabilizing soluble oligomeric intermediates.

Mature RBCs lack lysosomes and are not engaged in the synthesis of lysosomal enzymes, including Glcase, thus providing a model appropriate for the study of possible interactions between a-Syn and lipids. In view of the above reports, we sought in this study to investigate whether the observed increased dimerization of α-Syn in the RBC membrane of patients with GD is related to lipid abnormalities and the oxidative stress described in GD [16].

2. Patients–methods

2.1. Patients

Study was made of 27 patients with GD (24 type I, age at diagnosis 4.5–77 years and 3 type II, age at diagnosis 7 days–7.5 months) and 13 control subjects of a similar age range. The genotypes and ages of the patients studied are shown in Table 1.

2.2. Methods

RBCs were isolated from heparinized blood samples, which in the case of patients with GD were obtained prior to the initiation of any form of treatment. α-Syn was studied in membrane-enriched lysates of RBCs using western blotting, as described elsewhere [11]. The samples reported here represent a subset of those reported previously [11]. The levels of plasmalogens, GlcCer, ceramide (Cer), malonyldialdehyde (MDA) and the GlcCer/Cer ratio had already been studied in these patients and control subjects and reported earlier [16]. All samples were collected at the Institute of Child Health.

2.3. Statistical analysis

The results were statistically evaluated by non-parametric tests and Spearman's correlations were applied.

3. Results

In agreement with our previous report [11] a statistically significant increase in the dimerization of α-Syn, as shown by the increased α-Syn dimer/monomer ratio, was observed in