Reduced Content of α-Synuclein in Peripheral Blood Leukocytes of Patients with *LRRK2*-Associated Parkinson's Disease

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Measurement of α-synuclein level in the peripheral blood was proposed as a diagnostic test for Parkinson's disease. However, the results of these studies remain contradictory, probably because the examined samples included patients with different etiology of Parkinson's disease. To verify this assumption we studied the levels of α-synuclein in peripheral blood leukocytes of patients with Parkinson's disease associated with mutations in the gene of leucine-rich kinase 2 (*LRRK2)*. The mean α-synuclein level was significantly lower in patients with *LRRK2*-associated Parkinson's disease (N=8) than in patients with sporadic form of the disease (N=33; p<0.02) and in controls (N=18; p<0.05). On the other hand, we found no differences in the level of α-synuclein level between patients with sporadic form of the disease and controls. We hypothesize that the level of α-synuclein in the peripheral blood largely depends on the etiology of the disease and cannot be used as a universal diagnostic test for Parkinson's disease.

Key Words: *Parkinson's disease; α-synuclein; lymphocytes; leucine-rich kinase 2*

Parkinson's disease (PD) is a prevalent neurodegenerative disease with heterogeneous etiology. The overwhelming majority of patients have sporadic PD and familial forms constitute only 10% cases [11]. PD symptoms correlate with the death of dopaminergic neurons of the substantia nigra and the formation of protein aggregations, Levy bodies, in neuronal cytoplasm. It is believed that the disease is caused by neurotoxic effects of aggregations of a small presynaptic protein α-synuclein detected in the aggregated form in Levy bodies in familial and sporadic forms of the disease [3]. At the same time, the role of α-synuclein in the pathogenesis of PD remains disputable, because PD in many cases is not associated with the formation of Levy bodies [1].

The absence of reliable laboratory tests for PD is the main obstacle for preclinical diagnostics of the disease and in some cases it leads to misdiagnosis [2]. Much attention is now attracted to measurement of α-synuclein content in the peripheral blood, which, despite contradictory results, is considered as a potential diagnostic test for PS. We suppose that the observed contradictions can be determined by heterogeneous etiology of PD in patients in the examined groups [6-8].

In light of this, the use of etiologically homogeneous groups of patients can be a promising approach in the search of diagnostic marker for PD. Mutations in the gene encoding leucine-rich kinase 2 (*LRRK2*) are the most common cause of familiar PD [4]. In northwestern regions of Russia, the major mutation G2019S

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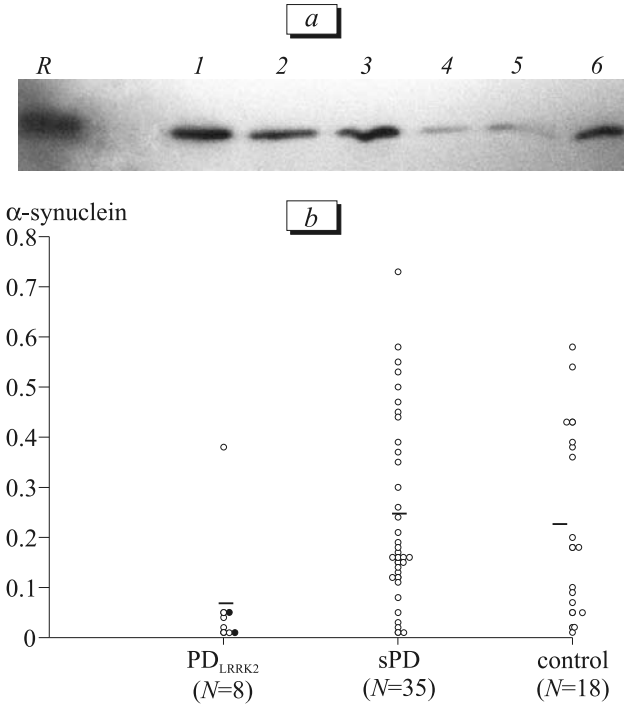

Fig. 1. Content of α-synuclein in extracts of peripheral blood lymphosytes. *a*) western blotting (*R*: recombinant α-synuclein; *1,2,6*: patients with sporadic PD, *5*: patient with G2019S *LRRK2*-associated PD; *3,4*: control); *b*) relative content of α-synuclein in peripheral blood lymphocytes. Horizontal lines: mean value for each group. PD$_{LRRK2}$: patients with *LRRK2*-associated PD (dark symbols: patients with V1613A mutation of *LRRK2* gene; light symbols: with G2019S mutation of *LRRK2* gene); sPD: sporadic form of PD.

LRRK2 was previously found by us in patients with PD and a new pathogenetic mutation V1613A *LRRK2* was found in one family [12,13].

Here we compared the level of α-synuclein in peripheral blood leukocytes of patients with *LRRK2*-associated PD, sporadic PD, and in the control group.

MATERIALS AND METHODS

The study included 8 patients with *LRRK2*-associated PD, 35 patients with sporadic PD, and 18 individuals without history of neurological diseases (control group, Table 1). Multiplication of α-synuclein gene (*SNCA*) was excluded in all PD patients by quantitative real-time PCR on ABI Prism7000 device (Applied Biosystems) [10].

Lymphocytes were isolated from 2 ml peripheral blood by ultracentrifugation in Ficoll density gradient (ρ=1.077) at 1600*g*, washed twice in cold phosphate buffered saline, and frozen at -80°C. Lymphocytes were lyzed in 10 mM tris solution (pH 7.4) with 2% sodium dodecylsulfate (SDS, TX-100) containing protease inhibitors (Sigma P8340). Total protein was measured by the method of Lowry using the corresponding kits (Sintakon) on a SmartSpecTMPlus spectrophotometer (BioRad). The content of α-synuclein was measured by western blotting. To this end, leukocyte lysate proteins were separated by electrophoresis in 12% PAAG (SDS-PAGE; 5 μg per row) and transferred onto PVDF-membrane (Millipore) in tris-glycine buffer (20% ethanol, 0.05% SDS). The membrane was incubated overnight with antibodies to α-synuclein (1:1000; BD Transduction Labs) and than with second peroxidase-conjugated rabit anti-mouse antibodies 1:5000; Amersham Pharmacia Biotech). Membrane-bound antibodies were identified using Lumigen^{TM}PS-3 kit (GE Healthcare UK Limited). The results of western blotting (Fig. 1) were analyzed using ImageJ 1.38a for Windows software (http://rsb.info.nih.gov/ij/). The amount of the studied protein was standardized by recombinant α-synuclein (5 ng). The mean values in the studied groups were compared by the Mann—Whitney test using SPSS 12.0 software. The differences were significant at $p<0.05$.

RESULTS

Immunoreactive proteins with a molecular weight of 16 kDa coincided by electrophoretic mobility with recombinant α-synuclein and corresponded to its monomers (Fig. 1). Individual content of α-synuclein in lymphocytes varied in a great extent in PD patients and controls.

Comparison of the mean values in different groups revealed a significant decrease in α-synuclein content in patients with *LRRK2*-associated PD compared to

TABLE 1. Characteristics of PD Patients and Healthy Volunteers

Group	N	Sex	Age (*M*±*m*)	Age of PD onset (range)
Healthy volunteers	18	6 men, 12 women	71.2±5.3	—
Patients with *LRRK2* gene mutations				
G2019S mutation	6	4 men, 2 women	65.7±11.6	52.5±13.9 (35-65)
V1613A mutation	2	1 men, 1 women	76.50±3.54	67.00±9.90 (60-74)
Patients with sporadic PD	35	12 men, 23 women	65.3±9.8	56.5±13.2 (36-75)

both sporadic PD (p<0.02) and controls (p<0.05). The level of α-synuclein in patients with sporadic PD did not differ from that in the control group (p=0.7). The level of α-synuclein in patients with PD (N=6) determined by G2019S mutation associated with increased kinase activity of LRRK2 [5] was also reduced compared to that in the group with sporadic PD (p<0.05).

An assumption was made in some studies that the level of peripheral α-synuclein can be a marker of PD development. Thus, some authors revealed reduced plasma level of α-synuclein in PD patients [7]. On the contrary, in study [8] highly variable α-synuclein concentrations in platelets from PD patients were demonstrated and this parameter did not differ from the control. It should be noted that these studies were performed on a heterogeneous groups of patients with sporadic PD of unknown etiology. In one patient with familial PD caused by mutation in *PARK2* gene, blood level of α-synuclein did not differ from the control [9].

We studied a etiologically homogeneous group of PD patients. Since the level of α-synuclein in peripheral blood lymphocytes was reduced only in patients with *LRRK2*-associated PD compared to patients with sporadic PD and control group, we believe that this parameter depends on etiology of the disease and cannot be used as a universal diagnostic marker of idiopathic PD.

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