Alpha-Synuclein mRNA Levels Correspond to Beck Depression Inventory Scores in Females with Eating Disorders

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Key Words

Eating disorders • Beck Depression Inventory •
 α -Synuclein • Major depressive disorder • Gene expression

Abstract

α -Synuclein (α -Syn) is a neuronal protein involved in the regulation of brain serotonin and dopamine levels. We analyzed the peripheral expression of α -Syn mRNA and Beck Depression Inventory scores in female patients suffering from anorexia nervosa ($n = 18$) or bulimia nervosa ($n = 24$). We found a significant positive association between α -Syn mRNA expression and the total scores of the Beck Depression Inventory (linear regression; $R^2 = 0.20$; $p = 0.003$). α -Syn may play a pathophysiological role in depressive symptoms associated with eating disorders. Further investigations in patients with depression as a sole diagnosis are needed to support its role in the pathogenesis of major depression.

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Introduction

Eating disorders such as anorexia nervosa (AN) and bulimia nervosa (BN) are often accompanied by depression as a comorbid disorder [1]. The lifetime prevalence rates of depression in patients with eating disorders range from 35 to 85% [1]. It has been proposed that depressive symptoms occur due to neuroendocrinological disturbances induced by starvation. Recent studies have revealed dysfunctions in corticotropin-releasing hormone and serotonin metabolism that were partially due to starvation. These dysfunctions are likely to be linked to depressive pathology [1].

We recently reported that expression of α -synuclein (α -Syn) is reduced in the blood of females with both AN and BN, and that this downregulation is caused by an epigenetic hypermethylation of the promoter-related DNA of the α -Syn gene [2]. Several lines of evidence suggest that α -Syn could be implicated in the pathophysiology of depression: (1) the group of synucleinopathies, including Parkinson's disease, multiple system atrophy and dementia with Lewy bodies, is characterized by abnormal α -Syn aggregation and these diseases have a high prevalence of comorbid depression (30–60%) [3]; (2) α -Syn is important for the trafficking of the dopamine and

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Table 1. Characteristics of the study population

	rAN (n = 7)	bAN (n = 11)	Purging-type BN (n = 21)	Nonpurging- type BN (n = 3)	F/ χ^2	p
Body mass index	14.92 (2.2)	16.10 (1.6)	22.10 (2.9)	23.42 (1.2)	25.7	<0.001
Age, years	25.71 (10.8)	28.18 (11.3)	25.81 (8.3)	24.00 (4.0)	0.23	0.87
Duration of illness, years	8.50 (12.2)	11.09 (11.4)	8.76 (6.1)	10.00 (4.4)	0.20	0.90
Comorbid depression, %	14.92	18.18	28.57	0	1.74	0.62
BDI	24.86 (13.0)	21.09 (7.4)	19.33 (8.4)	16.7 (14.4)	0.78	0.51
α -Syn, ΔC_T value	-1.43 (1.6)	-2.65 (2.5)	-3.20 (3.3)	-2.62 (2.9)	0.68	0.57

Figures in parentheses are SD. Prevalence of MDD according to DSM-IV criteria. Statistical details are summarized in the Results section.

serotonin transporters to the cell surface; thus, α -Syn has an impact on dopaminergic and serotonergic functioning in the brain, 2 neurotransmitter systems inevitably involved in depressive pathology [4, 5]. α -Syn is expressed in presynaptic neurons in the cortex, basal ganglia, hippocampus and the brain stem [6].

We conducted the present study to investigate whether there is an association between α -Syn expression and comorbid depression or depressive symptomatology in a well-characterized sample of patients suffering from eating disorders. For this purpose, we reanalyzed the original data of the Homocysteine and Eating Disorders (HEaD) study [7].

Materials and Methods

This study was part of an observational study on homocysteine in eating disorders (HEaD) [2, 7]. It was approved by the local Ethics Committee (University of Münster, Münster, Germany). All patients met DSM-IV criteria for AN or BN. Psychiatric diagnoses were assessed using the German version of the Structured Clinical Interview for DSM-IV diagnoses. Written informed consent was obtained from all patients after the procedure had been fully explained to them. All patients were inpatients in a psychosomatic hospital (Korso Hospital, Bad Oeynhausen, Germany). Patients were acutely ill at inclusion. Patients with current psychotropic medication were excluded from the investigation. Only for 42 of the originally reported 45 patients of the HEaD study, Beck Depression Inventory (BDI) data and blood samples were available. Therefore, we included only those patients into the analysis, among them 7 women with restrictive AN (rAN), 11 with the bulimic (purging) subtype of AN (bAN), 21 with the purging subtype of BN and 3 women with nonpurging BN. The characteristics of the study population are summarized in table 1.

Depressive symptomatology was assessed on the day after admission to the hospital using the German version of the BDI [8]. The BDI is a 21-item self-report scale measuring the severity of depression (range 0–63). The absence of depressive symptoms is

defined by a total score of less than 12, moderate depression by a score between 12 and 18, and a clinically relevant depression by a score above 18. Total BDI scores of above 30 are rated as severe depression. Apart from comorbid depression according to the ICD-10 criteria, the BDI was the only measure of depression that had been assessed in the study population.

The results of the measurement of α -Syn have already been published and the methods used are described in detail elsewhere [2, 9]. All parameters were normally distributed according to the Kolmogorov-Smirnov test. Between-group comparisons were done using either Student's *t* test (for 2 groups) or one-way analysis of variance with Tukey's post hoc test for 3 or more groups. Categorical data were assessed using Pearson's χ^2 test. To further analyze the results, we used linear regression analyses.

The results are presented as means (with SD in parentheses). $p < 0.05$ (two-tailed) were considered to indicate statistical significance. Data were analyzed employing SPSSTM for Windows 14.0 (SPSS Inc., Chicago, Ill., USA) and Graph Pad Prism 4 (Graph Pad Software Inc., San Diego, Calif., USA).

Results

We found a significant association between BDI sum scores and α -Syn mRNA expression in the linear regression analysis ($R^2 = 0.197$; $R^2_{\text{corr}} = 0.177$; $B = 1.472$; SE of $B = 0.470$; $\beta = 0.44$; $p = 0.003$; fig. 1).

A comorbid major depressive disorder (MDD) was present in 9 of the 42 patients. Patients with a comorbid MDD had higher BDI scores [MDD vs. no MDD: 26.0 (12.4) vs. 19.3 (7.9); $T = 2.06$; d.f. = 40; $p = 0.046$], but did not differ in α -Syn mRNA levels [MDD vs. no MDD: -1.82 (2.9) vs. -2.97 (2.8), ΔC_T β -actin; $T = 1.1$; d.f. = 40; $p = 0.28$].

We used multiple linear regression models to further analyze these results. The BDI score was computed as dependent variable, comorbid MDD, DSM-IV diagnosis of ED, age, duration of illness, BMI and α -Syn mRNA levels

Fig. 1. **a** Association of α -Syn mRNA expression and sum scores of BDI. Dotted lines represent the 95% confidence interval of the linear regression analysis. More statistical details are summarized in the Results section. **b** The same association plotted for rAN versus bulimic forms of eating disorders.

as covariates, as all of them were likely to influence BDI scores. Using the ENTER method, only α -Syn mRNA levels had a significant influence on BDI scores ($R^2 = 0.273$; $R^2_{\text{corr}} = 0.169$; α -Syn: $\beta = 0.450$; $p = 0.005$). We divided the sample according to the BDI score judged as 'clinically relevant' (>18) and found higher α -Syn mRNA levels for those patients with BDI score >18 when compared to those with BDI scores ≤ 18 (t test: $t = -2.35$; d.f. = 40; $p = 0.024$).

In a last step of analysis, we divided the sample according to the presence of 'bulimic behavior', that is, rAN versus bAN and BN. As plotted in figure 1b, the association between BDI scores and α -Syn expression only exists in the bulimic groups (linear regression: rAN: $\beta = 0.274$; $R^2 = 0.07$; $p = 0.55$; bAN, BN: $\beta = 0.44$; $R^2 = 0.19$; $p = 0.0075$; fig. 1b). However, due to the small size of the rAN group, the negative finding should be regarded with caution.

Discussion

We recently reported that α -Syn expression in leukocytes of females with AN and BN is reduced when compared with healthy controls without eating disorders or depression (controls had an average ΔC_T value of -0.12) [2]. In the present study, we aimed at possible clinical and pathophysiological implications of this finding. Periph-

eral α -Syn expression has already been associated with psychopathological findings in alcohol dependence, where it was correlated with the extent of craving [9]. In the present study we found an association between α -Syn expression and the total score of the BDI, a commonly used self-assessment scale for the severity of depressive symptoms.

α -Syn has received considerable attention during the last years, after missense mutations of its gene were found in autosomal dominant Parkinson's disease and after it was shown that abnormal α -Syn aggregates are present in several neurodegenerative disorders [10]. α -Syn is in several ways implicated in dopaminergic and serotonergic pathways, as it regulates the dopamine biosynthesis, and the trafficking of the serotonin and the dopamine transporter to the cell surface. α -Syn-expressing neurons are found in all parts of the limbic system and may play a crucial role in the regulation of the reward system, a system that has been implicated in the pathogenesis of both, depression and eating disorders [11, 12].

α -Syn regulates the synaptic availability of the dopamine and the serotonin transporter by subcellular protein-protein interactions, leading to a negative modulation of the intracellular trafficking and thus resulting in reduced levels of the proteins at the cell surface [5]. If peripheral expression of α -Syn is representative for the gene's expression in the central nervous system, as suggested by Miller et al. [13], our finding of a positive association be-

tween peripheral α -Syn levels and the severity of depressive symptoms may hint at an involvement of α -Syn in the pathophysiology of depression. However, our present finding of a positive association of α -Syn expression with depressive symptoms may seem contradictory to the previous finding of an overall reduction of its expression in females with eating disorders [2]. This paradox may be explainable by the assumption that the control subjects were more depressed than the patients with eating disorders. This can be ruled out, as all control subjects were carefully examined by a trained psychiatrist and any sign of depression was ruled out. At present, we are unable to solve this apparent paradox. One explanation, however, could be that the influence of α -Syn on self-reported depression is independent of the overall downregulation of the protein in eating disorders. If we follow that path, the following scenario of α -Syn expression and its relation to depressive symptoms seems possible: lack of monoamines leads to both depressive symptoms and a counterregulatory upregulation of α -Syn to reduce 5HTT and DAT trafficking, that is, α -Syn serves as an endogenous dual reuptake inhibitor. Further studies are clearly needed to shed some more light on the role of α -Syn in the pathophysiology of both, depression and eating disorders.

Our study has some limitations that should be kept in mind when discussing the findings. First, the cross-sectional design can only detect an association, no causality. Second, our findings were only valid regarding the self-rated depression, but not the presence of MDD according to DSM-IV criteria. However, the rate of moderate to severe depression according to the BDI score was more comparable to prevalence rates of depression in eating disorders given in the literature [14]. It may well be that DSM-IV criteria for MDD do not fit well the specific characteristics of depression during eating disorders or that eating disorder symptoms may counteract depressive symptoms (for example, hyperactivity and listless-

ness). These findings should therefore be replicated in a sample of patients suffering from MDD and different rating scales should be employed.

Another important confounding factor may be that the majority of serotonergic neurons are situated in the gastrointestinal system and may be affected by bingeing and vomiting. This behavior itself is associated with depressive symptomatology [15]. However, we were not able to show significant differences between those patients showing vomiting or purging behavior neither regarding presence of depressive symptoms nor α -Syn mRNA levels (data not shown). On the other hand, division of the study sample according to the presence of bulimic behavior, such as bingeing and purging, showed that the association was only present in the bulimic group. This may hint at a possible implication of the gastrointestinal tract in the observed dysregulation of α -Syn in eating disorders, especially with those of a bulimic type. Still, some caution should be allowed when speculating about the possible implications of this disease subtype effect – the rAN group consisted of only 7 probands, making the negative finding regarding the association in that group likely to be a second-kind error.

In conclusion, our study provides first hints derived from a clinical sample for an involvement of α -Syn in depressive pathology. Further studies are needed to evaluate possible contributions of α -Syn abnormalities to the pathogenesis of depression and depressive symptoms.

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