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

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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

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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serotonin transporters to the cell surface; Thus, α-Syn has
an impact on dopaminergic and serotonergic function-
ing 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, hippo-
campus 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 eat-
ing disorders. For this purpose, we reanalyzed the origi-
nal 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 Eth-
ics 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 ex-
plained to them. All patients were inpatients in a psychosomatic
hospital (Kors 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 De-
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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 χ² 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 sig-
nificance. Data were analyzed employing SPSS™ 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 (R² = 0.197; R²corr = 0.177; B = 1.472; SE of B = 0.470; β = 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 a dependent variable, comorbid MDD, DSM-IV diagnosis of ED, age, duration of illness, BMI and α-Syn mRNA levels

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>rAN (n = 7)</th>
<th>bAN (n = 11)</th>
<th>Purging-type BN (n = 21)</th>
<th>Nonpurging-type BN (n = 3)</th>
<th>F/χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>14.92 (2.2)</td>
<td>16.10 (1.6)</td>
<td>22.10 (2.9)</td>
<td>23.42 (1.2)</td>
<td>25.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>25.71 (10.8)</td>
<td>28.18 (11.3)</td>
<td>25.81 (8.3)</td>
<td>24.00 (4.0)</td>
<td>0.23</td>
<td>0.87</td>
</tr>
<tr>
<td>Duration of illness, years</td>
<td>8.50 (12.2)</td>
<td>11.09 (11.4)</td>
<td>8.76 (6.1)</td>
<td>10.00 (4.4)</td>
<td>0.20</td>
<td>0.90</td>
</tr>
<tr>
<td>Comorbid depression, %</td>
<td>14.92</td>
<td>18.18</td>
<td>28.57</td>
<td>0</td>
<td>1.74</td>
<td>0.62</td>
</tr>
<tr>
<td>BDI</td>
<td>24.86 (13.0)</td>
<td>21.09 (7.4)</td>
<td>19.33 (8.4)</td>
<td>16.7 (14.4)</td>
<td>0.78</td>
<td>0.51</td>
</tr>
<tr>
<td>α-Syn, ΔC_T value</td>
<td>–1.43 (1.6)</td>
<td>–2.65 (2.5)</td>
<td>–3.20 (3.3)</td>
<td>–2.62 (2.9)</td>
<td>0.68</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Figures in parentheses are SD. Prevalence of MDD according to DSM-IV criteria. Statistical details are summarized in the Results section.
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 ($R^2 = 0.273; R^2_{corr} = 0.169; \alpha$-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. Peripheral 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-
between 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 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 listlessness). 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 binging 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 binging 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.

Acknowledgements

The authors gratefully acknowledge the support by a grant (H.F.) from IZKF (Interdisciplinary Center for Clinical Research), Friedrich Alexander University of Erlangen-Nuremberg, Erlangen, Germany. This study was supported by a grant from the German Research Fund (Deutsche Forschungsgemeinschaft, DFG, No. BL 577/2-1).

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