

## CSF alpha synuclein and late-life depression

### CSF alpha synuclein correlates with CSF neurogranin in late-life depression

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Abstract

Major depressive disorder (MDD) in late life is linked to increased risk of subsequent dementia, but it is still unclear exactly what mechanisms underpin this link. A cause for elevated risk of dementia in MDD is increased levels of  $\alpha$ -synuclein ( $\alpha$ -Syn), a protein found in presynaptic neuronal terminals. In this study, we examined cerebrospinal fluid (CSF) levels of  $\alpha$ -Syn in conjunction with biomarkers of neurodegeneration (amyloid- $\beta$  42, total and phospho tau) and synaptic dysfunction (neurogranin), and measures of memory ability, in 27 cognitively intact older individuals with MDD and 19 controls. Our results show that CSF  $\alpha$ -Syn levels did not significantly differ across depressed and control participants, but  $\alpha$ -Syn was directly associated with neurogranin levels, and indirectly linked to poorer memory ability. All in all, we found that  $\alpha$ -Syn may be implicated in the association between late life MDD and synaptic dysfunction, although further research is needed to confirm these results.

## Introduction

Links between depressive symptoms and dementing illnesses, including Alzheimer's disease (AD), have been reported in several studies (1-4). Individuals with depression have been reported to carry more than double the risk of converting to AD than controls (5), while individuals with mild cognitive impairment and dementia are more than twice as likely as controls to be depressed (6). However, understanding the exact nature of this relationship has proven a challenging task. Not all individuals with depressive symptoms will progress to dementia and for those who do, it is not always clear whether the depressive symptoms are a consequence of dementia, or contributing to its emergence.

In the past, we have proposed that a possible mediator of the link between late-life MDD and AD is the role played in the central nervous system by amyloid beta ( $A\beta$ ). Pomara and Doraiswamy (7; see also 8) were first to suggest that chronic depression may lead to elevation of circulating  $A\beta$  levels independently of AD, specifically via increased platelet activation. In turn, due to bidirectional receptor-mediated active transport of  $A\beta$  across the blood-brain barrier, increased circulating  $A\beta$  levels will result in higher brain concentration. Consistent with this claim, we reported (4) that cerebrospinal fluid (CSF) levels of  $A\beta_{42}$ , which are thought to inversely reflect brain amyloid deposits, were lower in older (at least 60 years of age), cognitively healthy individuals with MDD compared to controls. However, a later follow up study from our group (8) showed that, although CSF  $A\beta_{42}$  levels remained sensitive to depressive symptoms, they tended to fluctuate longitudinally over a three-year span, and were not associated to cognitive health in subjects with late-life MDD.

An alternative, although not exclusive, potential mechanism for increased risk of dementia in individuals with late life MDD is related to increased levels of  $\alpha$ -synuclein ( $\alpha$ -Syn).  $\alpha$ -Syn is a pre-synaptic protein that is highly expressed in cortical and sub-cortical areas (9). The protein may aggregate into Lewy bodies, a pathological protein misfolding process that potentially may start in the gut and spread to the CNS (10).  $\alpha$ -Syn aggregation in the brain is a core feature of Parkinson's disease and dementia with Lewy bodies (DLB) (11), but it may be implicated also in the pathophysiology of depression, leading to alterations in dopaminergic and serotonergic neurotransmission (12). A recent report looking at serum levels of  $\alpha$ -Syn (13) showed that individuals with MDD had higher levels than

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controls, regardless of age. In turn,  $\alpha$ -Syn has been found to associate with synaptic dysfunction, as determined by CSF neurogranin (Ng) concentration, in Parkinson's disease (11), suggesting that  $\alpha$ -Syn may also reflect or promote cognitive impairment. Finally, depression has been reported to be a risk factor for Parkinson's disease (14).

Considering the evidence above, it may be possible that while amyloid  $\beta$  plays a role in influencing the severity of depression in late life (4, 8), and perhaps earlier, other mechanisms, namely mechanisms related to aggregation of  $\alpha$ -Syn, are at play in affecting cognitive decline and emergence of neurodegenerative symptoms. To test this hypothesis, we analysed cerebrospinal fluid (CSF) data of cognitively intact individuals with late life MDD and controls, and memory performance. We carried out cross-sectional analyses and correlations within each clinical group. The CSF biomarkers included in the analyses were  $\alpha$ -Syn, the A/T/N biomarkers (15), A $\beta$ 42, total (t) and phospho (p) tau), and Ng. Memory performance was measured by Buschke Selective Reminding Test (BSRT) total recall and the recency ratio (Rr; 16, 17). Our hypothesis was that higher levels of CFS  $\alpha$ -Syn would be associated with increased CSF Ng (indexing more synaptic dysfunction) and poorer memory ability.

### Methods

*Participants.* A total of 133 participants were recruited initially, but only the 47 individuals who allowed lumbar puncture for collection of CSF, presented no MRI evidence of confluent deep or periventricular white matter hyperintensities, and had a Mini-Mental State Examination (MMSE; 18) score of 28 or above were included in this study. Twenty-eight participants had a diagnosis of MDD, confirmed by a board-certified psychiatrist based on clinical evaluation and the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), and 19 were healthy controls. No one was diagnosed as having cognitive impairment. Demographic characteristics are summarised in Table 1 (see also 4). Participants received up to \$450.00 in compensation for their time.

*Biomarkers.* A $\beta$  levels were measured with electrochemiluminescence technology using the MS6000 Human Ab Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, Md.). T-tau levels were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) (INNOTEST hTAU-Ag, Innogenetics, Ghent, Belgium), whereas P-tau was measured with a sandwich ELISA method

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(INNOTEST Phospho-Tau [181P], Innogenetics). CSF Ng concentration was measured using an in-house sandwich ELISA (19). CSF  $\alpha$ -Syn levels were determined with a ELISA method (Tecan Sunrise, Salzburg, Austria), following the procedure described by van Geel et al. (20).

*Procedure.* The study procedure has been detailed previously (4). Briefly, the study consisted of four visits on successive weeks. On visit 1, all participants provided consent and were assessed for: medical history, vital signs, and general cognitive ability (MMSE). Severity of depression was measured at this stage with the Hamilton Depression Scale (HAM-D, 21 items). On visit 2, participants received an MRI scan of the head, and a physical examination, including routine laboratory tests. On visit 3, participants underwent a comprehensive neuropsychological assessment that included memory evaluation with the BSRT (21, 22). The BSRT (standard administration) involves the oral presentation of 16 unrelated nouns, which participants are asked to recall over several trials. In the first trial, participants are asked to free recall as many words as possible immediately after presentation of the study list; after this, participants are reminded of the items that were not retrieved and asked to recall all items again, over six subsequent trials; in a final delayed trial, participants are then asked to free recall the original study list, following a period of approximately 20 minutes from initial learning. Finally, a lumbar puncture was performed on a fourth visit, between 9am and 10am, after overnight fasting. This study was ethically approval by the institutional review boards of the Nathan Kline Institute for Psychiatric Research, and the New York University School of Medicine.

*Design and Analysis.* First we examined whether there were differences across late life MDD and control groups in Ng and  $\alpha$ -Syn levels (note that differences in A $\beta$ 42 were already reported in xxx), and memory scores. Due to the non-normal nature of these data, we performed non-parametric analyses. Second, we carried out a series of Spearman correlations separately in individuals with late-life MDD and controls. The variables included in the analyses were: CSF  $\alpha$ -Syn, Ng, A $\beta$ 42, t and p tau, total recall and Rr. Total recall was calculated by adding the number of all recalled items over the seven learning trials of the BSRT; and Rr is provided by the ratio between recall of the last four presented items in the first learning trial and in the delayed trial, using the adjustment provided here (16). Of note, whereas lower total and delayed recall are indicative of more memory loss, the opposite

is true of Rr. To avoid repeating previous findings, and to take into account and limit the high volume of tests, we focused only on correlations involving either Ng,  $\alpha$ -Syn or both. We report unadjusted p values and, when reaching conventional significance ( $p \leq 0.05$ ), we qualify the finding with adjusted (with False Discovery Rate; 23), p values.

## Results

Table 1 reports cross-sectional comparisons of demographic characteristics. Depression severity was higher in the MDD group, and thus the original diagnosis was consistent with the depressive state of the sample. None of the other demographic characteristics differed across groups.

*Comparisons across groups.* Except for A $\beta$ 42 (see 4), none of the variables of interest differed significantly between depressed individuals and controls (p values  $\geq 0.200$ , Z scores  $\leq 1.3$ ). Means and standard deviations are reported in Table 2, except for biomarker data reported elsewhere (see 4).

*Correlations late-life MDD.*  $\alpha$ -Syn correlated with Ng ( $\rho = 0.444$ ,  $p = 0.018$ ; see Figure), but not when adjusting the p value for multiple tests (0.198).  $\alpha$ -Syn did not correlate with A $\beta$ 42 levels ( $\rho = 0.199$ ,  $p = 0.310$ ), tau levels (p values  $\geq 0.500$ ), or memory (p values  $\geq 0.200$ ). Ng displayed a correlation with P-tau ( $\rho = 0.374$ ,  $p = 0.050$ ), which was not significant according to adjustment (adjusted p = 0.348). Ng was also positively correlated with Rr ( $\rho = 0.593$ ,  $p = 0.001$ ), surviving the adjustment (adjusted p value = 0.022). Ng was not otherwise correlated with the other variables (p values  $\geq 0.069$ ). See Figure.

*Correlations controls.* None of the correlations in this set approached significance (p values  $\geq 0.145$ ; see Figure).

Put Tables and Figure about here

## Discussion

To our knowledge, this was the first report examining CSF  $\alpha$ -Syn in individuals with late life MDD. We hypothesised that higher levels of CFS  $\alpha$ -Syn would be associated with increased CSF Ng, which signals more synaptic dysfunction, and poorer memory ability. The results of our study only confirmed our hypothesis in part. First, we did find that elevated levels of CSF  $\alpha$ -Syn in depressed individuals were associated with increased CSF Ng, but this finding did not survive statistical checks for multiple testing. This suggests that this relationship should be considered exploratory, at this time,

and requires further confirmation. Second, we did not observe a direct association between CSF  $\alpha$ -Syn and memory ability in the depressed subjects, but we did see an indirect association, i.e., that memory ability, as indexed by Rr, was poorer when CSF levels of Ng were higher.

We did not observe any association between CSF  $\alpha$ -Syn and the ATN biomarkers in either cohort. This finding is not entirely consistent with that in a recent report by Vergallo et al. (24), who found that CSF  $\alpha$ -Syn was positively correlated with CSF t- and p-tau levels as well as brain amyloid  $\beta$  levels. A few notable differences across the two studies are the following. First, our subjects were diagnosed with MDD, whereas Vergallo et al.'s participants are free of clinical psychiatric diagnoses: in that light, our results are consistent since we did not find any association between CSF  $\alpha$ -Syn and ATN biomarkers in controls either. However, it should be noted that Vergallo et al.'s subjects reported subjective memory complaints, which often are linked with mood disturbances (25). Second, our participants were generally younger, and age may have an impact on the relationship between CSF  $\alpha$ -Syn and other biomarkers.

Elevations of CSF Ng, a post-synaptic protein, have been reported to reflect neuronal degeneration in AD and MCI and are associated with greater cognitive deficits and longitudinal decline (19, 26). A lack of significant associations between CSF  $\alpha$ -Syn and neurodegenerative markers in depression, in conjunction with a positive correlation between CSF  $\alpha$ -Syn and CSF Ng concentrations, may suggest that changes in presynaptic aggregations of  $\alpha$ -Syn impact on neuronal activity and Ng release as reported for PD (11).

We did not observe a difference in CSF  $\alpha$ -Syn levels across individuals with late-life MDD and controls. This finding is in contrast with what previously reported by Ishiguro et al. (13), who examined serum  $\alpha$ -Syn levels. It is possible, therefore, that, while depression-related differences are detectable in serum, they are harder to identify when examining CSF. However, a more likely explanation, in our estimation, is that our sample was smaller compared to that of Ishiguro et al. (47 total subjects vs. 235) and, hence, we may have failed to detect a difference in  $\alpha$ -Syn levels across groups due to lack of power. More research is needed to confirm these results.

A final point should be made to note that Rr was sensitive to CSF Ng levels in depression, but not BSRT total recall. As we have suggested in recent papers (16), Rr, which can be extracted from



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most neuropsychological tests of memory, compares favourably to most conventional scores employed to estimate memory ability in older individuals.

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Conflicts of Interest

HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics. HZ has served at scientific advisory boards for Samumed, Wave, CogRx and Roche Diagnostics, and has given lectures in symposia sponsored by Alzecure and Biogen.

No other conflicts of interests are disclosed.

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