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 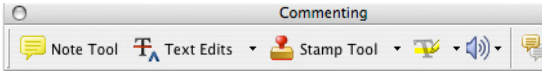

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 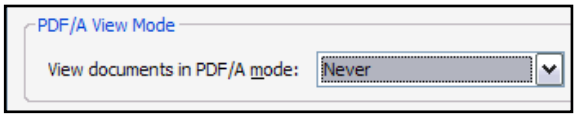

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 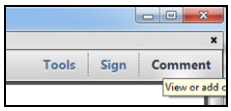

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 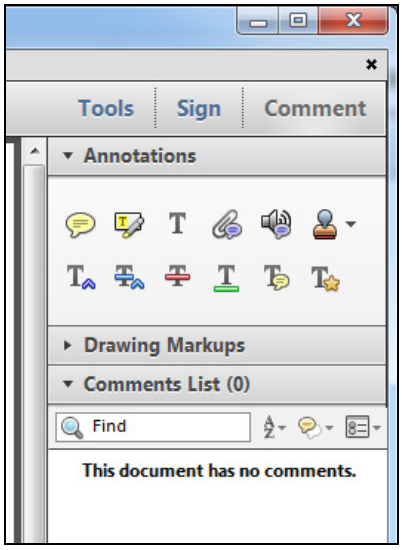
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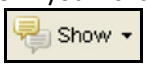
Review	To review your changes, click on the 'Show' button  on the Commenting tool bar. Choose 'Show Comments List'. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.	Your changes will appear automatically in a list below the Comment tool bar. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.
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Depression and Synaptic Zinc Regulation in Alzheimer Disease, Dementia with Lewy Bodies, and Parkinson Disease Dementia

Q13 David R. Whitfield, Ph.D., Julie Vallortigara, Ph.D., Amani Alghamdi, M.Sc., Tibor Hortobágyi, M.D., Ph.D., Clive Ballard, M.D., Alan J. Thomas, Ph.D., Q2 John T. O'Brien, M.D., Dag Aarsland, M.D., Paul T. Francis, Ph.D.

Objective: *Depression is a common symptom in dementia with Lewy bodies (DLB), Parkinson disease dementia (PDD), and Alzheimer disease (AD), yet its molecular basis remains unclear and current antidepressants do not appear to be effective. Cerebral zinc has been implicated in depression and synaptic dysfunction. We investigated the relationship between synaptic zinc regulation (for which zinc transporter 3 [ZnT3] is responsible) and depression in a large clinicopathologic study.* **Methods:** *We examined brains from people with PDD (N = 29), DLB (N = 27), and AD (N = 15) and comparison subjects without depression or dementia (N = 24). Individuals were categorized according to the presence and severity of depression (on a scale of 0–3) based on standardized assessments during life (principally Neuropsychiatric Inventory). Western blotting was used to determine ZnT3 levels in Brodmann area 9 (BA9), and regression analysis was used to determine the relationship between ZnT3 and depression.* **Results:** *Reductions in ZnT3 in BA9 were significantly associated with elevated depression scores in the study cohort ($\beta = -0.351$, $df = 93$, $t = -3.318$ $p = 0.0004$). This association remained when only individuals with DLB, PDD, and no dementia or depression were examined ($\beta = -0.347$, $df = 78$, $t = -3.271$, $p = 0.002$) or only individuals with AD and no dementia or depression were examined ($\beta = -0.433$, $df = 37$, $t = -2.924$, $p = 0.006$).* **Conclusion:** *Although decreased zinc levels have been implicated in the genesis of depression in animal models and in major depressive disorder in humans, this study provides the first evidence of a role for zinc in depression in people with dementia and highlights zinc metabolism as a therapeutic target.* (Am J Geriatr Psychiatry 2014; ■:■–■)

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Key Words: Depression, Alzheimer disease, Lewy body dementia, Parkinson disease dementia, zinc

INTRODUCTION

Depression is common in dementia, with a reported prevalence of up to 20% of people with Alzheimer disease (AD) and 30% or more of people with dementia with Lewy bodies (DLB) and Parkinson disease dementia (PDD), and is more frequent and persistent in DLB and PDD than in AD.[1,2] This is of critical importance because of the additive disability and impairment of function, reduced quality of life, distress, and increased mortality associated with depression in dementia.[3] Depression has also been identified as a major risk factor for dementia.[4,5]

Currently available antidepressants, which are effective in treating depression in people without dementia, do not appear to confer benefit in people with AD or Parkinson disease (PD).[6] A meta-analysis indicated uncertainty regarding the benefit of antidepressants in AD,[7] and subsequent large randomized controlled trials in the United States[8] and United Kingdom[9,10] have substantiated their lack of efficacy in treating depression in AD, with the U.K. study also indicating an increase in adverse events. Although no randomized controlled trials have investigated antidepressants in DLB or PDD, there is evidence that in PD antidepressants confer a similar lack of benefit.[11] This body of work raises the possibility that depression in dementia has a different molecular pathology. Depression is a frequent syndrome with significant impact, and a new and effective pharmacologic treatment is a high clinical priority.

Zinc homeostasis in neurons is primarily managed by zinc transporters (ZnTs) and Zn^{2+} importing proteins. In general, ZnTs remove Zn^{2+} from the cytosol and Zn^{2+} importing proteins import Zn^{2+} into the cytosol, and different members of each family are specific to certain organelles.[12] Zinc transporter 3 (ZnT3) is responsible for sequestering Zn^{2+} into glutamatergic vesicles before its release as a cotransmitter and modulator of synaptic strength.[13–16] Dietary zinc deficiency in rodents leads to reduced concentrations of synaptic Zn^{2+} in the hippocampus and has been consistently reported to cause depressive-like symptoms. Administration of antidepressants alleviated this effect, which the authors speculate may be due to an elevation of cerebral Zn^{2+} concentrations.[17–19] Electroconvulsive therapy, a treatment for depression, has also been shown to elevate synaptic Zn^{2+}.[18]

A number of studies have associated reduced serum Zn^{2+} concentration with depression in humans (reviewed by Swardfager et al.[17]), an association that strengthens with increased severity of depression. Additionally, evidence from small-scale clinical trials showed that zinc administration can be beneficial in depression, independently or alongside pharmacologic treatments.[20]

In this study we wanted to measure ZnT3 levels in the brain of patients with AD and DLB/PDD and its association with depression. The dorsolateral prefrontal cortex was selected because of the well-recognized role of frontal-subcortical circuits in the anatomy of depression and specifically because of reported reductions in volume and metabolic activity in this region in depression and its key role in many of the symptoms and in the pathology of the Lewy body dementias.[21–23] Two other regions were selected for comparison: the cingulate gyrus and parietal cortex. We hypothesized that reductions in ZnT3 in the prefrontal cortex, causing a loss of regulation of synaptic zinc, would be associated with depression in DLB/PDD and AD.

METHODS

Participants, Diagnosis, and Assessment

Postmortem brain tissue was obtained from several sources within the Brains for Dementia Research Network: University Hospital Stavanger (Norway), the MRC London Neurodegenerative Diseases Brain Bank, the Thomas Willis Oxford Brain Collection, and the Newcastle Brain Tissue Resource (Table 1). All patients were prospectively assessed during life

TABLE 1. Demographic Characteristics of the Cases Used in This Study According to Clinical Diagnosis

Diagnosis	Gender (M/F) (%)	Age at Death (mean ± SD)	Postmortem Delay (mean hours ± SD)	pH (mean ± SD)	MMSE (before death) (mean ± SD)
Comparison group (N = 24)	58/42	80.4 ± 7.1	39.5 ± 23.3	6.46 ± 0.3	N/A
PDD (N = 29)	55/45	79.1 ± 6.0	33.3 ± 16.3	6.48 ± 0.3	12.2 ± 7.7
DLB (N = 27)	56/44	81.2 ± 5.8	54.9 ± 29.3	6.26 ± 0.4	14.3 ± 6.9
AD (N = 15)	33/67	87.0 ± 7.0	36.4 ± 23.9	6.30 ± 0.3	8.6 ± 7.6

Notes: Age at death was significantly different between diagnostic groups (one-way ANOVA F = (df 3,91) 5.248, p = 0.002). The following were determined by Bonferroni post-hoc tests accompanying this ANOVA: age at death of AD subjects was significantly higher than individuals without dementia or depression (comparison group) (Bonferroni post-hoc multiple comparisons t test: mean difference 6.625, p = 0.013), PDD patients (Bonferroni post-hoc multiple comparisons t test: mean difference 7.862, p = 0.001), and DLB patients (Bonferroni post-hoc multiple comparisons t test: mean difference 5.778, p = 0.037). Postmortem delay was significantly higher (one-way ANOVA F = (df 3,89) 4.248, p = 0.007) in DLB patients compared with PDD patients (Bonferroni post-hoc multiple comparisons t test accompanying this ANOVA: mean difference 21.6, p = 0.05). There were no other significant differences between diagnostic groups for the variables shown. SD: standard deviation.

with standardized diagnostic and clinical rating scales. Informed consent was obtained from all participants, and the study had ethics approval from the National Research Ethics Service (08/H1010/4).

Individuals were categorized according to the duration and severity of depression on a scale of 0–3, where 0 was no depression, 1 was intermittent and mild depression, 2 was moderate (intermittent but significant) depression, and 3 was persistent and/or severe depression. This method was used partly to enable severity and persistence to be combined in the overall rating and to harmonize depression data from different behavioral tests across the cohorts from the various brain banks. For all individuals with dementia, scores from standardized tests or semi-structured interviews were used to derive this depression score; principally this was the Neuropsychiatric Inventory mood item (N = 41, 58% of dementia cases) and the Montgomery-Asberg Depression Rating Scale (N = 25, 21% of dementia cases). The thresholds for Montgomery-Asberg Depression Rating Scale were as follows: 15 and higher for a score of severe/persistent, 7–14 for a score of moderate, and 6 or lower for a score of mild. The thresholds for the Neuropsychiatric Inventory were as follows: 7 or higher for a score of severe/persistent, 4–6 for a score of moderate, and 3 or lower for a score of mild. However, in some instances only CAMDEX scores were available (N = 15, 21% of dementia cases), which rates depression as absent, mild/moderate, and severe, and longitudinal assessments enable the persistence element of the criteria to be applied. In all cases a score of 0 was treated as no depression.

Preparation of Tissue Samples for Western Blotting

Briefly, 500 mg of frozen tissue was taken from the frontal cortex (Brodmann area 9 [BA9]), cingulate cortex (Brodmann area 24 [BA24]), and parietal cortex (Brodmann area 40 [BA40]). Approximately 200 mg of gray matter was dissected from the tissue and homogenized, at a ratio of 2 mL to every 100 mg of tissue, in ice cold buffer containing 50 mM Tris-HCl, 5 mM EGTA, 10 mM EDTA, "complete protease inhibitor cocktail tablets" (1 tablet per 50 ml of buffer; Roche), and 2 µg/mL pepstatin A dissolved in ethanol-to-DMSO 2:1 (~~Sigma~~). An IKA Ultra-Turrax mechanical probe (~~KIA Werke, Germany~~) was used for homogenization.

Protein concentration was established using the Coomassie (Bradford) Protein Assay Kit (~~Thermo Scientific~~). Briefly, 10 µL crude homogenate was diluted 1:50 and read in triplicate at 595 nm using a FlexStation 3 (Molecular Devices).

Western Blotting

The homogenate was boiled for 5 minutes in 5× sample buffer (Genscript MB01015) before storage at −20°C. Samples were loaded at 20 µg/mL total protein on 10% SDS-polyacrylamide gel for protein separation, transferred to nitrocellulose membrane (Hydrobond-C; ~~Amersham~~), and probed with anti-ZnT3 (Synaptic Systems, 1:5,000) or anti-beta-III-tubulin (1:12,000; Abcam) and donkey anti-rabbit secondary antibody (IRDye; LI-COR). Bands were detected using an infrared fluorescent scanner (Odyssey), the integral of intensity quantified using

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infrared imaging systems application software (version 3.0.16; Odyssey) and expressed as ratios of human sample to rat cortex in arbitrary units. It was determined that rat cortex was more appropriate as a loading control becasue beta-III-tubulin was altered in other brain regions (data not shown). Western blotting was undertaken blind to the depression scores.

Statistical Analysis

Gender significantly predicted ZnT3 values in BA9 ($R^2 = 0.043$, $\beta = -0.207$, df = 113, t = −2.254, p = 0.026), and so regression analysis (SPSS software, IBM) was used to create a residual value (unstandardized) for ZnT3 to remove this effect of gender. This was applied to all ZnT3 values from all cases. Because this analysis was undertaken as a screening process before analysis of the subset of cases with depression scores, the N values are slightly higher. Furthermore, when the comparison cases were separated according to gender, there was no significant difference between ZnT3 values (Mann-Whitney U test, Z = −0.527, p = 0.625, two-tailed). Regression analysis (by entering variables without stepping) was then used to determine the relationship between depression scores and ZnT3 values in BA9. Differences in ZnT3 values between depression score groups were analyzed by one-way ANOVA and Bonferroni post-hoc tests after confirming that the homogeneity of variance (Levene F statistic 2.033, df 3,91, p = 0.115) was not significantly different between these groups. Once calculated, group means of residuals were compared using a one-way ANOVA followed by the Bonferroni post-hoc multiple comparison test if the ANOVA was <0.05.

RESULTS

Ninety-five individuals were included. There were significant differences in depression scores between diagnostic groups (Fig. 1; one-way ANOVA F = (3,91) 16.817, p <0.001). The accompanying post-hoc tests showed individuals with DLB (N = 27) were characterized by significantly lower depression scores than those with PDD (Bonferroni post-hoc multiple comparisons t test, mean difference −0.893, p = 0.001, N = 29) or AD (Bonferroni post-hoc multiple comparisons t test, mean difference −1.044, N = 15, p = 0.005), whereas PDD and AD had significantly higher depression scores than the comparison group (Bonferroni post-hoc multiple comparisons t test, mean differences 1.448 and 1.6, respectively, N = 24, p <0.001).

Reduced ZnT3 in BA9 Was Associated With Higher Depression Scores

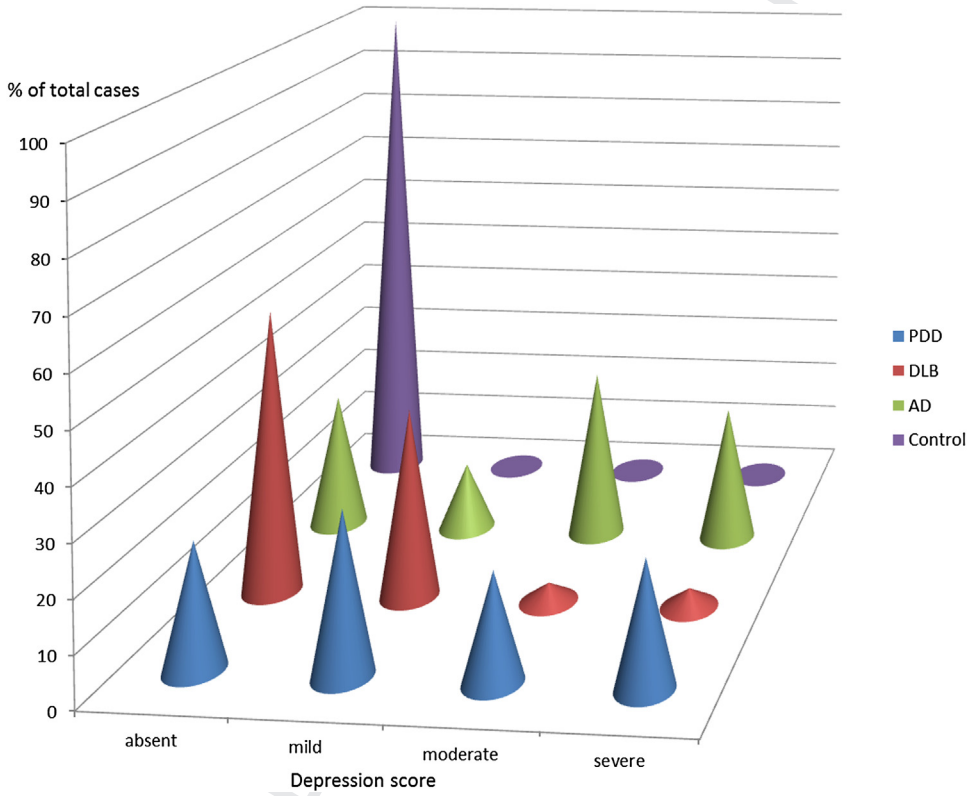
Reductions in ZnT3 in BA9 were associated with severity of depression (Fig. 2; $\beta = -0.351$, df = 93, t = −3.318 p = 0.0004). Dementia patients with severe depression had significantly lower ZnT3 levels than individuals without depression (Bonferroni post-hoc multiple comparisons t test, p = 0.018), a reduction of 22%. The accompanying one-way ANOVA values for all depression score groups were (F(3,91) = 4.534, p = 0.005). This association remained when only individuals with DLB, PDD, and no dementia or depression were examined ($R^2 = 0.121$, $\beta = -0.347$, df = 78, t = −3.271, p = 0.002) or only individuals with AD and no dementia or depression were examined ($R^2 = 0.188$, $\beta = -0.433$, df = 37, t = −2.924, p = 0.006). Individuals without depression or dementia were included in all analyses.

Thus, the association between ZnT3 and depression did not appear be influenced by the type of dementia. However, it was associated with the prefrontal cortex; there was no significant relationship between depression scores and ZnT3 levels in the cingulate gyrus or the parietal cortex (Pearson's correlation two-tailed BA24: r = −0.062, N = 96, p = 0.552; BA40: r = −0.081, N = 90, p = 0.445, data not shown). When ZnT3 was expressed as a ratio to beta-III-tubulin (which was unchanged across diagnostic groups in BA9; see Supplementary Figure 1; available online), the relationship to depression was still observed ($R^2 = 0.082$, $\beta = -0.286$, df = 88, t = −2.805, p = 0.006).

Depression and Gender

ZnT3 levels predicted depression scores in both men and woman when cases were separated by gender (woman: $R^2 = 0.158$, $\beta = -0.397$, df = 43, t = −2.838, p = 0.007, N = 45; men: $R^2 = 0.093$, $\beta = -0.305$, df = 48, t = −2.218, p = 0.031, N = 50).

FIGURE 1. **Percentage of individuals with each depression score according to diagnosis. Subjects without dementia or depression (or comparison group): absent N = 24, 100%. PDD: absent N = 7, 24.1%; mild N = 9, 31%; moderate N = 6, 20.7%; severe N = 7, 24.1%. DLB: absent N = 15, 55.6%; mild N = 10, 37%; moderate N = 1, 3.7%; severe N = 1, 3.7%. AD: absent N = 4, 26.7%; mild N = 2, 13.3%; moderate N = 5, 33.3%; severe N = 4, 26.7%. The difference in mean depression scores between diagnostic groups was determined using one-way ANOVA: F = (3,91)16.817, p <0.001. The accompanying post-hoc test showed depression scores were significantly higher in individuals with PDD and AD compared with subjects without dementia or depression (Bonferroni post-hoc multiple comparisons t test: mean difference −1.1448 and −1.6, p <0.001) and significantly lower in individuals with DLB compared with PDD (Bonferroni post-hoc multiple comparisons t test: mean difference −0.893, p = 0.001) and AD (Bonferroni post-hoc multiple comparisons t test: mean difference −1.044, p = 0.002).**



DISCUSSION

We identified a significant association between reduced ZnT3 levels in the prefrontal cortex and depression in individuals with AD, DLB, and PDD. This relationship was not observed in either the cingulate cortex or the parietal cortex and cannot be explained by cell loss because beta-III-tubulin levels were not significantly altered in BA9 and when ZnT3 was expressed as a ratio to beta-III-tubulin the relationship to depression was maintained.

This relationship is consistent with previous studies that linked depressive symptoms to the prefrontal cortex.[22,23] There is debate as to the relative prevalence of depression in these dementias[24]; in the individuals used in this study, depression was more frequent in PDD and AD than in DLB, which is unusual and likely reflects the smaller sample size for this postmortem study compared with clinical studies.

The authors were fortunate to have access to a substantial cohort of postmortem cases from across the United Kingdom and Norway, yet this came with the disadvantage common to such studies in that collection of data before death had been undertaken by others and not in a uniform manner across the brain banks. To resolve this, two authors (CB and JTO), experienced old-age psychiatrists, created a

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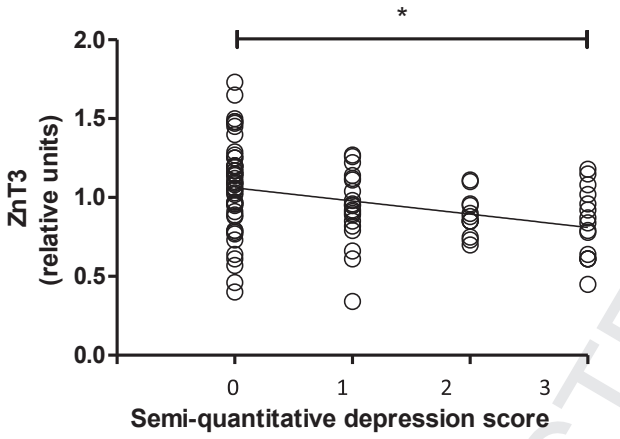
FIGURE 2. **ZnT3 levels, obtained from semiquantitative Western blotting in BA9, predicted depression in all cases. Using regression analysis, the semiquantitative depression score was found to be significantly predicted by ZnT3 values from semiquantitative Western blotting in BA9 (R^2 = 0.123, β = −0.351, df = 93, t = −3.318, p = 0.0004; absent (1) N = 50, mild (2) N = 21, moderate (3) N = 12, severe (4) N = 12). In addition, the ANOVA for this model was significant (p <0.000). One-way ANOVA, F(3,91) = 4.534, p = 0.005, and an accompanying post-hoc analysis showed ZnT3 levels to be significantly higher in cases without depression (1) compared with cases with severe depression (4) (Bonferroni post-hoc multiple comparisons t test p = 0.018).**



composite score using operationalized criteria to allow comparison of clinical data between cohorts.

Depression in these dementias is a significant cause of burden for both carers and people with dementia and is not benefited by treatment with antidepressants[3,7–9]; thus, there exists a critical need for effective pharmacologic treatment for depression in dementia.

A gender bias is recognized in some dementias; for example, AD is reported to have a higher prevalence in women.[25–27] Therefore, because we observed a relationship between gender and ZnT3 levels in BA9, we examined whether the observed relationship between ZnT3 and depression differed between men and women. Accordingly, we found the significant relationship between ZnT3 levels and depression was maintained when cases were separated by gender.

Depression has been frequently associated with Lewy body dementia and with cognitive decline[21,28]; however, there is less evidence concerning its molecular origin, despite a report linking depression in AD to serotonergic deficits.[29] Reduced cortical Zn^{2+} has been implicated in depression in humans, in animal models of depression, and been shown to be addressed by antidepressants.[18,30,31] Reduced ZnT3 corresponds to a loss of regulation of synaptic Zn^{2+}, which could have a similar consequence as a more general loss of cortical Zn^{2+} in terms of generating depressive behavior. Furthermore, it is probable that reduced release of Zn^{2+} at the synapse impacts on the glutamatergic and serotonergic systems, both of which are implicated in the molecular pathophysiology of depression.[32,33] This may explain why administration of Zn^{2+} alongside antidepressants in animal models improves their efficacy.[34]

It remains unclear why ZnT3 should be reduced in these dementias and what the relationship is between changes in Zn^{2+} levels and changes in ZnT3 expression; this is of particular interest because both age and central and peripheral inflammation, key correlates of dementia, are associated with reduced serum Zn^{2+} levels.[17,35] That the effect size is not larger is most likely a reflection of the complex etiology of depression in dementia; ZnT3 is only one of many significant contributing factors. A limitation of this study was the lack of a group of individuals with depression but no dementia; however, a suitably matched cohort was not available. Furthermore, there may have been a loss of some detail caused by the composite scores used for assimilating the different depression assessments, although the CAMDEX assessments did not suggest this was the case.

The modulation of Zn^{2+} has shown some potential in clinical trials as a treatment for AD, based on a compound PBT2, which acts to increase the Q6 bioavailability of Zn^{2+} at the synapse, thereby compensating for some of the effects brought about by the loss of ZnT3.[36–38] The findings presented here suggest a similar approach might be appropriate for DLB/PDD due to the contribution of Zn^{2+} dyshomeostasis to depression in these dementias. In conclusion, we present evidence that a loss of regulation of synaptic Zn^{2+} may have a role in depression in DLB/PDD and AD and raise the possibility of modulating Zn^{2+} as a candidate treatment approach for depression in these individuals.

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