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## Kynurenic Acid Levels in Cerebrospinal Fluid from Patients with Alzheimer's Disease or Dementia with Lewy Bodies

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**ABSTRACT:** Kynurenic acid (KYNA) is implicated in cognitive functions. Altered concentrations of the compound are found in serum and cerebrospinal fluid (CSF) of patients with Alzheimer's disease (AD). Further studies to determine whether KYNA serves as a biomarker for cognitive decline and dementia progression are required. In this study, we measured CSF KYNA levels in AD patients (n = 19), patients with dementia with Lewy bodies (DLB) (n = 18), and healthy age-matched controls (Ctrls) (n = 20) to further explore possible correlations between KYNA levels, cognitive decline, and well-established AD and inflammatory markers. Neither DLB patients nor AD patients showed significantly altered CSF KYNA levels compared to Ctrls. However, female AD patients displayed significantly higher KYNA levels compared to male AD patients, a gender difference not seen in the Ctrl or DLB group. Levels of KYNA significantly correlated with the AD-biomarker P-tau and the inflammation marker soluble intercellular adhesion molecule-1 (sICAM-1) in the AD patient group. No associations between KYNA and cognitive functions were found. Our study shows that, although KYNA was not associated with cognitive decline in AD or DLB patients, it may be implicated in AD-related hyperphosphorylation of tau and inflammation. Further studies on larger patient cohorts are required to understand the potential role of KYNA in AD and DLB.

**KEYWORDS:** Alzheimer's disease, astrocyte markers, cognition, dementia with Lewy bodies, kynurenic acid, inflammation

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### Introduction

Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) constitute the majority of cases with neurodegenerative dementia. Both diseases inevitably lead to severe impairment of cognitive functions and premature death. Neuropathologically, AD and to a large extent also DLB are characterized by accumulation of aggregated A $\beta$  ie senile plaques in the brain parenchyma.<sup>1–3</sup> Also neurofibrillary tangles (NFTs) are characteristics in AD (aggregations of hyperphosphorylated tau protein),<sup>1,3</sup> whereas patients with DLB, in addition to senile plaque, display Lewy bodies and Lewy neurites (intraneuronal accumulation of mainly  $\alpha$ -synuclein).<sup>2</sup> Accumulation of senile

plaques and Lewy bodies is associated with inflammatory processes, and neuropathological studies describe activated astrocytes and microglia adjacent to senile plaques<sup>4</sup> and Lewy bodies<sup>5,6</sup> in AD and DLB patients, respectively. Also increased levels of pro-inflammatory cytokines have been found in the cerebrospinal fluid (CSF) of AD patients.<sup>7</sup> In both AD and DLB patients, CSF levels of factors mediating inflammatory processes such as acute phase proteins and soluble adhesion molecules are elevated.<sup>8,9</sup> The dementia-related inflammatory processes appear to play a critical role already at an early stage of the diseases. Indeed, several epidemiological studies have shown reduced risk of AD in individuals medicated with



anti-inflammatory drugs at early age.<sup>10</sup> Also, individuals more than 90 years with elevated levels of C-reactive protein (CRP) have a five-fold risk of developing dementia,<sup>11</sup> and individuals with a parental history of AD have higher production capacity of pro-inflammatory cytokines compared to individuals without a family history of dementia.<sup>12</sup>

Inflammatory processes in the brain may influence several systems underlying disease progression. One of these, the kynurenine pathway of tryptophan degradation gives rise to several neuroactive metabolites. One branch of this pathway, taking place primarily in astrocytes, forms the neuroprotective metabolite kynurenic acid (KYNA). Another branch, primarily in resident and reactive microglia as well as in infiltrating macrophages, forms the excitotoxic metabolite quinolinic acid (QUIN). The latter is an *N*-methyl-D-aspartate (NMDA) receptor agonist, whereas KYNA blocks several receptors, including the glycine-site and the glutamate recognition-site of the NMDA receptor,<sup>13</sup> the  $\alpha$ 7-nicotinic acetylcholine receptor, as well as the  $\alpha$ -amino-3-hydroxy-5-methylisoxazol-4-propansyra (AMPA) receptor at higher concentrations.<sup>14</sup> KYNA is also known to stimulate the G protein-coupled receptor 35 (GPR35). Degradation of tryptophan into kynurenines is strongly influenced by inflammatory stimuli.<sup>15</sup> This may be particularly relevant in dementia, known to be driven by inflammatory processes. The potential involvement of KYNA in neurodegenerative disorders was discussed already three decades ago, as studies showed significantly reduced KYNA levels in CSF and brains of patients with Huntington's disease.<sup>16,17</sup> Studies from the same time period also demonstrated the presence and action of KYNA in the primate hippocampus,<sup>18</sup> a brain area crucial for memory storage. Additionally, experimental studies demonstrate that KYNA can prevent neurodegeneration in rat striatum.<sup>19</sup> The neuroprotective aspect of KYNA together with its ability to impair cognitive function is particularly interesting from a dementia perspective, and has led to the idea that an inflammation-induced increase of endogenous KYNA production may contribute to the memory disruption and possibly be a mechanism to counteract the neurodegenerative processes seen in dementia.<sup>20</sup> However, reports on KYNA levels in AD patients are inconclusive. Decreased KYNA levels,<sup>13,21</sup> but response of endogenous antibodies directed against KYNA (indicating overproduction of KYNA), have been shown in blood from AD patients.<sup>14</sup> Further, elevated<sup>22</sup> or unchanged<sup>16</sup> KYNA concentrations in the brain of AD patients have been found, and an earlier study have described reduced CSF KYNA levels in AD patients compared to healthy individuals.<sup>15</sup> Additionally, experimental studies show cognitive deficits, such as impairments in working memory and contextual learning,<sup>23–25</sup> in response to increased brain KYNA levels. Conversely, a clinical study has shown a positive association between cognitive function and plasma KYNA levels in AD patients.<sup>26</sup> These contradictory reports highlight the need for studies correlating central KYNA levels in AD with

cognitive functions. In addition, less is known about potential alterations in the kynurenine pathway in DLB patients, the second most common neurodegenerative dementia form. In the current study, we therefore investigated levels of KYNA in AD and DLB patients compared to healthy age-matched elders. We also investigated if cognitive functions in AD and DLB patients are associated with changes in CSF KYNA concentrations and whether KYNA correlates to the well-established AD biomarkers A $\beta$ 1-42, T-tau, and P-tau in these patients. Finally, we investigated the potential relationships between KYNA and a range of astrocyte-derived inflammatory markers previously shown to be altered in patients with dementia, including alpha 1-antichymotrypsin (ACT),<sup>27,28</sup> monocyte chemotactic protein-1 (MCP-1),<sup>29,30</sup> soluble intercellular adhesion molecule-1 (sICAM-1),<sup>8,31</sup> and soluble vascular cell adhesion protein-1 (sVCAM-1).<sup>8,32</sup>

## Material and Methods

**Patients.** The studied groups, AD patients ( $n = 19$ ), DLB patients ( $n = 18$ ), and non-demented controls (Ctrls) ( $n = 20$ ), consist of samples from the Malmö Alzheimer Study randomly selected blindly to clinical, mental, genetic, and biomarker results. The entire study cohort has previously been described in detail.<sup>8,9</sup> In brief, clinical diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, by the American Psychiatric Association (DSM-IV, 1994) combined with National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Diseases Associations (NINCDS-ADRDA) diagnostic criteria<sup>15,16</sup> for probable AD. Diagnosis of probable DLB was made according to the DLB consensus criteria.<sup>2,17</sup> Cognitive status of patients and Ctrls was evaluated using the Mini Mental State Examination (MMSE).<sup>18</sup> The basic CSF AD-biomarker (A $\beta$ 1-42, T-tau, P-tau181) profile of the subjects included in the Malmö Alzheimer Study has been described before.<sup>8</sup> Height of AD patients was determined and described as length (meter). The ethics committee of Lund University approved the study, and the study procedures were in accordance with the Helsinki Declaration of 1975 (revised in 2000). All individuals (or their nearest relatives) gave informed consent to participate in the study.

**Analysis of KYNA levels in CSF.** CSF samples were collected as described before.<sup>8</sup> All samples were freeze-thawed equal amount of times (four times) before the KYNA analysis. Importantly, KYNA is a stable compound and is not degraded even by repeated thawing.<sup>19</sup> The analysis of KYNA was performed utilizing an isocratic reversed-phase high-performance liquid chromatography (HPLC) system, including a dual piston, high liquid delivery pump (Bischoff, Leonberg, Germany), a ReproSil-Pur C18 column (silica pore size, 3  $\mu$ m (4  $\times$  100 mm), Dr. Maisch GmbH, Ammerbuch, Germany), and a fluorescence detector (Jasco Ltd., Hachioji City, Japan) with an excitation wavelength of 344 nm and an emission

wavelength of 398 nm (18 nm bandwidth). A mobile phase of 50 mM sodium acetate (pH 6.2, adjusted with acetic acid) and 7.0% acetonitrile was pumped through the reversed-phase column at a flow rate of 0.5 mL/minute. A total of 50  $\mu$ L samples were manually injected (ECOM, Prague, Czech Republic). In all, 0.5 M zinc acetate (not pH adjusted) was delivered post column by a peristaltic pump (P-500, Pharmacia, Uppsala, Sweden) at a flow rate of 0.10 mL/minute. The signals from the fluorescence detector were transferred to a computer for analysis with Datalys Azur (Grenoble, France). The retention time of KYNA was approximately seven to eight minutes. The sensitivity of the HPLC system was verified by analysis of a standard mixture of KYNA with concentrations from 1 to 30 nM resulting in a linear standard plot. The precision of the HPLC method used in the present study was routinely tested within (intra-assay) and between days (interassay) during the days of these analyses. For the determination of intra-assay precision, aliquots ( $n = 8$ ) of KYNA standards at concentrations of 1 and 10 nM were analyzed. The precision of the assay was calculated from the percentage coefficient of variation (CV) of the mean, according to the equation  $CV (\%) = (\text{standard deviation}/\text{mean})100$ . The CV values for 1 and 10 nM were 3.0 and 4.1%, respectively. Interassay precision was calculated by analyzing aliquots of the same KYNA standard (1 and 10 nM on the days the CSF samples were analyzed ie three consecutive days). The CV for interassay precision was 4.3% for 1 nM and 3.9% for 10 nM. The samples were analyzed in singles. In all, 10 of 57 samples (17.5%) were analyzed in duplicates, and the mean CV was 2.8%.

**Analysis of additional CSF markers.** Complete results regarding CSF levels of sICAM-1, sVCAM-1, and ACT have previously been reported for the entire Malmö Alzheimer Study (Refs. 8 and 9). In addition, CSF levels of MCP-1 were determined using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions.

**Statistical analysis.** Statistical analysis was performed using the SPSS software (version 12.0.1 of Windows, SPSS Inc., Chicago, IL, USA). Normal distribution of the variables was tested using the Kolmogorov–Smirnov test. The independent sample  $t$ -test was used for comparisons between two groups, and the one-way ANOVA analysis, followed by Bonferroni post hoc test, was used for multiple comparisons. Correlations were investigated using the Pearson correlation test. The two-sided  $\chi^2$  test was used to test frequency differences among the groups. Results are presented as means  $\pm$  standard deviation or range (age and MMSE in Table 1).  $P < 0.05$  was considered significant.

## Results

**Characteristics of individuals included in the CSF analysis.** Table 1 gives the demographic data and MMSE scores of the investigated dementia patients and non-demented Ctrl (Table 1). Dementia patients had significantly

**Table 1.** Demographic data of individuals included in the CSF analysis.

VARIABLES	CTRL	AD	DLB
n (M/F)	10/10	9/10	8/10
Age <sup>a</sup>	76 (71–84)	75 (72–79)	77 (71–84)
MMSE <sup>a</sup>	29 (27–30)	***21 (14–30)	***21 (14–28)
T-tau (ng/L) <sup>b</sup>	336.55 $\pm$ 42.37	**583.79 $\pm$ 59.06	403.67 $\pm$ 44.10
P-tau (ng/L) <sup>b</sup>	62.70 $\pm$ 4.61	*76.95 $\pm$ 5.80	*81.17 $\pm$ 3.70
A $\beta$ 1–42 (ng/L) <sup>b</sup>	646.19 $\pm$ 43.62	***414.11 $\pm$ 20.64	**474.94 $\pm$ 39.67

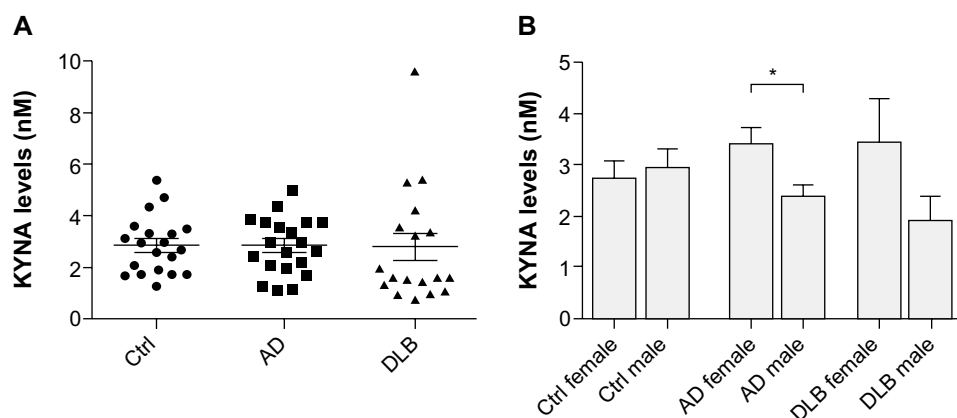
**Notes:** <sup>a</sup>Data are presented as means and (range). <sup>b</sup>Data are presented as means and standard deviation. \*Indicates a significant difference at the  $P < 0.05$  level compared to controls (CTRL). \*\*Indicates a significant difference at the  $P < 0.01$  level compared to CTRL. \*\*\*Indicates a significant difference at the  $P < 0.001$  level compared to CTRL.

lower MMSE scores ( $P < 0.001$ ), compared to Ctrl (Table 1). AD and DLB patients had significantly lower A $\beta$ 1–42 CSF levels and higher P-tau CSF levels compared to Ctrl, and AD patient had significantly higher T-tau levels compared to Ctrl (Table 1). Age and use of anti-hypertensive medication and NSAIDs were similar in all groups (data not shown).

**Cerebrospinal levels of KYNA.** KYNA values were normally distributed within all investigated groups regardless of whether groups were divided based on gender (Ctrl  $P = 0.953$ , female Ctrl  $P = 0.978$  and male Ctrl  $P = 0.905$ ; AD  $P = 0.978$ , female AD  $P = 0.982$  and male AD  $P = 1.000$ ; DLB  $P = 0.194$ , female DLB  $P = 0.787$  and male DLB  $P = 0.265$ ). As previous studies have shown age-dependent changes in CSF KYNA levels,<sup>20,23,24</sup> we first investigated whether age correlated with KYNA within the different analyzed groups. No correlation between the two variables was found, and thus age was not included as a covariate in the statistical analysis. Comparison analysis showed no difference in CSF KYNA levels when the groups were compared to each other (ANOVA  $P = 0.968$ ) (Fig. 1A). As earlier studies suggest a gender-dependent difference in CSF levels of KYNA,<sup>23</sup> we also analyzed CSF KYNA levels after subdividing the Ctrl and the patient groups into males and females. Comparison analysis showed no significant changes in CSF KYNA levels between female Ctrl and female DLB patients compared to their male counterparts ( $2.92 \pm 0.39$  vs  $2.75 \pm 0.33$  nM,  $P = 0.747$  and  $3.45 \pm 0.85$  vs  $1.91 \pm 0.43$  nM,  $P = 0.157$ , respectively). However, a significant increase in KYNA CSF levels was found in female AD patients compared to male AD patients ( $3.42 \pm 0.30$  vs  $2.39 \pm 0.26$  nM,  $P = 0.019$ ) (Fig. 1B). The gender-dependent difference between female and male AD patients was even greater when length was taken into account ( $2.11 \pm 0.50$  vs  $1.39 \pm 1.51$  nM/m,  $P = 0.007$ ). No significant changes in KYNA levels were seen when comparing female DLB or AD patients to female Ctrl, or male DLB or AD patients to male Ctrl (data not shown).

**CSF levels of markers for inflammatory processes.** Comparisons of the measured MCP-1 CSF concentrations showed no significant differences between non-demented





**Figure 1.** Graphs showing (A) KYN levels in CSF of healthy Ctrl, and patients with AD and DLB and (B) KYN levels in CSF of Ctrl, AD, and DLB divided according to gender. Each bar represents the mean  $\pm$  SEM.

**Note:** \*Indicates a significant difference at the  $P < 0.05$  level.

Ctrl, AD patients, and DLB patients ( $546.48 \pm 34.63$  ng/L,  $592.47 \pm 24.46$  ng/L, and  $555.24 \pm 27.70$  ng/L, ANOVA  $P = 0.506$ ). Individuals included in this study are selected from a large patient cohort where CSF levels of ACT, sICAM-1, and sVCAM-1 have been analyzed and published elsewhere.<sup>8,9</sup> Analysis of ACT, sICAM-1, and sVCAM-1 levels in individuals included in this smaller sample of the Malmö Alzheimer Study is inline with the previously presented results and shows significantly increased ACT levels in AD patients,<sup>9</sup> significantly increased sICAM-1 levels in DLB patients,<sup>8</sup> and significantly reduced sVCAM-1 levels in both DLB and AD patients compared to Ctrl.<sup>8</sup> Neither age nor gender was found to affect levels of MCP-1 or sVCAM-1, sICAM-1, and ACT in any of the investigated groups.

**Correlations analysis.** Next we investigated potential links between CSF KYN levels, AD biomarkers ( $A\beta 1-42$ , T-tau, and P-tau), and cognitive function (total MMSE scores). A positive correlation between KYN and P-tau (Table 2) and a trend to a significant correlation between KYN and T-tau (Table 2) was found in the AD patients group. The correlation between P-tau and CSF KYN levels and the tendency to correlate between T-tau and KYN was not found in the Ctrl group or DLB group, and no correlation between KYN CSF levels and the AD biomarkers  $A\beta 1-42$  and MMSE was detected in any of the investigated groups (Table 2). We also investigated the relationship between KYN levels and other inflammatory markers shown to be secreted by reactive astrocytes ie, MCP-1,<sup>25</sup> ACT,<sup>26</sup> sVCAM-1,<sup>33</sup> and sICAM-1.<sup>34</sup> Our correlation analysis showed a positive correlation between KYN and sICAM-1 (KYN;  $r = 0.597$ ,  $P = 0.007$ ) in the AD group, but not in the DLB or Ctrl group. No correlations between sVCAM-1, ACT, or MCP-1 levels and KYN levels were found in any of the investigated groups.

## Discussion

The present study shows that CSF KYN levels are not significantly changed when comparing a small patient cohort of

AD and DLB to age- and gender-matched Ctrl. Our study further shows that KYN levels are significantly higher in female AD patients compared to male AD patients, a result not found in Ctrl or in DLB. Finally, correlation analysis demonstrates a significant correlation between CSF KYN levels and CSF P-tau and between CSF KYN and CSF sICAM-1 in the AD group. CSF KYN was not significantly correlated to CSF T-tau, CSF  $A\beta 1-42$ , or the cognitive test battery MMSE in the any of the investigated groups.

Notably, our analysis of CSF KYN levels revealed a mean concentration just below 3 nM in healthy elders (71–84 years), a concentration almost two-folded higher compared to values found in younger (18–66 years) healthy individuals (approximately 1.5 nM).<sup>35</sup> This result is in line with a previous study demonstrating an age-dependent increase in CSF KYN levels, as individuals more than 50 years were shown to display a significant increase in CSF KYN concentrations compared to individuals below 50 years.<sup>22</sup> The CSF KYN levels in our study did not significantly correlate with age, but because the individuals included in the study were carefully age matched, it may well be that the age range was too narrow to yield a significant correlation between age and CSF KYN levels. Although a previous study reports decreased CSF levels of KYN in AD patients,<sup>27</sup> we were unable to confirm this finding. It should however be noted, as mentioned in the Introduction, that consensus regarding KYN alterations in AD is still lacking. This inconsistency may highlight the heterogeneity of disease, which the high variances of KYN values within the analyzed groups in our study may indicate. Notably, we found no evidence of a direct involvement of KYN alterations in DLB pathogenesis, as no significant changes in CSF KYN levels were detected in this patient group.

Levels of KYN in CSF from female AD patients were significantly higher compared to those of male AD patients, and similar findings were seen in DLB females compared to males although this difference was not significant. This finding is in line with previous studies demonstrating higher CSF and

**Table 2.** Correlations between KYN A and AD biomarkers in AD and DLB patients and healthy elders.

DIAGNOSIS	CTRL	AD	DLB
A $\beta$ 1–42	$r = 0.106$ , $p = 0.657$	$r = -0.225$ , $p = 0.355$	$r = -0.268$ , $p = 0.281$
P-tau	$r = -0.035$ , $p = 0.885$	$r = 0.571$ , $p = 0.011^*$	$r = 0.030$ , $p = 0.906$
T-tau	$r = 0.157$ , $p = 0.509$	$r = 0.449$ , $p = 0.054$	$r = 0.287$ , $p = 0.249$

**Notes:** \*indicates a significant correlation ( $r$ ) between HA and AD biomarker at the  $P < 0.05$  level.

KYN A levels in younger females (age less than 47 years),<sup>23,28</sup> which indicates a gender-dependent regulation of KYN A secretion. Previous studies have shown a negative correlation between CSF KYN A concentrations and body height,<sup>23</sup> and it is assumed that a tall person, because of a longer spinal compartment, exhibits a larger surface for resorption of metabolites from the CSF, which results in lower concentrations. However, the gender difference in CSF KYN A in AD patients was even greater when taking height into account. Interestingly, we found no difference in CSF KYN A levels between healthy elder females or males. The significance of this result is elusive, and because the number of individuals included in the gender divided groups is small, it may be that the lack of differences is because of low statistic power. However, given that the measured levels of KYN A in our healthy elder individuals are much higher than what is usually seen in younger individuals (as described above), it is tempting to speculate that age-dependent increase in KYN A may even out the difference. Another potential explanation is the fact that the elder women have passed their menopause and thereby lack estrogen production. Interestingly, previous studies have shown that estrogen affects the activity of tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), two rate-limiting enzymes of tryptophan metabolism along the L-Kynurenine pathway.<sup>29</sup> Activity of TDO is enhanced by estrogen via the hypothalamic–pituitary–adrenal axis,<sup>30</sup> and IDO expression has been shown to be upregulated in immune cells in response to estrogen.<sup>31</sup> If lack of estrogen production underlies the absence of difference in elder healthy Ctrl s, it also indicates that altered KYN A in female patients is a consequence of a gender-dependent pathology-induced increase in KYN A secretion. Notably, DLB has a male preponderance,<sup>32</sup> and thus our cohort is atypical for this disorder, which could affect the results. Nevertheless, we found no significant difference between male DLB patients and male Ctrl s ( $P = 0.157$ ), which may be a result of insufficient statistical power ( $n = 8$  DLB patients and  $n = 10$  Ctrl s).

Despite previous findings describing an association between MMSE and serum KYN A levels in AD patients,<sup>21</sup> we found no correlation between CSF KYN A levels and MMSE, regardless of gender, in any of the investigated groups. It should however be pointed out

that the MMSE test consists of different tasks evaluating hippocampal-dependent cognition such as spatial orientation, memory, and also calculation, language, and construct ability.<sup>36</sup> Previous experimental rodent studies foremost links altered KYN A levels to hippocampal-dependent cognition. Spatial discrimination, passive avoidance and object exploration/recognition, is enhanced in KAT II knock-out mice with reduced brain KYN A levels<sup>37</sup> and impaired memory, passive avoidance and elevated brain KYN A, is associated with reduced cognitive flexibility in offspring of dam rats fed with KYN A-containing chow under the gestational period.<sup>38,39</sup> In bypass patients, KYN A has been shown to function as a predictor of poor cognitive performance related to frontal executive functions and memory.<sup>40</sup> The lack of a correlation between CSF KYN A and MMSE in the present study could thus be related to the design of the cognitive test.

Our correlation analysis yielded no significant correlations with the AD biomarkers T-tau or A $\beta$ 1–42, but we found a significant positive correlation between P-tau and KYN A levels. CSF levels of P-tau are routinely used to aid clinical diagnostics, as increased levels of P-tau are indicative of intraneuronal hyperphosphorylation of tau<sup>41</sup> causing NFTs. Previous in vitro studies have shown an increase of tau hyperphosphorylation in neurons exposed to QUIN,<sup>42</sup> another metabolism of the kynurenine pathway, and in vivo studies have shown hyperphosphorylation of cytoskeletal intermediate filament proteins in astrocytes and neurons in rats after intrastriatal administration of QUIN.<sup>43</sup> Moreover, an immunohistological postmortem study shows co-localization of P-tau and QUIN positive NFTs with TDO, a key enzyme regulating the kynurenine pathway.<sup>44</sup> Although, there are no previous reports describing a direct impact of KYN A on neuronal tau hyperphosphorylation or a relationship between KYN A production and P-tau formation, our data suggest that also KYN A might be implicated in this pathological event. The previously demonstrated link between P-tau and QUIN highlights the importance of analyzing this metabolite also in dementia pathology. QUIN is a NMDA receptor agonist<sup>45</sup> and is foremost produced in microglia.<sup>46</sup> Production of QUIN is strongly affected by inflammatory actions, as cytokines have been shown to enhance the production of QUIN in macrophages.<sup>47</sup> Furthermore, QUIN is known to stimulate astrocyte secretion of chemokines<sup>48</sup> and cytokines.<sup>49</sup> Immunohistological studies on brain tissue from AD patients have shown increased immunoreactivity of QUIN in senile plaques,<sup>50</sup> and increased levels of QUIN levels have been found in plasma from AD patients.<sup>13,21</sup> Unfortunately, because of lack of sufficient amount of CSF we were unable to analyze QUIN in this study, but would like to stress the importance of including this metabolite in future studies for their role in kynurenine pathway in dementia.

Finally, we analyzed the relationship between KYN A levels and factors secreted by astrocytes in response to inflammatory stimuli. We found no relationship between ACT, MCP-1,



or sVCAM-1 and KYNA levels, which may be explained by the fact that these variables, in particular MCP-1 and sVCAM-1,<sup>51,52</sup> are secreted not solely from reactive astrocytes but also by a range of other cell types. Thus, KYNA may, in response to inflammatory actions, be produced independently of ACT, MCP-1, or sVCAM-1. A significant correlation was however found between sICAM-1 and KYNA in the AD group. This glycoprotein is expressed by endothelial cells, astrocytes, and infiltrating immune cells (macrophages and leukocytes)<sup>51</sup> and is implicated in the migration of immune cells across the endothelium.<sup>53</sup> Inflammatory events and elevated levels of cytokines upregulate the expression of sICAM-1.<sup>54</sup> Further in vitro studies have shown a direct correlation between levels of shedded sICAM-1 and cell surface ICAM-1<sup>55</sup> suggesting that increased levels of sICAM-1 in the CSF is indicative of up-regulation of surface-bound ICAM-1 in the brain. Interestingly, KYNA is also produced in endothelial cells<sup>56</sup> and elicits firm arrest of immune cells on the endothelium, an event mediated by ICAM-1.<sup>57</sup> Hence, although the positive correlation between KYNA and sICAM-1 does not prove a direct causality between increased KYNA levels and increased sICAM-1, it is tempting to speculate that the correlation between KYNA levels and sICAM-1 levels mirrors this intimate relationship.

## Conclusion

Our study showed no significant alterations in CSF KYNA levels in either AD or DLB patients compared to those of Ctrl. Further, no correlation between KYNA levels and cognitive decline in these patients was observed. However, the number of individuals included in this study was relatively small, and further studies on larger patient cohorts are required to understand the potential role of KYNA in AD and DLB. The inconsistency of KYNA alterations in AD may also be because of the heterogeneity of the disease. Furthermore, our correlation analysis shows that KYNA may be implicated in AD-related hyperphosphorylation of tau and infiltration of immune cells.

## Author Contributions

Conceived and designed the experiments: MW, SE. Analyzed the data: MW, HN, FO. Wrote the first draft of the manuscript: MW. Contributed to the writing of the manuscript: HN, SE. Agree with manuscript results and conclusions: LM, EL. Jointly developed the structure and arguments for the paper: MW, SE. Made critical revisions and approved final version: HN, EL. All authors reviewed and approved of the final manuscript.

## DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their

compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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