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RESEARCH ARTICLE

Higher concentrations of kynurenic acid in CSF are associated with the slower clinical progression of Alzheimer's disease

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Abstract

INTRODUCTION: The kynurenine pathway's (KP) malfunction is closely related to Alzheimer's disease (AD), for antagonistic kynurenic acid (KA) and agonistic quinolinic acid act on the *N*-methyl-D-aspartate receptor, a possible therapeutic target in treating AD.

METHODS: In our longitudinal case–control study, KP metabolites in the cerebrospinal fluid were analyzed in 311 patients with AD and 105 cognitively unimpaired controls.

RESULTS: Patients with AD exhibited higher concentrations of KA ($\beta = 0.18$, $P < 0.01$) and picolinic acid ($\beta = 0.20$, $P < 0.01$) than the controls. KA was positively associated with tau pathology ($\beta = 0.29$, $P < 0.01$), and a higher concentration of KA was associated with the slower progression of dementia.

DISCUSSION: The higher concentrations of neuroprotective metabolites KA and picolinic acid suggest that the activation of the KP's neuroprotective branch is an adaptive response in AD and may be a promising target for intervention and treatment.

KEYWORDS
Alzheimer's disease, biomarkers, cerebrospinal fluid, kynurenic acid, kynurenine pathway, longitudinal case control study, quinolinic acid, tryptophan

Highlights

- Patients with Alzheimer's disease (AD) exhibited higher concentrations of kynurenic acid and picolinic acid than controls.
- Higher concentrations of kynurenic acid were associated with slower progression of AD.
- Potential neurotoxic kynurenines were not increased among patients with AD.
- Activation of the kynurenine pathway's neuroprotective branch may be an adaptive response in AD.

1 | BACKGROUND

With a fundamental role in energy metabolism, cell signaling, neuronal protection, and brain health, the kynurenine pathway (KP) is the primary metabolic pathway that degrades L-tryptophan (Trp) into several intermediary metabolites, all collectively termed kynurenines.[1] The KP is expressed in multiple organ systems, especially in the liver, immune system, and brain, where the metabolites produced prompt a host of immunoregulatory and neuronal signaling events.[2] The KP is dysregulated in several neurodegenerative diseases, including Alzheimer's disease (AD) and Huntington's disease, and was recently linked to delirium.[3] As such, a derailed KP can effect a wide range of cellular malfunctions and pathologies, including nicotinamide adenine dinucleotide (NAD+) reduction, oxidative stress, neuroinflammation, tau phosphorylation, and amyloid aggregation.[2]

The essential amino acid Trp plays an important role in synthesizing proteins, serotonin, and melatonin. Approximately 90% of Trp's degradation occurs in the liver,[4] where Trp is a precursor of numerous biologically active metabolites.[2,4] Whereas the chief neuroprotective metabolites include kynurenic acid (KA) and picolinic acid (Pic), those considered to be neurotoxic include 3-hydroxykynurenine (HK), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), and, in higher concentrations, quinolinic acid (QA).[5] Kynurenine (Kyn) is a pivotal metabolite of the KP in the brain, where up to 70% of it is of systemic origin.[6] Whereas Trp, HK, and AA also pass through the blood–brain barrier,[2] KA and QA are presumed not to.[7,8] Because the rate-limiting enzymes converting Trp to Kyn show low expression in the brain, the pathway is especially dependent on Kyn's availability.[7]

The neuroactive abilities of metabolites in the KP differ considerably. Two of the most important neuroactive metabolites are the neuroprotective KA and QA, the latter of which is potentially neurotoxic in high concentrations (>100–300 nM).[9] KA and QA have opposing effects on the *N*-methyl-D-aspartate receptor (NMDAR), which plays an important role in regulating synaptic plasticity.[7,10] Further, KA influences the immune response through agonistic effects on receptors as the G protein-coupled receptor 35 (GPR35) and the aryl hydrocarbon receptor (AHR) and is an antagonist of the α (7) nicotinic acetylcholine receptor (α 7 nAChR) of the cholinergic system also known to be dysregulated in AD.[11] With key physiological functions, QA also ranks among known precursors of endogenous NAD+ synthesis.[12] In mammals, the KP is the most important pathway for NAD+,[1] a vital metabolite for cell health and survival. NAD+ abundance declines in normal aging,[12,13] particularly in patients with dementia and some consider it to be a promising pathway for intervention.[1]

As the KP changes with age, the concentrations of Kyn, KA, and QA increase, whereas that of Trp drops.[14,15] However, most KP studies have been performed in blood and far fewer in cerebrospinal fluid (CSF), while most studies on KP metabolites in neurodegenerative disorders have been small and their results mixed regarding concentrations of Trp,[15–17] KA,[18–20] and QA.[19,21–23] However, some KP metabolites have been associated with AD's progression, especially due to interacting with the phosphorylation of tau and the formation of amyloid plaques.[2] QA's role as a precursor of NAD+ and its neurotoxic ability enable it to not only exert direct neurotoxic effects in higher concentrations but also increase the phosphorylation of tau in neurons.[24] QA also has an agonistic effect on the NMDAR, which seems to be important for the production of amyloid beta (Aβ) and direct neurotoxic effects given higher glutamate concentrations in the extracellular space.[5,24] However, owing to diverse results found in clinical populations, the role of the KP in patients with symptomatic AD needs to be further explored.

Against that background, the aim of our study was to explore changes in concentrations of KP metabolites in CSF among patients with AD by comparing patients with symptomatic AD and cognitively unimpaired (CU) controls. To determine the significance of such metabolites in AD, we also sought to ascertain the association between the metabolites' concentration and AD's clinical progression.

2 | METHODS

2.1 | Study design

Our longitudinal study included 311 patients from two memory clinics in Norway—one at Oslo University Hospital in Oslo (n = 178), the other at St. Olavs Hospital,

Trondheim University Hospital in Trondheim (n = 133)—and 105 CU controls included from Oslo University Hospital and Diakonhjemmet Hospital in Oslo. The patients and the CU controls were followed for up to 5 years.

2.2 | Patients with AD

From 2010 to 2018, a total of 311 patients were recruited from the Norwegian Registry of Persons Assessed for Cognitive Symptoms (the NorCog registry).[25] After referral, each patient received a comprehensive assessment based on a standardized research protocol that consisted of an interview with them and their caregiver(s), cognitive testing, a physical examination, and CSF and blood sampling and imaging with computed tomography, magnetic resonance imaging, or fluorodeoxyglucose positron emission tomography. No patient was found to be using symptomatic anti-dementia drugs at the time of inclusion, but were offered these drugs during follow-up.

According to the National Institute on Aging and the Alzheimer's Association's criteria,[26,27] all patients had probable or possible AD or AD mixed with cerebrovascular disease; 59 patients had mild cognitive impairment (AD-MCI), whereas 252 had mostly mild AD dementia. Patients with other mixed pathologies were excluded from the study. In patients with at least one clinical follow-up after initial diagnosis (n = 252), AD's progression was analyzed using the Clinical Dementia Rating (CDR) scale. The level of cognitive and functional impairment was evaluated post hoc by certified CDR raters based on all information available from the patients' clinical records. The categories of memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care were scored on a 4-point scale ranging from 0 to 3, with higher values indicating more severe impairment. The scores for all categories were totaled to yield the CDR Sum of Boxes (CDR-SB), ranging from 0 to 18.[28]

Core biomarkers of AD in CSF—namely, CSF Aβ42, phosphorylated tau (p-tau181), and total tau (t-tau)—were analyzed at Akershus University Hospital in Nordbyhagen, Norway, using enzyme-linked immunosorbent assays (Innotest ELISA hTau Ag, phoshoTau [181P], and β-amyloid 1-42 Fujirebio Europe, Ghent, Belgium). According to the specific cutoff points provided by the laboratory, the results were considered to be abnormal as follows: Aβ42 < 700 pg/mL and p-tau181 > 80 pg/mL for all patients and t-tau > 300 pg/mL for patients < 50 years old, t-tau > 450 pg/mL for patients 50 to 70 years old, and t-tau > 500 pg/mL for ones > 70 years old (Table 1).

2.3 | CU controls

Meanwhile, 105 CU controls were recruited for the study when assessed at the hospital for elective surgery involving spinal anesthesia for gynecological, orthopedic, or urological conditions. Their CSF was collected at the onset of spinal anesthesia but before anesthetic agents were administered, and all were cognitively tested before surgery using the same test battery used with the patients with AD, followed by repeated cognitive testing annually for up to 5 years. Core biomarkers of AD in CSF were analyzed at Sahlgrenska University Hospital in Mölndal, Sweden, using Innotest ELISAs also used with patients with AD but with laboratory-specific pathological cutoff values: Aβ42 < 530 pg/mL, p-tau181 > 60 pg/mL, and t-tau > 350 pg/mL. Details about the cohort have previously been published.[29]

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors began with a review of literature found on PubMed; all relevant publications are cited in the article. The kynurenine pathway's (KP) importance in Alzheimer's disease (AD) is becoming evident given the counter-effects of metabolites found on the *N*-methyl-D-aspartate receptor. However, most studies on the topic, with diverse results in clinical populations, have been small and performed in blood.
2. **Interpretation**: In our longitudinal case–control study, kynurenic acid (KA) in cerebrospinal fluid, a part of the KP's neuroprotective branch, was found to be activated and associated with the slower progression of AD. Those findings suggest an adaptive response.
3. **Future Directions**: KA may warrant attention as a potential biomarker of AD's properties and progression. Although KA may be a promising therapeutic target in treating AD, our results should be replicated in other cohorts of patients.

2.4 | KP metabolites

CSF samples were collected in sterile cryotubes, centrifuged for 10 minutes at 2000 × g, allocated into 0.5 mL cryotubes, and immediately frozen at −20°C. Within a week, the samples were transported to the biobank for long-term cryopreservation at −80°C. After one freeze-and-thaw cycle, liquid chromatography–tandem mass spectrometry was performed to measure kynurenine metabolites at Bevital A/S in Bergen, Norway. Using targeted metabolomics, we analyzed nine KP metabolites: Trp, Kyn, KA, AA, HK, xanthurenic acid (XA), HAA, Pic, and QA.

2.5 | Statistical analyses

We used the Mann–Whitney U test for descriptive analyses, Pearson's χ^2 test for the categorical variables, and Spearman's ρ for correlation analyses. The KP metabolites were not normally distributed but approximated a log-normal distribution when log-transformed prior to parametric analyses to keep in line with the assumption of normally distributed errors in multivariate analyses. Due to multiple comparisons, two-sided P values less than .01 were considered statistically

TABLE 1 Characteristics of the whole cohort.

Patient characteristics	CU controls $N = 105$	AD patients $N = 311$	P values	AD-MCI $N = 59$	AD dementia $N = 252$	P values
Age (years)	71 (67; 76)	71 (67; 75)	0.19	71.0 (68; 75)	71.0 (66; 75)	0.52
Women, *N* (%)	47 (44.8)	180 (57.7)	0.02	31 (52.5)	149 (59.1)	0.36
APOE ε4 positive* *N* (%)	37 (37)	209 (74.9)	<**0.001**	44 (77.2)	164 (74.2)	0.64
CSF AD core biomarkers						
$A\beta_{42}$ (pg/mL)	731.0 (533; 858)	550.0 (465; 660)	†	580.0 (503; 682)	544.5 (458.5; 656.5)	0.03
Phosphorylated tau_{181} (pg/mL)	57.0 (43; 69.5)	88.0 (61; 112)	†	77.0 (55; 106)	91.0 (64; 91)	0.02
Total tau (pg/mL)	335.3 (258;430.2)	660.0 (441; 920)	†	560.0 (342; 772)	672.0 (459.8; 930)	**.006**
Pathological CSF AD core biomarkers						
$A\beta_{42,}$ *N* (%)	26 (24.8)	254 (81.7)	<**0.001**	46 (78.0)	208 (82.5)	0.41
Phosphorylated $tau_{181,}$ *N* (%)	41 (39.0)	182 (58.5)	<**0.001**	26 (44.1)	156 (61.9)	0.01
Total tau, *N* (%)	47 (44.8)	218 (70.1)	<**0.001**	36 (61.0)	182 (72.2)	0.09
CSF kynurenine metabolites						
Tryptophan (μM)	2.8 (2.4; 3.3)	2.58 (2.3; 3.0)	**.001**	2.6 (2.2; 3.0)	2.6 (2.3; 3.0)	0.5
Kynurenine (nM)	53.8 (47.1; 69.7)	52.0 (43.3; 64.9)	0.14	52.2 (41.0; 66.4)	52.0 (43.8; 64.4)	1.0
Kynurenic acid (nM)	2.8 (2.0; 4.1)	3.5 (2.6; 4.9)	<**0.001**	4.0 (2.6; 5.5)	3.6 (2.5; 4.8)	0.56
Anthranilic acid (nM)	10.3 (7.5; 14.0)	8.2 (6.5; 11.3)	<**0.001**	8.2 (6.5; 11.3)	8.3 (6.4; 8.3)	0.88
3-Hydroxykynurenine (nM)	4.6 (3.5; 6.4)	4.4 (3.5; 6.5)	0.65	4.8 (3.7; 6.3)	4.4 (3.5; 6.6)	0.32
Picolinic acid (nM)	20.7 (16.0; 24.4)	23.2 (18.3; 31.0)	**.002**	23.8 (18.1; 32.7)	23.0 (18.3; 30.8)	0.71
Quinolinic acid (nM)	36.9 (27.8; 50.1)	33.2 (24.0; 44.25)	**.001**	36.2 (26.7; 44.5)	32.7 (23.7; 44.3)	0.16
Kyn/Trp ratio (KTR)	19.6 (16.9; 24;5)	20.6 (16.9; 24.1)	0.56	21.6 (17.7; 24.2)	20.5 (16.3; 23.9)	0.32
KA/QA ratio	0.08 (0.05; 0.1)	0.11 (0.08; 0.15)	<**0.001**	0.11(0.07; 0.15)	0.11 (0.08; 0.15)	0.24
Detectable kynurenine metabolites						
3-Hydroxyanthranilic acid *N* (%)	17 (16.2)	187 (60.1)	<**0.001**	38 (64.4)	149 (59.1)	0.29
Xanthurenic acid, *N* (%)	0 (0)	0 (0)		0 (0)	0 (0)	

Notes: Data are presented as *N* (%) using χ^2 test and median (Q1; Q3) using Mann–Whitney *U* test, significant differences $P < 0.01$ in **bold**. % = valid percent without missing, * = missing genotype data in $n = 38$ patients, † = comparison not possible due to inter-laboratory variability.
Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; AD, Alzheimer's disease; CSF, cerebrospinal fluid; CU, cognitively unimpaired; KA, kynurenic acid; Kyn, kynurenine; MCI, mild cognitive impairment; p-tau, phosphorylated tau; QA, quinolinic acid; Trp, tryptophan.

significant. Network graph visualizations were created with the qgraph package in R 4.1.1. (R Foundation for Statistical Computing).[30]

Concentrations of XA and HAA in CSF were less than the limit of detection in 100% and 51% of the samples (i.e., 84% among CU controls and 40% among patients with AD), respectively, and were excluded from subsequent statistical analyses. The other metabolites were detectable in all samples.

Because core biomarkers of AD in CSF were analyzed at two laboratories, the results were dichotomized (i.e., normal or pathological) in analyses including both patients and controls.

There was a strong correlation between t-tau and p-tau181 in CSF (patients: r = 0.89, $P < 0.001$; CU controls: r = 0.93, $P < 0.001$). Accordingly, t-tau was excluded from subsequent analyses. Regression analyses of the whole sample, of patients, and of controls were performed with Trp, Kyn, KA, AA, HK, Pic, or QA as the dependent variables. For independent variables, age, sex, $A\beta_{42}$, and p-tau181 were included in all regression analyses, along with the apolipoprotein E (*APOE*) ε4 genotype when analyzing the full sample.

To estimate the association of baseline metabolites with clinical progression, we applied eight linear mixed-effects models to test the interaction of each KP metabolite in CSF and the KA-to-QA ratio × Time on the dependent variable of CDR-SB scores. The fixed effects included each KP metabolite in CSF or the KA-to-QA ratio × Time + $A\beta_{42}$ in CSF × Time + p-tau181 in CSF × Time + KP metabolite in CSF + $A\beta_{42}$ in CSF + p-tau181 in CSF + age + sex. Random effects included time and intercept, the former of which was the follow-up duration of CDR scores in years (M = 2.3 ± 1.6 years). The interactions of $A\beta_{42}$ in CSF × Time and p-tau181 in CSF × Time were included to explore the association between the progression rate and each metabolite in CSF relative to the effect of AD pathology over time. To visualize the results of the linear mixed-effects models, we estimated plots of predictive margins using margins and marginsplot.

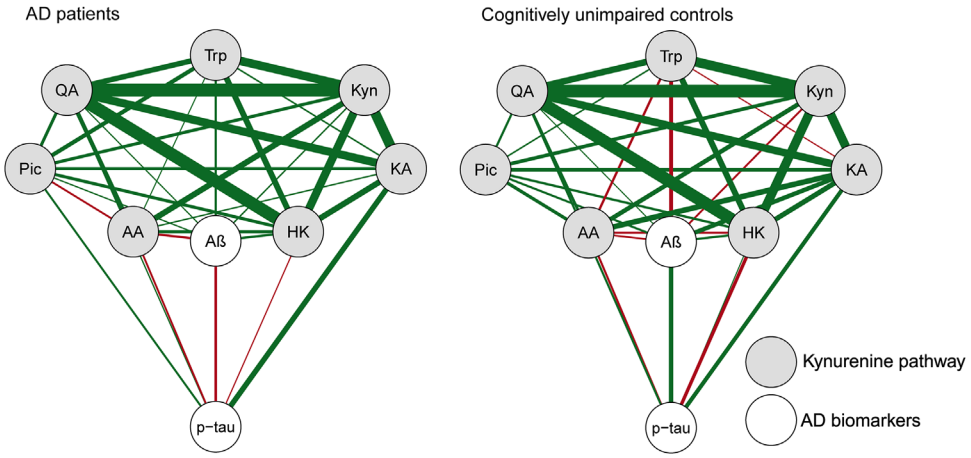

FIGURE 1 Spearman's correlation matrix for the CSF kynurenine pathway metabolites and AD biomarkers in the AD patients and the cognitively unimpaired controls. Thicker lines signify stronger relationships. Green lines represent positive correlations, red lines represent negative correlations. AA, anthranilic acid; Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; HK, 3-hydroxykunurenine; KA, kynurenic acid; Kyn, kynurenine; Pic, picolinic acid; p-tau, phosphorylated tau; QA, quinolinic acid; Trp, tryptophan.

3 | RESULTS

3.1 | Characteristics of the sample

Of the study's 416 participants, 311 were patients with AD, whereas 105 were CU controls (Table 1). The median age in both groups was 71 years. There were more women among the patients (n = 180, 57.7%) than among the controls (n = 47, 44.8%; P = .02); however, no differences in age emerged between men and women in either group (both P > .10).

3.2 | KP metabolite concentrations in CSF

Concentrations of Trp, AA, and QA were significantly lower among patients with AD than the CU controls, whereas KA and Pic concentrations were higher (Table 1). Although no difference in the Kyn-to-Trp ratio emerged between the groups, the KA-to-QA ratio was higher in the patients with AD. Among them, however, no significant differences in concentrations surfaced between patients with AD-MCI and ones with AD dementia, so the two subgroups were treated as one in regression analyses.

Of the entire sample, 402 (96.6%) participants had concentrations of QA < 100 nM, 14 (3.4%) had concentrations between 100 and 300 nM (i.e., six patients and eight controls), and none had concentrations > 300 nM.

3.3 | Bivariate correlations in CSF

As for the KP metabolites in CSF, the same correlation patterns were observed among patients with AD and the CU controls (Figure 1). In both groups, the precursor Kyn was strongly correlated with QA (patients: ρ = 0.67; controls: ρ = 0.7) and moderately correlated with KA (patients: ρ = 0.41; controls: ρ = 0.39), as detailed in Table S1 in supporting information. Among all participants, age was moderately correlated with QA (ρ = 0.45); weakly correlated with Kyn (ρ = 0.27), KA (ρ = 0.22), HK (ρ = 0.21), and AA (ρ = 0.15); but not correlated with Trp, Pic, Aβ_{42}, or p-tau181 (Table S2 in supporting information).

3.4 | Determinants of kynurenines according to multiple regression analyses

After adjusting for age, sex, *APOE* ε4 genotype, Aβ_{42}, and p-tau181 in the multiple regression analyses for the entire sample, the associations of AD diagnosis with higher concentrations of KA and Pic and lower concentrations of AA remained significant (Table 2A). Also among the patients, p-tau181 was positively associated with KA but not with any other KP metabolites, whereas Aβ_{42} was not associated with any of the metabolites (Table 2B).

Age was positively associated with Kyn, KA, AA, HK, and QA among patients with AD (Table 2B) but with Trp, Kyn, KA, and QA among the CU controls (Table 2C). Last, among the patients, maleness was associated with higher concentrations of Trp, Kyn, Pic, and QA (Table 2B).

3.5 | Kynurenine metabolites and the clinical progression of AD

Higher concentrations of KA at baseline were significantly associated with the slower progression of CDR-SB with coefficient = −0.12, P = 0.009), as shown in Figure 2 and Table 3. The same trend was observed for Kyn and QA but did not reach significance (Table 3).

TABLE 2 Determinants of kynurenines by adjusted multiple regression analyses.

A. Whole cohort	Trp	Kyn	KA	AA	HK	Pic	QA
Diagnoses	−0.12	−0.02	**0.18****	**−0.25****	−0.01	**0.20****	−0.11*
Age	0.11*	**0.30****	**0.26****	**0.2****	**0.17****	0.003	**0.45****
Sex	**0.18****	**0.14****	0.03	−0.1*	0.09	**0.23****	**0.18****
APOE ε4 genotype	0.006	−0.08	−0.01	0.04	0.04	−0.01	0.01
$A\beta_{42}$	−0.03	−0.01	−0.08	0.08	0.002	−0.06	−0.03
P-tau_{181} dichotomized	−0.006	−0.02	**0.22****	−0.03	0.004	0.08	0.01
Adjusted R^2	0.06	0.12	0.13	0.08	0.02	0.07	0.27
B. Alzheimer's disease patients							
Age	0.04	**0.22****	**0.22****	**0.16****	**0.19****	−0.01	**0.42****
Sex	**0.16****	**0.16****	0.05	−0.12*	0.07	**0.26****	**0.22****
$A\beta_{42}$	0.1	0.02	0.04	−0.12*	0.02	−0.02	0.04
P-tau_{181} continuous	0.07	−0.01	**0.29****	−0.11	−0.01	0.04	0.03
Adjusted R^2	0.03	0.07	0.12	0.05	0.03	0.06	0.23
C. Cognitively unimpaired controls							
Age	**0.36****	**0.46****	**0.33****	0.18	0.11	0.05	**0.49****
Sex	0.19*	0.11	0.05	−0.09	0.15	0.12	0.14
$A\beta_{42}$	0.05	−0.02	0.19*	0.002	0.03	0.1	0.004
P-tau_{181} continuous	−0.24*	−0.17	0.08	−0.11	−0.02	−0.02	−0.003
Adjusted R^2	0.16	0.19	0.14	0.003	−0.001	−0.01	0.24

Notes: Diagnoses, CU controls = 0, AD = 1: Sex, women = 0, men = 1.
APOE ε4 genotype, neg = 0, pos = 1; Whole cohort (A): $A\beta_{42}$ and p-tau_{181} dichotomized, (normal = 0, pathological = 1) according to laboratory-specific cutoff values; Alzheimer's disease patients (B) and cognitively unimpaired controls (C): $A\beta_{42}$ and P-$tau_{181,}$ as continuous variables. The adjusted *R*-squared measures how well a model explains the data, accounting for the number of predictors. Unlike the regular *R*-squared, it only increases with significant predictor additions, avoiding the issue of overfitting.
Abbreviations: AA, anthranilic acid; Aβ, amyloid beta; *APOE*, apolipoprotein E; CU, cognitively unimpaired; HK, 3-hydroxykunurenine; KA, kynurenic acid; Kyn, kynurenine; Pic, picolinic acid; p-tau, phosphorylated tau; QA, quinolinic acid; Trp, tryptophan.
*$P < 0.05$, **$P < 0.01$, significant differences in **bold**, standardized β-coefficients are presented.

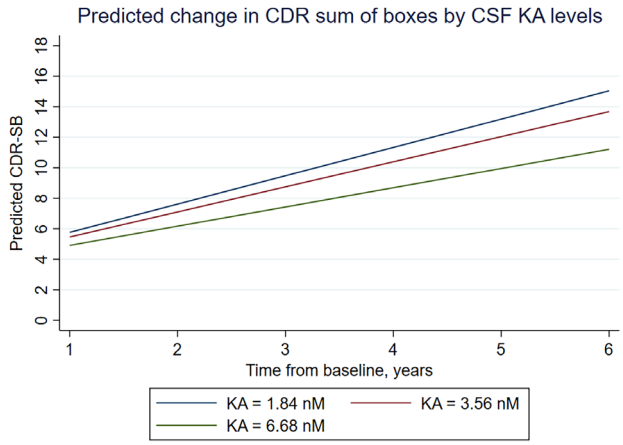

FIGURE 2 Marginsplot of predicted change in CDR-SB by CSF concentrations of KA. Predicted change of the CDR-SB scores of a patient with CSF KA = 1.84 nM (equals the 10th percentile, blue line), KA = 3.56 nM (equals the 50th percentile, red line), and KA = 6.68 nM (equals the 90th percentile, green line). Patients' numbers are 28, 123, 126, and 34 for KA < 1.84, = 1.84–3.55, = 1.85–6.67, and > 6.68 nM, respectively. CSF, cerebrospinal fluid; CDR-SB; Clinical Dementia Rating Scale Sum of Boxes; KA, kynurenic acid; nM, nanomolar.

4 | DISCUSSION

We investigated alterations in the KP among both patients with symptomatic AD and CU controls. Although we expected an increased concentration of the neurotoxic metabolite QA driven by microglial activation, concentrations of the neuroprotective metabolites KA and Pic were higher among the patients than the controls. Underscoring the significance of those changes in the KP, higher concentrations of KA were associated with the slower progression of AD.

Past studies investigating concentrations of KA among patients with AD compared to controls have shown increased concentrations in the CSF but lower concentrations of KA in plasma.[22,23,31] Meanwhile, other research has shown reduced concentrations of KA in CSF. Unlike QA, KA in CSF is seldom reported to be increased in neurodegenerative diseases other than AD.[8] KA is primarily produced locally in astrocytes activated in response to neuroinflammation as a compensatory process to neutralize neurotoxicity.[7] KA executes its effect among other mechanisms by antagonistically affecting the NMDAR to prevent further damage[32] although KA concentrations required to antagonize the NMDAR in experimental models are higher than observed in this study.[11] However, KA's effects may be bidirectional, given animal

TABLE 3 Results of the eight linear mixed-effects regression models assessing the association between each CSF KP metabolite and the rate of change in CDR-SB.

	Coefficient	95% CI	*P*		Coefficient	95% CI	*P*
TRP	−0.13	−0.66, 0.40	0.64	**Trp x time**	−0.13	−0.49, 0.22	0.46
KYN	−0.00	−0.02, 0.01	0.74	**Kyn x time**	−0.01	−0.02, 0.00	0.05
KA	−0.05	−0.19, 0.09	0.45	**KA x time**	−0.12	−0.22, −0.03	**0.009****
HK	0.05	−0.02, 0.11	0.16	**HK x time**	−0.03	−0.07, 0.01	0.19
PIC	−0.02	−0.04, 0.01	0.19	**Pic x time**	0.00	−0.01, 0.02	0.75
AA	0.04	−0.02, 0.10	0.16	**AA x time**	0.01	−0.04, 0.05	0.27
QA	−0.01	−0.02, 0.01	0.50	**QA x time**	−0.01	−0.02, 0.00	0.06
KA/QA	3.60	−0.89, 8.00	0.12	**KA/QAx time**	−1.77	−4.76, 1.21	0.24

Notes: Linear mixed effects regression models. Each line represents one model. In every model fixed effects includes CSF KP metabolite × time, CSF $A\beta_{42}$ × time, CSF p-tau_{181} × time, CSF KP metabolite, CSF Aβ42, CSF p-tau_{181}+, age, and sex. Random effects include time and intercept. $^{**}P < 0.01$.
Abbreviations: AA, anthranilic acid; Aβ, amyloid beta; CDR-SB, Clinical Dementia Rating Sum of Boxes; CI, confidence interval; CSF, cerebrospinal fluid; HK, 3-hydroxykunurenine; KA, kynurenic acid; KP, kynurenine pathway; Kyn, kynurenine; Pic, picolinic acid; p-tau, phosphorylated tau; QA, quinolinic acid; Trp, tryptophan.

models showing that high levels of KA can weaken cognition.[7,32] It has been speculated if KA's antagonistic effect on the α7 nAChR contribute, as α7 nAChR agonists have shown positive effects on cognitive function and memory in rats.[33]

Along with KA, concentrations of Pic, also assumed to be neuroprotective, were higher among the patients with AD. Pic is known to mildly inhibit QA's neurotoxic effects by chelating zinc.[34] Because reduced Pic is associated with the loss of neurons and astrocytes in patients with AD,[34] we speculate that Pic may exert a compensatory effect similar to KA's. Furthermore, concentrations of Pic likely reflect the increased expression of amino-ß-carboxymuconate-semialdehyde-decarboxylase (ACMSD), a KP enzyme correlated with the synthesis of NAD+ from Trp,[35] one with multiple beneficial effects linked to mitochondrial function by way of NAD+.[36]

The concentration of AA was lower among the patients with AD than the CU controls. AA is an intermediate metabolite modulated by KA, one whose role in the brain remains unclear.[4] Although the concentration of QA in CSF among patients with AD was lower than among the CU controls, its association with AD was not significant after adjustment. By comparison, Jacobs et al. detected no differences in QA in CSF or plasma between patients with AD and age-matched controls,[21] whereas van der Velpen et al. found increased QA in both CSF and plasma among patients with AD.[19] Both studies were quite small, and, in the latter, the controls were younger than the patients, which might have influenced the results given that concentrations of QA increase with age,[15] as our results confirm. QA may both increase the phosphorylation of tau and have direct neurotoxic effects by serving as an NMDAR agonist.[24] However, the concentrations of QA in our study, both among patients with AD (median = 33.2 nM) and the CU controls (median = 36.9 nM), were well below concentrations considered to be neurotoxic (> above 100–300 nM).[9,24] Only 3.4% of all participants had concentrations between 100 and 300 nM. Astrocytes clear QA from the synaptic space and direct QA toward the production of NAD+,[32] which has several functions in all cells to optimize a huge number of cellular pathways, including mitochondrial homeostasis. As an important coenzyme in redox reactions and involved in energy metabolism, DNA repair, and inflammation, NAD+ is reduced in normal aging, possibly due to reduced production and increased metabolism.[1] In dysfunctional mitochondria, the depletion of NAD+ increases the amount of NAD + hydrogen, which consequently forces cells into senescence.[37] Because that process is accentuated in neurodegenerative brain disorders, NAD+ plays a critical role in their development.[1] Lower concentrations of QA among patients with AD in our study, well below the threshold associated with neurotoxicity, may reflect the weakened synthesis of NAD+.

Among other results, concentrations of Trp in CSF were lower among the patients with AD than the CU controls, though the association was not significant after adjustment. Those results align with the findings of Fathi et al.'s review and meta-analysis, in which the cohorts of six CSF-focused studies showed no differences in such concentrations of Trp between patients with AD and controls.[31] That outcome sharply contrasts typical findings of reduced Trp in the blood of patients with AD, as summarized in the same meta-analysis.[31] As for the cause of reduced circulating Trp in AD, malnutrition with reduced protein intake has been proposed, along with the inflammation-induced catabolism of Trp along the KP.[38]

The concentrations of KP metabolites did not differ between patients with AD-MCI and AD dementia, meaning that the stage of AD did not influence the baseline results among symptomatic patients. One explanation may be that most patients with dementia had mild dementia. Although including patients with more severe dementia may have yielded different results, our findings align with those of another small study examining patients with MCI and mild or moderate to severe AD. Such consistency may indicate that alterations in the KP are already established when AD becomes symptomatic.

As others have found,[20,21] we did not detect any association between concentrations of $A\beta_{42}$ and the KP metabolites in any group. A smaller study with 40 patients with AD revealed a positive correlation between KA and $A\beta_{42}$ in CSF—that is, patients with highest soluble $A\beta_{42}$ had the highest concentrations of KA.[19] Although that finding could reflect KA's neuroprotective property in the brain in serving as a NMDAR antagonist and antioxidant,[7] those results were not replicated

in our study. Instead, we found that p-tau181 was positively associated with KA only among patients with AD and not with any other KP metabolite. A similar correlation was found in another small study on AD,[39] and some researchers have even reported a strong correlation between p-tau and HK[21] or an association between p-tau and QA after adjusting for age and sex.[19] Most studies have also shown a strong association between tauopathy and the rate of progression in AD[40–42] as we previously found in the same sample of patients with AD.[43] The same clear association was not found for $A\beta_{42}$, however.[40] When adjusting for the known relationship between p-tau and disease progression, we found that higher concentrations of KA were associated with a slower decline in functional and cognitive impairment, as measured by CDR-SB. There was also a trend of slower clinical progression with higher concentrations of Kyn and QA.

As other researchers have also found, we observed that the concentrations of KP metabolites increased with age independently of underlying neurodegenerative disease,[2,15,23] which may be important when considering the usefulness of biomarkers in clinical practice. The concentrations of Kyn and QA especially increase, and both age and AD have previously been shown to modify the KP metabolites in the same direction.[15]

A strength of our study was the large number of clinically well-characterized patients with AD and age-matched CU controls. Both the patients and the controls were assessed with the same comprehensive cognitive test battery, and most patients were followed up for several years. A limitation of our findings is that the core biomarkers of AD for patients were analyzed at one laboratory but at another for the controls, and the results had to be dichotomized in statistical analyses including both cohorts. Concurrent analyses in blood may have strengthened the results.

5 | CONCLUSION

Contrary to our expectations, the potentially neurotoxic kynurenines produced by microglia (i.e., HK,and QA) were not increased among patients with AD. Instead, higher concentrations of neuroprotective metabolites (i.e., KA and Pic) were found. Increased KA production in patients was possibly an adaptive mechanism secondary to neuronal loss engaged to prevent further damage. The potential significance of such protection was underscored by slower clinical progression among patients with high concentrations of KA, an endogenous NMDAR antagonist, in CSF. KA may be of interest as a potential biomarker of both properties of AD and its progression. Based on our data, maintaining the activity of kynurenine aminotransferases and thus the levels of endogenous neuroprotective KA seems to be a promising therapeutic target in treating AD.

AUTHOR CONTRIBUTIONS

Anne-Brita Knapskog: Designed the study, conducted the clinical assessment and diagnoses of the memory clinic patients, performed statistical analyses, interpreted the data, and wrote the manuscript. Mari Aksnes: contributed in planning of the study, performed statistical analyses, contributed to the interpretation of the data, and contributed to revising the manuscript. Trine Holt Edwin: conducted the clinical assessment and diagnoses of the memory clinic patients and rated CDR at follow-up, performed statistical analyses, contributed to the interpretation of the data, and contributed to revising the manuscript. Per Magne Ueland: performed biomarker analyses in CSF at Bevital, interpreted the data, and contributed to revising the manuscript. Arve Ulvik: performed biomarker analyses in CSF at Bevital, interpreted the data, and contributed to revising the manuscript. Evandro Fei Fang: contributed guidance on the NAD+ synthetic pathway, interpreted the data, and contributed to revising the manuscript. Rannveig Sakshaug Eldholm: conducted the clinical assessment and diagnoses of the memory clinic patients, interpreted the data, and contributed to revising the manuscript. Nathalie Bodd Halaas: collected data from the CU controls, interpreted the data, and contributed to revising the manuscript. Ingvild Saltvedt: conducted the clinical assessment and diagnoses of the memory clinic patients, interpreted the data, and contributed to revising the manuscript. Lasse M. Giil: contributed to planning of the study, interpreted the data and contributed to revising the manuscript. Leiv Otto Watne: contributed in designing the study, collected data from the CU controls, interpreted the data, and contributed to revising the manuscript. All authors approved the final manuscript for publication.

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CONFLICT OF INTEREST STATEMENT

A.B.K. has been principal investigator on the Roche drug trial BN29553, on the Boehringer-Ingelheim drug trial 1346.0023, and the Novo Nordisk drug trial NN6535-4730. T.H.E. has been investigator on the clinical trial Roche BN29553; and T.H.E. and I.S. have worked on the Boehringer-Ingelheim clinical trial 1346.0023. E.F.F. has an MTA with LMITO Therapeutics Inc. (South Korea), a CRADA arrangement with ChromaDex (USA), and a commercialization agreement with Molecule AG/VITADAO, and is consultant to Aladdin Healthcare Technologies

(UK and Germany), the Vancouver Dementia Prevention Centre (Canada), Intellectual Labs (Norway), and MindRank AI (China). The other authors declare that they have no competing interests, Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

Due to legal restrictions, imposed by the registry owners and the ethical committee, the data are not publicly available for publicly sharing as the de-identified dataset include sensitive patient information. The clinical data on the memory clinic patients may be requested from the Norwegian Registry of Persons Assessed for Cognitive Symptoms (NorCog) at e-mail: post@aldringoghelse.no. The demographic data for the CU controls and results of the KP analyses are available upon reasonable request to the authors. All data accessibility is dependent on the approval from the REC South East, contact at e-mail: post@helseforskning.etikkom.no

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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