Kynurenines, Neuropsychiatric Symptoms, and Cognitive Prognosis in Patients with Mild Dementia

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ABSTRACT

INTRODUCTION: Circulating tryptophan (Trp) and its downstream metabolites, the kynurenines, are potentially neuroactive. Consequently, they could be associated with neuropsychiatric symptoms and cognitive prognosis in patients with dementia.

OBJECTIVE: The objective of this study was to assess associations between circulating kynurenines, cognitive prognosis, and neuropsychiatric symptoms.

METHODS: We measured baseline serum Trp, neopterin, pyridoxal 5′-phosphate (PLP), and 9 kynurenines in 155 patients with mild dementia (90 with Alzheimer's disease, 65 with Lewy body dementia). The ratios between kynurenine and Trp and kynurenic acid (KA) to kynurenine (KKR) were calculated. The Mini-Mental State Examination (MMSE) and the Neuropsychiatric Inventory (NPI) were administered at baseline and annually over 5 years. Associations between baseline metabolite concentrations with MMSE and the NPI total score were assessed using a generalized structural equation model (mixed-effects multiprocess model), adjusted for age, sex, current smoking, glomerular filtration rate, and PLP. Post hoc associations between KKRs and individual NPI items were assessed using logistic mixed-effects models. False discovery rate (0.05)–adjusted *P* values (*Q* values) are reported.

RESULTS: Kynurenine had a nonlinear quadratic relationship with the intercept of the MMSE scores over 5 years ($Q < 0.05$), but not with the slope of MMSE decline. Kynurenine was associated with a higher NPI total score over time ($Q < 0.001$). Post hoc, both KKR and KA were associated with more hallucinations ($Q < 0.05$).

CONCLUSIONS: Kynurenine has a complex relationship with cognition, where both low and high levels were associated with poor cognitive performance. A higher KKR indicated risk for neuropsychiatric symptoms, especially hallucinations.

KEYWORDS: Kynurenines, hallucinations, neuropsychiatric symptoms, kynurenic acid, kynurenine, Alzheimer's disease, Lewy body dementia

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Introduction

The essential amino acid tryptophan (Trp) is degraded through the kynurenine pathway (Figure 1), giving rise to metabolites referred to as kynurenines.[1] The kynurenine pathway is most highly expressed not only in liver and monocytes[2] but also in muscle, brain, and intestine.[3] The kynurenines and the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) of the kynurenine pathway have been implicated in experimental cognitive dysfunction in mice,[4-7] and kynurenines are lower in Alzheimer's disease (AD) compared with healthy controls.[8]

Tryptophan 2,3-dioxygenase (TDO) and IDO generate kynurenine (Kyn) from Trp,[9] which gives rise to downstream metabolites that have shown neuroprotective (kynurenic acid [KA])[10] and neurotoxic properties (quinolinic acid [QA]).[11] Both KA and QA act as antagonist and agonist, respectively, at the *N*-methyl-D-aspartate receptor (NMDAR), suggesting a potential role of kynurenines in relation to signal transduction pathways related to cognitive dysfunction.[12] The key enzymes IDO and kynurenine 3-monooxygenase (KMO) are induced by pro-inflammatory cytokines. KMO converts Kyn

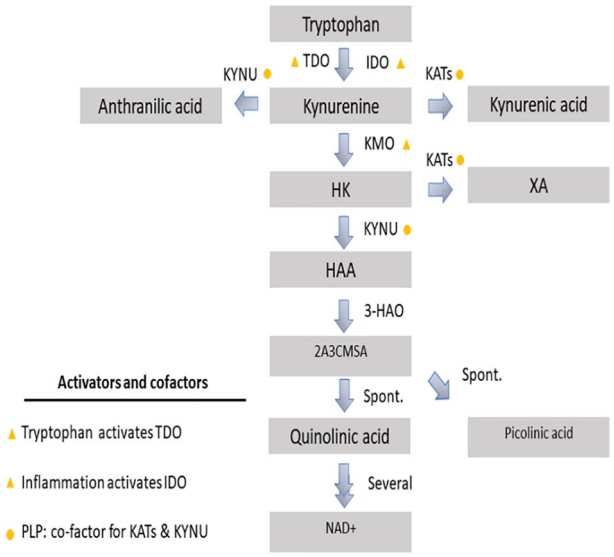

Figure 1. The kynurenine pathway. TDO and IDO convert tryptophan to kynurenine (Kyn). HK (3-hydroxykynurenine) is converted to 3-hydroxyanthranilic acid (HAA) by kynureninase (KYNU), and subsequently to quinolinic acid (QA), catalyzed by quinolinate phosphoribosyl transferase. QA is converted to nicotinamide adenosine dinucleotide (NAD^+), the final product of the pathway. Anthranilic acid (AA) is produced from Kyn by KYNU. Kynurenine aminotransferases (KATs) generate KA from Kyn and xanthurenic acid (XA) from HK. Picolinic acid (PIC) is produced by spontaneous conversion of HAA. Both KYNU and KATs have pyridoxal 5′-phosphate (PLP) as a cofactor.[9] IDO indicates indoleamine 2,3-dioxygenase; TDO, tryptophan 2, 3-dioxygenase; KMO, kynurenine 3-monooxygenase; KATs, kynurenine aminotransferases; 3-HAO, 3-hydroxyanthranilic acid 3, 4-dioxygenase; Spont., spontaneous; NAD^+, nicotine adenine dinucleotide; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid.

to 3-hydroxykynurenine (HK).[13] Interferon gamma (IFN-γ) is the most potent activator.[2] Higher circulating levels of kynurenine metabolites are associated with depression[14] and elevated postmortem brain levels of kynurenines, and relevant enzymes have been observed in patients with psychotic and mood disorders.[15-19] Cerebrospinal fluid (CSF) levels of KA were not significantly altered in patients with dementia with Lewy bodies (DLB) compared with controls.[20] The kynurenine-to-tryptophan ratio (KTR) and neopterin, which are biomarkers of cellular immune activation, have been associated with reduced cognitive performance in community-dwelling older adults.[21]

We aimed to assess whether the levels of circulating kynurenines at baseline predicted cognitive prognosis and neuropsychiatric symptoms over 5 years in patients with AD and Lewy body dementia (LBD).

Methods

Study participants

The Dementia Study of Western Norway (DemVest) is a multicenter longitudinal cohort study with annual follow-up until death. The study recruited 155 participants from specialist clinics of neurology and old-age psychiatry situated in the Norwegian counties Hordaland and Rogaland with available blood samples in a biobank. Participant recruitment during 2005 to 2007 relied on fulfillment of the inclusion criteria: patients diagnosed with mild dementia for the first time and a minimal Mini-Mental State Examination (MMSE) score of 20.[22] Thereafter, selective recruitment of patients with either DLB or Parkinson disease with dementia (PDD) was undertaken. Thus, the latter 2 patient groups are overrepresented in the study. Due to similar pathologies, DLB and PDD were classified together as LBD.

Independently, 2 physicians experienced in the diagnostic workup of dementia made a clinical diagnosis using the NINCDS-ADRDA criteria for AD (National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association)[23] and the revised consensus criteria for DLB (2005).[24] A detailed study protocol has been published.[22] Briefly, a physician interviewed the patient together with a caregiver who provided complementary information. Medical history was also obtained from electronic records and a clinical neurological examination was performed. In addition to a global cognitive assessment of cognition by the MMSE, and dementia severity using Clinical Dementia Rating, patients were assessed with a standardized neuropsychological test battery. In situations with diagnostic uncertainty, physicians discussed each case until consensus. In addition, after 5 years, 3 specialists in geriatrics and psychiatry revised the diagnoses in consensus meetings. All patients were followed longitudinally with annual assessments with MMSE and the Neuropsychiatric Inventory (NPI), mostly until death. Due to the progressive nature of dementia, most patients followed over time will reach a point where they score 0 on the MMSE on each consecutive follow-up. This is called the floor effect. At this point, the MMSE can no longer measure further disease progression, and for a statistical model, it will look as if disease progression has stopped. Furthermore, variance will be reduced at follow-ups with many 0 scores. This will result in the introduction of a range of statistical biases, which are not easily compensated for, especially if a substantial proportion of patients reach a floor or ceiling effect.[25] Therefore, a decision was made to censor the study on biomarkers after the fifth follow-up.

Postmortem studies from the full DemVest study (56 autopsies) found that the concordance rate for a clinical diagnosis compared with a pathological diagnosis was 83% for AD and 80% for LBD.[26]

Some data during follow-up were missing. Most were observed in an intermittent pattern, meaning that the patient missed one appointment and later returned to the study. The proportion of missing measurements that was not due to death was small. The MMSE and NPI were assessed at the same visit and thus had largely corresponding missing measurements. Accordingly, missing measurements for the MMSE are listed. For the MMSE, there were no missing measurements at baseline, 6 missing measurements at the first follow-up, 11 at the second follow-up, 9 at the third follow-up, 6 at the fourth

follow-up, and 8 at the fifth follow-up. During the study period, several patients died prior to their planned follow-up. At the second follow-up, 15 patients had died, 34 at the third, 55 at the fourth, and 78 at the fifth follow-up.

The Mini-Mental State Examination

The MMSE has maximum score of 30 and a minimum of 0 and consists of a variety of questions, grouped into 7 categories representing different cognitive domains. The categories are orientation to time, orientation to place, registration of 3 words, attention and calculation, recall of 3 words, language, and visual construction.[27] A decline of 2 to 4 points is considered a reliable change,[28] and about 3 points is also the expected annual decline.[29]

The Neuropsychiatric Inventory

The NPI evaluates 12 neuropsychiatric symptoms common in dementia: delusions, hallucinations, agitation, apathy, dysphoria, anxiety, irritability, euphoria, disinhibition, motor disturbances, and sleep- and appetite disturbances. A caregiver familiar with the patient rates the severity and frequency of each neuropsychiatric symptom using a standardized questionnaire. A combined score for each symptom is calculated by multiplying the frequency by severity. The total score is determined by adding all the domain scores together.[30] We used the NPI total score to limit the number of outcomes.

Measurement of metabolic biomarkers

Baseline levels of Trp, anthranilic acid (AA), 3-hydroxyanthranilic acid, HK, KA, Kyn, picolinic acid, QA, xanthurenic acid (XA), pyridoxal 5′-phosphate (PLP), and neopterin were measured using liquid chromatography-tandem mass spectrometry in serum samples, collected between 2005 and 2009, and stored at −80°C until analysis in 2018. The ratio between Trp and Kyn (KTR) was defined as Kyn (μM)/Trp (μM)*100 and the kynurenic acid-to-kynurenine ratio (KKR) was estimated. The limit of detection for neopterin and the kynurenines ranged from 0.5 to 7 nmol/L, whereas the limit of detection for Trp was 0.4 μmol/L. Within-day and between-day coefficients of variation were 5.7% to 16.9% and 3.0% to 9.5%, respectively. The biochemical analyses were performed at the laboratory of Bevital AS (Bergen, Norway; http://bevital.no). We did not detect any significant correlations between metabolite levels and storage time using Spearman rank order correlations (data not shown).

Statistics

Univariate differences between AD and LBD were assessed using *t* tests, Pearson χ^2, and Mann-Whitney *U* tests for normal, categorical, and skewed variables, respectively. Metabolite concentrations were transformed to approximate normality using Tukey's ladder of powers.[31] A constant of one was added prior to logarithmic transformation for all metabolites with a minimum concentration below 1 to avoid an uneven spread of the data after logarithmic transformations. Associations between cognitive deterioration and neuropsychiatric symptoms over 5 years and baseline metabolite levels were examined in a multiprocess model or joint model. Of note, although patients underwent 5 annual follow-up examinations, there were occasional delays, and some patients were followed for 6 years. The MMSE test scores were transformed by the square root of errors, $\sqrt{}$(30-MMSE), thereby higher values indicate poorer performance. The MMSE raw scores were right skewed toward higher scores, which is problematic in statistics, as transformations typically work best to obtain normality with left-skewed data. Thus, the reciprocal of MMSE (30 − MMSE) was calculated to obtain a right-skewed distribution of the number of errors committed by patients on the MMSE (an MMSE score of 24 is 30 − 24 = 6 errors). After this, the square root transformation of the MMSE errors resulted in an approximately normal distribution as assessed by quantile-quantile plots and histograms.[32] However, 37 measurements of the transformed MMSE test scores reached a ceiling effect. Thus, right censoring was implemented using a linear mixed-effects Tobit model with random intercepts and slopes.

The NPI total score was best fitted using a negative binomial random intercept model, according to the Bayesian information criterion. Random slopes could not be fitted, likely due to considerable individual deviation from a linear slope. The MMSE and NPI total models were linked by correlated random effects, implemented using a generalized structural equation model framework (Stata 15 package "gsem"). Each metabolite measured at baseline was entered in a separate multiprocess model, with years in study (time), age, age*time interaction, sex, AD vs LBD, AD vs LBD*time interaction, current smoking, glomerular filtration rate, and PLP as independent variables in the MMSE model. The independent variables were the same in the NPI total model, without a nonsignificant age*time interaction. Nonlinearity was checked using orthogonal polynomials of the transformed metabolite levels.

Post hoc, we compared the multiprocess models stratified by diagnosis. We further assessed the association between metabolite concentrations and the presence of individual NPI items (domain score $\geq$1) using a logistic random intercept model with time, age, sex, AD vs LBD, AD vs LBD*time interaction, current smoking, glomerular filtration rate, and PLP as independent variables. Finally, all study findings were adjusted for multiple comparisons, using the tail area–based false discovery rate (FDR) due to dependency, and adjusted *P* values are reported (*Q* values or *Q*). This was done separately for post hoc tests (R package: fdrtool).[33,34] The statistical analyses, besides FDR correction, were conducted in Stata (version 15; StataCorp, College Station, TX, USA).

Table 1. Participant demographics of the Dementia Study of Western Norway and serum metabolite concentrations at baseline.

CLINICAL CHARACTERISTICS	DEMENTIA (N = 155)	AD (N = 90)	LBD (N = 65)	*P* VALUE
Age, y, mean (SD)	75.1 (7.31)	75.1 (7.8)	75.1 (6.3)	.694[a]
Education, y, mean (SD)	9.7 (3.0)	9.7 (3.1)	9.6 (2.8)	.738[a]
Female, %	56.1	67.8	40.0	.001[b]*
Lewy body disease, %	42.3			
Current smokers, %	20.0	23.3	15.4	.222[b]
MMSE, score, mean (SD)	23.7 (2.8)	23.6 (2.5)	23.8 (3.1)	.597[a]
GFR[c], mean (SD)	79.2 (20.4)	79.2 (22.4)	80.7 (25.4)	.459[a]
METABOLITES				
Trp[d,e]	66.2 (22.4)	66.0 (22.8)	66.6 (15.5)	.547[f]
Kyn[d,e]	1.74 (0.67)	1.74 (0.58)	1.74 (0.73)	.582[f]
HK[e,g]	50.0 (33.7)	48.0 (28.8)	54.4 (34.3)	.033[f]*
KA[e,g]	51.1 (24.4)	51.5 (21.7)	50.2 (25.8)	.772[f]
XA[e,g]	12.3 (9.0)	12.5 (8.9)	12.3 (9.9)	.558[f]
AA[e,g]	21.7 (10.7)	20.1 (11.5)	22.5 (8.6)	.539[f]
HAA[e,g]	36.1 (16.5)	35.2 (16.2)	39.2 (16.3)	.360[f]
PIC[e,g]	35.9 (22.4)	33.0 (18.0)	38.1 (26.5)	.143[f]
QA[e,g]	474 (312)	465 (312)	481 (309)	.736[f]
KTR[e]	2.59 (1.06)	2.49 (1.02)	2.69 (0.96)	.244[f]
KKR[e]	7.97 (0.43)	8.00 (0.40)	7.95 (0.41)	.357[f]
Neopt[e,g]	19.7 (14.0)	18.7 (11.3)	20.6 (15.3)	.276[f]
PLP[e,g]	31.6 (33.9)	34.0 (33.7)	29.6 (24.9)	.021[f]*

Abbreviations: AA, anthranilic acid; AD, Alzheimer's disease; GFR, glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; LBD, Lewy body dementia; MMSE, Mini-Mental State Examination; Neopt, neopterin; PIC, picolinic acid; PLP, pyridoxal 5´-phosphate; Trp, tryptophan; QA, quinolinic acid; XA, xanthurenic acid.
[a]Student *t* test.
[b]Pearson χ^2 test.
[c]Metabolite levels in median and interquartile range.
[d]Micromoles per liter.
[e]Milliliters per minute per 1.73 m^2 surface area.
[f]Mann-Whitney *U* test.
[g]Nanomoles per liter.
*$P < .05$.

Ethics

The Regional Committee for Medical and Health Research Ethics approved the study protocol and a notification of change relating to biomarker analyses (REC number: 2010/633). All participants provided signed informed consent at baseline after a detailed explanation of the procedures.

Results

Study participants

The study included 155 patients (56% women) with dementia (90 AD, 65 LBD). The baseline mean MMSE score was 23.7 and mean educational level was 9.7 years. A total of 20% of the patients were current smokers at baseline (Table 1).

Kynurenines and cognitive performance

Kynurenine measured at baseline had a significant nonlinear, quadratic, relationship with the average MMSE score over the 5 follow-up examinations (Table 2, Figure 2), but not with the rate of change. Using orthogonal polynomials, the first polynomial of kynurenine, representing a linear relationship, was not significant (estimate [Est.] −0.023, $Q = 0.840$), whereas the second, representing a nonlinear relationship, was significant (Est. 0.10, $Q = 0.035$).

Table 2. Associations between serum kynurenines and neopterin at baseline and 5-year prognosis in dementia.[a]

COGNITIVE PERFORMANCE (MMSE)					NEUROPSYCHIATRIC SYMPTOMS (NPI TOTAL SCORE)				
	EST.	SE	*P* VALUE	*Q*		EST.	SE	*P* VALUE	*Q*
Trp	0.059	0.044	.185		Trp	0.010	0.055	.852	
Kyn	−0.023	0.052	.656		Kyn	−0.036	0.065	.569	
Kyn2	0.102	0.030	.006*	.046*					
AA	−0.080	0.048	.096		AA	−0.097	0.058	.099	
KA	0.072	0.057	.209		KA	−0.049	0.087	.575	
					KA*T	0.051	0.022	.021*	.080
HK	−0.099	0.060	.099		HK	−0.005	0.077	.950	
XA	0.017	0.050	.728		XA	−0.101	0.077	.190	
					XA*T	0.051	0.021	.017	.075
HAA	−0.004	0.048	.932		HAA	0.027	0.058	.636	
QA	−0.014	0.052	.795		QA	−0.087	0.063	.170	
PIC	0.011	0.045	.808		PIC	0.022	0.057	.701	
Neopt	0.023	0.050	.647		Neopt	−0.086	0.060	.149	
KTR	0.023	0.051	.648		KTR	−0.074	0.064	.247	
KKR	0.092	0.046	.046	.133	KKR	−0.050	0.074	.501	
					KKR*T	0.063	0.021	.003	.045*

Abbreviations: AA, anthranilic acid; Est., estimate; GFR, glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KKR, kynurenic acid-to-kynurenine ratio; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; Kyn2, second degree orthogonal polynomial of Kyn; MMSE, Mini-Mental State Examination; Neopt, neopterin; NPI, Neuropsychiatric Inventory; PIC, picolinic acid; PLP, pyridoxal 5´-phosphate; SE, standard error; Trp, tryptophan; *Q*, *Q* value; QA, quinolinic acid; XA, xanthurenic acid.

[a]Generalized structural equation model linking 2 mixed models by their random effects: Model 1: Tobit mixed-effects model with MMSE as the outcome, measured at baseline and for 5 consecutive years. Model includes random intercepts and slopes. MMSE transformed to $\sqrt{(30-\text{MMSE})}$. Model 2: Negative binomial mixed-effects model with NPI total (sum of items 1 through 10) measured at baseline and for 5 consecutive years. Model includes random intercepts. Link: Random intercepts and slopes of MMSE correlated with random intercepts of NPI total. Covariates: Time, age (also *time for MMSE), sex, Lewy body dementia vs Alzheimer disease (also *time), current smoking, GFR, and PLP as independent variables.

*$P < .05$ or $Q < 0.05$.

Kynurenines and neuropsychiatric symptoms

The kynurenic acid-to-kynurenine ratio was positively associated with the rate of change per year in neuropsychiatric symptoms, specifically with more neuropsychiatric symptoms over time ($Q = 0.045$; see Figure 3). There was a trend for KA and XA to also be positively associated with more neuropsychiatric symptoms over time, but these findings were not significant after adjustment for multiple comparisons (Table 2).

Post hoc analyses

Differences in prognostic associations of kynurenines between AD and LBD. The associations between the kynurenines, cognitive prognosis, and neuropsychiatric symptoms over 5 years did not differ between AD and LBD after corrections for multiple comparisons (no significant interaction by clinical diagnosis [AD versus LBD]; Supplementary Table 1, and Supplementary Figure 1).

Individual neuropsychiatric symptoms. The kynurenic acid-to-kynurenine ratio was significantly associated with an increasing probability of hallucinations over time (odds ratios in Figure 4 indicate increased odds per year), whereas KA was significantly associated with more hallucinations, on average, over 5 years ($Q < 0.001$) with no change over time. There were several other observed trends. Of note, KA, KKR, and XA displayed trends for increasing agitation over time. Kyn, AA, QA, neopterin, and KTR showed trends for association with reduced average test scores on the item for irritability, whereas Trp, Kyn, HK, and neopterin displayed trends for association with higher average probabilities of experiencing apathy (Figure 4).

Discussion

In this study, Kyn had a nonlinear relationship with the participants' average MMSE test performance over 5 years. This relationship suggests that both low and high levels of Kyn are associated with poorer MMSE test performance, as compared

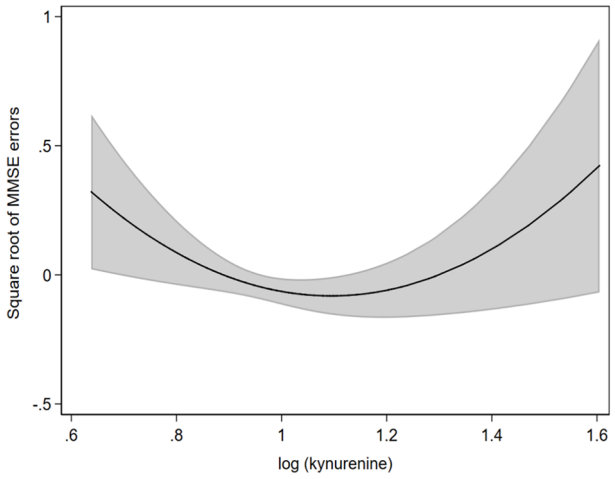

Figure 2. Nonlinear association between MMSE and kynurenine. Low levels of kynurenine are associated with more errors on the MMSE on average (intercept). At mean kynurenine levels, there is no association with MMSE, whereas high or low serum concentrations are associated with more average MMSE errors. The model was estimated as a multiprocess model together with a model for the NPI total score (see statistics). Of note, a constant of 1 was added to kynurenine prior to logarithmic transformation to avoid an uneven spread below and above a kynurenine level of 1, shifting the log (mean) from 0.55 to 1.02. MMSE indicates Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

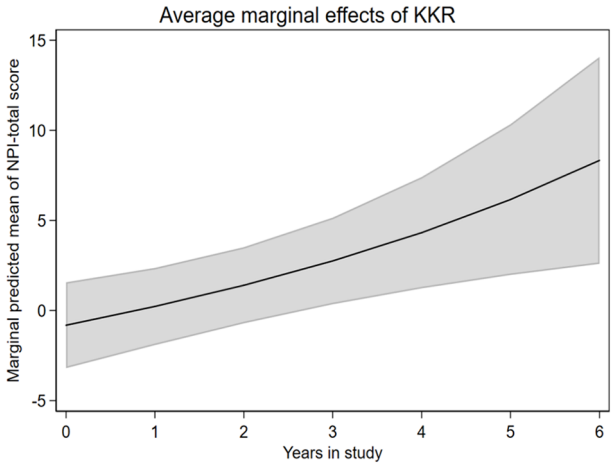

Figure 3. Kynurenic acid-to-kynurenine ratio and neuropsychiatric symptoms. The graph shows how a change in 1 standard deviation of the transformed and standardized levels of KKR, the reciprocal of $1/\sqrt{(KKR)}$ is associated with an increase in neuropsychiatric symptoms over time, using a negative binomial random intercept model, adjusted for age, sex, current smoking, glomerular filtration rate, and PLP in the model for MMSE. KKR indicates kynurenic acid-to-kynurenine ratio; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

with values around the mean. Kynurenine was not associated with the rate of MMSE decline over time. A higher KKR was significantly associated with increasing neuropsychiatric symptoms over time. In post hoc analyses, we found that KKR and KA were significantly associated with more hallucinations. The associations between the kynurenines, cognitive prognosis, and neuropsychiatric symptoms over 5 years did not differ between patients with AD or LBD. However, several trends were observed, which should be investigated in a study with statistical power for subgroup analyses.

Kynurenine showed a nonlinear association with average MMSE score over 5 years (Figure 2), but not with the rate of change. Previously, we observed a similar nonlinear trend between Kyn and cognitive function in a cohort of community-dwelling older adults.[21] This may suggest that a homeostatic level of Kyn around the mean value can be beneficial for cognitive function. One might speculate that the lack of association between kynurenines and the rate of cognitive decline suggests that circulating kynurenines are not related to strong drivers of cognitive deterioration, such as synaptic loss[35] and tau pathology.[36] Availability of precursors of neuroactive kynurenines which are linked to both nicotinamide adenosine dinucleotide (NAD^+) metabolism[9] and low-grade inflammation,[13] could lead to cognitive differences that are stable throughout the disease course. Circulating Kyn, which crosses the blood-brain-barrier (BBB), may affect kynurenines in the brain, as both TDO and IDO converting Trp to Kyn have low activity in the brain.[9] Furthermore, Kyn is induced by pro-inflammatory cytokines, but notably also gives rise to metabolites that suppress inflammation, indicating a complex relationship.[37] There is ample evidence that IDO activation has a negative impact on cognitive function in rodent models[4-7] and can exacerbate AD pathology in amyloid knock-in mice.[5] However, it is less clear how IDO activity outside the brain relates to cognitive function. Peripheral interferon alpha may increase both blood and CSF levels of Kyn.[38] Kynurenine could be a marker of IFN-γ activity, but neopterin and KTR, which are more strongly related to IFN-γ induction,[39,40] were not associated with cognitive function in older humans.[21] Whereas high Kyn levels may signify inflammation,[13] low levels may limit the availability of a key precursor of neuroactive kynurenines and perhaps NAD^+.[9] Deficiency of kynurenines may explain poor outcomes with low Kyn levels, by decreasing levels of NAD^+ leading to neuronal degeneration in dementia. Reduced availability of NAD^+ may impair the activity of the NAD^+-dependent enzymes, such as the sirtuins, resulting in accumulation of amyloid-beta plaques and tau tangles.[41]

A higher KKR was significantly associated with more neuropsychiatric symptoms over time. A similar association was found in post hoc analysis, suggesting that KKR was related to hallucinations with a similar trend for delusions and disinhibition, indicative of psychotic symptoms. Kynurenic acid was significantly associated with hallucinations independent of time in post hoc analysis, with a similar trend for agitation. The NMDAR antagonism, a function of KA, is a known trigger of psychosis,[42] and KA is increased in the brain[19] and CSF of patients with schizophrenia,[43] making this finding intriguing. Increased KA levels, indicating higher kynurenine aminotransferase (KAT) activity, may produce symptoms of

Figure 4. Post hoc: neuropsychiatric symptoms and metabolites. The bubble diagram shows associations between individual neuropsychiatric symptoms over 5 years and metabolites assessed by logistic random intercept models. The KKR was significantly associated with an increasing probability of hallucinations over time, whereas KA was significantly associated with more hallucinations, on average, over 5 years. The analyses were adjusted for using the Benjamini-Hockberg procedure with a false discovery rate of 0.05, and *Q* values, representing adjusted *P* values, were estimated. The bubble sizes are proportional to $-\log^{10}$ *P* values. Odds ratios (ORs) are depicted inside bubbles with thin dark borders representing significant *P* values and thick dark borders representing significant *Q* values. Light blue coloring represents an OR of <1, whereas pink represents an OR >1. Odds ratios are stratified by color transparency as 0% (OR: 0.60-0.69/1.75-2.00), 20% (OR: 0.70-0.79/1.50-1.74), 40% (OR: 0.80-0.89/1.25-1.49), 60% (OR: 0.90-0.99, 1.00-1.24). AA indicates anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KA*T, kynurenic acid interaction with time; KKR, kynurenic acid-to-kynurenine ratio; KKR*T, kynurenic acid-to-kynurenine ratio interaction with time; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; Neopt, neopterin; OR, odds ratio; PIC, picolinic acid; Trp, tryptophan; QA, quinolinic acid; XA, xanthurenic acid; XA*T, xanthurenic acid interaction with time.

schizophrenia in experimental animals.[44] In addition, mice with genomic deletion of the KAT II enzyme show improved cognitive function.[45] Furthermore, KA may lead to decreased levels of the neurotransmitters glutamate,[46] dopamine,[47] and acetylcholine,[48] and KA has been linked to elevated dopaminergic activity in the brain.[49] KKR might reflect the activity of peripheral KATs in the periphery. Notably, KATs also generate XA, which was associated at trend toward more neuropsychiatric symptoms over time, specifically, agitation in post hoc analyses. However, contrary to Kyn, KA does not cross the BBB, but is formed in the brain from Kyn catalyzed by KATs.[50] Accordingly, follow-up studies measuring CSF kynurenines would be highly informative. In addition, KA is an agonist for the aryl hydrocarbon receptor[51,52] and is an antagonist of α7 nicotinic acetylcholine receptors (α7nAChR), both implicated in schizophrenia.[53,54]

There were several nonsignificant associations in post hoc analyses indicating that in particular AA and QA, but also KTR, Kyn, and neopterin, could be associated with less irritability and motor disturbances. It is interesting that increased concentrations of many of these metabolites may indicate metabolic flux away from KA. Reduced activity of KMO, linked to higher KA,[55] has been shown in schizophrenia.[56]

Our study suggests that increased circulating KA and KKR, potentially related to KAT activity, could be biomarkers of an increased risk of neuropsychiatric symptoms in dementia. Furthermore, several direct and indirect effects of kynurenines on neurotransmitter receptors[51-54] suggest the possibility of a potential role in the pathogenesis of such symptoms. There are several important regulators of the kynurenine pathway in the periphery, such as IFN-γ[40] and interleukin 1β (IL-1β).[57] Furthermore, IL-1β can affect the activity of KAT.[57] Thus, both clinical and experimental studies are needed to confirm and elaborate on our findings.

Strengths of the study include its longitudinal design with annual follow-up examinations until death, a low dropout rate among the participants and centralized laboratory analyses of all metabolites. The main limitations are a relatively small sample size, use of nonfasting blood samples, lack of longitudinal measurements of kynurenines, and KKR might not accurately reflect KAT activity. Furthermore, we could

not conclude that the associations with cognition are confined to patients with dementia, due to the absence of an age-matched longitudinal control group. Our previous study on community-dwelling older adults indeed found a similar association between Kyn and cognitive function.[21] Kynurenines in the brain may mostly be derived from circulating kynurenines with Kyn as the main precursor.[3] Still, synthesis of the potentially neuroprotective KA is confined to astrocytes, whereas the potentially neurotoxic QA is synthesized in microglia.[3] Thus, our assessment of kynurenines in dementia is incomplete without measurements of CSF and/or brain samples.

In summary, circulating Kyn concentrations around the mean level may be beneficial for cognitive function in patients with dementia. Serum Kyn concentrations which diverge from the mean in either direction (higher or lower) may be associated with poorer global cognitive function. We observed an association of KA and KKR with neuropsychiatric symptoms, which adds to existing literature suggesting a role of kynurenines in mental health.[3]

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Author Contributions

All authors have approved of the final manuscript to be published and agrees to be accountable for all aspects of the work. S-EHS contributed to the planning of the study, performed statistical analyses and interpretation of the results, and was responsible for drafting the manuscript. JEN was involved in planning of the study, preparing an analytic protocol, and interpretation of the results. JEN critically revised the manuscript. DA is the principal investigator of DemVest and was involved in the conception of the study, interpretation of the results, and critically revised the manuscript. JL was involved in the planning of the study, organized the biological samples, contributed to the interpretation of measurements and results, and revised the manuscript. PMU was involved in the planning of the study, measurements and quality control of metabolic biomarkers, interpretation and critically revised the manuscript. ØM performed measurements and quality control of metabolic biomarkers in sera, participated in the interpretation of the results, and critically revised the manuscript. AM was involved in measurements and quality control of metabolic biomarkers in sera, interpretation of the results and critically revised the manuscript. GST was involved in the planning of the study, statistical analyses, participated in the interpretation of the results, and critically revised the manuscript. LMG was involved in the conception of the study, assessed and checked all statistical analyses and their presentation, interpreted the results and critically revised the manuscript.

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Supplemental Material

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