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Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau

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ABSTRACT

Chronic kynurenine pathway (KP) activation is implicated in Alzheimer's disease (AD) pathophysiology and results in quinolinic acid–induced excitotoxic stimulation of the N-methyl-D-aspartate receptor. However, most studies focus on plasma and it is unclear if peripheral concentrations reflect brain concentrations and how these may correlate to the AD biomarkers amyloid-β, total-tau (t-tau), or phosphorylated-tau (p-tau). We characterized the KP in matched plasma and cerebrospinal fluid (CSF) samples from 20 AD patients and 18 age-matched control subjects. Plasma concentrations of kynurenine (KYN), 3-hydroxykynurenine, anthranilic acid, picolinic acid, and neopterin significantly correlated with their respective CSF levels. In patients with AD, plasma KYN ($r = -0.48$, $p = 0.033$) and picolinic acid ($r = -0.57$, $p = 0.009$) inversely correlated with CSF p-tau and t-tau, respectively. Furthermore, in AD CSF, increased 3-hydroxykynurenine/KYN ratio correlated with t-tau ($r = 0.58$, $p = 0.009$) and p-tau ($r = 0.52$, $p = 0.020$). These data support KP involvement in AD pathogenesis and add to the case for the therapeutic modulation of the KP in AD.

1. Introduction

Neuropathological features of AD include activated microglia and reactive astrocytes surrounding plaques (Medeiros and LaFerla, 2013; Meyer-Luehmann et al., 2008). Initially, induction of these cells probably constitutes an endogenous repair/defense mechanism against neurotoxic amyloid fibrils that later form plaques. However, chronic activation, particularly of microglia, likely exacerbates neuroinflammation that has a known role in AD pathogenesis (Heneka et al., 2015; Osborn et al., 2016).

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A significant contributor to the neuroinflammatory environment is overactivation of the kynurenine pathway (KP) that results in excessive production of the excitotoxin quinolinic acid (QUIN) by activated microglia and perivascular and infiltrating macrophages (Guillemin et al., 2003). Although the numbers of perivascular and infiltrating macrophages will be much lower than activated microglia present in the brain, it is known that activated macrophages produce 40-fold more QUIN than activated microglia (Guillemin et al., 2004). The KP is the main route of tryptophan (TRP) metabolism, and its downstream metabolites are involved in many physiological processes including neurotransmission and immune response. Alterations in the balances of these metabolites have been observed in several major neurodegenerative conditions (Guidetti et al., 2004, 2006; LeWitt et al., 2013; Lim et al., 2017; Ogawa et al., 1992; Schwarz et al., 2013).

The major KP neurotoxin QUIN acts primarily as an N-methyl-D-aspartate (NMDA) receptor agonist. Elevation of extracellular glutamate and excitotoxic damage due to enhanced influx of Ca^{2+} ions is a well-described phenomenon particularly in the context of neurodegenerative disease (Dong et al., 2009). Other known mechanisms of QUIN-induced neurotoxicity include activation of neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS), leading to nitric oxide (NO) production (for review see Guillemin, 2012). Another key KP metabolite is kynurenic acid (KYNA), which is primarily produced by astrocytes (Guillemin et al., 2001). KYNA is a broad-spectrum endogenous inhibitor of ionotropic glutamate and α7 nicotinic acetylcholine receptors (Albuquerque and Schwarcz, 2013). Accordingly, KYNA possesses neuroprotective effects by competitive inhibition of QUIN binding at the NMDA receptor, thereby preventing glutamate excitotoxicity. Other important downstream metabolites of this pathway include 3-hydroxykynurenine (3-HK), anthranilic acid (AA), and 3-hydroxyanthranilic acid (3-HAA), all of which possess redox-modulating activity (Jacobs et al., 2017). Rebalancing the ratio of neuroprotective/neurotoxic KP metabolites by modulation of the KP enzyme kynurenine 3-monooxygenase (KMO) has been viewed as a viable therapeutic strategy in AD and other neurodegenerative conditions, since Zwilling et al. first demonstrated that KMO inhibition improved symptomology in murine models of neurodegenerative disease (Zwilling et al., 2011).

Several studies have reported significant alterations in the levels of KP metabolites and activity of KP pathway enzymes in AD patient serum/plasma compared with age-matched normal controls (Giil et al., 2017; Gulaj et al., 2010; Schwarz et al., 2013). Most recently, significant decreases in TRP, 3-HAA, and xanthurenic acid (XA) were reported in plasma of histopathologically confirmed AD patients (Giil et al., 2017). Furthermore, in this cohort, increased QUIN correlated with reduced cognitive performance in elderly patients with AD. In preclinical AD characterized by neocortical Aβ (NAL), increased kynurenine (KYN) and AA were reported in female participants compared with age-matched females who were at no apparent risk of AD, suggesting use as a prognostic AD biomarker (Chatterjee et al., 2018).

Although links between the KP and AD are well recognized, it is unclear if KP status in the periphery reflects that in the central nervous system, where disease processes are occurring. Only 2 reports have evaluated KP parameters in cerebrospinal fluid (CSF) from patients with AD, but these did not also assess matched patient plasma/serum samples. To fill this gap, we assessed the relationship between plasma and CSF levels of KP metabolites and whether these can be linked to plasma neopterin, a marker of cellular immune activation, and the AD CSF biomarkers $Aβ_{42}$, total-tau (t-tau), and phosphorylated-tau (p-tau) reflective of amyloid aggregation and deposition and tauopathy in AD (Blennow et al., 2010).

2. Materials and methods

2.1. Study design, subject characterization, and sample collection

We quantified plasma and matching CSF levels of KP metabolites in samples from 20 patients with AD and 18 age-matched non-AD controls (NCs) sourced from the Department of Psychiatry and Neurochemistry at Institute of Neuroscience and Physiology Gothenburg University. The AD group included patients with cognitive disturbances indicative of AD and a core AD CSF biomarker profile typical for AD, including CSF $Aβ_{1–42} < 550$ ng/L, CSF t-tau > 400 ng/L, and CSF p-tau > 60 ng/L. Individuals in the control group had minor or suspected cognitive symptoms, but a normal CSF biomarker profile, thereby excluding AD-type pathology and ongoing neurodegeneration. The CSF samples used were de-identified aliquots from clinical routine analyses (where patients had provided informed written consent), following a procedure approved by the Ethics Committee at University of Gothenburg (EPN 140811).

2.2. Kynurenine metabolite analysis by UHPLC, HPLC, and GC/MS

2.2.1. Plasma and CSF preparation

Blinded plasma samples were deproteinized with the addition of equal volumes of 10% w/v trichloroacetic acid followed by centrifugation at 3600 g at 4 °C for 15 minutes. The supernatants were then collected and filtered through 0.45 μm polytetrafluoroethylene syringe filters (Merck-Millipore, CA, USA). Blinded CSF samples were filtered through 0.45 μm polytetrafluoroethylene syringe filters and stored at −80 °C before analysis.

2.2.2. Quantification of TRP, KYN, 3-HK, 3-HAA, AA, KYNA, PIC, and QUIN

TRP, KYN, 3-HK, 3-HAA, and AA were quantified by ultra-high-performance liquid chromatography (UHPLC) in accordance with published methods (Jones et al., 2015).

KYNA analysis was conducted using an Agilent 1260 high-performance liquid chromatography (HPLC) system. A reverse phase C18 column (ZORBAX XDB, 4.6 × 100 mm; Agilent Technologies, CA, USA) was used for compound separation at 38 °C. The mobile phase (0.05 M sodium acetate, 0.05 M zinc acetate, 5% v/v HPLC grade acetonitrile, pH 5.2) was run isocratically at 1.00 mL/min. KYNA was quantified via fluorescence (ex: 344 nm and em: 388 nm).

Picolinic acid (PIC) and QUIN quantification was performed using an Agilent 7890 gas chromatograph (GC) coupled with an Agilent 5975 mass spectrometer (MS) adapted from published methods (Guillemin et al., 2007). Deuterated internal standard QUIN-d3 and PIC-d4 were sourced from Medical Isotopes Inc (Pelham, NH). Samples were injected into an HP-5MS GC capillary column (Agilent Technologies), and analysis carried out with the MS operating in negative chemical ionization mode. Selected ions for fluorinated esters of PIC and QUIN (m/z 273 for PIC, m/z 277 for d4-PIC, m/z 467 for QUIN, and m/z 470 for d3-QUIN) were simultaneously monitored.

2.3. Quantification of neopterin

Neopterin was quantified by UHPLC using an Agilent 1290 HPLC system. A reverse phase C18 column (ZORBAX Rapid Resolution High Definition C18 reverse phase 2.1 × 150 mm column with pore size of 1.8 μm; Agilent Technologies) was used for compound separation at 38 °C. The mobile phase (0.1 M sodium acetate, pH 4.65) was run isocratically at 0.75 mL/min. Neopterin was quantified by fluorescence (ex: 353 nm and em: 438 nm).

2.4. Immunoassay protocols

CSF levels of t-tau were determined using an enzyme-linked immunosorbent assay (ELISA) method designed to measure all forms of tau, irrespective of phosphorylation status (Blennow et al., 1995), whereas p-tau levels were measured using a sandwich ELISA method based on the AT270 antibody recognizing tau phosphorylated at threonine 181 (Vanmechelen et al., 2000). CSF $A\beta_{1-42}$ was measured using a sandwich ELISA specifically constructed to measure Aβ starting at amino acid 1 and ending at amino acid 42 (Andreasen et al., 1999). Analyses were performed by board-certified laboratory technicians who were blinded to clinical data, following strict rules for internal quality control, run approval, and batch-bridging of reagents (Palmqvist et al., 2014).

2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism, version 7.02, Minitab 18, and Matlab (version R2018A). The normality of the variables was checked by the Anderson-Darling normality test ($\alpha = 0.05$, Minitab) and visual examination of residual/Q-Q plots. Outliers were identified by the ROUT analysis (Q = 1%; GraphPad Prism). Data were log-transformed, as required, to approximate a normal distribution. Student *t*-tests and Mann-Whitney *U* tests were performed to identify significant differences in KP metabolite concentrations between groups in both plasma and CSF ($\alpha = 0.05$). Selected variables were modeled using logistic regression (Matlab, version R2018a) and presented using the receiver operating characteristic curve analysis.

Before correlation analysis between KP metabolites and neopterin in plasma and CSF, linear regression models were fit using diagnosis (AD or NC) as a binary predictor. Correlations between gold-standard CSF AD biomarkers (tau, p-tau, and Aβ) and demographic variables were evaluated using Pearson's correlation (normally distributed data) and the Spearman's rho correlation coefficient (nonparametric analysis). Deming linear regression was used to fit linear models after correlation analysis.

3. Results and discussion

3.1. Demographic and clinical characteristics

There was no significant difference in age (unpaired *t*-test, $p = 0.064$) between the AD and NC groups. Furthermore, there was no significant correlation between age and KP metabolites, tau, Aβ, or p-tau in the AD and NC groups, and thus, age was not included as a covariate in the statistical analysis. However, there were significant differences in gender composition (Chi-square statistic, $p = 0.014$). By definition, CSF levels of t-tau (Mann-Whitney, $p < 0.001$),$A\beta_{42}$ (unpaired *t*-test, $p < 0.001$), and p-tau (Mann-Whitney, $p < 0.001$) differed significantly between the AD and NC groups, as summarized in Table 1.

Diagnosis (AD or NC) did not significantly contribute to the linear regression results for all KP metabolites and product/substrate ratios except KYNA, indicating correlations between plasma and CSF KP metabolites were independent of disease status.

3.2. Kynurenine pathway metabolites are significantly modulated in CSF of patients with AD

Concentrations of KP metabolites in plasma and CSF from patients with AD and NCs are summarized in Table 2. In plasma of patients with AD, a 30% decrease in 3-HAA ($p = 0.035$) was evident compared with that of controls; an analogous change was not observed in the CSF. Similarly, a significant 30% decrease in 3-HAA was recently reported in plasma of histopathologically confirmed AD patients (Giil et al., 2017). Significant reductions in 3-HAA were also reported in the plasma of patients with advanced-stage Huntington's disease; the authors speculated this may reflect a mechanism whereby QUIN production is reduced following inhibition of upstream 3-hydroxyanthranilic acid oxygenase (3-HAO) by AA (Darlington et al., 2010).

In AD patient CSF, the metabolite KYNA was significantly elevated 1.7-fold ($p = 0.005$) compared with that of controls. The QUIN/KYNA ratio was decreased by approximately 50% ($p = 0.003$). Notably, KYNA was significantly increased 192% and 177% in the caudate nucleus and putamen of AD patient brains. Furthermore, increased activity of KAT-I was also reported in the aforementioned brain regions in AD (Baran et al., 1999). Although KYNA is usually considered neuroprotective, increases in the CSF may suggest engagement of a compensatory mechanism directed at reducing the effects of chronic excitotoxicity at the NMDA receptor.

To determine whether the differences in KP metabolites between groups could be used to discriminate the patients with AD from NCs, we modeled the variables with logistic regression and presented the findings using the receiver operating characteristic curve analysis (Supplementary Fig. 1). CSF KYNA (area under curve [AUC] = 0.806) outperformed plasma 3-HAA (AUC = 0.613) and CSF kynurenine/tryptophan ratio (K/T; AUC = 0.764).

3.3. Correlations between plasma and CSF concentrations of KP metabolites and neopterin

A common criticism of studies aimed at the identification of blood-based AD biomarkers is whether the concentrations of the target(s) in the periphery are truly reflective of concentrations in the brain and can therefore be accurate biomarkers of disease process. Plasma concentrations of the KP metabolites, KYN ($r = 0.70$, $p < 0.001$), 3-HK ($r = 0.33$, $p = 0.044$), AA ($r = 0.63$, $p < 0.001$), PIC ($r = 0.54$, $p < 0.001$), and neopterin ($r = 0.62$, $p < 0.001$, Supplementary Fig. 2), significantly correlated with their respective CSF concentrations (Fig. 1). The product/substrate ratios, K/T ($r = 0.76$, $p < 0.001$) and 3-HK/KYN ($r = 0.58$, $p = 0.001$; Fig. 2), also significantly correlated with their respective CSF values. Plasma concentrations of all other KP metabolites failed to correlate with CSF levels. This is particularly interesting for TRP as it was previously shown to correlate in multiple sclerosis patient plasma/CSF samples (Lim et al., 2017) and may suggest a disease or age-dependent relationship. TRP is known to be actively transported

Table 1
Demographic and clinical characteristics of patients with AD and NCs

	AD (n = 20)	NC (n = 18)	*p* value
Age (y; mean ± SD)	77.9 ± 7.5	73.1± 7.9	0.064
Gender (n), female/male	11/9	3/15	0.014
CSF t-tau (ng/L; 25th/75th percentile)	611 (470/723)	271 (203/330)	<0.001
CSF $A\beta_{42}$ (ng/L, mean ± SD)	449 ± 83	719 ± 148	<0.001
CSF p-tau (ng/L; 25th/75th percentile)	77 (66/79)	43 (35/53)	<0.001

Key: AD, Alzheimer's disease; CSF, cerebrospinal fluid; NC, non-AD controls.

Table 2
Median KP metabolite and neopterin concentrations (interquartile range) in plasma and CSF of patients with AD and NCs

	Plasma			CSF		
	AD	NC	*p* value	AD	NC	*p* value
TRP, μM	51.2[a] (8.3)	53.1[a] (14.8)	0.837	2.26 (0.72)	2.39 (0.54)	0.251
KYN, μM	2.95 (0.84)	2.85 (1.02)	0.390	0.088 (0.027)	0.081 (0.039)	0.900
3-HK, nM	111.8[a] (39.2)	98.0[a] (38.3)	0.718	21.2 (6.4)	22.4 (11.0)	0.742
3-HAA, nM	18.3[a] (17.7)	27.9[a] (15.5)	**0.035**	15.0 (7.6)	13.6 (5.5)	0.228
AA, nM	3.93 (1.96)	3.82 (2.66)	0.535	0.77[a] (0.45)	0.84[a] (0.42)	0.936
KYNA, nM	17.2 (13.5)	20.6 (7.9)	0.835	2.67[a] (2.15)	1.52[a] (1.16)	**0.005**
PIC, nM	164.7[a] (87.9)	159.6[a] (58.4)	0.253	43.1 (14.4)	38.5 (12.5)	0.155
QUIN, nM	687.1[a] (433.5)	651.5[a] (254.4)	0.391	322.3 (137.1)	364.7 (250.6)	0.335
K/T	59.1[a] (19.3)	50.9[a] (23.2)	0.194	42.4 (13.8)	33.6 (14.0)	0.059
3-HK/KYN	40.8 (12.7)	41.0 (17.9)	0.830	25.5[a] (7.8)	23.5[a] (15.2)	0.442
3-HAA/AA	4.75 (3.53)	6.46 (5.76)	0.131	18.3 (12.2)	17.2 (9.3)	0.277
PIC/QUIN	26.4 (14.3)	27.1 (16.9)	0.551	14.5 (16.5)	12.5 (10.0)	0.929
QUIN/KYNA	38.5 (37.5)	33.9 (13.4)	0.573[b]	116.5 (114.9)	228.0 (267.5)	**0.003**
Neopterin, nM	26.4 (6.75)[a]	21.66 (11.93)[a]	0.165	7.20 (4.24)	6.46 (2.90)	0.540

Where necessary, data were log-transformed to approximate a normal distribution before statistical analysis (unpaired *t*-test). AD: n = 20, NC: n = 18. Significant *p*-values are highlighted in bold.
Key: 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; AD, Alzheimer's disease; CSF, cerebrospinal fluid; KP, kynurenine pathway; KYN, kynurenine; KYNA, kynurenic acid; K/T, kynurenine/tryptophan ratio; NC, non-AD control; PIC, picolinic acid; QUIN, quinolinic acid; TRP, tryptophan; QUIN/KYNA, quinolinic acid/kynurenic acid ratio.
[a] indicates variables that were log-transformed before statistical analysis.
[b] Mann-Whitney test.

across the blood-brain barrier via the larger neutral amino acid (LNAA) transporter. Under physiological conditions, the LNAA transporter is nearly saturated and changes in the ratio between TRP and other competing LNAA, for example, phenylalanine that is increased in AD, could significantly affect brain concentrations of TRP (Liu et al., 2014; Richard et al., 2009). 3-HK is also transported into the brain by the LNAA transporter and is likely subject to similar competition; however, a significant correlation may occur because of strong correlations in precursor KYN (Fukui et al., 1991). The significant correlation between plasma and CSF KYN concentrations agrees with previous studies examining patients with hepatitis C and multiple sclerosis (Lim et al., 2017; Raison et al., 2010). Conversely, 3-HAA, AA, KYNA, and QUIN passively diffuse into the brain (Fukui et al., 1991). The uptake of AA occurs at a significantly increased rate relative to 3-HAA, KYNA, and PIC (Fukui et al., 1991) and is a likely explanation of the significant correlation between concentrations of AA in the CSF and plasma.

The correlation between plasma and CSF PIC is less easily explained because its low LogP value suggests that it is blood brain barrier impermeable (Grant et al., 2009). A potential explanation may lie in a strong correlation in the activity of amino-carboxymuconate semialdehyde decarboxylase (ACMSD) in the brain and periphery. Although ACMSD expression in the brain is very low relative to the liver (1:30), its low K_m value indicates that it is likely readily saturated at physiological concentrations of its substrate, 2-amino-3-carboxymuconate 6-semialdehyde (ACMS; Pucci et al., 2007). When ACMSD becomes saturated, excess ACMS is converted nonenzymatically to QUIN, limiting synthesis of PIC (Bender and McCreanor, 1985).

3.4. CSF and plasma concentrations of kynurenine pathway metabolites correlate with Aβ, tau, and p-tau levels

Further analysis identified correlations between CSF and plasma KP metabolite concentrations and the level of AD biomarkers t-tau and p-tau. In AD patient plasma, the immunomodulator KYN ($r = -0.48$, $p = 0.033$, Fig. 3A) and neuroprotective PIC ($r = -0.57$, $p = 0.009$, Fig. 3B) inversely correlated with p-tau and t-tau, respectively, whereas these observations were not seen in NCs. Therefore, although increased PIC in plasma/CSF were not significantly different between patients with AD and NCs ($p = 0.253$ and 0.155, respectively), the subset of patients with AD with the lowest plasma PIC had the highest CSF tau, suggesting that increased PIC production in AD could be protective against tau formation.

The strongest positive correlations identified were between t-tau and CSF levels of 3-HK ($r = 0.66$, $p = 0.003$; Fig 4A) and 3-HK/KYN ($r = 0.57$, $p = 0.009$; Fig. 4B). Notably, 3-HK/KYN also positively correlated with p-tau in AD ($r = 0.52$, $p = 0.020$; Fig. 4C) and approached significance in AD patient plasma ($r = 0.44$, $p = 0.054$). Although not replicated in this work, Schwarz et al. previously reported a 3-fold increase in 3-HK in the plasma of patients with AD compared with those with major depression or subjective cognitive impairment (Schwarz et al., 2013). Increased 3-HK-mediated protein modifications (suggesting increased 3-HK) have also previously been reported in the hippocampus of patients with AD but were not apparent in AD with diffuse Lewy body patients (Bonda et al., 2010). The Lewy body variant of AD, or mixed dementia, tends to present with numerous senile plaques but limited NFT pathology compared with classical AD (Hansen et al., 1993). Our results are consistent with this as patients with AD with increased t-tau and p-tau were also seen to have higher 3-HK concentrations. 3-HK has both pro-oxidant and antioxidant activity inducing reactive oxygen species (ROS)–dependent apoptosis (Okuda et al., 1996) but preventing lipid peroxidation and decreasing peroxyl radical production in vitro (Colín-González et al., 2014b; Leipnitz et al., 2007). Most recently, increased KMO activity and in turn accumulation of 3-HK were shown to result in mitochondrial dysfunction, leaving cells vulnerable to oxidative damage and energy deficits (Castellano-Gonzalez et al., 2019).

CSF levels of 3-HAA were shown to positively correlate with p-tau in both patients with AD and NCs ($r = 0.45$ and 0.64, respectively; $p = 0.044$ and 0.004, respectively, Fig. 4D), highlighting a disease-independent relationship. Although, a direct link between 3-HAA and p-tau has not been previously reported, similarly to 3-HK, 3-HAA is known to produce ROS including superoxide and hydrogen peroxide in a copper-dependent manner (Goldstein et al., 2000). Oxidative stress is linked with major AD pathological processes, including Aβ deposition, mitochondrial dysfunction, tau phosphorylation, and iron accumulation (Su et al., 2010; Zhao and Zhao, 2013; Zhu et al., 2005). Redox active 3-HAA is also known to induce the expression of hemeoxygenase-1 (HO-1) in astrocytes and macrophages (Krause et al., 2011), the chronic overactivation of

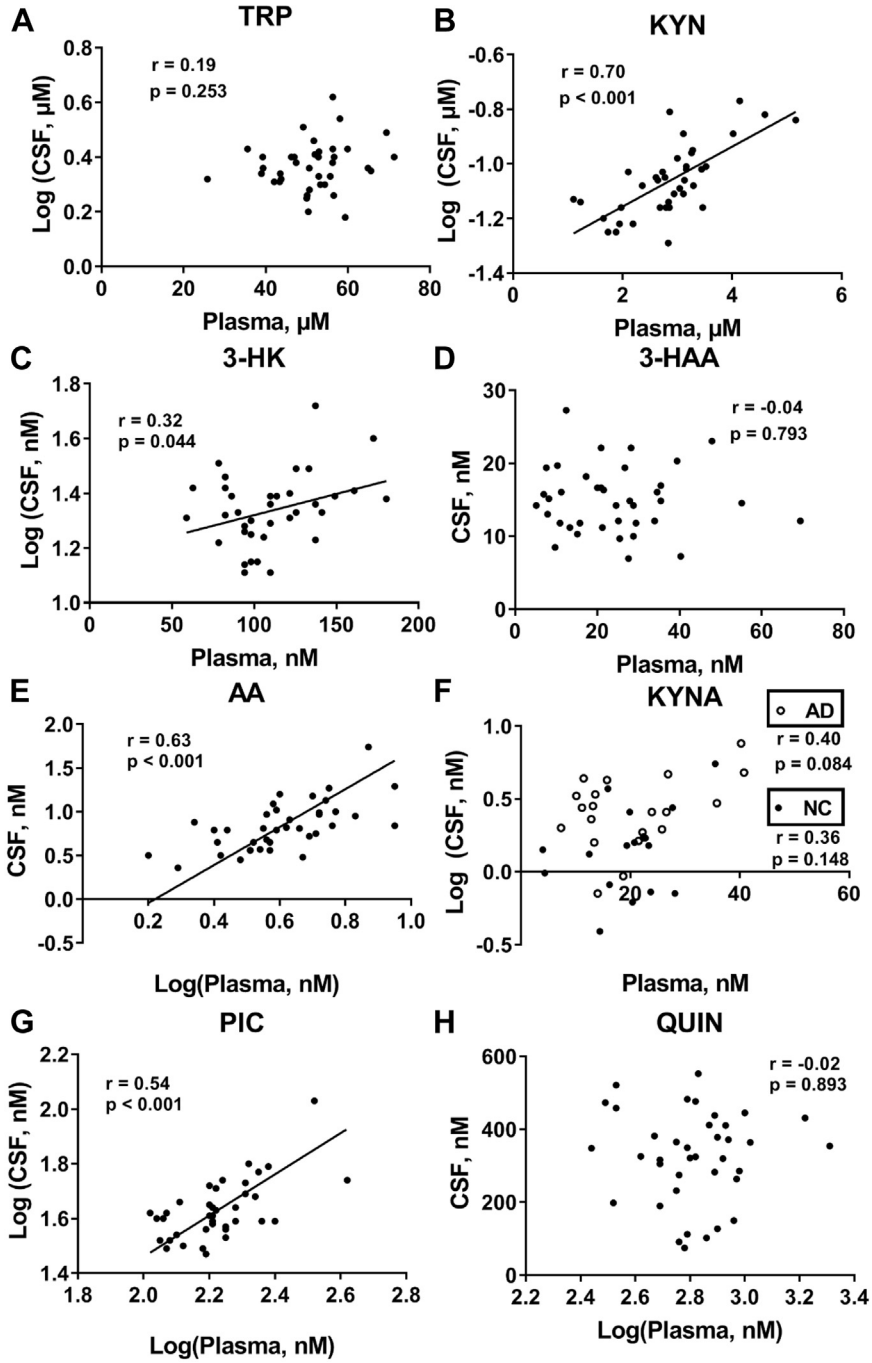

Figure 1. Positive correlations and Deming linear regression results between concentrations of KP metabolites in plasma and CSF were found for KYN (B), 3-HK (C), AA (E), and PIC (G). Significant correlations were not found for TRP (A), 3-HAA (D), KYNA (F), and QUIN (H). All correlations analyzed using Pearson's correlation except for TRP and PIC (Spearman's correlation). Diagnosis (AD/NC) did not significantly contribute to the linear regression for all metabolites except KYNA. Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; AD, Alzheimer's disease; CSF, cerebrospinal fluid; KYN, kynurenine; KYNA, kynurenic acid; NC, non-AD control; PIC, picolinic acid; QUIN, quinolinic acid.

which promotes iron deposition and mitochondrial dysfunction (Melov et al., 2007; Schipper, 2000) further contributing to ROS production. Finally, increased concentrations of 3-HAA may contribute to increased levels of downstream QUIN, which was also shown to result in dose-dependent tau phosphorylation in primary human astrocytes (Rahman et al., 2009).

3-HAA in the CSF of NCs positively correlated with CSF $A\beta_{42}$ ($r = 0.551$, $p = 0.0118$; Fig. 4E), signifying decreased brain Aβ deposition. This agrees with studies showing that 3-HAA dose dependently inhibits the in vitro aggregation of Aβ (Meek et al., 2013). However, in AD, mechanistic biomarkers are not expected to correlate with CSF Aβ, as at disease onset, Aβ is already very decreased. Accordingly, we did not find any significant correlations between CSF $A\beta_{42}$ and KP metabolites in AD patient samples.

Studies in dominantly inherited patients with AD demonstrate that CSF t-tau continues to increase after onset of disease (Bateman et al., 2012). Furthermore, the baseline levels of CSF p-tau and t-tau were significantly higher in patients with mild cognitive

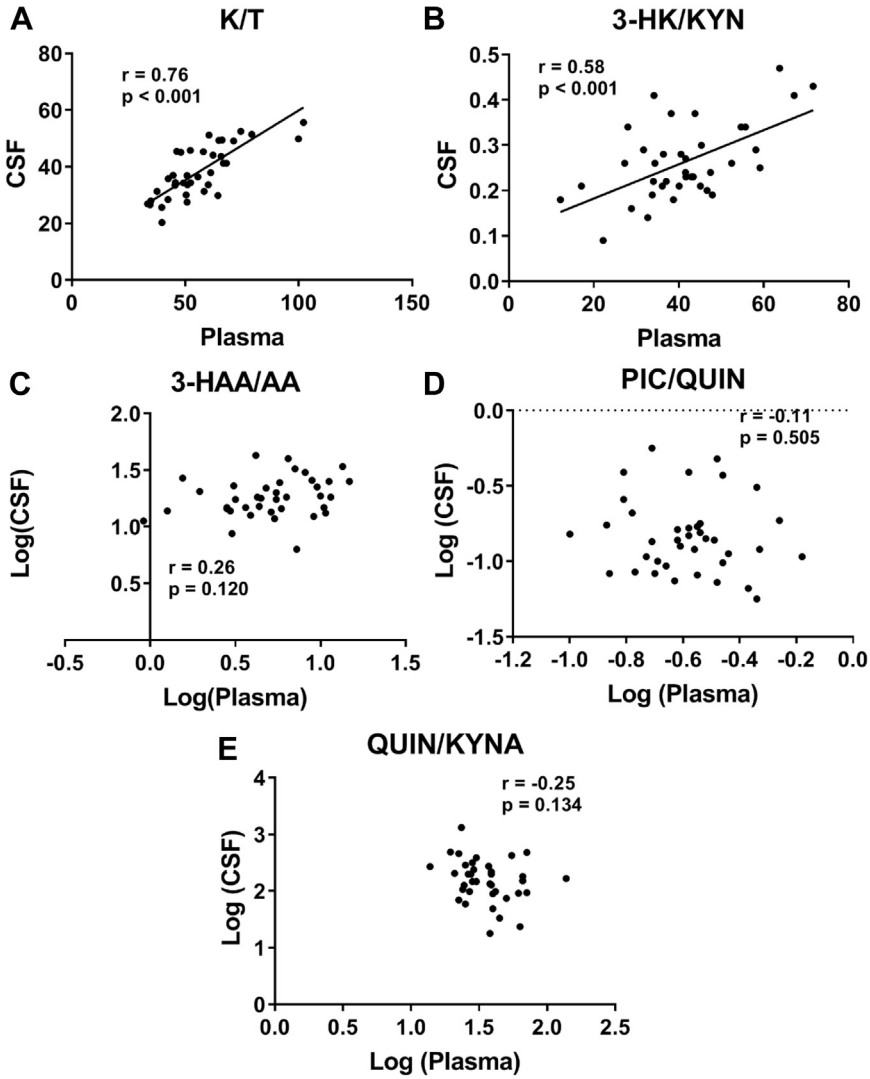

Fig. 2. Positive correlations and Deming linear regression results between KP product/substrate ratios in plasma and CSF were found for K/T (A) and 3-HK/KYN (B) but not for 3-HAA/AA (C), PIC/QUIN (D), and QUIN/KYNA (E). All correlations analyzed using Pearson's correlation. Abbreviations: 3-HAA/AA, 3-hydroxyanthranilic acid/anthranilic acid ratio; 3-HK/KYN, 3-hydroxykynurenine/kynurenine ratio; CSF, cerebrospinal fluid; KP, kynurenine pathway; K/T, kynurenine/tryptophan ratio; PIC/QUIN, picolinic acid/quinolinic acid ratio; QUIN/KYNA, quinolinic acid/kynurenic acid ratio.

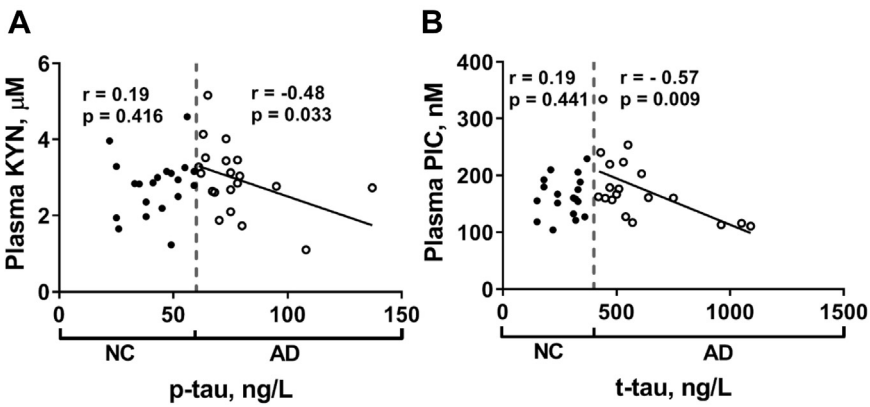

Fig. 3. Negative correlations between plasma KYN (A) and PIC (B) and AD biomarkers t-tau and p-tau in AD patient (n = 20) plasma. Similar trends were not seen in NCs (n = 18). The dashed vertical line indicates the biomarker concentration cutoff for classification as AD or NC. All correlations analyzed using Spearman's correlation. Abbreviations: AD, Alzheimer's disease; NC, non-AD control; KYN, kynurenine; PIC, picolinic acid.

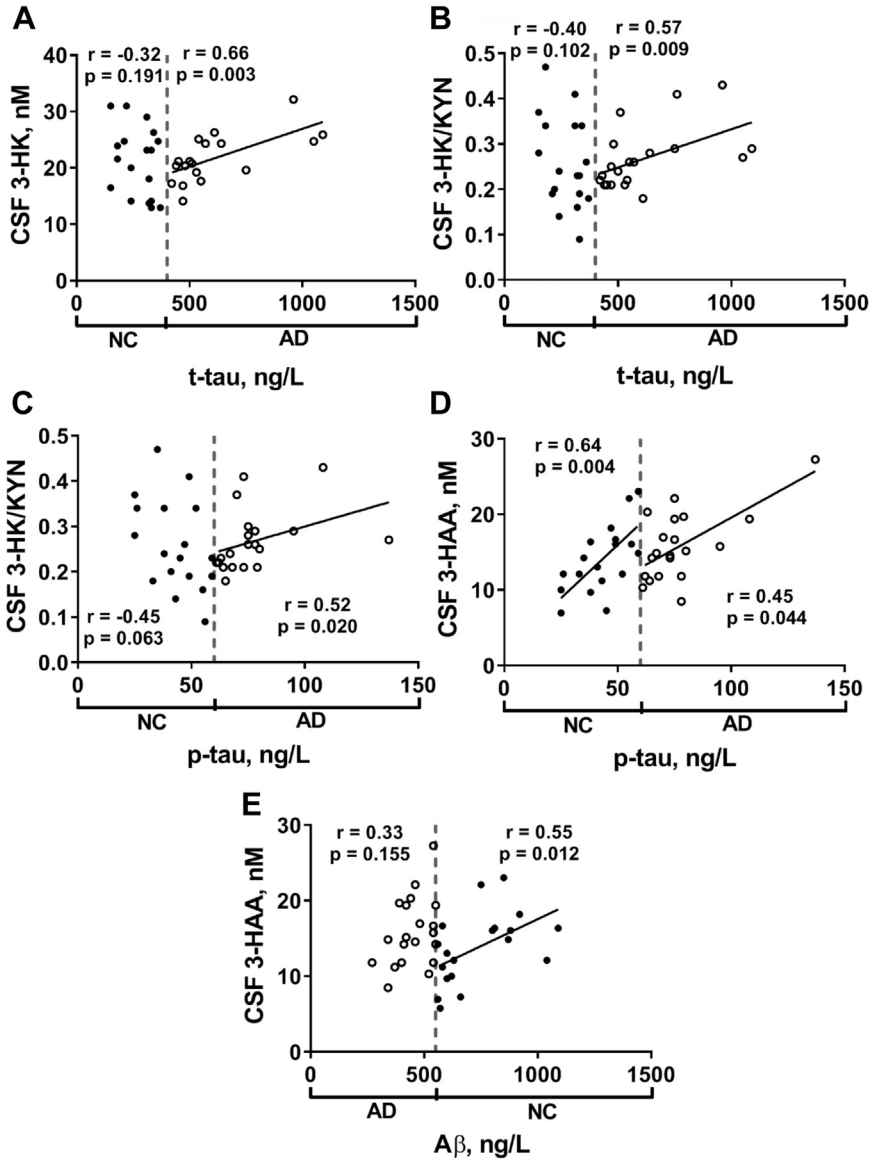

Fig. 4. Positive correlations between CSF 3-HK (A), 3-HK/KYN (B and C), and 3-HAA (D and E) and AD hallmarks t-tau, p-tau, and $A\beta_{42}$ in patients with AD (n = 20) and NCs (n = 18). Linear models were fit using Deming linear regression. The dashed vertical line indicates the biomarker concentration cutoff for classification as AD or NCs. All correlations analyzed using Spearman's correlation. Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 3-HK/KYN, 3-hydroxykynurenine/kynurenine ratio; Aβ, amyloid-beta; AD, Alzheimer's disease; NC, non-AD control; p-tau, phosphorylated-tau; t-tau, total-tau.

impairment that transitioned to AD more quickly than slow processors (0–2.5 years vs. 5–10 years; Jack et al., 2013). Therefore, further investigation of these metabolites in a longitudinal clinical cohort may highlight mechanistic links and their use as prognostic markers. A schematic illustrating the key results and potential mechanistic implications is shown in Fig. 5.

3.5. CSF and plasma concentrations of neopterin do not correlate with Aβ, tau, and p-tau levels

There was no significant difference in the concentration of neopterin in the CSF and plasma of patients with AD and NCs ($p = 0.540$ and $p = 0.165$, respectively). Correlations between AD biomarkers (Aβ, t-tau, and p-tau) and neopterin, a marker for immune activation, were investigated to assess if correlations between KP metabolites and AD biomarkers resulted from increased inflammation. Plasma neopterin significantly correlated with p-tau in NCs (r = 0.48, $p = 0.042$), but no other correlations were found, despite significant positive correlations between plasma neopterin and plasma KYN (r = 0.66, $p < 0.001$), AA (r = 0.46, $p = 0.004$), KYNA (r = 0.39, $p = 0.016$), QUIN (r = 0.67, $p < 0.001$), and K/T (r = 0.65, $p < 0.001$) and negative correlations with 3-HK/KYN (r = −0.50, $p = 0.001$) and PIC/QUIN (r = −0.51, $p = 0.001$) as summarized in Supplementary Fig. 3. Similarly, CSF neopterin positively correlated with CSF KYN (r = 0.38, $p = 0.021$), AA (r = 0.38, $p = 0.021$), KYNA (r = 0.35, $p = 0.034$), and K/T (r = 0.37, $p = 0.023$) and negatively correlated with QUIN/KYNA (r = −0.39, $p = 0.016$) as summarized in Supplementary Fig. 4. Correlations between KYN and K/T are unsurprising as the activity of indoleamine 2,3-dioxygenase is upregulated by inflammatory cytokines, including INF-γ, and molecules, such as amyloid peptides (Jacobs et al., 2017). Similarly, neopterin is produced by INF-γ–activated macrophages and monocytes (Widner et al., 1999). Correlations with downstream metabolites AA and KYNA likely

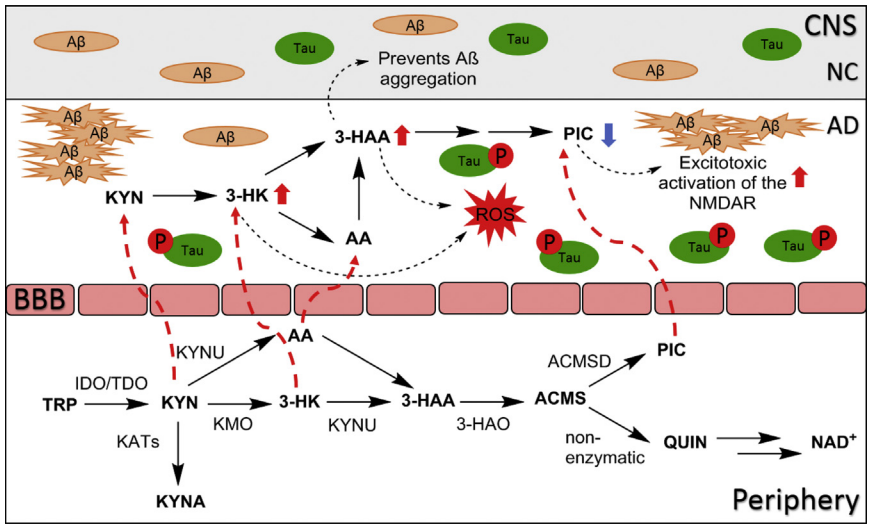

Fig. 5. Summary of results and potential mechanistic implications. Plasma concentrations of KYN, 3-HK, AA, and PIC were shown to positively correlate with concentrations in the CSF (dashed red arrows). Increased 3-HK/KYN in the CSF, generally indicative of increased KMO activity, was shown to positively correlate with t-tau and p-tau. Increases in 3-HAA positively correlated with increases in p-tau in both AD and NCs. In contrast, increased 3-HAA correlated with increased Aβ (and therefore decreased Aβ deposition) in NCs. 3-HK and 3-HAA are reported to contribute to oxidative stress after the production of ROS. Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilic acid oxygenase; 3-HK, 3-hydroxykynurenine; 3-HK/KYN, 3-hydroxykynurenine/kynurenine ratio; Aβ, amyloid-beta; AA, anthranilic acid; ACMS, 2-amino-3-carboxymuconate semialdehyde; ACMSD, 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; AD, Alzheimer's disease; CSF, cerebrospinal fluid; NC, non-AD control; IDO, indoleamine 2,3-dioxygenase; KATs, kynurenine aminotransferases; KMO, kynurenine 3-monooxygenase; KYN, kynurenine; KYNA, kynurenic acid; KYNU, kynureninase; PIC, picolinic acid; p-tau, phosphorylated-tau; ROS, reactive oxygen species; t-tau, total-tau; TDO, tryptophan dioxygenase; TRP, tryptophan; QUIN, quinolinic acid. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

result from significant increases in precursor KYN after activation of indoleamine 2,3-dioxygenase.

Correlations between CSF 3-HK and 3-HAA with t-tau and p-tau but not neopterin suggest that increased inflammation is not the key mechanism linking these metabolites and biomarkers. However, work investigating correlations with other inflammatory markers and cytokines may provide further insight.

3.6. Study limitations and significance

Limitations of this study include small sample size and lack of multivariate adjustment for cofactors and renal function. In addition, the control group had minor or suspected cognitive symptoms, which may have contributed to and/or masked some of the expected changes to KP metabolite and neopterin concentrations commonly reported in AD (e.g., increased K/T ratio (Widner et al., 2000) and neopterin (Casal et al., 2003; Savas et al., 2016). However, considering the uniqueness of this cohort of matched CSF and plasma samples and the strong correlations between plasma and CSF metabolite concentrations, this work should inform the analysis of data in other studies. Furthermore, correlations between these metabolites and well-established AD biomarkers (tau and p-tau) suggest mechanistic links that warrant further investigation.

4. Conclusion

Dysregulation of the KP has been widely reported in AD patient plasma. However, it is still unclear to what extent peripheral concentration of KP metabolites mirrors those found in the central nervous system. The present study highlights disease-independent correlations between KYN, 3-HK, AA, PIC, K/T, and 3-HK/KYN in plasma and CSF, providing confidence that plasma measures of these metabolites may obviate the need for intrusive CSF sampling. Furthermore, strong clinical correlations between 3-HK and 3-HK/KYN and t-tau may support further studies examining KMO as a potential drug target for neurodegenerative disease with significant tauopathy. However, although mechanistic data support the role of KP metabolites in AD pathogenesis, for example, via QUIN neurotoxicity and 3-HAA mediated ROS production, it is unclear whether these are truly causative of disease hallmarks or simply byproducts of chronic neuroinflammation, and further work must be done to demonstrate causality.

Disclosure

KB has served as a consultant or at advisory boards for Alzheon, CogRx, Biogen, Novartis, and Roche Diagnostics and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures–based platform company at the University of Gothenburg, all unrelated to this work. HZ has served at scientific advisory boards for Eli Lilly, Roche Diagnostics, Wave, CogRx, and Samumed; has received travel support from Teva; and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures–based platform company at the University of Gothenburg, all unrelated to this work. All other authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.03.015.

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