

**Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid- $\beta$  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 AD pathophysiology reflected by the AD biomarkers amyloid- $\beta$ , total-tau (t-tau) or phosphorylated-tau (p-tau). We characterised 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 (3-HK), anthranilic acid and picolinic acid (PIC) significantly correlated with their respective CSF levels. In AD participants, plasma KYN ( $r = -0.479$ ,  $p = 0.033$ ) and PIC ( $r = -0.650$ ,  $p = 0.009$ ) inversely correlated with CSF p-tau and t-tau, respectively. Further, in AD CSF, increased 3-HK/KYN ratio correlated with t-tau ( $r = 0.567$ ,  $p = 0.009$ ) and p-tau ( $r = 0.515$ ,  $p = 0.020$ ). These data support KP involvement in AD pathogenesis and add to the case for the therapeutic modulation of the KP in AD.

## **Highlights (3-5, max 85 characters including spaces per bullet point)**

- Kynurenine pathway (KP) metabolites correlate in matched plasma and CSF samples
- CSF KP metabolites correlate with AD biomarkers, t-tau and p-tau
- KP correlation with t-tau and p-tau may suggest mechanistic links

## **Keywords (maximum of 6)**

Alzheimer's disease, kynurenine pathway, amyloid- $\beta$ , tau protein, cerebrospinal fluid, disease biomarkers

## **1. Introduction**

Classic hallmarks of Alzheimer's disease (AD) are accumulation of extracellular amyloid plaques formed by secreted amyloid-beta ( $A\beta$ ) and intracellular neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau (Masters et al., 2015). Additional neuropathological features of AD include activated microglia and reactive astrocytes surrounding plaques (Meyer-Luehmann et al., 2008; Medeiros and LaFerla, 2013). Initially, induction of these cells probably constitutes an endogenous repair/defence mechanism against neurotoxic amyloid fibrils that later form plaques. However, chronic activation, particularly of microglia, probably exacerbates neuroinflammation that has a known role in AD pathogenesis (Heneka et al., 2015; Osborn et al., 2016).

A significant contributor to the neuroinflammatory environment is over-activation of the kynurenine pathway (KP) that results in excessive production of the excitatory neurotoxin quinolinic acid (QUIN) by activated microglia and perivascular and infiltrating macrophages (Guillemin et al., 2003). Although 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 has been observed in several major neurodegenerative conditions (Ogawa et al., 1992; Guidetti et al., 2004; Guidetti et al., 2006; LeWitt et al., 2013; Schwarz et al., 2013). More recently, attention has also focused on the KP in the context of neuropsychiatric disorders such as suicidality (Brundin et al., 2016; Bryleva and Brundin, 2017), depression (Réus et al., 2015; Ogyu et al., 2018) and schizophrenia (Plitman et al., 2017). As neuropsychiatric symptoms are common among

dementia patients, with up to 80% of dementia patients reporting at least one neuropsychiatric symptom (apathy, 36%; depression, 32% and agitation/aggression, 30%; Lyketsos et al., 2002), this suggests that in AD, that the neuroinflammatory sequelae of chronic KP activation may also be a factor in the cognitive and mood changes developed by AD patients.

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  $\text{Ca}^{2+}$  ions is a well-described phenomenon particularly in the context of neurodegenerative disease (Dong et al., 2009). More directly, QUIN has been shown to enhance  $\text{Ca}^{2+}$  influx in astrocytes (Lee et al., 2010) but this has not yet been reported in neurons. 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 and other free radicals, which in turn, promotes DNA damage, decreased mitochondrial membrane potential leading to mitochondrial destabilization, poly ADP-ribose polymerase (PARP) activation and nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) depletion (Braidly et al., 2009). QUIN is also capable of forming redox-enhancing iron complexes and has been shown to enhance phosphorylation of structural proteins including tau (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  $\alpha 7$  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 Zwillig et al. first demonstrated that KMO inhibition improved symptomology in murine models of neurodegenerative disease (Zwillig 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 to age matched normal controls (Gulaj et al., 2010; Schwarz et al., 2013; Giil et al., 2017). Most recently, significant decreases in TRP, 3-HAA and xanthurenic acid (XA) were reported in plasma of histopathologically confirmed AD patients (REF here instead of 1 line below?). Furthermore, in this cohort increased QUIN correlated with reduced cognitive performance in elderly AD patients (Giil et al., 2017). In pre-clinical AD characterised by neocortical A $\beta$  (NAL), increased KYN and AA were reported in female participants compared to age matched females who were at no apparent risk of AD, suggesting use a prognostic AD biomarker (Chatterjee et al., 2018). Although links between the KP and AD are well recognised, it is unclear if KP status in the periphery reflects that in the CNS, where disease processes are occurring. Only two reports have evaluated KP parameters in cerebrospinal fluid (CSF) from AD patients, 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 the AD CSF biomarkers A $\beta$ 42, total tau (t-tau) and phosphorylated tau (p-tau) biomarkers reflective of amyloid aggregation and deposition, neurodegeneration and tauopathy in AD (REF).

## **2. Materials and Methods**

### **2.1 Study design, subject characterisation and sample collection**

We quantified plasma and matching CSF levels of KP metabolites in samples from 20 AD patients and 18 age-matched non-AD controls (NC) 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 A $\beta$  1-42 <550 ng/L CSF t-tau >400 ng/L and CSF p-tau >60 ng/L, while all individuals in the control group had minor neurological or psychiatric symptoms, but a normal CSF biomarker profile. The CSF samples used were de-identified aliquots from clinical routine analyses, 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 deproteinised with the addition of equal volumes of 10% w/v trichloroacetic acid (TCA) followed by centrifugation at 3600 g at 4°C for 15 min. The supernatants were then collected and filtered through 0.45  $\mu$ m PTFE syringe filters (Merck-Millipore, CA, USA). Blinded CSF samples were filtered through 0.45  $\mu$ m PTFE syringe filters and stored at -80°C prior to 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) system 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 x 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).

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 in onto a HP-5MS GC capillary column (Agilent Technologies, CA, USA) and analysis carried out with the MS operating in negative chemical ionization (NCI) 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 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), while 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 A $\beta$  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).

## 2.4 Statistical analysis

Statistical analyses were performed using Minitab 18 and GraphPad Prism version 7.02. The normality of the variables was checked by Anderson-Darling normality test ( $\alpha = 0.05$ ) and visual examination of residual/Q-Q plots. Outliers were identified by ROUT analysis ( $Q = 1\%$ , GraphPad Prism). Data was 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 ( $\alpha = 0.05$ ). Prior to correlation analysis between KP metabolites 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  $A\beta$ ) 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 following 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,  $A\beta$  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,  $p < 0.0001$ ), A $\beta$ 42 (Unpaired t-test,  $p < 0.0001$ ) and p-tau (Mann-Whitney,  $p < 0.0001$ ) differed significantly between the AD and NC groups, as summarised 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 AD patient CSF

Concentrations of KP metabolites in plasma and CSF from AD patients and normal controls (NC) are summarised in Table 2. In AD plasma, a 30% decrease in 3-HAA ( $p = 0.035$ ) was evident compared to NC, an analogous change was not observed in the CSF. Similarly, a significant 30% decrease in 3-HAA was recently reported in histopathologically confirmed AD patient plasma (Giil et al., 2017). Significant reductions in 3-HAA were also reported in the plasma of advanced-stage Huntington's disease (HD) patients; the authors speculated this may reflect a mechanism whereby QUIN production is reduced following inhibition of upstream 3-hydroxyanthranlic 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 to 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. Further, 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.

### 3.3 Correlations between plasma and CSF concentrations of KP metabolites

A common criticism of studies aimed at the identification of blood-based AD biomarkers is whether the concentration 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 ( $r = 0.700$ ,  $p < 0.0001$ ), 3-HK ( $r = 0.328$ ,  $p = 0.044$ ), AA ( $r = 0.634$ ,  $p < 0.0001$ ) and PIC ( $r = 0.5358$ ,  $p = 0.0005$ ) significantly correlated with their respective CSF concentrations (Figure 1). The product/substrate ratios, K/T ( $r = 0.757$ ,  $p < 0.0001$ ) and 3-HK/KYN ( $r = 0.579$ ,  $p = 0.0001$ ; Figure 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 MS patient plasma/CSF (Lim et al., 2017) and may suggest a disease or age-dependent relationship. TRP is known to be actively transported 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 which is increased in AD, could significantly affect brain concentrations of TRP (Richard et al., 2009; Liu et al., 2014). 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 due to strong correlations in precursor KYN (Fukui et al., 1991). The significant correlation between plasma and CSF KYN concentrations agrees with previous studies examining hepatitis C and multiple sclerosis patients (Raison et al., 2010; Lim et al., 2017). 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 since its low LogP value suggests that it is BBB impermeable (Grant et al., 2009). A potential explanation may lie in a strong correlation in the activity of aminocarboxymuconate 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 non-enzymatically to QUIN, limiting synthesis of PIC (Bender and McCreanor, 1985).

#### 3.4 CNS and plasma concentrations of kynurenine pathway metabolites correlate with A $\beta$ , tau and p-tau levels.

Further analysis identified correlations between CSF and plasma KP metabolite concentrations and the level of AD biomarkers A $\beta$ , t-tau and p-tau. In AD patient plasma, the immunomodulator KYN ( $r = -0.650$ ,  $p = 0.0328$ , Figure 3A) and neuroprotective PIC ( $r = -0.479$ ,  $p = 0.0094$ , Figure 3B) inversely correlated with p-tau and t-tau, respectively, while these observations were not seen in NCs. Therefore, while increased PIC in AD plasma/CSF were not significantly different between in AD patients and normal controls ( $p = 0.253$  and  $0.155$ , respectively), the subset of AD patients 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 ( $r = 0.658$ ,  $p = 0.003$ ; Figure 4A) and 3-HK/KYN ( $r = 0.567$ ,  $p = 0.009$ ; Figure 4B). Notably, 3-HK/KYN also positively correlated with p-tau in AD ( $r = 0.515$ ,  $p = 0.0200$ , Figure 4C) and approached significance in AD plasma ( $r = 0.437$ ,  $p = 0.054$ ). Although not replicated in this work, Schwarz *et al.* previously reported a 3-fold increase in 3-HK in the plasma of AD patients compared to 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 AD hippocampus but were not apparent in AD with diffuse Lewy body patients (Bonda *et al.*, 2010). The Lewy body variant (LBV) of AD tends to present with numerous senile plaques, but limited NFT pathology compared to classical AD (Hansen *et al.*, 1993). Our results are consistent with this as AD patients with increased t-tau and p-tau were also seen to have higher concentrations of 3-HK.

CSF levels of 3-HAA were shown to positively correlate with p-tau in both AD patients and NCs ( $r = 0.4543$  and  $0.6423$  respectively;  $p = 0.0442$  and  $0.0040$  respectively, Figure 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 reactive oxygen species (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 A $\beta$  deposition, mitochondrial dysfunction, tau phosphorylation and iron accumulation (Zhu *et al.*, 2005; Su *et al.*, 2010; Zhao and Zhao, 2013). 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 which promotes iron deposition and mitochondrial dysfunction (Schipper, 2000; Melov *et al.*, 2007) 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).

However, in contrast, 3-HAA in the CSF of NC positively correlated with CSF A $\beta$ 42 ( $r = 0.551$ ,  $p = 0.0118$ ; Figure 4E), signifying decreased brain A $\beta$  deposition. This agrees with studies showing that 3-HAA dose-dependently inhibits the *in-vitro* aggregation of A $\beta$  (Meek et al., 2013). As a similar relationship is not seen in AD, this may suggest that other mechanisms leading to A $\beta$  aggregation overwhelm the capacity of 3-HAA to decrease A $\beta$  deposition. A schematic illustrating the key results and potential mechanistic implications is shown in Figure 5.

#### **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 mirror those found in the CNS. 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, although mechanistic data exists supporting 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 causative of disease hallmarks, or simply by-products of chronic neuroinflammation which further exacerbate disease process is yet to be determined. Strong correlations between 3-HK and 3-HK/KYN and t-tau, may support the former argument, and could be an argument in support of further studies examining KMO as a potential drug target for neurodegenerative disease with significant tauopathy.

## **Disclosure statement**

KB has served as a consultant or at advisory boards for Alzheon, CogRx, Biogen, Novartis, and Roche Diagnostics, and is a co-founder 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 co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to this work.

The other authors report no conflicts of interest.

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**Table 1: Demographic and Clinical characteristics of AD patients and Normal Controls**

|   | AD (n=20)              | NC (n=18)              | P value |
|---|------------------------|------------------------|---------|
| Age (y; mean $\pm$ SD) (Range)                                  | 77.9 $\pm$ 7.5 (66-96) | 73.1 $\pm$ 7.9 (60-86) | 0.064   |
| Gender (n) Female/Male  | 11/9                   | 3/15                   | 0.014   |
| CSF t-tau (ng/L; 25 <sup>th</sup> /75 <sup>th</sup> percentile) | 611 (470/723)          | 271 (203/330)          | <0.0001 |
| CSF A $\beta$ 42 (ng/L, mean $\pm$ SD)                          | 449 $\pm$ 83           | 719 $\pm$ 148          | <0.0001 |
| CSF p-tau (ng/L; 25 <sup>th</sup> /75 <sup>th</sup> percentile) | 77 (66/79)             | 43 (35/53)             | <0.0001 |

Abbreviations: AD: Alzheimer's disease, NC: Normal controls

**Table 1: Median KP metabolite concentrations (25<sup>th</sup> Percentile/75<sup>th</sup> Percentile) in plasma and CSF of AD patients and normal controls (NC). Where necessary data was log transformed to approximate a normal distribution prior to statistical analysis (unpaired t-test) AD: n = 20, NC: n = 18.**

|               | Plasma                  |                         |                    | CSF                    |                        |              |
|---------------|-------------------------|-------------------------|--------------------|------------------------|------------------------|--------------|
|               | AD                      | NC                      | P value            | AD                     | NC                     | P value      |
| TRP / $\mu$ M | 51.2*<br>(47.7/56.0)    | 53.1*<br>(43.7/58.5)    | 0.837              | 2.26<br>(1.83/2.55)    | 2.39<br>(2.14/2.68)    | 0.251        |
| KYN / $\mu$ M | 2.95<br>(2.62/3.46)     | 2.85<br>(2.14/3.16)     | 0.390              | 0.088<br>(0.071/0.098) | 0.081<br>(0.067/0.106) | 0.900        |
| 3-HK /nM      | 111.8*<br>(95.1/134.3)  | 98.0*<br>(89.2/127.5)   | 0.718              | 21.2<br>(19.3/25.7)    | 22.4<br>(14.1/25.1)    | 0.742        |
| 3-HAA /nM     | 18.3*<br>(9.9/27.6)     | 27.9*<br>(19.3/34.8)    | <b>0.035</b>       | 15.0<br>(11.8/19.4)    | 13.6<br>(10.9/16.4)    | 0.228        |
| AA /nM        | 3.93<br>(3.26/5.22)     | 3.82<br>(3.16/5.82)     | 0.535              | 0.77*<br>(0.65/1.10)   | 0.84*<br>(0.56/0.98)   | 0.936        |
| KYNA /nM      | 17.2<br>(12.9/26.4)     | 20.6<br>(15.5/23.4)     | 0.835              | 2.67*<br>(1.89/4.04)   | 1.52*<br>(0.79/1.95)   | <b>0.005</b> |
| PIC /nM       | 164.7*<br>(134.8/222.7) | 159.6*<br>(131.2/189.6) | 0.253              | 43.1<br>(38.8/53.2)    | 38.5<br>(32.6/45.1)    | 0.155        |
| QUIN /nM      | 687.1*<br>(486.6/920.1) | 651.5*<br>(539.8/794.2) | 0.391              | 322.3<br>(266.5/403.6) | 364.7<br>(223.4/474.0) | 0.335        |
| K/T           | 59.1*<br>(46.2/65.5)    | 50.9*<br>(41.7/64.9)    | 0.194              | 42.4<br>(34.4/48.2)    | 33.6<br>(28.3/42.3)    | 0.059        |
| 3-HK/KYN      | 40.8<br>(34.2/46.9)     | 41.0<br>(31.7/49.6)     | 0.830              | 25.5*<br>(21.2/29.0)   | 23.5*<br>(18.8/34.0)   | 0.442        |
| 3-HAA/AA      | 4.75<br>(2.87/6.40)     | 6.46<br>(3.84/9.60)     | 0.131              | 18.3<br>(14.2/26.4)    | 17.2<br>(13.7/23.0)    | 0.277        |
| PIC/QUIN      | 26.4<br>(20.1/34.4)     | 27.1<br>(18.9/35.8)     | 0.551              | 14.5<br>(11.0/27.5)    | 12.5 (8.0/18.0)        | 0.929        |
| QUIN/KYN<br>A | 38.5<br>(25.1/62.6)     | 33.9<br>(26.6/40.0)     | 0.573 <sup>M</sup> | 116.5<br>(78.8/193.7)  | 228.0<br>(132.7/400.2) | <b>0.003</b> |

Table note: Significant p-values are highlighted in bold. \* indicates variables that were log transformed prior to statistical analysis. <sup>M</sup> Mann-Whitney test.

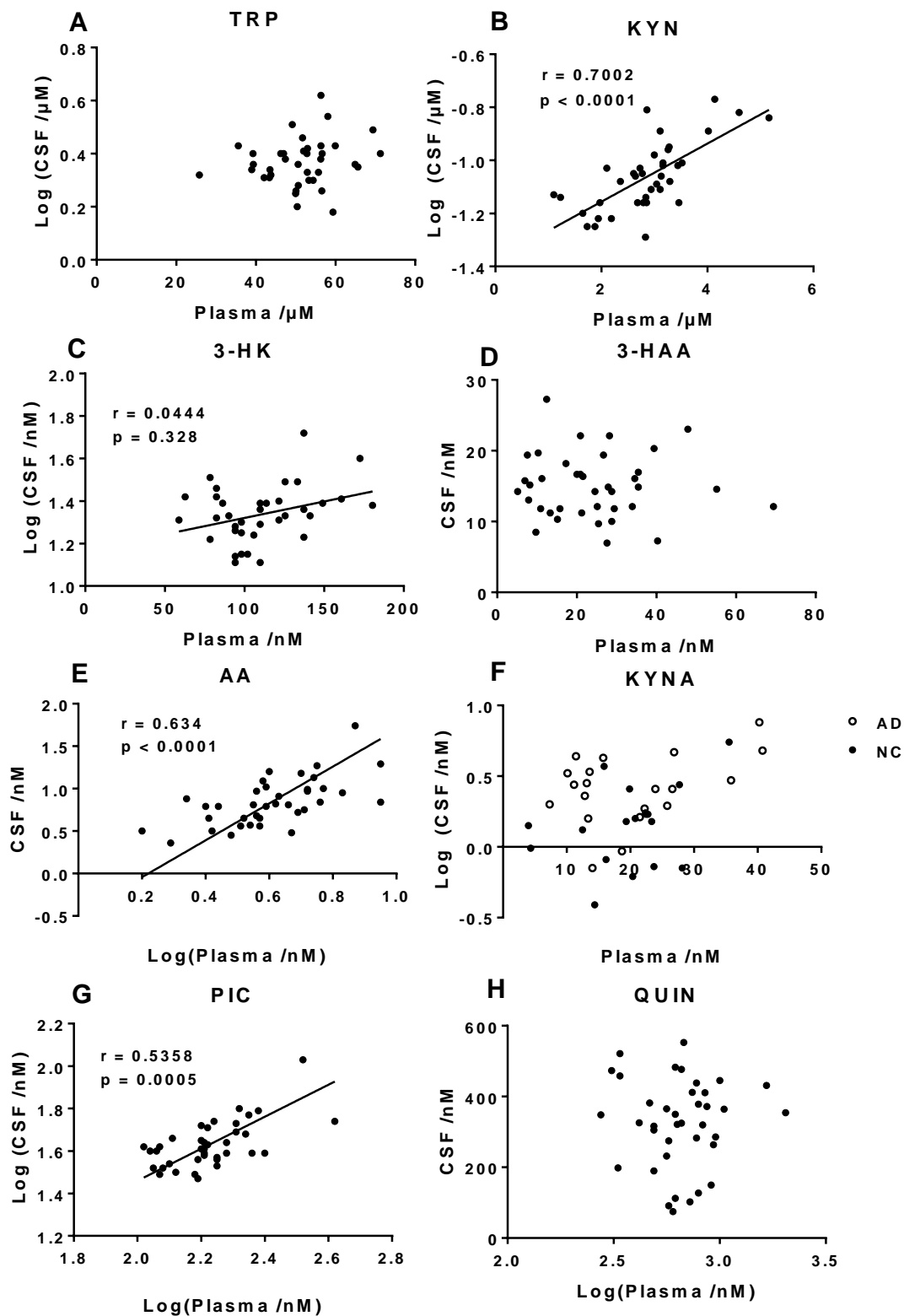


Figure 1: Positive Correlations and Deming Linear regression results between concentrations of KP metabolites in plasma and CSF were found for KYN (B; Kynurenine), 3-HK (C; 3-hydroxykynurenine), AA (E; Anthranilic acid) and PIC (G; Picolinic acid). Significant correlations were not found for TRP (A; Tryptophan), 3-HAA (D; 3-hydroxyanthranilic acid), KYNA (F; Kynurenic acid) and QUIN (H; Quinolinic acid)



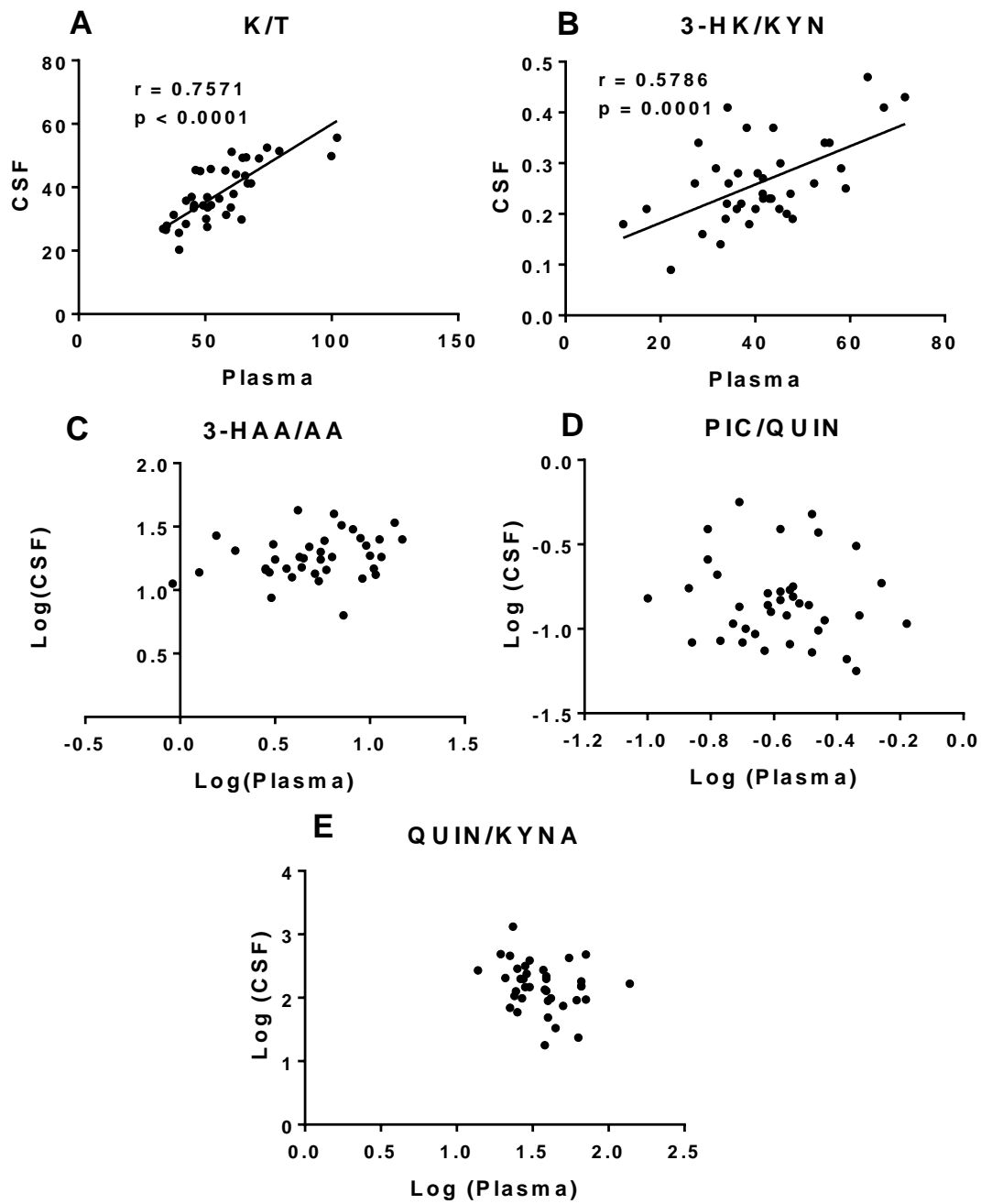


Figure 2: Positive Correlations and Deming Linear regression results between KP product/substrate ratios in plasma and CSF were found for K/T (A) and 3-HK/KYN (B), but not 3-HAA/AA (C), PIC/QUIN (D) and QUIN/KYNA (E).

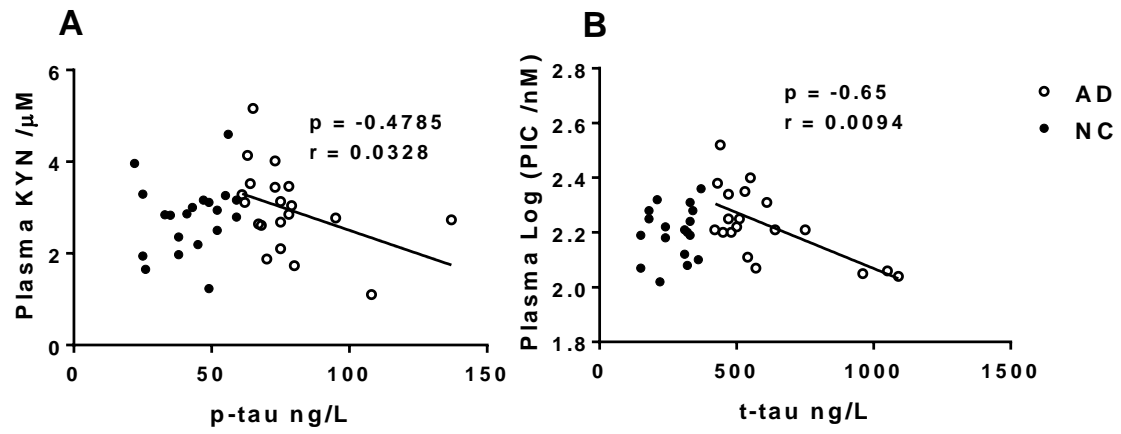


Figure 3: Negative correlations between plasma KYN (A) and PIC (B) and AD biomarkers t-tau and p-tau in AD patient ( $n = 20$ ) plasma. Similar trends were not seen in NCs ( $n = 18$ ).

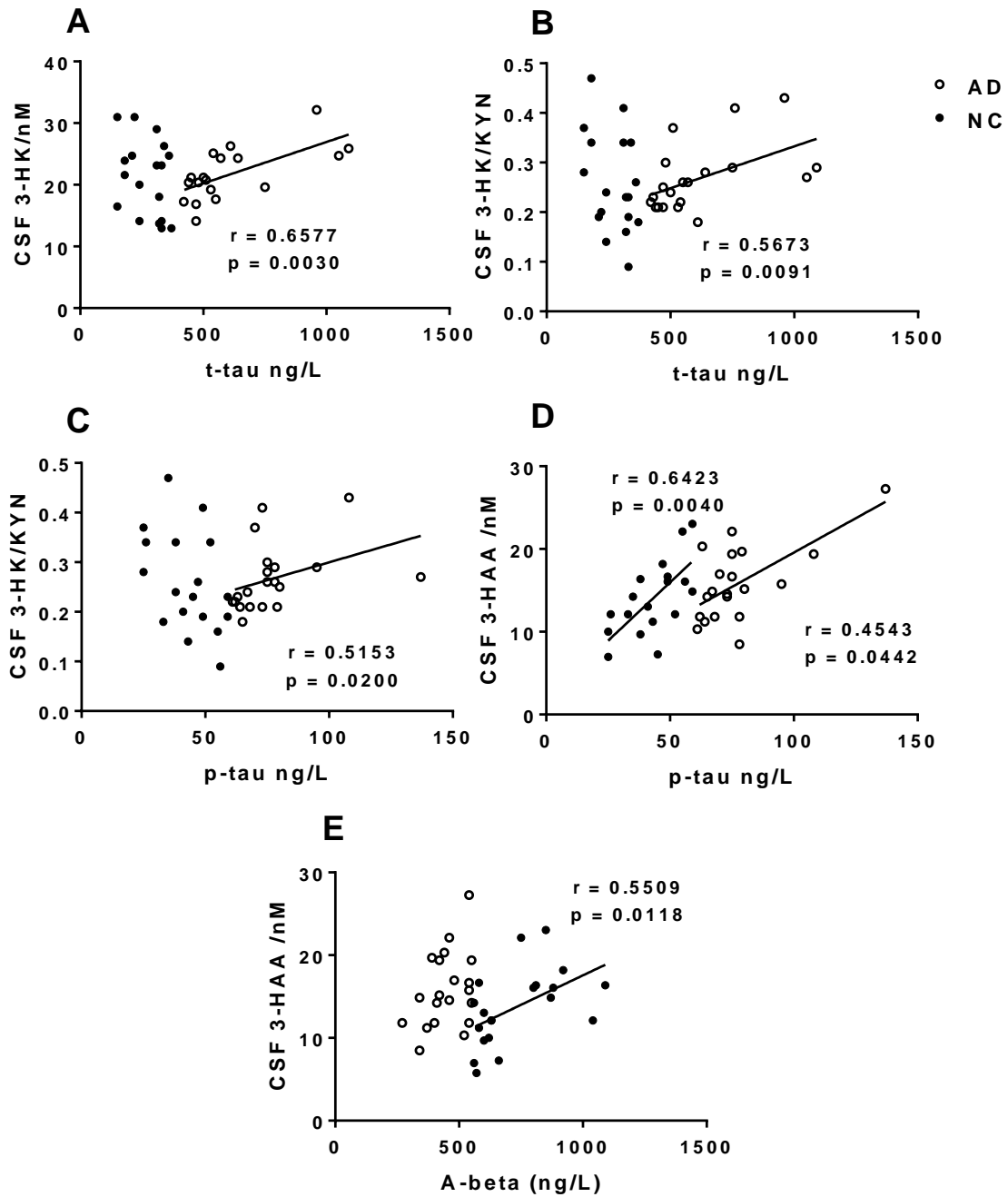


Figure 4: Positive Correlations between CSF 3-HK (A), 3-HK/KYN (B and C) and 3-HAA (D and E) and AD hallmarks t-tau, p-tau and A $\beta$ 42 (A-beta) in AD patients (n = 20) and NC (n = 18). Linear models were fit using Deming linear regression.

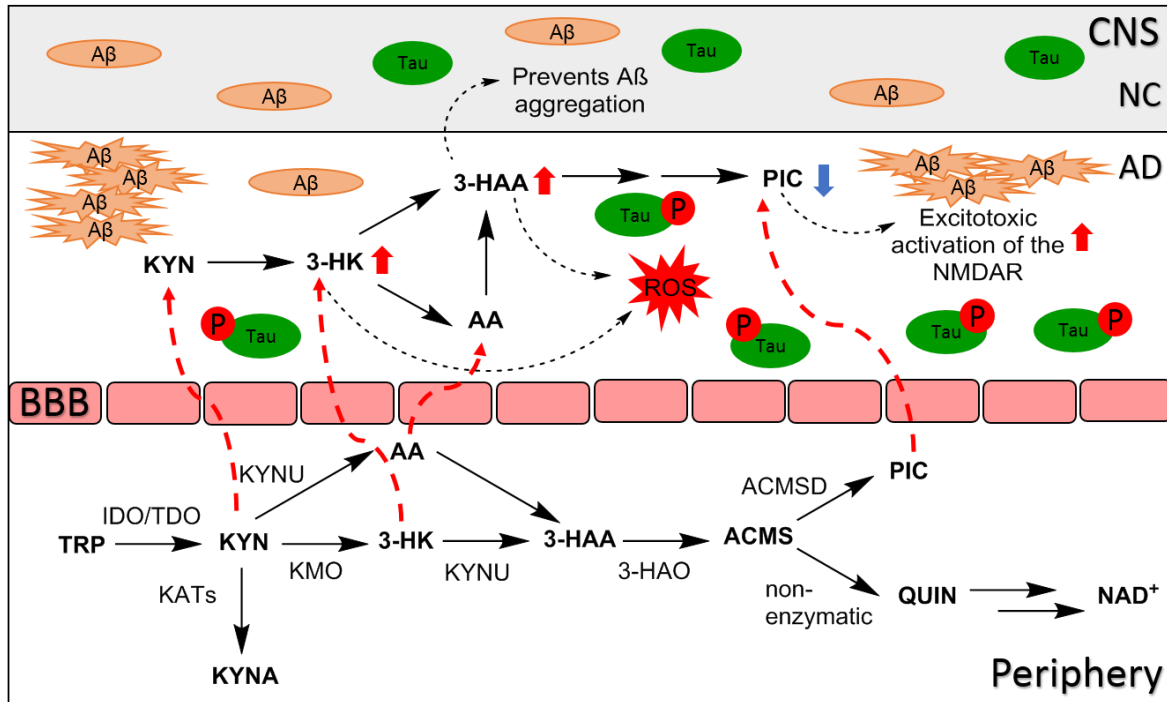


Figure 5: Plasma concentration of kynurenine (KYN), 3-hydroxykynurenine (3-HK), anthranilic acid (AA) and picolinic acid (PIC) were shown to positively correlate with concentrations in the CSF (dashed red arrows). Increased 3-HK/KYN ratio in the CSF, generally indicative of increased kynurenine 3-monooxygenase (KMO) activity was shown to positively correlate with *t*-tau and *p*-tau. Increases in 3-hydroxyanthranilic acid (3-HAA) positively correlated with increases in *p*-tau in both AD and NC. In contrast increased 3-HAA correlated with increased A $\beta$  (and therefore decreased A $\beta$  deposition) in NCs. 3-HK and 3-HAA are reported to contribute to oxidative stress following the production of reactive oxygen species (ROS). 3-HAO = 3-hydroxyanthranilic acid oxygenase; A $\beta$  = amyloid beta; ACMS = 2-amino-3-carboxymuconate semialdehyde; ACMSD = 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; AD = Alzheimer's disease; NC = normal control; IDO = indoleamine 2,3-dioxygenase; KATs = kynurenine aminotransferases; KYNU = kynureninase; KYNA = kynurenic acid; TDO = tryptophan dioxygenase; TRP = tryptophan; QUIN: quinolinic acid.