

REVIEW ARTICLE

The kynurenine pathway in Alzheimer's disease: a meta-analysis of central and peripheral levels

Brisa S. Fernandes,¹ Mehmet Enes Inam,¹ Nitesh Enduru,^{1,2} Joao Quevedo,³ Zhongming Zhao^{1,2,3,4}

¹Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX, USA.

²Department of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX, USA. ³Faillace Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA. ⁴Human Genetics Center, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX, USA.

Objective: Changes in the kynurenine pathway are recognized in psychiatric disorders, but their role in Alzheimer's disease (AD) is less clear. We aimed to conduct a systematic review and meta-analysis to determine whether tryptophan and kynurenine pathway metabolites are altered in AD.

Methods: We performed a systematic review and random-effects meta-analyses. Inclusion criteria were studies that compared AD and cognitively normal (CN) groups and assessed tryptophan or kynurenine pathway metabolites in cerebrospinal fluid or peripheral blood.

Results: Twenty-two studies with a total of 1,356 participants (664 with AD and 692 CN individuals) were included. Tryptophan was decreased only in peripheral blood. The kynurenine-to-tryptophan ratio was only increased in peripheral blood of the AD group. 3-Hydroxykynurenine was decreased only in cerebrospinal fluid and showed higher variability in the CN group than the AD group. Kynurenic acid was increased in cerebrospinal fluid and decreased in peripheral blood. Finally, there were no changes in kynurenine and quinolinic acid between the groups.

Conclusions: Our results suggested a shift toward the kynurenine pathway in both the brain and in the periphery, as well as a shift towards increased kynurenic acid production in the brain but decreased production in peripheral blood. In addition, our analysis indicated dissociation between the central and peripheral levels, as well as between plasma and serum for some of these metabolites. Finally, changes in the kynurenine pathway are suggested to be a core component of AD. More studies are warranted to verify and consolidate our results.

Keywords: Biomarker; kynurenic acid; tryptophan; inflammation; neuroscience

Introduction

Alzheimer's disease (AD) affects 1 in 9 individuals over 65 years of age. Cognitive decline is becoming a major global health and economic hazard. One reason for this high burden is the lack of tests for early detection and insufficient pharmacological treatment, both of which derive from incomplete understanding of the pathophysiology of AD. Changes in the metabolism of tryptophan and its downstream metabolites in the kynurenine pathway have been implicated in neuroinflammation and dementia, including AD,¹ in addition to several psychiatric disorders,²⁻⁴ and could possibly represent new therapeutic targets and be used to guide treatment, enabling precision medicine.^{3,5}

More than 90% of available tryptophan is metabolized via the kynurenine pathway, with the remnant used to

produce serotonin. In the kynurenine pathway, tryptophan is initially metabolized to kynurenine via the rate-limiting enzymes tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase. In the microglia, kynurenine is converted to 3-hydroxykynurenine and then to quinolinic acid; this pathway has been linked with neurotoxicity via free radical generation, increased oxidative stress, and the excitotoxic effects of quinolinic acid as a glutamate N-methyl-D-aspartate (NMDA) receptor agonist. In astrocytes, kynurenine is converted to kynurenic acid, which has neuroprotective potential, both via NMDA and $\alpha 7$ nicotinic acetylcholine ($\alpha 7nACh$) receptor antagonism, as well as through anti-inflammatory and immunosuppressive functions.³ It has been hypothesized that both kynurenic and quinolinic acids are implicated in the regulation of glutamate in AD; accordingly, aging has been related to increased levels of kynurenine, kynurenic

Correspondence: Zhongming Zhao, The University of Texas Health Science Center at Houston School of Biomedical Informatics, 7000 Fannin Street, Suite 600, Houston, TX, 77030, USA.
E-mail: zhongming.zhao@uth.tmc.edu
Submitted Nov 15 2022, accepted Jan 16 2023, Epub May 29 2023.

How to cite this article: Fernandes BS, Inam ME, Enduru N, Quevedo J, Zhao Z. The kynurenine pathway in Alzheimer's disease: a meta-analysis of central and peripheral levels. Braz J Psychiatry. 2023;45:286-297. <http://doi.org/10.47626/1516-4446-2022-2962>

acid, and quinolinic acid in both cerebrospinal fluid (CSF) and blood compartments.⁶ Moreover, positive correlations for kynurenine pathway metabolites and amyloid-beta have been reported, notably for the kynurenine-to-tryptophan ratio. This ratio reflects indoleamine 2,3-dioxygenase activity and thus general activation of the kynurenine pathway.⁷ In addition, higher levels of kynurenic acid are linked to poorer cognition.^{1,8}

The results of studies on tryptophan and kynurenine pathway metabolites in AD are conflicting, with some studies finding them higher but others lower in AD. It is also unknown whether changes in the kynurenine pathway, if existent, are a core feature of AD or if there is heterogeneity in its dysregulation. In addition, not all kynurenine pathway metabolites cross the blood-brain barrier (BBB), and it is unknown whether the concentration of those metabolites in the peripheral blood mirrors the concentrations found in CSF. Thus, we performed a meta-analysis, aiming to determine whether: 1) tryptophan and kynurenine pathway metabolites are altered in AD compared to cognitively normal (CN) individuals; 2) the same changes are found in CSF and peripheral blood; 3) there are differences in plasma and serum compartments; and, finally, 4) whether there are subgroups of kynurenine pathway change in AD or if these changes are more uniformly present. This will help clarify the role of kynurenine pathway metabolites in the pathophysiology of AD and their possible role as biomarkers for guiding treatment and as drug targets in precision medicine.⁵

Methods

This study consists of a series of between-group meta-analyses comparing tryptophan and kynurenine pathway metabolites in AD and CN groups.

We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement.⁹ We conducted a systematic search for all potentially eligible English and non-English peer-reviewed articles to avoid language publication bias using MEDLINE and Embase from inception to March 2022. Two authors (BSF and MEI) performed the literature search, made inclusion decisions, and performed data extraction and quality control.

Study selection

The inclusion criteria for AD groups were: 1) pairwise comparison to a CN control group; and 2) studies assessing tryptophan or kynurenine metabolites (i.e., kynurenine, 3-hydroxykynurenine, kynurenic acid, quinolinic acid, or the kynurenine-to-tryptophan ratio); 3) studies assessing those metabolites in CSF or peripheral blood (serum or plasma). The exclusion criteria were: 1) duplicate reports; 2) lack of a control group; and 3) individuals with mild cognitive impairment. The decision to include studies in the meta-analysis was based on the above criteria through consensus among the authors (one with medical training). Whenever multiple reports pertained to the same groups of patients, we retained only the report with the largest sample size in the meta-analysis to avoid duplicate information.

Data extraction

Two investigators conducted the searches (BSF and MEI). The authors first screened the titles and abstracts according to the eligibility criteria. Articles for which this was unclear were carried forward into the full-text review. The same investigators extracted the data (n, mean, and SD) using a standardized Excel-based data extraction form in accordance with the Cochrane Collaboration Handbook.¹⁰ All disagreements were resolved by consensus. We extracted the following data, among other characteristics: diagnostic criteria, sex, age, medications, metabolite source (i.e., CSF, serum, or plasma), methods of laboratory analysis, country in which the sample was collected, and year of publication. Discrepancies in data entry were double-checked with the original publication and a consensus was reached. When the necessary information was graphically presented, we used the data extraction method of Siström et al.¹¹ Results described as median and interquartile range were converted to mean and SD according to a formula by Wan et al.¹² and Luo et al.¹³

Statistical analyses

Comprehensive Meta-Analysis v2 and R with the metafor package v3.0.2 were used for all meta-analyses. Because the studies used different measurement methods, standardized mean difference (SMD) estimates of metabolite differences between the AD and CN groups were used as the effect size (ES). This method provides an unbiased ES adjusted for small sample sizes. The 95% CI of the ES was also computed. An ES of 0.2 indicates a small effect, i.e., a small difference between subjects and controls, 0.5 indicates a moderate effect, and 0.8 a large effect. Meta-analysis was performed only when at least two studies examined a given metabolite. Random effect modeling assumes genuine diversity in the results of various studies and incorporates between-study variance in the calculations.¹⁰ In the between-group meta-analyses, the direction of the ES was positive if the AD group had higher metabolite values than the CN group and was negative when they were lower. Two-sided p values < 0.05 were considered statistically significant.

Studies with negative results are less likely to be published than those with positive results.¹⁴ To account for significant publication bias, we analyzed a funnel plot graph, a scatter plot of the ES against a measure of study size, using the Egger test when the number of studies in a given meta-analysis was ten or more, as per Cochrane Collaboration recommendations.^{15,16} Sensitivity analyses were conducted to ascertain whether the results of our analyses were strongly influenced by any single study. We performed sensitivity analyses and leave-one-out analyses to determine whether the results were due to a unique outlier, with the overall significance recomputed after removing individual studies. Duval and Tweedie's "trim-and-fill" method was applied to the random-effects models to re-calculate the pooled ES to account for any studies that could introduce publication bias. We assessed the heterogeneity across studies using Cochran's Q test, a weighted sum of the squares of the deviations of

individual ES estimates from the overall estimate, with $p < 0.10$ considered generally significant (i.e., showing heterogeneity).¹⁷ Inconsistency across studies was then quantified with the I^2 statistic, which can be interpreted as the percentage of total variation across several studies due to heterogeneity; it is considered moderate when between 50 and 75% and high when $> 75\%$.¹⁸ Since the analyses showed that the studies were heterogeneous, we pooled ES results from different studies using an inverse variance method that accounts for random effects, which allows population-level inferences and is more stringent than fixed-effect models.¹⁹ Risk of bias in the studies was analyzed using the Newcastle-Ottawa Scale, which assesses the quality of observational studies included in a meta-analysis.²⁰

Meta-analysis of variance

To measure variability and thus ascertain the existence of possible subgroups, we calculated the log variability ratio using, for each group, the natural log of the ratio of SD estimates; since variance commonly scales with the mean in biological systems, between-group differences in relative variability may, at least in part, be a function of between-group differences in the mean. Therefore, meta-analyses of the relative variability of metabolites in individuals with AD compared to individuals with CN were scaled to group means using the log coefficient of variation ratio (CVR) (the natural logarithm of the ratio of estimates of population coefficients of variation).²¹ When the mean is greater in the AD group than the CN group, CVR is a more conservative estimate of variability. To aid interpretation, summary ES for $\ln VR$ and $\ln CVR$ were transformed back to a linear scale. Thus, a CVR of 1 indicates no difference in variability between AD and CN, a CVR > 1 indicates greater relative variability in the AD group, and a CVR < 1 indicates lower relative variability in the AD group.

Results

The search strategy resulted in 1,149 non-duplicate studies, all of which were screened (Box S1, available as online-only supplementary material). A total of 115 went to full-text review, of which 22, comprising 1,356 participants (664 AD and 692 CN), were included (Figure S1, available as online-only supplementary material).^{1,22-43} Eight studies provided data on CSF^{1,31,33,34,38-41} and 17 on serum or plasma.^{1,23-33,35-37,42,43} Sample sizes for each study varied from 9 to 137. The mean participant age varied from 64 to 80 years old (Table S1, available as online-only supplementary material). The quality of the studies, according to the Newcastle-Ottawa Scale, varied from 1 to 6, with 10 studies reaching a score of 5 or more, denoting a low risk of bias (Table S2).

Tryptophan and kynurenine pathway metabolites in AD: discrepancy between central and peripheral levels

Altered peripheral levels of the kynurenine pathway were not mirrored in the CNS. Tryptophan was not altered in

CSF (SMD = -0.67, 95%CI -1.43-0.10, $p = 0.088$, $k = 6$, $Q = 25.35$, $I^2 = 84.50$, $n=221$), but was decreased in peripheral blood (SMD = -0.82, 95%CI -1.12 to -0.51, $p < 0.001$, $k = 16$, $Q = 91.66$, $I^2 = 79.60$, $n=1,086$). Interestingly, central levels of kynurenic acid were higher in the AD group than the CN group (SMD = 0.70, 95%CI 0.10-1.30, $p = 0.021$, $k = 3$, $Q = 6.09$, $I^2 = 66.80$, $n=161$), while peripheral levels were lower (SMD = -0.35, 95%CI -0.59 to -0.12, $p = 0.005$, $k = 8$, $Q = 12.58$, $I^2 = 43.70$, $n=597$). 3-Hydroxykynurenine levels were lower in CSF (SMD = -1.17, 95%CI -2.32 to -0.02, $p = 0.045$, $k = 3$, $Q = 11.87$, $I^2 = 82.40$, $n=87$) but not peripheral blood (SMD = 0.06, 95%CI -0.53-0.64, $p = 0.85$, $k = 7$, $Q = 46.69$, $I^2 = 90.80$, $n=613$). Kynurenine levels were unaltered centrally (SMD = -0.56, 95%CI -1.58-0.35, $p = 0.229$, $k = 4$, $Q = 15.07$, $I^2 = 80.40$, $n=109$) and peripherally (SMD = 0.03, 95%CI -0.27-0.33, $p = 0.81$, $k = 10$, $Q = 36.53$, $I^2 = 72.50$, $n=744$). Similarly to kynurenine, quinolinic acid levels were unaltered in any compartment (SMD = -0.16, 95%CI -0.55-0.23, $p = 0.412$, $k = 2$, $Q = 0.21$, $I^2 = 0$, $n=102$ in CSF; SMD = -0.04, 95%CI -0.60-0.53, $p = 0.86$, $k = 5$, $Q = 24.14$, $I^2 = 86.80$, $n=448$) of peripheral blood. Finally, the kynurenine-to-tryptophan ratio (kynurenine/tryptophan) was increased in peripheral blood (SMD = 0.54, 95%CI 0.03-1.04, $p = 0.03$, $k = 7$, $Q = 40.45$, $I^2 = 85.80$, $n=525$), suggesting activation of the kynurenine pathway, but not in CSF (SMD = 0.14, 95%CI -1.28-1.55, $p = 0.851$, $k = 2$, $Q = 7.13$, $I^2 = 86.00$, $n=62$) (Table 1, Figures 1 and 2).

When analyzing the metabolites in peripheral blood according to source (i.e., plasma vs. serum), we found that tryptophan remained significantly lower in both plasma and serum, with large and moderate effects, respectively. On the other hand, kynurenic acid remained lower and the kynurenine-to-tryptophan ratio remained higher only in plasma. Finally, quinolinic acid was significantly lower in serum, although this specific analysis only included two studies (Table 1).

Tryptophan and kynurenine pathway metabolite subgroups in AD

To verify the presence of subgroups related to tryptophan and kynurenine pathway metabolites, we performed meta-analyses of variance using the CVR. In the main meta-analyses, we found variation differences for 3-hydroxykynurenine in the CSF, with reduced variability in AD (CVR = 0.66, 95%CI 0.46-0.95, $p = 0.02$, $k = 3$, $n=87$), which suggests that this change is a more uniform feature in AD. Regarding peripheral blood, there was a trend towards significance in tryptophan, with higher variability in AD (CVR = 1.21, 95%CI 0.98-1.49, $p = 0.07$, $k = 16$, $n=1,086$), which might suggest the occurrence of subgroups in AD. There were no differences in any of the other metabolites in the CVR analyses (Figures 3 and 4).

Publication bias and sensitivity analyses

Since only the analyses of tryptophan and kynurenine in peripheral blood included ≥ 10 studies, the funnel plot

Table 1 Between-group meta-analyses of central and peripheral levels of the kynurenine pathway in AD

Group-wise Between-group meta-analyses	Subjects (n)		Heterogeneity		Meta-analysis		Egger's test p-value				
	Pairwise (n)	AD	CN	Q	I ²	p-value		95%CI	SMD	p-value	
Cerebrospinal fluid											
Tryptophan	6	136	85	25.35	84.50	< 0.001*		-0.67	-1.43-0.10	0.088	0.009*
Kynurenine	4	62	47	15.07	80.40	0.001*		-0.56	-1.58-0.35	0.229	0.037*
3-Hydroxykynurenine	3	49	38	11.87	82.40	0.002*		-1.17	-2.32 to -0.02	0.045*	0.035*
Kynureninic acid	3	100	61	6.09	66.80	0.050		0.70	0.10-1.30	0.021*	0.313
Quinolinic acid	2	55	47	0.21	0.00	0.650		-0.16	-0.55-0.23	0.412	-
Kynurenine/tryptophan	2	34	28	7.13	86.00	0.006*		0.14	-1.28-1.55	0.851	-
Peripheral blood (serum and plasma)											
Tryptophan	16	505	581	91.66	79.60	< 0.001*		-0.82	-1.12 to -0.51	< 0.001*	0.819
Plasma	9	284	308	63.65	87.43	< 0.001*		-0.99	-1.54 to -0.45	< 0.001*	-
Serum	7	221	273	13.22	54.61	0.040*		-0.61	-0.91 to -0.30	< 0.001*	-
Kynurenine	10	355	389	36.53	72.50	< 0.001*		0.03	-0.27-0.33	0.845	< 0.001*
Plasma	5	163	147	14.65	72.70	0.005*		0.23	-0.23-0.69	0.330	-
Serum	5	192	242	15.03	73.38	0.005*		-0.15	-0.57-0.27	0.490	-
3-Hydroxykynurenine	7	290	323	46.69	90.80	< 0.001*		0.06	-0.53-0.64	0.847	0.007*
Plasma	3	119	101	9.23	78.34	0.010*		-0.18	-0.82-0.46	0.587	-
Serum	4	171	222	37.83	92.07	< 0.001*		0.24	-0.61-1.08	0.584	-
Kynureninic acid	8	316	281	12.58	43.70	0.074*		-0.35	-0.59 to -0.12	0.003*	0.689
Plasma	5	173	152	7.09	43.57	0.131		-0.53	-0.84 to -0.21	0.001*	-
Serum	5	143	129	0.13	0.00	0.940		-0.13	-0.36-0.11	0.307	-
Quinolinic acid	5	242	206	24.14	86.80	< 0.001*		-0.04	-0.60-0.53	0.893	0.160
Plasma	3	119	101	18.78	85.35	< 0.001*		0.24	-0.68-1.17	0.608	-
Serum	2	123	105	0.36	0.00	0.549		-0.48	-0.74 to -0.21	< 0.001*	-
Kynurenine/tryptophan	7	279	246	40.45	85.80	< 0.001*		0.54	0.03-1.04	0.037*	0.004*
Plasma	4	135	116	22.58	86.72	< 0.001*		0.83	0.04-1.63	0.040*	-
Serum	3	144	130	10.34	80.66	0.006*		0.20	-0.45-0.85	0.551	-

Bold type denotes meta-analysis p < 0.05.

AD = Alzheimer's disease; CN = cognitively normal; SMD = standardized mean difference.

* p < 0.05.

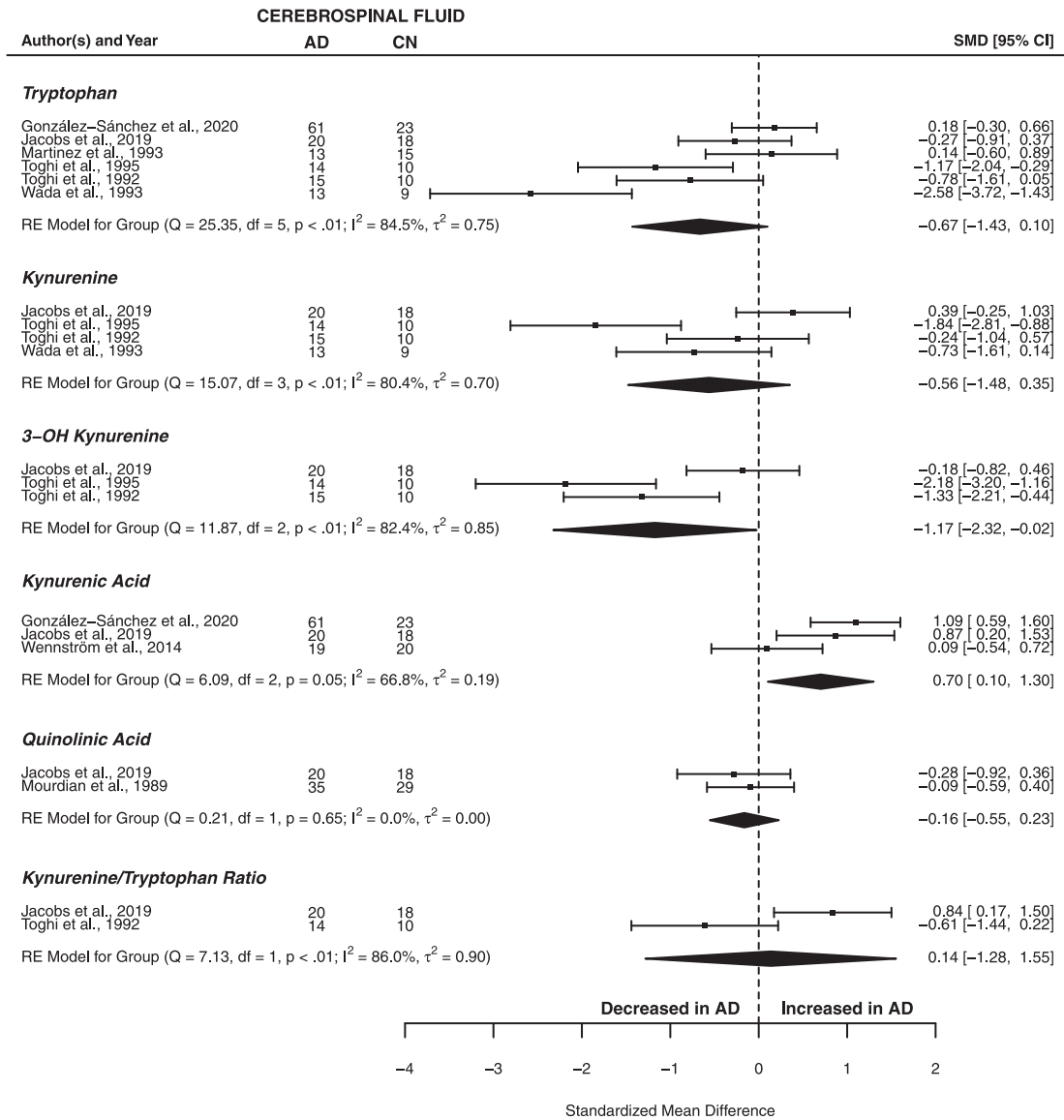


Figure 1 Forest plots with the summary effect size standardized mean difference [SMD] of kynurenine metabolites in cerebrospinal fluid in individuals with Alzheimer's disease (AD) compared to cognitively normal (CN) controls.

was not inspected for most metabolites, as per Cochrane guidelines. The Egger's test was significant, suggesting publication bias for tryptophan, kynurenine, and 3-hydroxykynurenine in CSF, and for kynurenine, and 3-hydroxykynurenine in peripheral blood. Although the funnel plot and the trim-and-fill procedure suggested a missing study for kynurenine in peripheral blood, the overall significance did not change after this potentially missing study was imputed. For tryptophan and kynurenic acid in the CSF, the trim-and-fill procedure suggested no missing study. However, for 3-hydroxykynurenine, the result was no longer significant after the potentially missing studies were imputed (Figures S2-S11, available as online-only supplementary material).

The heterogeneity of the whole analysis was moderate to high in all groups, with the exception of quinolinic acid

in CSF and kynurenic acid in peripheral blood. Thereafter, we conducted a sensitivity analysis in the meta-analyses, excluding studies one at a time to determine whether a particular study was responsible for the high heterogeneity. In the leave-one-out analyses, quinolinic acid in blood decreased significantly when Gulaj et al.²⁹ was excluded, and 3-hydroxykynurenine in blood also became significantly lower when Jacobs et al.³¹ was excluded. In peripheral blood, the kynurenine-to-tryptophan ratio also lost significance in several instances. In CSF, tryptophan became significant when Gonzalez-Sanchez et al.¹ was excluded. Kynurenic acid lost significance when Gonzalez-Sanchez et al.,¹ as well as when Jacobs et al.,³¹ were excluded. No other changes were detected in the leave-one-out analyses (Tables S3-12, available as online-only supplementary material).

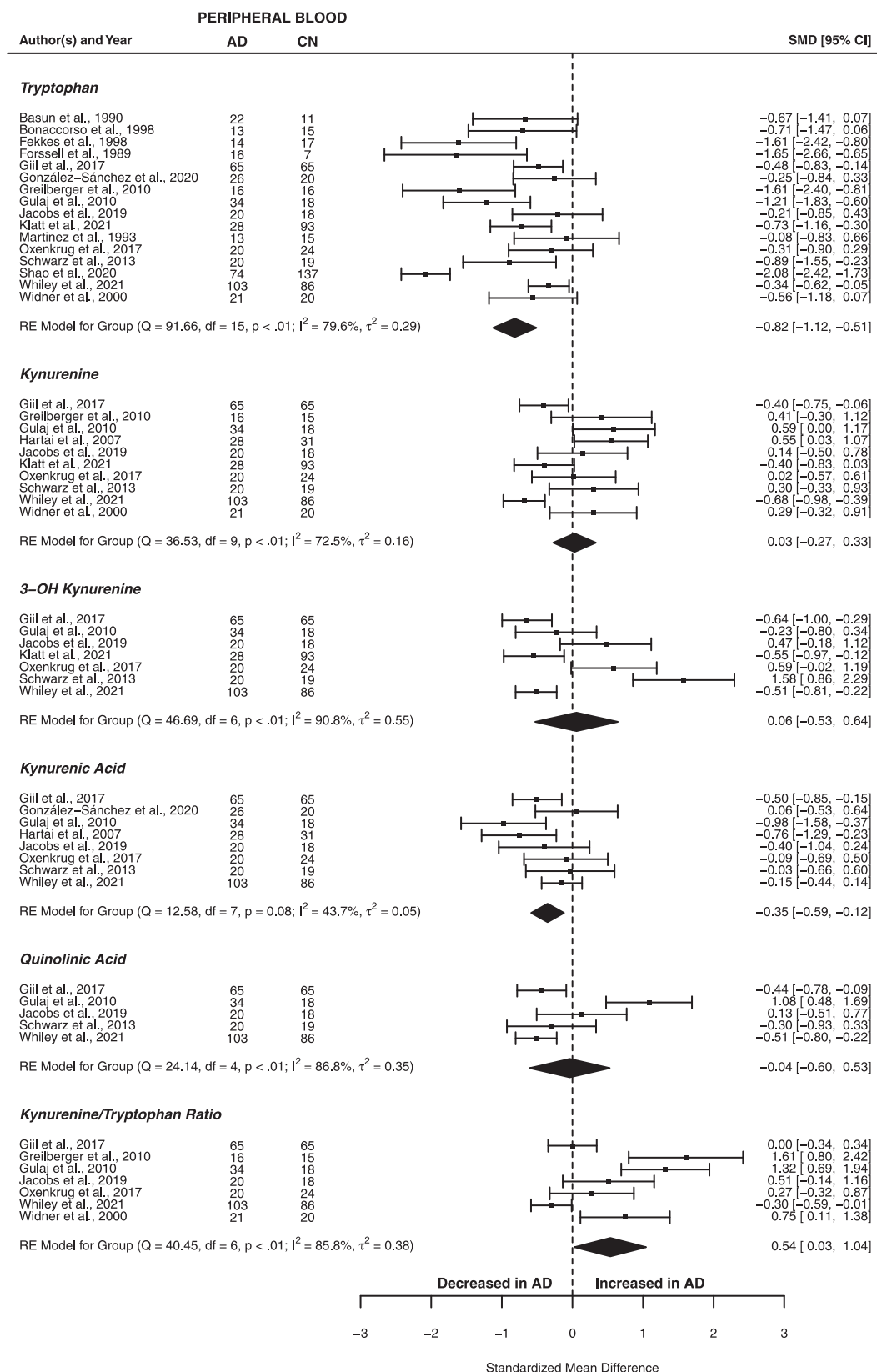


Figure 2 Forest plots with the summary effect size (standardized mean difference [SMD]) of kynurenine metabolites in peripheral blood (plasma and serum) in individuals with Alzheimer's disease (AD) compared to cognitively normal (CN) controls.

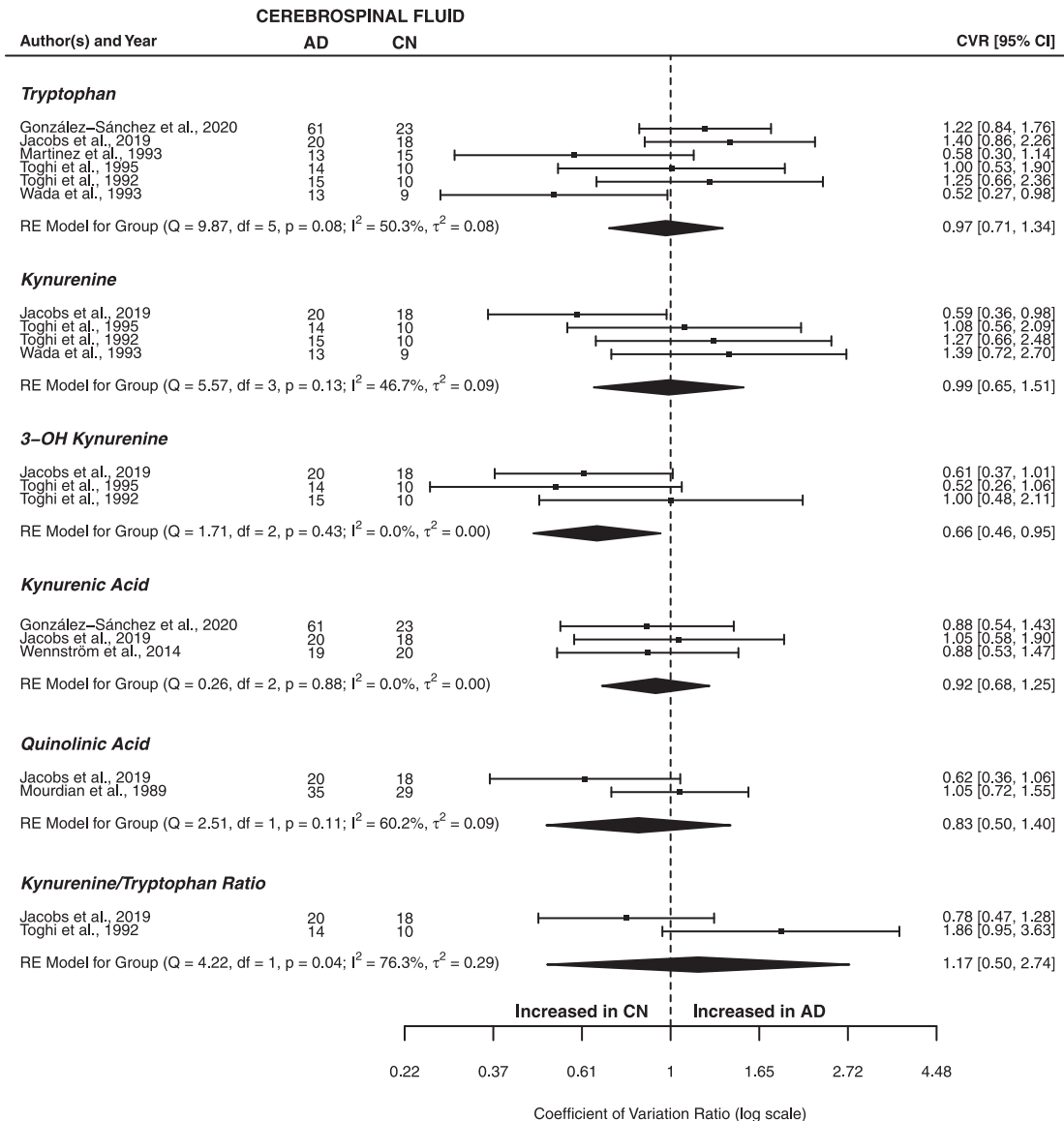


Figure 3 Variability of tryptophan and kynurenine pathway metabolites in a forest plot, showing summary effect sizes for the coefficient of variation ratio (CVR) of tryptophan and kynurenine pathway metabolites in cerebrospinal fluid. AD = Alzheimer's disease; CN = cognitively normal.

Discussion

Our meta-analyses of kynurenine pathway metabolites in AD included 22 studies with 1,356 participants (664 AD, 692 CN). Overall, there was a trend towards a shift in the kynurenine pathway metabolism, particularly regarding kynurenic acid production. Tryptophan was lower and the kynurenine-to-tryptophan ratio was higher only in peripheral blood in the AD group. 3-Hydroxykynurenine was lower only in CSF; there were no differences in kynurenine and quinolinic acid levels between the AD and CN groups. Importantly, regarding kynurenic acid, there was dissociation between the findings in CSF and peripheral blood, with kynurenic acid being higher in CSF and lower in peripheral blood. These findings show that peripheral levels of kynurenine pathway metabolites do

not exactly mirror central levels (Figure 5). Furthermore, even in peripheral blood, our subgroup analyses suggest that kynurenine pathway metabolite levels might vary between plasma and serum.

Shifting towards the kynurenine pathway and kynurenic acid in AD

Although the results of our meta-analyses are insufficient to draw definitive conclusions, there was a trend towards a shift in the kynurenine pathway in AD, both centrally and peripherally. Centrally, downstream kynurenine appears to shift towards kynurenic acid production, which does not occur in peripheral blood.

Kynurenic acid acts as an antagonist of NMDA and $\alpha 7$ nACh receptors. Activation of the NMDA and $\alpha 7$ nACh

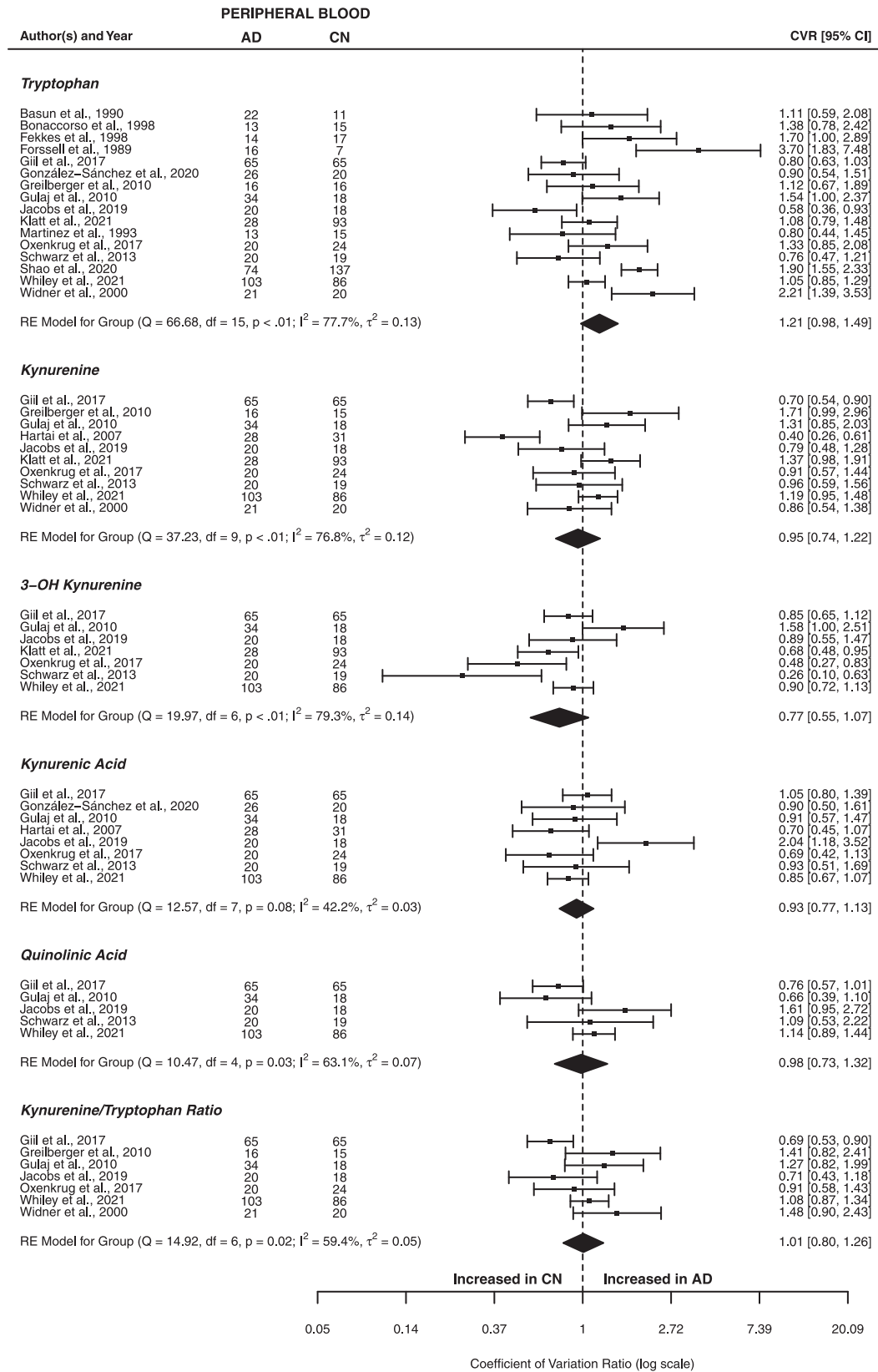


Figure 4 Variability of tryptophan and kynurenine pathway metabolites in Alzheimer's disease (AD). Forest plot showing summary effect sizes for the coefficient of variation ratio (CVR) of tryptophan and kynurenine pathway metabolites in peripheral blood (serum and plasma). CN = cognitively normal.

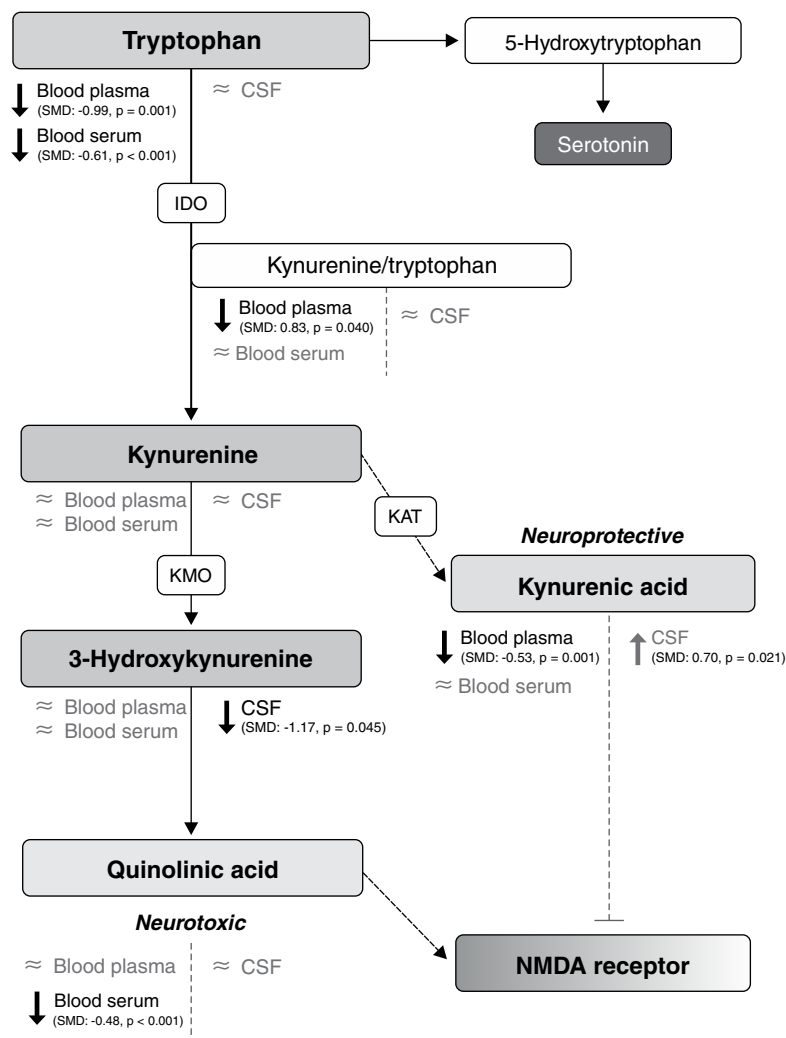


Figure 5 Summary representation of altered kynurenine pathway metabolites in Alzheimer's disease (AD). CSF = cerebrospinal fluid; SMD = standardized mean difference. IDO = indoleamine 2,3-dioxygenase; KAT = kynurenine aminotransferase; KMO = kynurenine 3-monooxygenase. ↓ Significantly lower levels of metabolites or ratios in the AD group than the cognitively normal (CN) group. ↑ Significantly higher levels of metabolites or ratios in AD than CN. ≈ Non-significant difference between AD and CN.

receptors initiates excitatory glutamatergic neurotransmission and is essential for synaptic plasticity and, hence, for memory and cognitive function.^{44,45} When even low concentrations of kynurenic acid are administered in the brain, glutamate levels decrease.⁴⁶ Although excess glutamate is neurotoxic, physiological levels are necessary to maintain synaptic plasticity. Accordingly, in animal models, increased kynurenic acid can result in cognitive abnormalities and affect learning and memory, while decreased kynurenic acid improves cognitive function.^{8,47} Thus, as seen in our meta-analysis, increased kynurenic acid in the brain might impair cognition and could be related to the memory and cognitive deficits that occur in AD.

Kynurenine pathway metabolites: the periphery does not mirror the brain

Not all kynurenine pathway metabolites cross the BBB.⁴⁸ It appears that peripheral levels of tryptophan are

connected to central levels, regardless of whether blood tryptophan is bound to albumin or not.⁴⁹ Kynurenine and 3-hydroxykynurenine can also cross the BBB. Conversely, the BBB is poorly permeable to both kynurenic and quinolinic acids, although passive diffusion occurs.⁵⁰ Such dissociation was reflected in our meta-analyses. Tryptophan, which is obtained through diet, was decreased in peripheral blood but not CSF, although there was a trend toward decreased tryptophan levels in CSF as well. However, tryptophan became significantly decreased in the leave-one-out analyses when a particular study was excluded. 3-Hydroxykynurenine, which can cross the BBB as well, was only decreased in CSF but, as in tryptophan, dropped significantly in blood when a particular study was excluded from the analysis. The kynurenine-to-tryptophan ratio was increased only in peripheral blood, showing a shift towards the kynurenine pathway in blood, but not the brain. However, the latter

analysis should be viewed with caution, since it included only two studies on the brain. Surprisingly, the opposite pattern occurred in kynurenic acid, i.e., increased in CSF but decreased in peripheral blood (plasma only). The fact that kynurenic acid was decreased in peripheral blood may indicate a shift toward toxic metabolites and away from neuroprotective ones in AD. In an inflammatory environment, kynurenic acid acts as an anti-inflammatory metabolite by reducing tumor necrosis factor alpha expression and Th17 cell differentiation.^{48,51} Kynurenic acid can also abort reactive oxygen species production, preventing tissue damage caused by excessive inflammation. Thus, a lower level of kynurenic acid in AD might indicate an insufficient anti-inflammatory response and consequent tissue damage in the periphery.^{48,52} Our findings indicate that strong caution should be exercised when inferring brain levels of kynurenine pathway metabolites based on those found in blood, since blood appears to poorly reflect their activity in the brain. Thus, peripheral blood levels of these metabolites are not a surrogate for brain levels, and according to the premises of precision medicine, blood is limited as a guide for pharmacological interventions.

Peripheral blood metabolites of the kynurenine pathway: plasma vs. serum

Regarding peripheral blood, there appears to be dissociation between plasma and serum levels. Only tryptophan was altered in both plasma and serum. In this case, it was decreased in AD, with a large ES in plasma and a moderate one in serum. Kynurenine and 3-hydroxykynurenine were unaltered, regardless of blood source. Kynurenic acid was decreased and the kynurenine-to-tryptophan ratio was increased in peripheral blood, although only in plasma. Conversely, quinolinic acid was only altered in serum, not plasma. However, again, caution should be exercised regarding this analysis since it consisted of only two studies. This dissociation between plasma and serum might be due to the clotting process that occurs in serum, a phenomenon in which both the sequestration and release of metabolites from platelets can occur.⁵³ Our findings suggest that peripheral blood is not a monolithic entity regarding the kynurenine pathway and that individualization in blood sources is necessary. It appears that more pronounced metabolite changes occur in plasma than serum. Future studies must take the blood compartment into consideration when analyzing kynurenine pathway metabolites. This is particularly important when these metabolites are to be clinically used in precision medicine for AD.^{3,5}

3-hydroxykynurenine as a core component in AD: meta-analyses of variation

If changes in the kynurenine pathway occur only in a subgroup of individuals with AD, then greater variability would be found in AD than CN, reflecting heterogeneity in the dysregulation of the kynurenine pathway. However, if dysregulation of the kynurenine pathway is a central component of the pathophysiology of AD, there would be

lower metabolite variability in AD than CN, reflecting homogeneity in the pathway's dysregulation.²¹ We found that the 3-hydroxykynurenine in CSF CVR levels were higher in CN than AD, with significantly lower variability in AD, suggesting that this metabolite is a more uniformly present, or even a central, element in the pathophysiology of AD. It should be noted that we found no changes in the CVR of other metabolites in CSF or peripheral blood, except a trend toward higher tryptophan variability in blood in the AD group, which might indicate differences in diet, since tryptophan is obtained entirely from food. Thus, our finding of lower 3-hydroxykynurenine variability in AD may support the hypothesis that a shift in tryptophan towards the kynurenine pathway and kynurenic acid in the brain, which can be inferred by lower 3-hydroxykynurenine levels, is a general component in AD.

Limitations

Since our analysis was based on a moderate sample size (22 studies with 1,356 participants), it involves several limitations. First and foremost, some analyses might have failed to achieve statistical significance due to a lack of power, leading to a false negative result. This might have particularly been the case for analyses involving CSF and subgroup analyses according to blood compartment (serum vs. plasma). In addition, the overall significance changed for some metabolites in the sensitivity analyses. For instance, tryptophan became significant in CSF when one study was excluded. Thus, more studies in CSF and peripheral blood (both plasma and serum) are necessary to draw firmer conclusions. Second, the meta-analyses compared AD and CN groups, providing pooled results from cross-sectional studies; therefore, we cannot draw any causal associations. Third, tryptophan is obtained through diet, and dietary information was missing from all included studies. Fourth, the majority of studies provided no information on AD severity. Thus, we could not take it into consideration in our analyses. Accordingly, we could not determine whether greater AD severity translates into more accentuated changes in kynurenine pathway metabolites.

Conclusions

This study provides meta-analytic evidence of changes in tryptophan and kynurenine pathway metabolites in AD. Our results suggest a shift toward the kynurenine pathway in both the brain and in the periphery, as well as a shift towards higher kynurenic acid production in the brain and lower production in peripheral blood. Our analysis also indicated dissociation between central and peripheral levels, as well as between plasma and serum levels of some of these metabolites. Finally, kynurenine pathway changes appear to be a core component in AD. Further studies are warranted to verify and consolidate our results.

Acknowledgements

This study was partially supported by National Institutes of Health grants (R01LM012806 and R03AG077191).

The authors are grateful for technical support from the Cancer Genomics Core and Data Science and Informatics Core, which is funded by the Cancer Prevention and Research Institute of Texas (CPRIT RP180734 and RP170668).

Disclosure

JQ has conducted clinical research at LivaNova and Janssen, has been a speaker for Myriad Neuroscience and AbbVie, has equity at the Instituto de Neurociencias Dr. Joao Quevedo, and receives book royalties from Artmed Editora, Artmed Panamericana, and Elsevier/Academic Press. The other authors report no conflicts of interest.

References

- González-Sánchez M, Jiménez J, Narváez A, Antequera D, Llamas-Velasco S, Martín AH, et al. Kynurenic acid levels are increased in the csf of Alzheimer's disease patients. *Biomolecules*. 2020;10:571.
- Serafini G, Adavastro G, Canepa G, Capobianco L, Conigliaro C, Pittaluga F, et al. Abnormalities in kynurenic pathway metabolism in treatment-resistant depression and suicidality: a systematic review. *CNS Neurol Disord Drug Targets*. 2017;16:440-53.
- Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenic pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Mol Psychiatry*. 2021;26:4158-78.
- Ullah I, Awan HA, Aamir A, Diwan MN, Filippis R, Awan S, et al. Role and perspectives of inflammation and c-reactive protein (CRP) in psychosis: an economic and widespread tool for assessing the disease. *Int J Mol Sci*. 2021;22:13032.
- Fernandes BS, Williams LM, Steiner J, Leboyer M, Carvalho AF, Berk M. The new field of 'precision psychiatry'. *BMC Med*. 2017;15:80.
- Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenic pathway in Parkinson's and Alzheimer's disease. *J Neurochem*. 2019;151:656-68.
- Chatterjee P, Zetterberg H, Goozee K, Lim CK, Jacobs KR, Ashton NJ, et al. Plasma neurofilament light chain and amyloid- β are associated with the kynurenic pathway metabolites in preclinical Alzheimer's disease. *J Neuroinflammation*. 2019;16:186.
- Pocivavsek A, Wu HQ, Potter MC, Elmer GI, Pellicciari R, Schwarcz R. Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. *Neuropsychopharmacology*. 2011;36:2357-67.
- Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
- Higgins JPT, Green S. *Cochrane handbook for systematic reviews of interventions*. Version 5.1.0 [Internet]. The Cochrane Collaboration; 2011. <https://handbook-5-1.cochrane.org/>
- Sistrom CL, Mergo PJ. A simple method for obtaining original data from published graphs and plots. *AJR Am J Roentgenol*. 2000;174:1241-4.
- Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. 2014;14:135.
- Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res*. 2018;27:1785-805.
- Egger M, Zellweger-Zahner T, Schneider M, Junker C, Lengeler C, Antes G. Language bias in randomised controlled trials published in English and German. *Lancet*. 1997;350:326-9.
- Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*. 2000;56:455-63.
- Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629-34.
- Bowden J, Tierney JF, Copas AJ, Burdett S. Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med Res Methodol*. 2011;11:41.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557-60.
- Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med*. 1997;127:820-6.
- Lo CK, Mertz D, Loeb M. Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. *BMC Med Res Methodol*. 2014;14:45.
- Pillinger T, Osimo EF, Brugger S, Mondelli V, McCutcheon RA, Howes OD. A meta-analysis of immune parameters, variability, and assessment of modal distribution in psychosis and test of the immune subgroup hypothesis. *Schizophr Bull*. 2019;45:1120-33.
- Baran H, Jellinger K, Deecke L. Kynurenic metabolism in Alzheimer's disease. *J Neural Transm (Vienna)*. 1999;106:165-81.
- Basun H, Forsell LG, Almkvist O, Cowburn RF, Eklof R, Winblad B, et al. Amino acid concentrations in cerebrospinal fluid and plasma in Alzheimer's disease and healthy control subjects. *J Neural Transm Park Dis Dement Sect*. 1990;2:295-304.
- Bonaccorso S, Lin A, Song C, Verkerk R, Kenis G, Bosmans E, et al. Serotonin-immune interactions in elderly volunteers and in patients with Alzheimer's disease (DAT): lower plasma tryptophan availability to the brain in the elderly and increased serum interleukin-6 in DAT. *Aging (Milano)*. 1998;10:316-23.
- Fekkes D, van der Cammen TJ, van Loon CP, Verschoor C, van Harskamp F, Koning I, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. *J Neural Transm (Vienna)*. 1998;105:287-94.
- Forsell LG, Eklof R, Winblad B. Early stages of late onset Alzheimer's disease. II. Derangements in protein metabolism with special reference to tryptophan, tyrosine and cystine. *Acta Neurol Scand*. 1989;79:27-42.
- Gill LM, Midtun Ø, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenic pathway metabolites in Alzheimer's disease. *J Alzheimer's Dis*. 2017;60:495-504.
- Greilberger J, Fuchs D, Leblhuber F, Greilberger M, Wintersteiger R, Tafeit E. Carbonyl proteins as a clinical marker in Alzheimer's disease and its relation to tryptophan degradation and immune activation. *Clin Lab*. 2010;56:441-8.
- Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenic and its metabolites in Alzheimer's disease patients. *Adv Med Sci*. 2010;55:204-11.
- Hartai Z, Juhász A, Rimanóczy A, Janáky T, Donkó T, Dux L, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. *Neurochem Int*. 2007;50:308-13.
- Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenic pathway metabolites in Alzheimer's disease and relationship to amyloid- β and tau. *Neurobiol Aging*. 2019;80:11-20.
- Klatt S, Doecke JD, Roberts A, Boughton BA, Masters CL, Horne M, et al. A six-metabolite panel as potential blood-based biomarkers for Parkinson's disease. *npj Parkinsons Dis*. 2021;7:94.
- Martinez M, Frank A, Diez-Tejedor E, Hernanz A. Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. *J Neural Transm Park Dis Dement Sect*. 1993;6:1-9.
- Mouradian MM, Heyes MP, Pan JB, Heuser IJE, Markey SP, Chase TN. No changes in central quinolinic acid levels in Alzheimer's disease. *Neurosci Lett*. 1989;105:233-8.
- Oxenkrug G, van der Hart M, Roeser J, Summergrad P. Peripheral tryptophan – kynurenic metabolism associated with metabolic syndrome is different in Parkinson's and Alzheimer's diseases. *Endocrinol Diabetes Metab J*. 2017.
- Schwarz MJ, Guillemin GJ, Teipel SJ, Buerger K, Hampel H. Increased 3-hydroxykynurenic serum concentrations differentiate Alzheimer's disease patients from controls. *Eur Arch Psychiatry Clin Neurosci*. 2013;263:345-52.
- Shao Y, Ouyang Y, Li T, Liu X, Xu X, Li S, et al. Alteration of metabolic profile and potential biomarkers in the plasma of Alzheimer's Disease. *Aging Dis*. 2020;11:1459-70.
- Tohgi H, Abe T, Takahashi S, Kimura M, Takahashi J, Kikuchi T. Concentrations of serotonin and its related substances in the cerebrospinal fluid in patients with Alzheimer type dementia. *Neurosci Lett*. 1992;141:9-12.

- 39 Tohgi H, Abe T, Takahashi S, Saheki M, Kimura M. Indoleamine concentrations in cerebrospinal fluid from patients with Alzheimer type and Binswanger type dementias before and after administration of citalopram, a synthetic serotonin uptake inhibitor. *J Neural Transm Park Dis Dement Sect.* 1995;9:121-31.
- 40 Wada H, Iijima S, Mizoguchi T, Mashige F, Orimo H. [Concentrations of multiple neurochemicals in the cerebrospinal fluid of patients with senile dementia and the relationship to alpha 1-antichymotrypsin]. *Nihon Ronen Igakkai Zasshi.* 1993;30:46-53.
- 41 Wennström M, Nielsen HM, Orhan F, Londos E, Minthon L, Erhardt S. Kynurenic acid levels in cerebrospinal fluid from patients with Alzheimer's disease or dementia with lewy bodies. *Int J Tryptophan Res.* 2014;7:1-7.
- 42 Whiley L, Chappell KE, D'Hondt E, Lewis MR, Jiménez B, Snowden SG, et al. Metabolic phenotyping reveals a reduction in the bioavailability of serotonin and kynurenine pathway metabolites in both the urine and serum of individuals living with Alzheimer's disease. *Alzheimers Res Ther.* 2021;13:20.
- 43 Widner B, Leblhuber F, Walli J, Titz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm (Vienna).* 2000;107:343-53.
- 44 Seibt J, Frank MG. Primed to sleep: the dynamics of synaptic plasticity across brain states. *Front Syst Neurosci.* 2019;13:2.
- 45 Niciu MJ, Kelmendi B, Sanacora G. Overview of glutamatergic neurotransmission in the nervous system. *Pharmacol Biochem Behav.* 2012;100:656-64.
- 46 Carpenedo R, Pittaluga A, Cozzi A, Attucci S, Galli A, Raiteri M, et al. Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur J Neurosci.* 2001;13:2141-7.
- 47 Kozak R, Campbell BM, Strick CA, Horner W, Hoffmann WE, Kiss T, et al. Reduction of brain kynurenic acid improves cognitive function. *J Neurosci.* 2014;34:10592-602.
- 48 Tanaka M, Bohár Z, Vécsei L. Are kynurenines accomplices or principal villains in dementia? Maintenance of kynurenine metabolism. *Molecules.* 2020;25:564.
- 49 Almulla AF, Supasitthumrong T, Amrapala A, Tunvirachaisakul C, Jaleel AKA, Oxenkrug G, et al. The tryptophan catabolite or kynurenine pathway in Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis.* 2022;88:1325-39.
- 50 Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J Neurochem.* 1991;56:2007-17.
- 51 Wirthgen E, Hoeflich A, Rebl A, Gunther J. Kynurenic acid: the janus-faced role of an immunomodulatory tryptophan metabolite and its link to pathological conditions. *Front Immunol.* 2017;8:1957.
- 52 Boros FA, Vecsei L. Immunomodulatory effects of genetic alterations affecting the kynurenine pathway. *Front Immunol.* 2019;10:2570.
- 53 Fernandes BS, Steiner J, Berk M, Molendijk ML, Gonzalez-Pinto A, Turck CW, et al. Peripheral brain-derived neurotrophic factor in schizophrenia and the role of antipsychotics: meta-analysis and implications. *Mol Psychiatry.* 2015;20:1108-19.