The tryptophan catabolite or kynurenine pathway in schizophrenia: meta-analysis reveals dissociations between central, serum and plasma compartments.

SHORT TITLE: TRYCATs in schizophrenia

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Abstract

The tryptophan catabolite (TRYCAT) pathway is implicated in the pathophysiology of schizophrenia (SCZ) since the rate-limiting enzyme indoleamine-dioxygenase (IDO) may be induced by inflammatory and oxidative stress mediators. This systematic review searched PubMed, Web of Science, and Google Scholar for papers published from inception until August 2021 and meta-analyzed the association between SCZ and TRYCATs in the central nervous system (CNS) and peripheral blood. We included 61 studies comprising 2813 patients and 2948 healthy controls. In the CNS we found a significant ($p<0.001$) increase in the

kynurenine/tryptophan (KYN/TRP) (standardized mean difference, SMD=0.769, 95% confidence interval, CI: 0.456; 1.082) and kynurenic acid (KA)/KYN+TRP (SMD=0.697, CI:0.478-0.917) ratios, KA (SMD=0.646, CI: 0.422; 0.909) and KYN (SMD=1.238; CI: 0.590; 1.886), while the 3OH-kynurenine (3HK) + KYN-3-monooxygenase (KMO)/KYN ratio was significantly reduced (SMD=-1.089, CI: -1.682; -0.496). There were significant differences between KYN/TRP, (KYN+KA)/TRP, (3HK+KMO)/KYN, KA, and KYN levels among the CNS and peripheral blood, and among serum and plasma KYN. The only useful peripheral marker of CNS TRYCATs findings was the increased KYN/TRP ratio in serum (SMD=0.211, CI: 0.056; 0.366, p=0.007), but not in plasma. There was no significant increase in a neurotoxic composite score based on KYN, 3HK, and picolinic, xanthurenic, and quinolinic acid. SCZ is accompanied by **i**ncreased IDO activity in the CNS and serum, and reduced KMO activity and a shift towards KA production in the CNS. This CNS TRYCATs profile indicates neuroprotective, negative immunoregulatory and anti-inflammatory effects. Peripheral blood levels of TRYCATs are dissociated from CNS findings except for a modest increase in serum IDO activity.

Keywords: Schizophrenia, indoleamine-dioxygenase, inflammation, neuro-immune, oxidative and nitrosative stress, biomarkers

Introduction

Smith and Maes (1995) proposed a new neuro-inflammatory theory of schizophrenia (SCZ) that combined immune-induced neurodevelopmental abnormalities with second immune injuries, resulting in activation of immune-inflammatory and oxidative and nitrosative (O&NS) pathways, which stimulate indoleamine-dioxygenase (IDO) and the tryptophan catabolite (TRYCAT) pathway [1]. IDO activation may result in the breakdown of TRP and an increase in the levels of different TRYCATs [1, 2]. This early theory was updated in 2020: various SCZ phenotypes are

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associated not only with an activated immune-inflammatory response system (IRS), as evidenced by increased M1 macrophage activity (elevated levels of interleukin (IL)-6 and IL-1), T-helper (Th)-1, and Th-17 cells, but also with an activated compensatory immune-regulatory system (CIRS), which dampens the immune response and prevents hyperinflammation, as evidenced by increased Th-2 and T regulatory (Treg) phenotypes (e.g. increased IL-10) [3-9].

Increased levels of Th-1 (interferon-γ, IFN-γ) and M1 (IL-1β) cytokines, lipopolysaccharides (LPS), as well as superoxide and reactive oxygen species (ROS), may stimulate the activity of IDO, the first and rate-limiting enzyme in the TRYCAT pathway [10-12]. Reduced TRP is a component of the innate CIRS system because reduced TRP levels protect against invading microorganisms and activation of the TRYCAT pathway plays a role in immunological tolerance and has strong antioxidant and immunosuppressive effects [13, 14]. Furthermore, most TRYCATs (e.g. KYN, KA, XA, QA) have anti-inflammatory activities by lowering the Th-1/Treg ratio [15] and certain TRYCATs, especially 3-hydroxyanthranilic acid (3HA) and KA, have neuroprotective properties [12, 16]. Furthermore, some TRYCATs are potent antioxidants, whereas some also have pro-oxidant characteristics [11, 15]. Nonetheless, TRYCATs in the neurotoxic branch of this pathway, such as KYN, 3HK, PA, QA, and XA have behavioral and neurotoxic characteristics when overproduced [14, 17].

Previous research has revealed a link between TRP in the CNS and either free (i.e. unbound to albumin) or total (i.e. free and bound to albumin) plasma/serum TRP [18, 19]. Because the competitive amino acids (CAA) leucine, isoleucine, valine, tyrosine, and phenyl alanine compete with TRP for transport through the blood brain barrier (BBB) via the large neutral amino acid transporter-1 (LAT-1), the brain levels of TRP are also governed by those CAA [18, 19]. Furthermore, KYN and 3HK are carried to the brain at a substantial rate via LAT-1, and anthranilic acid (AA)

is passively transported to the brain at a significant rate, whereas 3HA, KA, and QA have significantly lower passive diffusion rates [20]. In chronic kidney disease or renal insufficiency, KYN and QA may cumulate in the serum and also in the cerebro-spinal fluid (CSF) as a result of increased KYN and QA transfer across the BBB and QA production in the brain [21, 22]. Moreover, Kita et al. (2002) detected that peripheral blood KYN and QA determine in part brain KYN and QA concentrations [23]. To further complicate matters, KYN and TRP use the same transport system to cross the BBB [23]. It is estimated that around 60% of the KYN concentrations in the brain may be from peripheral origin [24].

Overall, it appears that peripheral IRS activation, particularly M1 and Th-1 activation, may result in IDO activation in the periphery and CNS, and that peripheral TRP and TRYCAT levels in part influence brain TRP, KYN, and QA levels. The several parameters that influence the availability of plasma/serum TRP/TRYCAT levels to the brain are depicted in **Figure 1**.

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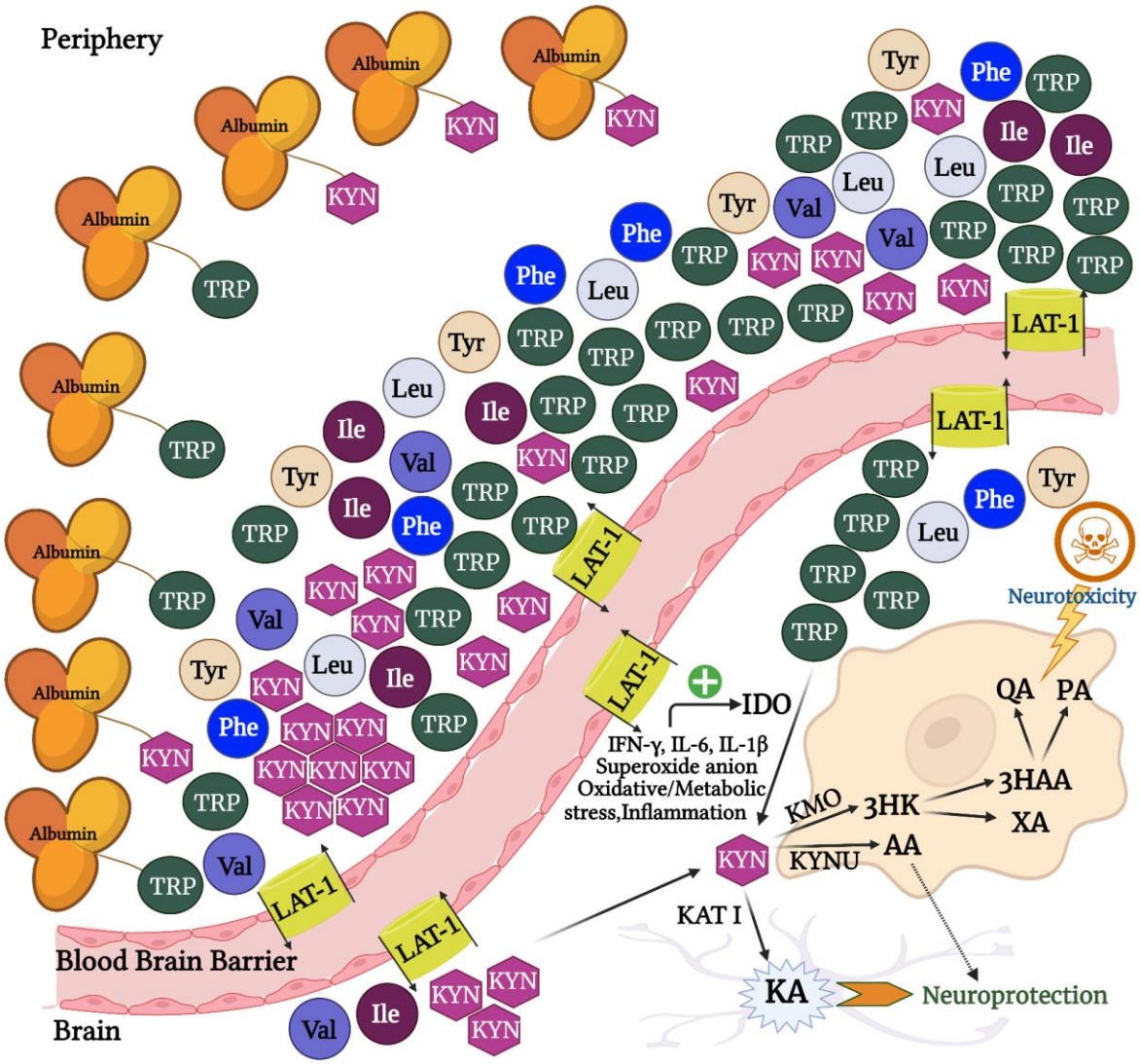

Figure 1: The relationship of essential amino acids and kynurenine in peripheral blood and brain tissues. This figure illustrates the transfer of tryptophan and kynurenine across the blood-brain barrier through the large neutral amino acid's transporter-1 (LAT-1).

TRP: Tryptophan, KYN: Kynurenine, Leu: Leucine, Ile: Isoleucine, Val: Valine, Phe: Phenylalanine, Tyr: Tyrosine, AA: Anthranilic acid, XA: Xanthurenic acid, 3HK: 3-Hydroxykynurenine, 3HAA: 3-Hydroxyanthranilic acid, QA: Quinolinic acid, PA: Picolinic acid, KA: Kynurenic acid, LAT-1: large neutral amino acid transporter 1, IFN-γ:

Interferon-gamma, IL-6: Interleukin-6, IL-1β: Interleukin-1 beta, KMO: Kynurenine 3-monooxygenase, KATI: Kynurenine aminotransferase I, KYNU: Kynureninase, IDO: Indoleamine 2, 3-dioxygenase.

TRYCAT pathway assays in the CNS and serum/plasma of SCZ patients indicate that changes in this pathway may contribute to its pathophysiology. Firstly, elevated levels of KYN and 3HK were observed in the brain tissues and CSF of SCZ patients [25, 26], implying that perhaps IDO activity is increased in the brain. Another study discovered decreased levels of KMO and increased levels of KYN aminotransferase (KAT), which catalyzes the permanent conversion of KYN into KA in the brains of SCZ patients [27]. These findings suggest a) an increased KA (neuroprotective) versus KYN (neurotoxic) ratio [28], and b) decreased levels of the upstream neurotoxic TRYCATs, 3HK and QA, due to decreased KMO activity [29]. Recently, four meta-analyses examined TRP and TRYCATs levels in serum/plasma in SCZ [30-33]. Plitman et al. investigates KA levels only and reported high KA levels in the CNS. Morrens et al. (2020) reported that some TRYCATs were downregulated in schizophrenia spectrum disorders, especially in the acute phase of the disease and older patients. Cao et al. (2021) reported that SCZ is accompanied by reduced TRP levels and a higher KYN/TRP ratio, indicating IDO activation [32] and significant group effects with differences in some TRYCATs between serum and plasma [32]. Marx et al. (2021) confirmed the increased KYN/TRP ratio and reported no significant shift towards production of neurotoxic TRYCATs in SCZ [33].

As such, the results on the TRYCAT pathway in SCZ are contradictory and do not reveal a uniform TRYCAT pattern. Hence, the present study aimed to examine whether IDO, KMO, KAT and the neurotoxic potential of TRYCATs are elevated in the central nervous system (CNS) (brain

tissues and CSF) and peripheral blood (serum and plasma) of SCZ patients versus controls. Towards this end, we conducted a systematic review and meta-analysis on the TRYCATs and TRP data. We examined the KYN/TRP and (KYN+KA)/TRP ratios (IDO proxies), (KA+KAT)/(KYN+TRP) and (KA+KAT)/KYN (KAT proxies), and (3HK+KMO)/KYN (KMO proxy) ratios or sum of all neurotoxic TRYCATs (XA, PA QA, KYN, 3HK). The specific hypotheses of the current meta-analysis are that IDO is activated, with increased levels of neurotoxic TRYCATs and lowered levels of TRP and this both in the CNS and peripheral blood.

Materials and methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 [34], the guidelines of the Cochrane Handbook for Systematic Reviews and Interventions [35], as well as the Meta-Analyses of Observational Studies in Epidemiology (MOOSE), were followed as standards guidelines to accomplished the methodology of the study. This meta-analysis investigates different TRYCATs profiles, which reflect IDO, KAT and KMO activity and the neurotoxic potential in the serum, plasma, CSF, and brain tissues, which are involved in the pathophysiology of SCZ namely prefrontal cortex[36] and frontal cortex[26]. The KYN/TRP, (KYN+KA)/TRP, (KA+KAT)/KYN, (KA+KAT)/(KYN+TRP), and (3HK+KMO)/KYN ratios along with TRP, KYN, KA, and anthranilic acid (AA) were examined. During all stages of this study, there was no direct or indirect participation of the population or patients' representatives.

Search strategy

On August 11th, 2021, we began exploring the data in preparation for a systematic review, and the required data collection concluded on 15th October 2021. All related articles published

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within the electronic databases PubMed/MEDLINE, Google Scholar, and Web of Science were searched using precise keywords and mesh terms for the different databases to extract TRYCATs data in SCZ or schizophrenia spectrum disorder patients. All the terms and the number of identified articles is shown in the Electronic Supplementary file (ESF) Table 1. In addition, we manually checked the reference lists of the identified studies and the previous meta-analysis to ensure all published studies were included in our study.

Table 1. The outcomes and number of patients and controls along with the side of standardized mean difference (SMD) and the 95% confidence intervals with respect to zero SMD

Outcome profiles	n studies	Side of 95% confidence intervals				Patient Cases	Control Cases	Total number of participants
		< 0	Overlap 0 and SMD < 0	Overlap 0 and SMD > 0	> 0			
KYN/TRP	52	3	16	21	12	2244	2483	4727
(KYN+KA)/TRP	61	6	20	21	14	2772	2855	5627
(KA+KAT)/(KYN+TRP)	62	8	18	22	14	2806	2890	5696
KA	34	8	7	9	10	1615	1659	3274
TRP	44	14	19	7	4	1921	2181	4102
KYN	37	6	14	9	8	1670	1865	3535
(KA+KAT)/KYN	49	7	18	15	9	2367	2386	4753

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(3HK+KMO)/KYN	41	11	12	13	5	1860	2036	3896
(KYN+3HK+PA+QA+XA)	44	10	16	12	6	2042	2151	4193

KYN: Kynurenine, TRP: Tryptophan, KA: Kynurenic acid, 3HK: 3-Hydroxykynurenine, PA: Picolinic acid, QA: Quinolinic acid, XA: Xanthurenic acid, KMO: Kynurenine 3-monooxygenase, KAT: Kynurenine aminotransferase.

Eligibility criteria

The English language and publication in peer-reviewed journals were the primary criteria for including manuscripts, but grey literature, papers in other languages (Thai, French, Spanish, German, Italian, Arabic) and the reference lists of the extracted papers were also examined. Inclusion criteria comprise a) observational case-control and cohort studies which investigate the concentrations of TRYCATs (and TRP in the same studies) in serum, plasma, CSF, and brain tissues of the patients with SCZ or schizophrenia spectrum disorders; b) the patients should be diagnosed according to any version of the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Classification of Diseases (ICD) criteria as SCZ or psychotic spectrum disorders; and c) studies that include a normal control group. Exclusion criteria were: a) studies conducted on animal samples and genetic and translational studies; b) lack of a normal control group; c) systematic reviews or meta-analyses; d) articles with duplicate data, and e) studies not reporting the means and standard deviation (SD)/standard error (SEM) values, and f) studies that utilized saliva or whole blood to assess TRYCATs. Nevertheless, we contacted the authors of studies that did not show mean with SD/SEM values but showed geometric means, median

(interquartile range) or graph format data. When the author did not respond, and the median with either interquartile range (IQR) or minimum/maximum values was presented, we estimated the mean and SD values according to a method suggested by [37] , or we used the Web Plot Digitizer to estimate mean and SD/SEM from graphs (https://automeris.io/WebPlotDigitizer/).

Primary and secondary outcomes

The primary outcome measures of the present study are the KYN/TRP and (KYN+KA)/TRP ratios, reflecting IDO activity [28], in patients with SCZ versus controls (see **Table 1**). Secondary outcome variables were (KA+KAT)/KYN and (KA+KAT)/(KYN+TRP) (KAT activity proxies), and (3HK+KMO)/KYN ratio (KMO activity proxy). In addition, if there were any differences in these ratios, we also conduct meta-analyses on the solitary TRYCATs (KYN, 3HK, KA, AA) and TRP. The meta-analysis estimated the neurotoxic potential of the produced TRYCATs by entering a composite score based on KYN, 3HK, XA, QA, and PA, and examining the (KA+KAT)/KYN ratio.

Screening and data extraction

The first author (AA) conducted a preliminary review of the studies to determine whether each study could be included in the current meta-analysis based on the inclusion criteria by inspecting the titles and abstracts of the papers. After eliminating studies based on the pre-defined exclusion criteria, the entire text of potentially eligible publications was downloaded. The same author also extracted the mean and SD, and other required data from the selected articles. They were first organized on a pre-defined excel spreadsheet designed specifically for this purpose.

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Once the first author (AA) had completed all information, another author (AV) double-checked the retrieved data. In the case of a conflict, the last author (MM) was consulted.

The key data in the pre-defined excel file comprised: author's name, year of publication, names of measured TRYCATs, mean and SD of the biomarkers, as well as the sample size for each group (patients and healthy controls, type of the study, demographic characteristics of the study population involved (mean±SD of age and sex distribution), rating scales for determining the severity of the disease, the medium, which was examined to measure the TRYCATs (brain tissue, CSF, serum, and plasma) as well the study's latitude and scores of methodological quality. The immune cofounder scale (ICS) [38] was utilized as a methodological quality score checklist, which the last author somewhat adjusted to be useful for TRYCATs data. Both scoring scale (quality and redpoint) checklists used in the present meta-analysis are shown in ESF Table 2. The major purpose of using such score scales is to evaluate the methodological quality of studies conducted to measure the TRYCATs concentrations. The first scale focuses on key quality domains of the articles consisting of sample size, confounder control, the exact time for collecting the samples, etc. The total scores range from 0 to 10, with values to ten scores denoting higher methodological quality. The second scale is the redpoints score scale which assesses the lack of control or adjustment for key confounders and thus increased analytical/biological bias in the TRYCATs assays and study designs. Total scores can vary from 0 to 26, with 0 indicating that all confounder variables were considered and 26 indicating that there was no control at all.

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Table 2. Results of meta-analysis performed on several outcome (TRYCATs) variables and different media, central nervous system (CNS), serum, and plasma, alone and together.

Outcome feature sets	n	Groups	SMD	95% CI	z	p	Q	df	p	I^2 (%)	τ^2	T
KYN/TRP	52	Overall	0.213	0.105; 0.321	3.86	< 0.0001	170.90	51	< 0.0001	70.15	0.117	0.343
	5	CNS	0.769	0.456; 1.082	4.82	< 0.0001	5.16	4	0.272	22.44	0.029	0.169
	21	Serum	0.211	0.056; 0.366	2.68	0.007	42.16	20	0.003	52.57	0.058	0.240
	26	Plasma	0.046	-0.126; 0.218	0.52	0.600	97.16	25	< 0.0001	74.27	0.133	0.365
(KYN+KA)/TRP	61	Overall	0.177	0.069; 0.285	3.20	0.001	243.94	60	< 0.0001	75.40	0.153	0.391
	10	CNS	0.714	0.465; 0.963	5.63	<0.0001	15.33	9	0.082	41.30	0.063	0.252
	22	Serum	0.167	-0.034; 0.367	1.63	0.103	84.80	21	< 0.0001	75.24	0.148	0.385
	29	Plasma	-0.013	-0.163; 0.137	-0.17	0.866	94.37	28	< 0.0001	70.33	0.108	0.328
(KA+KAT)/(KYN+TRP)	62	Overall	0.150	0.026; 0.274	2.37	0.018	273.23	61	< 0.0001	77.67	0.174	0.417
(KA+KAT)/KYN	49	Overall	0.072	-0.069; 0.212	0.99	0.320	236.21	48	< 0.0001	79.68	0.184	0.429
(3HK+KMO)/KYN	41	Overall	0.023	-0.109; 0.155	0.33	0.735	231.93	40	< 0.0001	82.75	0.225	0.474
	6	CNS	-1.089	-1.682; -0.496	-3.59	<0.0001	28.40	5	< 0.0001	82.40	0.444	0.666
	16	Serum	-0.182	-0.441; 0.077	-1.37	0.169	67.11	15	< 0.0001	77.65	0.187	0.433
	19	Plasma	0.179	0.021; 0.338	2.21	0.027	52.87	18	< 0.0001	65.95	0.075	0.274
KA	34	Overall	0.288	0.119; 0.456	3.34	0.001	332.43	33	< 0.0001	90.07	0.436	0.661
	10	CNS	0.676	0.442; 0.909	5.67	<0.0001	13.78	9	0.130	34.68	0.047	0.217
	14	Serum	0.042	-0.380; 0.464	0.19	0.846	192.61	13	< 0.0001	93.25	0.562	0.750
	10	Plasma	-0.225	-0.524; 0.074	-1.47	0.141	46.96	9	< 0.0001	80.83	0.180	0.425
TRP	44	Overall	-0.213	-0.364; -0.088	-3.21	0.001	194.32	43	< 0.0001	77.87	0.173	0.416
KYN	37	Overall	-0.107	-0.235; 0.021	-1.64	0.101	216.13	36	< 0.0001	83.34	0.236	0.486
	5	CNS	1.238	0.590; 1.886	3.74	<0.0001	20.29	4	< 0.0001	80.29	0.432	0.657
	16	Serum	0.152	-0.111; 0.416	1.13	0.257	69.85	15	< 0.0001	78.53	0.197	0.443
	16	Plasma	-0.263	-0.414; -0.113	-3.44	0.001	32.55	15	0.005	53.91	0.045	0.212
(KYN+3HK+PA QA+XA)	44	Overall	-0.052	-0.198; 0.093	-0.70	0.481	255.75	43	< 0.0001	83.19	0.237	0.487

KYN: Kynurenine, TRP: Tryptophan, KA: Kynurenic acid, 3HK: 3-Hydroxykynurenine, PA: Picolinic acid, QA: Quinolinic acid, XA: Xanthurenic acid, KAT: Kynurenine aminotransferase, KMO: Kynurenine 3-monooxygenase, CNS: Brain and CSF, SMD: standardized mean difference, 95% CI: 95% confidence intervals

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Data analysis

The CMA V3 program was used to conduct a PRISMA-based meta-analysis. **Table 1** shows the outcome biomarker profiles examined in our systematic review and meta-analysis. Meta-analyses were conducted for variables that were presented in at least three studies. The synthetic scores reflecting IDO, KMO and KAT activity and neurotoxic potential were compared among SCZ patients and healthy controls by computing the mean values of the markers of their respective profiles while assuming dependence. To estimate IDO activity, KYN and TRP were entered in the analysis with the direction of increasing KYN and decreasing TRP favoring SCZ. The (KA+KAT)/KYN or (KA+KAT)/(KYN+TRP) ratios (estimating KAT) were estimated by entering KA and KAT (direction set at positive, favoring SCZ), and KYN and TRP (direction: negative, favoring SCZ) were entered in the meta-analysis. The (3HK+KMO)/KYN ratio was estimated by entering 3HK and KMO, direction positive, favoring SCZ, and KYN, direction set at negative, favoring SCZ, in the analysis. The total neurotoxic potential of the TRYCAT pathway was estimated by entering all neurotoxic TRYCATs (XA, PA, KYN, 3HK and QA) in the meta-analysis.

We conducted the meta-analysis employing a restricted maximum-likelihood random-effects model under the assumption that the features of the included studies varied. The effect size was estimated by computing the standardized mean difference (SMD) with 95% confidence intervals (95% CI). The results were statistically significant at $p < 0.05$ (two-tailed tests). SMD values of 0.2, 0.5 and 0.8 indicate small, moderate, and large effect sizes, respectively [39]. We used tau-squared values to denote heterogeneity, as previously described, and we also calculated Q and I^2 metrics [40, 41]. This meta-analysis examined subgroups (brain tissue, CSF, serum, plasma) and used those subgroups within the study as unit of analysis. Brain tissue and CFS were combined into a “central nervous system (CNS)” subgroup if there were no significant differences in the outcome variables between these groups. The meta-analysis was also performed across the subgroup levels and we compared the effects at the different levels within the study.

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Results

Search results

We examined 2025 research papers according to the specific keywords and search sentences listed in ESF, table 1 during the search process. **Figure 2** shows the Prisma flow chart and displays the search method's outcomes and details for inclusion-exclusion articles. After removing 1957 records from the initial search outcome, 68 full-text research papers were eligible for the systematic review. Seven of the 68 articles were eliminated for the reasons listed in ESF, table 4 and therefore the meta-analysis consists of 61 records [25-27, 36, 42-98].

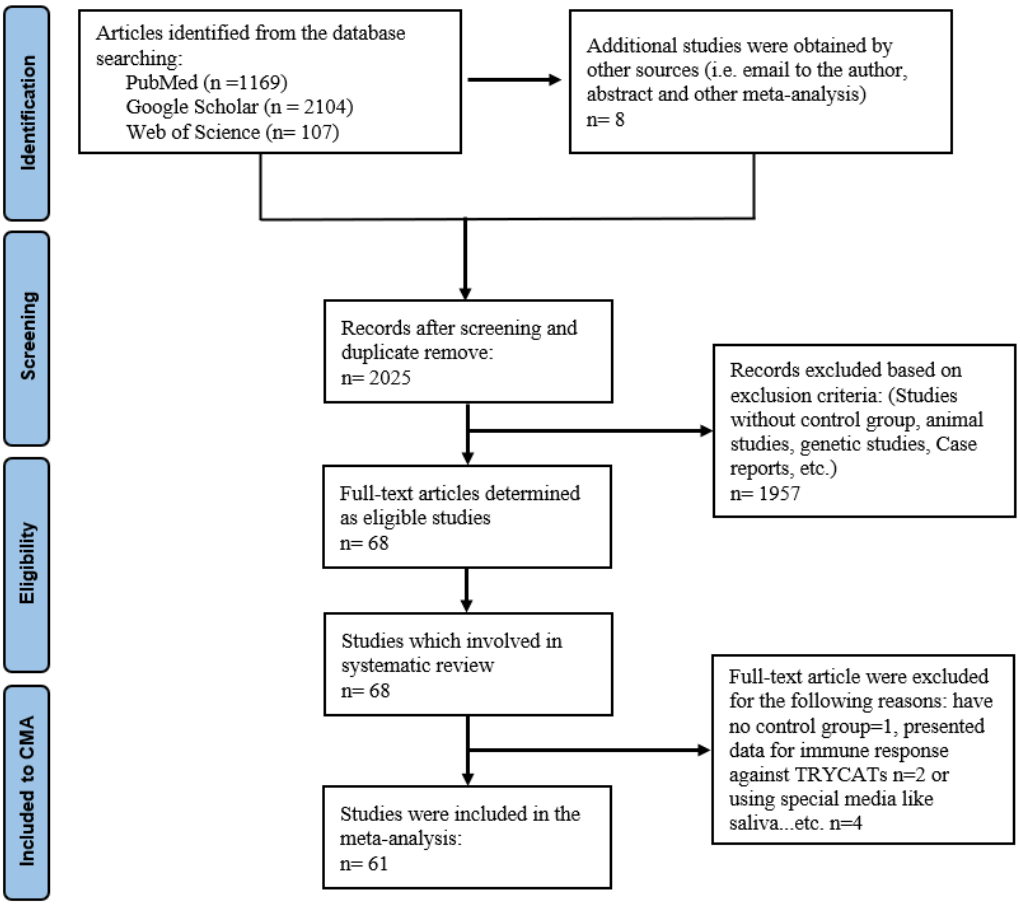

Figure 2: Prisma flow-chart of the meta-analysis study of tryptophan catabolites in schizophrenia

The total number of participants included in the current meta-analysis is 5761, namely 2813 SCZ patients and 2948 healthy controls. This systematic review and meta-analysis only included case-control studies. Seven studies were excluded from the meta-analysis and ESF, table 4 shows the reasons. Twelve studies assayed the TRYCAT pathway in the CNS (8 CSF and 4 brain tissue studies), 27 studies in the plasma, and 22 in the serum. The included papers investigated the TRYCAT pathway in the CNS of 677 individuals (318 patients versus 359 healthy controls), serum of 2186 participants (1054 patients versus 1132 healthy controls), and plasma of 2899 subjects (1441 patients and 1458 healthy subjects). The most common method for assaying TRYCATs was high-performance liquid chromatography (HPLC) used in 31 studies. In 19 research papers, liquid chromatography-mass spectrometry (LCMS) was utilized, while other studies used alternative test procedures indicated in ESF, table 5.

Overall, there were 12 studies conducted on SCZ spectrum disorder patients and 49 studies on pure SCZ patients. We included 2065 SCZ patients versus 2005 healthy controls and 747 patients with SCZ spectrum disorders versus 943 healthy controls. The participants' ages ranged from 20 to 60 years old. Most of the participants in the current meta-analysis are from the United States (19 studies), Sweden (8 studies), China (8 studies), Japan (4 studies), Italy (3 studies), Netherlands (3 studies), Brazil (2 studies), South Korea (2 studies), Germany (2 studies), Australia, Austria, Belgium, Estonia, India, Ireland, Norway, Poland, Switzerland, UK (each one). The quality scores along with redpoint scores, are shown in ESF, table 5 as median (min-mix), namely 4 (min= 1, max=8) and 12 (min=7.0, max=25.0), respectively.

The primary outcome variables

KYN/TRP

Table 1 shows that in 12 studies (4 CNS, 3 plasma, 5 serum studies), the 95% CI intervals of KYN/TRP were totally on the positive side of zero while 3 studies (2 plasma, 1 serum) reported 95% CI

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which were entirely on the negative side of zero. Additionally, 36 intervals crossed the zero distributed as 21 studies with SMD values greater than zero and 16 studies with SMD smaller than zero. The forest plot of KYN/TRP in patients versus controls is shown in **Figure 3**. There were no significant differences (p=0.407) in the ratio between brain tissue (SMD=0.606; 95% CI: 0.175, 1.038, p=0.006) and CSF (SMD=0.877; 95%CI: 0.406, 1.348, p=0.001) and, therefore, we examined the CNS subgroup combining CSF and brain tissues. The meta-analysis performed on 5 CNS, 26 plasma, and 21 serum studies showed a statistically significant SMD with a small effect size. However, high heterogeneity was observed when considering all media together, and, therefore, subgroup analysis was carried out. There were significant differences between the three media (p<0.0001) with a high effect size in the CNS and a small but significant effect size in serum, whereas plasma yielded non-significant results (see **Table 2**). There were significant differences among CNS and serum (p=0.002) and plasma (p<0.0001) and a trend toward a non-significant difference between plasma and serum (p=0.162). The heterogeneity in CNS and serum was lower than in the total sample and plasma. ESF, table 6 displays that no bias was present when all data were combined and the CNS, serum and plasma did not show any signs of bias.

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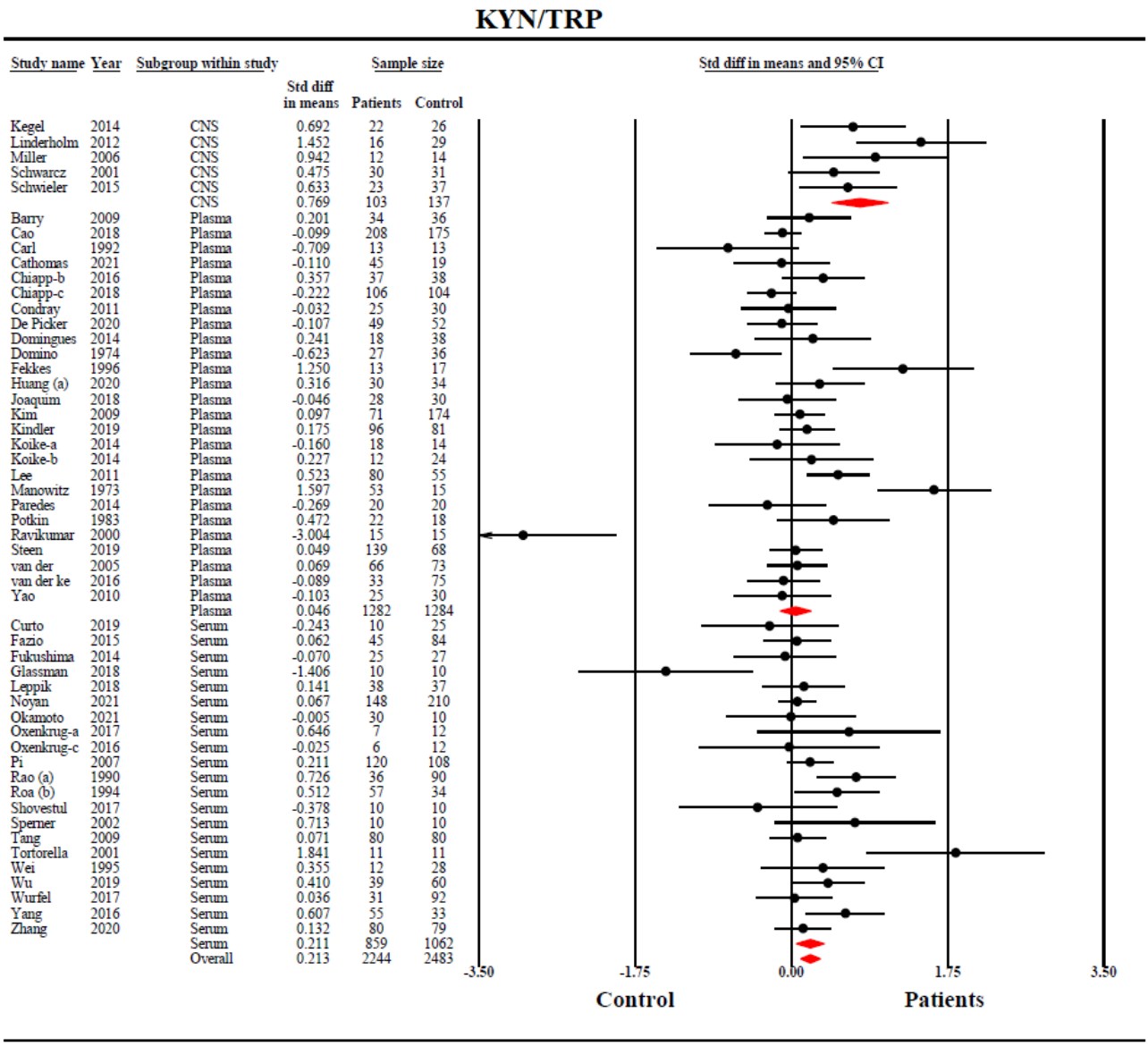

Figure 3: Forest plot of the kynurenine/tryptophan (KYN/TRP) ratio reflecting indoleamine-2,3-dioxygenase (IDO) activity with results of a meta-analysis conducted upon 52 studies.

The (KYN+KA)/TRP ratio

There were no significant differences (p=0.562) in the (KYN+KA)/TRP ratio between brain tissue (SMD=0.890; 95% CI: 0.177, 1.603, p=0.014) and CSF (SMD=0.665; 95% CI: 0.444, 0.940, p<0.001). **Table 1** shows that fourteen studies (6 CNS, 3 plasma, 5 serum) showed 95% CI that were totally on the

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positive side of zero while 6 studies (4 plasma, 2 serum) reported CI values that were entirely on the negative side of zero. Additionally, 41 intervals crossed the zero, distributed as 21 studies with SMD values greater than zero and 20 studies with SMD smaller than zero. ESF, Figure 1 shows the forest plot of the (KYN+KA)/TRP ratio. The results indicate an overall significant SMD of 0.177, although heterogeneity was considerable and again partly explained by highly significant intergroup differences ($p<0.0001$) with a high effect size in the CNS and no significant effects sizes in serum and plasma (see **Table 2**). There were highly significant differences between CNS and serum ($p=0.001$) and plasma ($p<0.0001$). As shown in ESF, table 6, publication bias occurs when all data are combined, resulting in a decrease in the adjusted point estimate (0.132; 95% CI: 0.008; 0.255). Significant bias was detected in plasma as displayed in ESF, table 6, and the adjusted point estimate was 0.05 with 95% CI (-0.104; 0.205). In contrast, there was no bias when considering CNS and serum.

Secondary outcome variables

(KA+KAT)/(KYN+TRP) ratio

There were no significant differences ($p=0.530$) in the (KA+KAT)/(KYN+TRP) ratio between brain tissue (SMD=0.503; 95% CI: -0.217, 1.222, $p=0.171$) and CSF (SMD=0.246; 95% CI: -0.106, 0.897, $p=0.171$). **Table 2** shows that the (KA+KAT)/(KYN+TRP) ratio was significantly higher in SCZ than in controls with a very modest effect size. There were no significant differences between CNS, serum, and plasma ($p=0.463$). Nevertheless, the ratio in CNS was significantly higher in SCZ as compared with controls, with a moderate effect size (SMD=0.325, 95% CI: 0.010; 0.639, $tau^2=0.184$), whereas the effects sizes were not significant in serum (SMD=0.082, 95% CI: -0.153; 0.317, $tau^2=0.229$) and plasma (SMD=0.136, 95% CI: -0.030; 0.301, $tau^2=0.142$). ESF, Figure 2 shows the forest plot of the (KA+KAT)/(KYN+TRP) ratio and ESF, table 6 shows that probably no bias was present.

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(KA+KAT)/KYN

There were no significant differences (p=0.642) in the (KA+KAT)/KYN ratio between brain tissue (SMD=0.446; 95% CI: -0.630, 1.521, p=0.417) and CSF (SMD=0.172; 95% CI: -0.245, 0.589, p=0.419). The results displayed that overall, there was no significant difference in the (KA+KAT)/KYN ratio between SCZ patients and controls (SMD=0.072, 95% CI: -0.069; 0.212, tau^2= 0.184) and that no significant differences were detected between CNS, serum and plasma (p=0.217). After adjusting for 2 missing studies the overall SMD was 0.091 (95% CI: -0.050; 0.232).

(3HK+KMO)/KYN

Table 2 shows that the (3HK+KMO)/KYN ratio was not significantly different between SCZ patients and controls. Since there were highly significant differences between CNS, serum, and plasma (p<0.0001) we performed subgroup analysis and found that the CNS (3HK+KMO)/KYN ratio was significantly lower in SCZ than in controls (SMD=-1.089, 95%CI: -1.682; -0.496, tau^2= 0.444), whereas plasma showed an increased ratio in SCZ (SMD= 0.179, 95%CI: 0.021; 0.338, tau2= 0.075). There were highly significant differences between CNS and plasma (p<0.0001) and CNS and serum (p=0.006). In plasma, imputation of 6 studies changed the SMD to 0.323 (95% CI: 0.167; 0.479).

TRP, KYN, KA and AA

Table 2 and ESF, Figure 3 show that TRP was significantly lower in SCZ patients than in controls with a very modest effect size. There were no significant differences in the TRP effect sizes among serum, plasma, and CSF. Kendall's tau and Egger's regression did not suggest that bias could be present.

Nevertheless, after imputing three missing studies on the right site, the adjusted estimated point estimate (-0.081) was no longer significant (95% CI: -0.241; 0.078).

Table 2 shows that in all studies combined there was no significant change in KYN in SCZ versus controls. Nevertheless, group analysis showed highly significant differences between CNS, serum and plasma ($p<0.0001$) with significant differences between CNS and either serum ($p=0.002$) and plasma ($p<0.0001$) and between serum and plasma ($p=0.007$). While in the CNS (high effect size) and serum (non-significant) there was a positive association with SCZ, in plasma a highly significant inverse correlation was established (see **Table 2**). **Figure 4** shows the forest plot of the KYN data in SCZ. Kendall's tau and Egger's regression did not suggest that bias was present in CNS, serum, or plasma.

Table 2 and ESF, Figure 4 show that KA was significantly higher in all SCZ patients combined than in controls with a very modest effect size. Group analysis showed highly significant differences between CNS, serum, and plasma ($p<0.0001$) with significant differences between CNS and either plasma ($p<0.0001$) and serum ($p=0.01$). ESF, table 6 shows the results on publication bias in the KA data which show a bias in the plasma but not serum levels. Anthranilic acid (AA) data were obtained in three serum studies and show significantly increased AA levels in SCZ as compared with controls with a medium effect size (SMD= 0.590, 95% CI: 0.045; 1.136, $p=0.034$).

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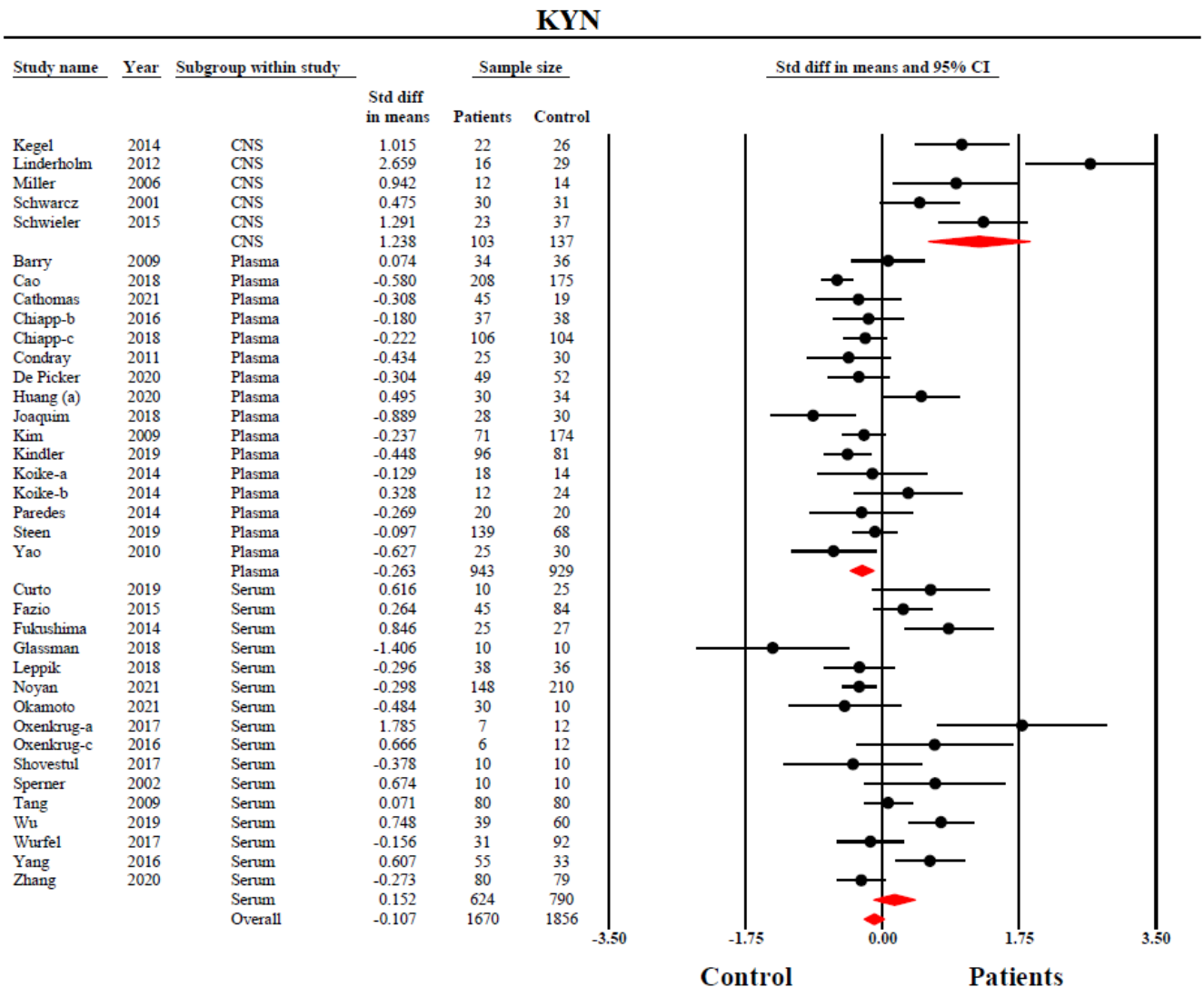

Figure 4. Forest plot with results of the meta-analysis performed on 37 studies reporting on kynurenine (KYN) in schizophrenia.

(KYN+3HK+PA+QA+XA) composite

The composite score of KYN,3HK, PA, QA and XA was not significant different between SCZ patients and controls. In addition, group analysis showed no significant difference (p=0.344) among CNS,

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serum, and plasma. Publication bias was detected with 4 missing studies and the adjusted SMD was 0.134 (95% CI: -0.048; 0.318).

Meta-regression analyses

Meta-regression (ESF, table 7) reveals that after adjusting for the differences between CNS, serum and plasma, there were significant effects of latitude (p=0.032) on the KYN/TRP ratio and KYN. Age explained part of heterogeneity in the KYN/TRP ratio and KYN. Female gender affects the results of (KYN+KA)/TRP ratio, KA and KYN. The total number of participants affected heterogeneity in KYN.

Discussion

The KYN/TRP ratio in SCZ

The current study's first major finding is that the KYN/TRP ratio was significantly increased in SCZ patients when compared to controls, implying that IDO may be activated in SCZ patients. Nonetheless, subgroup analysis across the CNS, serum, and plasma reveals a large effect size (0.769) in the CNS and a small effect size (0.211) in the serum, whereas the effect size in the plasma is non-significant. As such, serum measurements reflect changes in the CNS in part, whereas plasma measurements are completely unrelated to CNS observations. A such, the current study discovered a more widespread IDO activation in the CNS and serum of SCZ patients. It appears that the plasma KYN/TRP ratio cannot be used as a proxy for IDO activity in the brain or peripheral blood. Moreover, computing the (KYN+KA)/TRP ratio showed significant differences in CNS but not in serum, suggesting that it is more adequate to interpret the serum KYN/TRP ratio when evaluating peripheral blood IDO activity. The findings are in agreement with the theories (see Introduction) that activated M1, Th-1, and O&NS pathways in SCZ stimulate IDO, resulting in TRYCAT pathway activation [99-102].

Secondary analyses to determine whether KYN or TRP or both are altered in SCZ revealed that KYN levels were significantly increased in SCZ patients compared to controls, and that there were highly significant differences between CNS and serum or plasma and between plasma and serum. Thus, KYN levels were significantly increased in the CNS of SCZ patients and slightly increased in the serum, whereas the plasma assay revealed a significant decrease in KYN. As a result, the findings in plasma are diametrically opposed to those in the CNS. Morrens et al. [31], on the other hand, did not account for plasma-serum differences, and thus their KYN and KYN/TRP results are uninterpretable. Two other meta-analyses also detected increased serum KYN but decreased plasma KYN levels in SCZ [32 33]. Nonetheless, the primary distinction between our study and those two meta-analyses is that we calculated the mean of all available data on KYN and TRP in all studies as well as a composite score for the KYN/TRP ratio [40, 103]. On the other hand, Cao et al. and Marx et al. used only a few KYN/TRP ratios when the original articles computed this ratio. Consequently, the current meta-analysis findings far surpass those of the earlier studies.

Group analysis performed on the TRP levels, on the other hand, did not show significant differences between CNS, plasma, and serum. Although the overall meta-analysis showed lower TRP levels in SCZ than in controls, these differences were no longer significant after imputing missing values. Three previous meta-analyses [31-33] reported an overall significant reduction in TRP level in SCZ.

Because KYN can cross the BBB during systemic inflammation, blood derived KYN is the primary source of brain KYN levels (see Introduction and **Figure 1**). Additionally, the brain concentrations of other TRYCATs, such as KA, 3HK, and QA, are determined in part by peripheral TRYCATs levels [23, 104]. As such, alterations in peripheral blood TRYCATs induced by peripheral inflammation contribute to a variety of immune, redox, and behavioral effects in the brain (see Introduction).

KAT activity in SCZ

The second major finding of this study is that the (KA+KAT)/(KYN+TRP) ratio and KA levels, but not the (KA+KAT)/KYN ratio, are significantly increased in the CNS of SCZ patients, whereas serum and plasma showed no significant effects or even an inverse association. These findings extend those of Plitman et al. (2017) and Cao et al. [30, 32], who discovered increased brain KA levels in SCZ but no significant changes in peripheral KA. Marx et al. [33] reported that SCZ patients had stable KA levels and a decreased KA/KYN ratio. Morrens et al. [31] found no statistically significant differences in peripheral blood KA levels between SCZ and controls, but as previously stated, these results are not interpretable. As discussed above, our findings regarding ratios, including the (KA+KAT)/KYN ratio, are much more appropriate and should be considered when interpreting those ratios.

Our findings suggest that increased KA formation from its precursors KYN and TRP may occur in the CNS, whereas no such effects were observed in the peripheral blood. Such disparities between the brain and peripheral blood have also been observed during aging, which is associated with increased KAT activity in the brain but no changes in the periphery [105]. KA is unlikely to cross the BBB [20], and the majority of brain KA is derived from KYN [104]. Thus, blood borne KYN, which is transported from the peripheral blood to the brain, may stimulate KAT activity in the CNS (see Introduction). This explains that blood-derived levels of KYN are correlated with CNS KA concentrations [25, 26]. Astrocytes are another critical determinant of KA level in the CNS because they lack KMO (KYN hydroxylase) and produce KA and KYN in response to IRS-induced IDO activation [106]. For instance, elevated KA levels may be detected following IL-6 administration to cultured astrocytic cells [44].

KMO in SCZ

The third major finding of this meta-analysis is that the (3HK+KMO)/(KYN+TRP) ratio (a proxy for KMO activity) was significantly decreased in the CNS, whereas no significant changes were observed

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in plasma or serum. Not only increased IDO activity, but also decreased KMO activity may account for some of the increases in KYN and KA levels in the CNS of SCZ patients [107, 108]. The onset of inflammation and infection are accompanied by depletion of intracellular riboflavin resulting in lowered KMO activity [109], while reduced KMO activity induces elevated levels of AA concentrations and an increase in the utilization of KYN, resulting in increased KA production [109, 110]. Our meta-analysis revealed that AA levels were significantly elevated in SCZ, even though only three studies assessed AA levels. Notably, AA serves as a protective agent because this TRYCAT may inhibit the formation of QA and PA from 3HA [109, 111]. Moreover, 3HA has many potent anti-inflammatory and negative immune-regulatory effects [109].

Is there increased neurotoxicity in SCZ?

The fourth major finding of our study is that SCZ was not accompanied by a significant increase in the composite KYN + 3HK + XA + PA + QA score, a neurotoxicity index. Reduced KMO activity and increased AA concentrations may have contributed to the absence of significant increases in neurotoxic TRYCATs such as PA and QA despite increased IDO activity. Additionally, as mentioned previously, the (KA+KAT)/KYN ratio, a measure of neuroprotection/neurotoxicity, was significantly increased in SCZ, implying a net neuroprotective effect. KA appears to have more antioxidant than pro-oxidant properties, exhibiting significant ROS scavenging activity against hydroxyl, peroxinitrite, and superoxide, as well as inhibiting lipid peroxidation and subsequent aldehyde formation and protein oxidation [105]. In addition, KA is a CIRS component that has anti-inflammatory properties and aids in the resolution of inflammation [112]. KA also exerts neuroprotective effects [12, 113] by inhibiting extra synaptic NMDA-receptors and the 7 nicotinic acetylcholine receptor (7nAChr) thereby preventing the release of glutamate and thus further stimulation of postsynaptic NMDA-receptors [114]. The preceding demonstrates that KA has a multitude of protective properties and may act as a buffer against the neurotoxic effects of QA and KYN, while the latter

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has also some anti-inflammatory and antioxidant properties which contribute to neuroprotection and CIRS activity [102, 105]. These protective effects should be added to the pathway's intrinsic functions comprising CIRS, anti-inflammatory, and antioxidant activities (see Introduction).

At first glance, the findings of this meta-analysis contradict prior reviews' claims that SCZ is associated with greater TRYCATs neurotoxicity [12]. However, new research using a more sensitive TRYCATs assay which measures serum IgA levels directed to TRYCATs shows that only first episode schizophrenia (FES)/multiple episode schizophrenia (MES) with worsening and consequent deficit SCZ are characterized by increased TRYCAT-associated neurotoxicity, as evidenced by the measurement of IgA levels to XA, PA, and 3HK [115]. This IgA response evaluates changes in TRYCATs levels as identified by the immune system. According to these recent findings, elevated neurotoxic TRYCATs may play a role in the neuroimmune toxicity linked to the worsening of FES/MES and SCZ deficiency [115]. As a result, one of the major flaws in TRYCATs studies in SCZ is that they failed to account for the worsening of MES and FES, as well as the resulting deficit SCZ [115].

Sources of heterogeneity in TRYCATs studies

Apart from the major differences in TRYCATs levels between CNS, plasma and serum, subgroup analyses showed that latitude, age, gender, and the number of participants in the studies may contribute to the heterogeneity. Moreover, significantly increased KA, KYN, and KYN/TRP and (KYN+KA)/TRP ratios in patients who are treated with antipsychotics although these results may be confounded by to differences among serum, plasma and CNS. The effects of antipsychotics in modulating TRYCATs is still ambiguous.

Our findings show that the plasma assay of TRYCATs and plasma KYN may be prone to increased sources of pre-analytical or analytical error. First, the dilution effects of anticoagulants may impact lower concentrations of analytes [116]. Furthermore, thermally decomposition following EDTA may increase α-

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amino products [117] and carbonyl-containing compounds such as EDTA may induce degradation of KYN in biological samples [118].

There are some studies suggesting that - to achieve the most precise TRP assay - it is preferable to collect serum instead of plasma to avoid pre-analytical and analytical errors emerging from using plasma anti-coagulant tubes based on EDTA, heparin, and citrate [119, 120]. These materials may interfere with the metabolites thereby producing contamination especially in HPLC and spectrophotometric methods. Furthermore, the probability of oxidative degradation of TRP is high, and carbonyl-containing compounds including EDTA may contribute to TRP degradation [118]. Moreover, at room temperature (> 4 ^{o}C), serum TRP and the CAA may increase in plasma (personal observations), which may be explained by increased protease activity which release amino acids. Moreover, also heparin tubes may not be appropriate to assay TRP as heparin may interact with binding of albumin to TRP [121, 122]. Another source of variation in plasma/serum TRP levels is associated with blood platelets, which have high concentrations of TRP and 5-HT and may release TRP when activated. Patients with SCZ show altered platelet reactivity due to O&NS [123], which may impact 5-HT levels in platelets and consequently impact peripheral TRP concentrations [124].

Apart from the effects of the above sources of (pre-)analytical variability, the TRYCAT pathway products are also prone to considerable biological variability. First, the ratio of free or total plasma/serum TRP / the sum of the 5 CAA is probably the best index predicting brain TRP concentrations [14]. Second, TRP is bound (80%) to albumin [19, 125] and, thus, any changes in albumin (a negative acute phase protein which is decreased in inflammatory conditions) are accompanied by changes in TRP levels. Moreover, TRP is stripped off from albumin in the microcirculation when transported through the BBB [126]. Third, insulin levels indirectly effect this binding by decreasing plasma levels of non-esterified fatty acids which compete with TRP for albumin binding [19, 127].

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Limitations

The current systemic review and meta-analysis' findings should be addressed in terms of their limitations. Firstly, to delineate whether TRYCAT-associated neurotoxicity (especially in PA, XA, and QA) plays a role in SCZ, we would need more brain tissue, CSF and serum levels of the neurotoxic TRYCATs including in the FES and MES worsening phenotypes and deficit SCZ. Some TRYCATs were largely missing in the studies included here, e.g. 3HA, and therefore we were unable to compute the 3HA/AA ratio which is important to understand the protective properties of the TRYCAT pathway. Third, in the present study we combined TRYCAT measurements in CFS and relevant brain tissues into a new subgroup, namely CNS, because we could not detect any differences between both compartments and to increase the number of studies to be included in the meta-analysis. Nevertheless, more studies on both compartments are needed to further examine possible differences and effects of for example the organic anion transporter which is involved in the transport of KA into CSF [128]. Fourth, preanalytical studies should scrutinize why there are significant differences in some TRYCATs between plasma and serum. Meanwhile we recommend measuring peripheral levels of TRYCATs in serum and not plasma.

Conclusions

Figure 5 summarizes the findings of the current meta-analysis. Increased IDO and decreased KMO activity accompany SCZ, showing that the TRYCAT pathway's first part is activated. This leads to increased KA production in the CNS, which improves neuroprotection, antioxidant protection, and CIRS activities, as well as enhanced AA production, which may reduce neurotoxic TRYCATs like QA and PA. SCZ does not show a substantial rise in the composite score, which includes all neurotoxic TRYCATs. Only the serum KYN/TRP ratio appears to be linked to increased KYN levels and IDO activity in the CNS. The other TRYCAT levels in the peripheral blood have no diagnostic significance. Even worse, when compared to the

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CNS, plasma TRYCAT levels demonstrate the opposite results. The TRYCATs profile established in SCZ patients is predominantly neuroprotective (increased KA and AA) and should be considered in addition to the pathway's intrinsic CIRS, anti-inflammatory, and antioxidant capabilities. Overall, there is no evidence that TRYCAT-associated neurotoxicity causes SCZ, while it is not excluded that neurotoxic TRYCATs cause a worsening of FES/MES and hence a deficit SCZ.

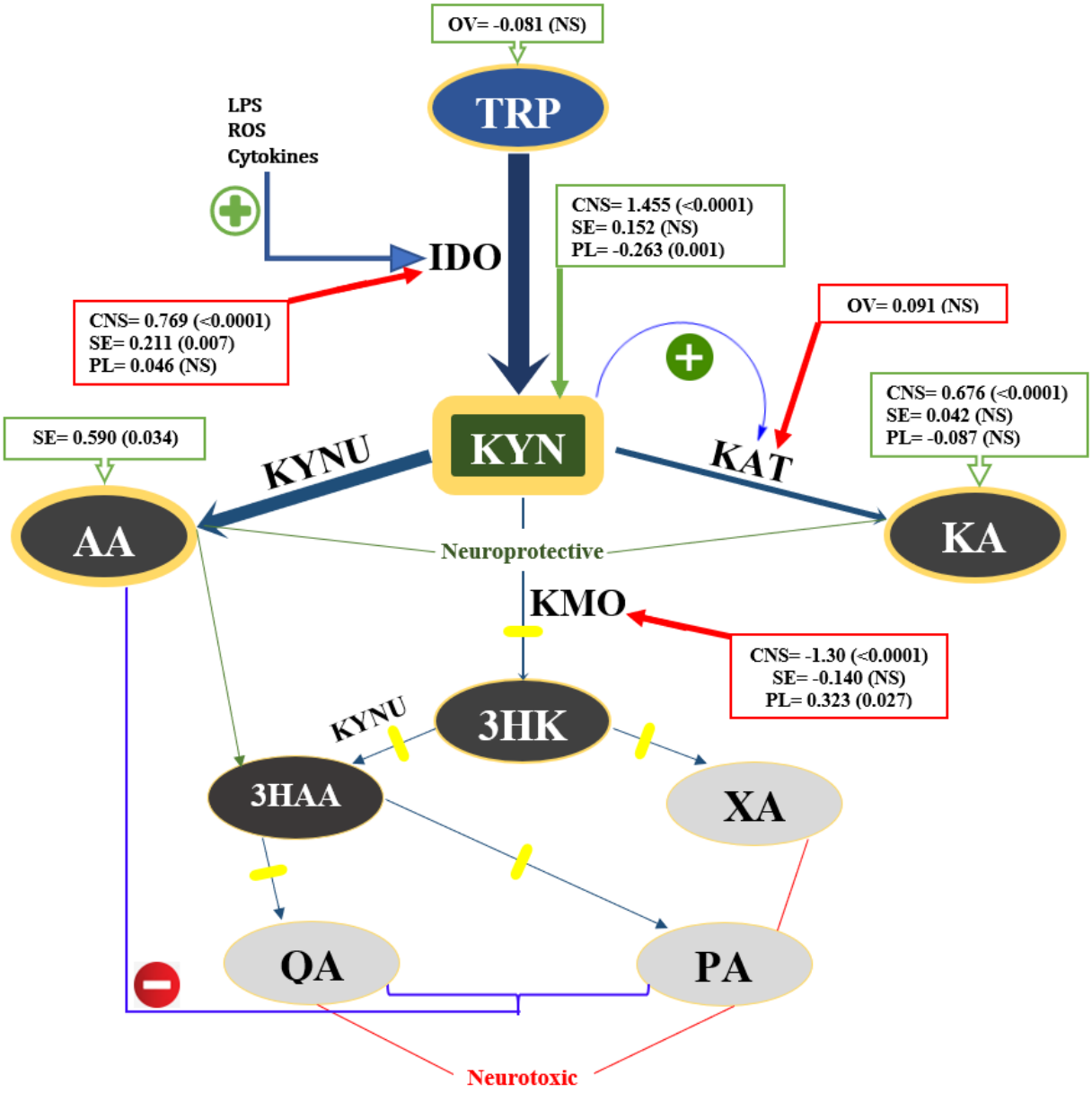

Figure 5: Summary of the findings in schizophrenia.

TRP: Tryptophan, KYN: Kynurenine, KA: Kynurenic acid, AA: Anthranilic acid, 3HK: 3-Hydroxy kynurenine, 3HAA: 3-Hydroxy anthranilic acid, XA: Xanthurenic acid, QA: Quinolinic acid, PA: Picolinic acid.

IDO: Indoleamine 2,3-dioxygenase, KAT: Kynurenine aminotransferase, KMO: Kynurenine 3-monooxygenase, KYNU: Kynureninase, LPS: Lipopolysaccharides, ROS: Reactive oxygen species.

In this graph we also show the effect sizes (as standardized mean difference (SMD) with p-values) for the tryptophan catabolites, namely as OV: Overall, when there were no group differences and as CNS: Central nervous system, SE: Serum, and PL: Plasma, when there were significant group differences. The activities of IDO, KAT and KMO enzymes were estimated by computing ratios, i.e., IDO: KYN/TRP ratio, KAT: (KA+KAT)/KYN ratio, and KMO: (3HK+KMO)/KYN ratio.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. MS received honoraria and has been a consultant for Angelini, Lundbeck.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset (excel file) generated during and/or analyzed during the current study will be available from MM upon reasonable request and once the dataset has been fully exploited by the authors.

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Author's contributions

All authors contributed to the writing up of the paper. The work was designed by AA and MM. Data were collected by AA and AV. Statistical analyses were performed by AA and MM. All authors revised and approved the final draft.

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Not applicable.

Supplementary Information

Supplementary information is available at MP's website.

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