Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

International Journal of Tryptophan Research

Imbalanced Kynurenine Pathway in Schizophrenia

Magdalena E. Kegel[1], Maria Bhat[2,3], Elisabeth Skogh[4], Martin Samuelsson[4], Kristina Lundberg[4], Marja-Liisa Dahl[5], Carl Sellgren[6], Lilly Schwieler[1], Göran Engberg[1], Ina Schuppe-Koistinen[2,3] and Sophie Erhardt[1]

[1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. [2]AstraZeneca, Research and Development, Innovative Medicines, Personalised Healthcare and Biomarkers, Translational Science Centre, Science for Life Laboratory, Solna, Sweden. [3]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. [4]Department of Clinical and Experimental Medicine, Section of Psychiatry, Faculty of Health Sciences, Linköping University, Linköping, Sweden. [5]Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden. [6]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

ABSTRACT: Several studies suggest a role for kynurenic acid (KYNA) in the pathophysiology of schizophrenia. It has been proposed that increased brain KYNA levels in schizophrenia result from a pathological shift in the kynurenine pathway toward enhanced KYNA formation, away from the other branch of the pathway leading to quinolinic acid (QUIN). Here we investigate the levels of QUIN in cerebrospinal fluid (CSF) of patients with schizophrenia and healthy controls, and relate those to CSF levels of KYNA and other kynurenine metabolites from the same individuals. CSF QUIN levels from stable outpatients treated with olanzapine (n = 22) and those of controls (n = 26) were analyzed using liquid chromatography-mass spectrometry. No difference in CSF QUIN levels between patients and controls was observed (20.6 ± 1.5 nM vs. 18.2 ± 1.1 nM, $P = 0.36$). CSF QUIN was positively correlated to CSF kynurenine and CSF KYNA in patients but not in controls. The CSF QUIN/KYNA ratio was lower in patients than in controls ($P = 0.027$). In summary, the present study offers support for an over-activated and imbalanced kynurenine pathway, favoring the production of KYNA over QUIN in patients with schizophrenia.

KEYWORDS: NMDA receptor, cerebrospinal fluid, psychosis, kynurenic acid, quinolinic acid, inflammation

CITATION: Kegel et al. Imbalanced Kynurenine Pathway in Schizophrenia. *International Journal of Tryptophan Research* 2014:7 15–22 doi: 10.4137/IJTR.S16800.

RECEIVED: May 12, 2014. **RESUBMITTED:** June 23, 2014. **ACCEPTED FOR PUBLICATION:** June 24, 2014.

ACADEMIC EDITOR: Gilles Guillemin, Editor in Chief

TYPE: Original Research

FUNDING: This work was supported by grants from the Swedish Medical Research Council (Prof. Engberg: 2009–3068, 2011–4789; Dr Erhardt: 2009–7053, 2013–2838; and Dr Svensson 2009–3068; Prof. Dahl 2008–2578, 2012–2592), the Swedish Brain foundation, Petrus och Augusta Hedlunds Stiftelse, Östergötland County Council; the AstraZeneca-Karolinska Institutet Joint Research Program in Translational Science, the Karolinska Institutet (KID). The clinical research study was sponsored by Linköping University Hospital with an AstraZeneca in-kind contribution to sample analysis for the present paper. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: sophie.erhardt@ki.se

This paper was subject to independent, expert peer review by a minimum of two blind peer reviewers. All editorial decisions were made by the independent academic editor. All authors have provided signed confirmation of their compliance with ethical and legal obligations including (but not limited to) use of any copyrighted material, compliance with ICMJE authorship and competing interests disclosure guidelines and, where applicable, compliance with legal and ethical guidelines on human and animal research participants.

Introduction

Schizophrenia is one of our world's most devastating and complex psychiatric disorders. It is characterized by periods of delusional psychotic states and thought disturbance interlaced with a general state of anhedonia, avolition, and social withdrawal. Cognitive deficits are considered a core feature of the disorder. It follows that this lifelong disability often is accompanied by social and occupational exclusion.

In the past decade, the kynurenine pathway has been implicated in schizophrenia pathophysiology.[1,2] Thus, elevation of brain kynurenic acid (KYNA), a neuroactive metabolite of this pathway, is one of the most consistently found biochemical aberrations in schizophrenia and bipolar disorder with psychotic features.[3–10] Apart from being an antagonist at the glycine site of the N-methyl-D-aspartate (NMDA) receptor,[11] KYNA also antagonizes the cholinergic α7 nicotinic

receptor (α7nAChR).[12] Similar to synthetic NMDA receptor antagonists such as phencyclidine or ketamine, elevation of endogenous KYNA in rodents produces aberrant behavior thought to model certain aspects of schizophrenia.[13–18]

The kynurenine pathway accounts for approximately 95% of tryptophan metabolism and involves several neuroactive metabolites (Fig. 1). There are two main branches of the pathway diverting from the common precursor L-kynurenine. In a dead-end branch, KYNA is produced from kynurenine via the astrocytic kynurenine aminotransferase (KAT) enzymes. In the other branch of the pathway, guarded by the enzyme kynurenine 3-monooxygenase (KMO), 3-hydroxykynurenine is produced from kynurenine and further metabolized to 3-hydroxyanthranilic acid by kynureninase. This ultimately leads to the formation of the intermediate quinolinic acid (QUIN), which subsequently is transformed to nicotinamide adenine dinucleotide (NAD^+). QUIN is considered neurotoxic because of its agonistic action on the NMDA receptor and has mainly been studied with regard to neurodegeneration.[19] Thus, elevated levels of cerebrospinal fluid (CSF) QUIN have previously been reported in neurodegenerative disorders such as Huntington's disease[20] and Alzheimer's disease.[21] Recently though, QUIN was found to be elevated also in patients with severe depression and suicide attempters.[22,23] The kynurenine pathway is critically regulated by inflammatory stimuli where the two rate-limiting enzymes tryptophan-2,3-dioxygenase (TDO) and indolamine-2,3-dioxygenase (IDO) are induced by proinflammatory cytokines.[24–26] The cellular source of QUIN in the brain is microglia, a cell type responsible for most inflammatory signaling in the brain.

The literature on central levels of QUIN in schizophrenia is sparse. One early study found normal CSF QUIN levels in a small cohort of patients with schizophrenia.[27] Similarly, according to a postmortem study 3-hydroxykynurenine, a precursor of QUIN, was unchanged in patients with schizophrenia, while kynurenine and KYNA were elevated.[4] No studies hitherto have investigated KYNA and QUIN in the same schizophrenia patient cohort.

The aim of the present study is to further characterize the kynurenine pathway in schizophrenia. We therefore analyzed

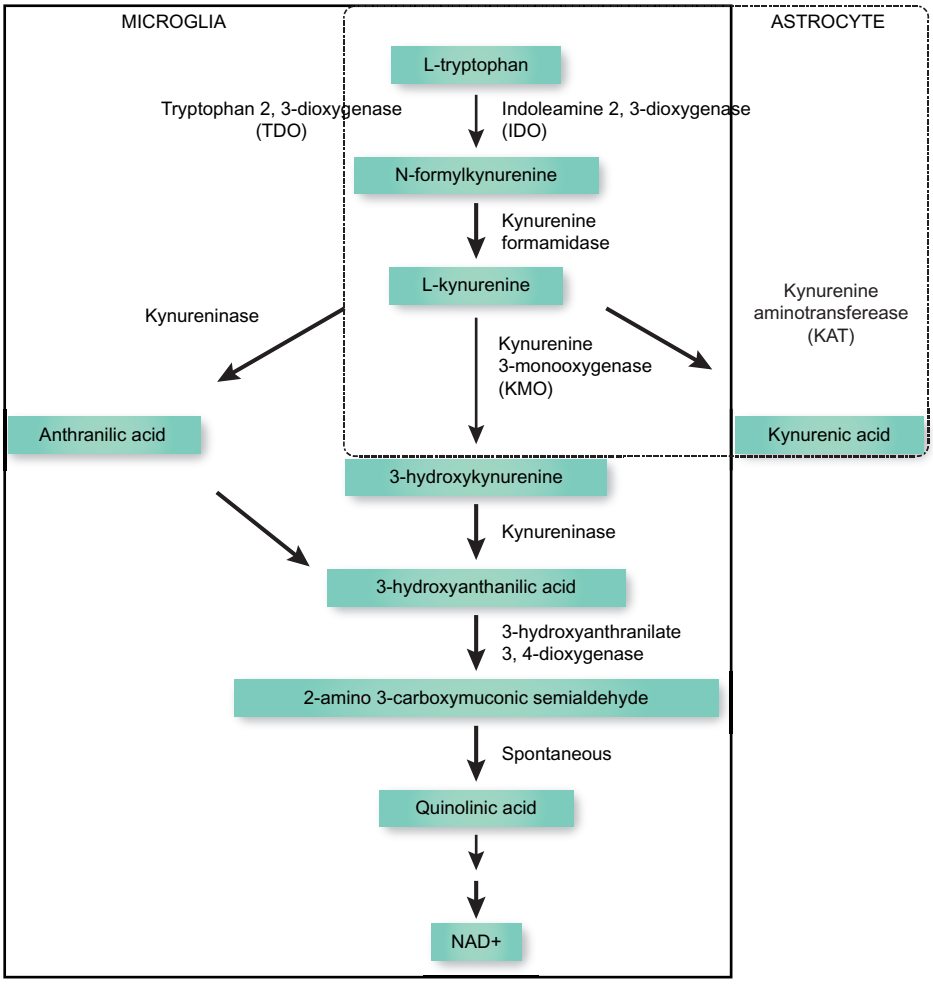

Figure 1. The kynurenine pathway of tryptophan degradation. KYNA is produced mainly in astrocytes (dashed box), whereas QUIN is produced mainly in microglia (solid box).

CSF QUIN and related the levels to CSF KYNA, CSF kynurenine, and CSF tryptophan from the same patients with schizophrenia and from healthy controls.

Materials and Methods

Patients. Twenty-two Swedish Caucasian outpatients diagnosed with schizophrenia ($n = 18$; 11 males, 7 females) or schizoaffective disorder ($n = 4$; 2 males, 2 females), according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*)[28] criteria, were included in the study. Dr Elisabeth Skogh and Dr Martin Samuelsson were the responsible psychiatrists for the DSM-IV diagnosis. Full details of the study design and patient characteristics, including serum and CSF concentrations of olanzapine, have been published elsewhere.[29] All of the patients were prescribed olanzapine as the only antipsychotic drug. The patients had been on medication with olanzapine for between 0.1 and 11 years (median 2 years) using the same dose (5–25 mg/day) for at least 14 days prior to CSF sampling. All patients were somatically healthy, as judged by routine laboratory analyses (electrolytes, hematology, kidney, liver, and thyroid function) and a physical examination. The Brief Psychiatric Rating Scale (BPRS)[30] and Global Assessment of Functioning (GAF)[28] index were used to evaluate symptoms and the level of function, respectively. Mean (± SD) age of patients was 37.1 ± 7.6 years (range 23–50 years).

Controls. As controls, 26 healthy Caucasian volunteers (18 males, and 8 females in the follicular phase of the menstrual cycle) were recruited among medical students, hospital staff, and their relatives. All controls were somatically healthy, as judged by routine laboratory analyses (electrolytes, hematology blood, kidney, liver, and thyroid function) and physical examination. Volunteers were subjected to a semi-structured interview using the Structured Clinical Interview of DSM-IV disorders (SCID Axis I)[31] and a questionnaire for personality disorders (SCID Axis II)[32] or interviewed by a psychiatrist to exclude mental illness. None of them had a family history of major psychosis or suicide in first- or second-degree relatives, and they were all found to be free from current signs of psychiatric morbidity or difficulties in social adjustment at the time of sampling. Controls were not allowed to use any medication for at least one month prior to sampling; however, coffee and smoking were allowed. The mean (± SD) age of the controls was 24.9 ± 5.8 years (range 18–49 years).

CSF levels of kynurenine, KYNA, and tryptophan from a subsample of patients ($n = 11$, all males) and controls ($n = 18$, all males) have previously been published.[6] KYNA is a stable compound, neither degraded nor affected by storage time, nor by repeated thawing.[33] Since the sample size of the present study differs from our previously published paper,[6] levels of CSF kynurenine, KYNA, and tryptophan in the patients and controls included here are shown in Table 1.

Ethical consideration. The patients and controls were recruited at Linköping University Hospital. The study was approved by the Ethics Committee of Linköping University and the Swedish Medical Products Agency. The study was performed according to the Declaration of Helsinki for experiments involving human subjects. All patients and healthy volunteers received written as well as verbal information and provided written consent prior to engaging in the study. AstraZeneca did neither influence nor sponsor the clinical research performed at Linköping University. These data arise from a sample collection, not from a prospective trial, and this work is therefore not recorded in any clinical trial registry.

CSF sampling. For lumbar puncture, a disposable needle (BD Whitacre Needle 0.7 × 90 mm) was inserted at the L 4–5 level with the subject in the right decubitus position. CSF was allowed to drip into a plastic test tube. The CSF samples were protected from light, centrifuged at 1438 *g* for 10 minutes (Sigma 203 centrifuge) within 30 minutes after the puncture, and divided into 2- to 3-ml aliquots. Samples were stored at –70 °C pending analysis.

Analysis of olanzapine and laboratory variables. In order to analyze olanzapine in CSF and serum, a validated liquid chromatography/tandem mass spectrometry method was used as described in detail elsewhere.[34]

Analysis of kynurenines. For QUIN, standard curves were prepared in the range of 0.005 to 0.5 µmol/L QUIN (Sigma-Aldrich), dissolved in Dulbecco´s phosphate buffered saline (PBS; Gibco®, Life Technologies, Carlsbad, CA, USA), aliquoted, and stored at –70 °C until use. CSF and standard samples (50 µL) were diluted 2× with internal standard solution in 5% formic acid and filtered by centrifugation at 3000 *g* for 60 minutes at 10 °C using 10 kDa Ultracel®-10 filter plates (Merck Millipore, Darmstadt, Germany). Internal standard ($^{13}C_3{}^{15}N_1$-QUIN; Synfine research Inc., Ontario, Canada) was added to each standard and CSF sample to a final concentration of 0.5 µmol/L.

Following centrifugation, 7.5 µL of the filtrate was injected into a Waters Acquity high-performance liquid chromatography (HPLC) system equipped with a SymmetryShield™

Table 1. CSF levels of tryptophan,kynurenine and KYNA in the present sample, presented as mean ± SEM. Part of these data have previously been published.[6]

	PATIENT	CONTROL
N	21	26
Tryptophan (µM)	1.7 ± 0.03	1.8 ± 0.07
	$P = 0.652$	
N	21	26
Kynurenine (nM)	57.2 ± 3.5	37.3 ± 4.3
	$P = 0.001$	
N	19	26
KYNA (nM)	2.1 ± 0.2	1.6 ± 0.1
	$P = 0.012$	

RP18 2.1 × 100 mm, 3.5 µm particle column. The detection was performed using a Waters Xevo TQ-S triple quadrupole mass spectrometer operating in positive ionization MS/MS configuration. The mobile phase was run at a flow rate of 300 µL/minute and consisted of 2.1% formic acid (MS-grade, Sigma-Aldrich, St. Louis, MO, USA) in MilliQ water (A phase) and 95% acetonitrile (MS-grade, Sigma-Aldrich), 0.1% formic acid in MilliQ water (B phase), starting with 5% B for two minutes, following gradient elution up to 95% B, with a total run time of 10 minutes. The mass spectrometer was tuned for QUIN and set at capillary voltage of 3.0 V, cone voltage 25 V, source temperature 150 °C, desolvation temperature 500 °C, desolvation gas flow of 150 L/hour, and collision energy of 16 eV. Mass spectral transition for QUIN was m/z 168 > 106 and for the IS 172 > 110.

Calibration was performed using standards covering the range of the CSF concentration. Seven concentration points were used to establish a linear calibration curve and plotted using the ratio of analyte peak area over IS peak area after integration by Masslynx 4.1 software (Waters Corporation, Milford, MA, USA). Retention time for QUIN was 1.2 minutes.

The analysis of tryptophan, kynurenine, and KYNA was performed utilizing a reversed-phase HPLC system as previously described.[5,6] Fifty-microliter samples were manually injected, and some samples were analyzed in duplicate, and the inter-individual variation was less than 5%.

Statistical analysis. Plotting CSF QUIN residuals revealed one patient as an outlier in regard to CSF QUIN (standardized residuals > 3 SD). Data from this 29-year-old male patient (CSF QUIN = 85.1 nM) were removed from all further analyses. For one patient the QUIN levels were below the lowest level of detection (LLOD), and the QUIN value for that patient was substituted for the LLOD value (5 nM). Background characteristics between patients and controls were compared using *t*-tests or Chi-square tests. To study the effect of background characteristics on CSF QUIN concentration in the patient sample and in the controls sample we used correlation analyses or Mann–Whitney *U*-tests. All correlation analyses were performed using Spearman rank correlation tests. The comparisons of CSF kynurenine metabolites levels and the QUIN/KYNA ratio between patients and healthy volunteers were performed using *t*-tests. Logistic regression analyses with age and sex as covariates were also performed. All reported *P*-values are two sided. All analyses were made using IBM SPSS Statistics 21.0 software (IBM SPSS Inc., Chicago, IL, USA).

Results

Background characteristics. The mean age (±SD) among the 21 patients was 37.5 (±7.5) years, and in the 26 controls 24.9 (±5.8) years ($P < 0.0001$). Sex, smoking status, height, weight, and body mass index (BMI) did not differ significantly between patients and healthy controls (Table 2).

Age was correlated to QUIN levels in controls ($P = 0.012$) but not in patients; there was however a trend toward correlation also in patients ($P = 0.113$). Sex, height, weight, and BMI were not correlated to CSF QUIN in patients or in controls. In the patient group there was no correlation between CSF QUIN and psychiatric symptom ratings (BPRS and GAF); however, there was a tendency of positive correlation between CSF QUIN and serum levels of olanzapine ($\rho = 0.414$, $P = 0.062$). There was no correlation between CSF QUIN levels and CSF olanzapine levels ($\rho = 0.333$, $P = 0.140$). The mean CSF QUIN concentration did not differ between smokers/nonsmokers or male/females, in patients or in controls (Table 3).

CSF QUIN levels and the QUIN/KYNA ratio in patients and controls. The mean CSF QUIN concentration did not differ significantly between patients and controls (patients: 20.6 ± 1.51 nM vs. controls: 18.2 ± 1.08 nM; $P = 0.198$; Fig. 2). Adjusting the comparison for potential confounding by age and sex gave a similar result (Odds Ratio (OR) = 0.93, 95% confidence interval (CI) = 0.80–1.10; $P = 0.355$). The QUIN/KYNA ratio was however close to significantly decreased in patients compared to healthy controls ($P = 0.057$). Adjusting the analysis for differences in age and gender distribution in two groups strengthened the association between the patient group and a decreased QUIN/KYNA ratio (OR = 0.66, 95% CI 0.45–0.95; $P = 0.027$).

Relationship with other kynurenine metabolites. A correlation between CSF QUIN and CSF kynurenine levels was detected in patients ($\rho = 0.53$, $P = 0.014$) but not in the controls ($\rho = -0.32$, $P = 0.117$). A similar pattern was seen regarding CSF QUIN and CSF KYNA in patients ($\rho = 0.54$, $P = 0.016$) and in controls ($\rho = 0.12$, $P = 0.565$). No significant correlation between CSF tryptophan and CSF QUIN levels was detected in patients ($\rho = 0.32$, $P = 0.164$), or in controls ($\rho = -0.18$, $P = 0.373$). See Table 1 for levels of kynurenine, KYNA, and tryptophan.

Discussion

The present study offers support to the view of an imbalanced kynurenine pathway in patients with schizophrenia. Thus, levels of CSF kynurenine and CSF KYNA were found to be elevated whereas CSF QUIN levels were found to be in the same range in patients and in healthy controls. Furthermore, a reduced QUIN/KYNA ratio was observed in patients, a result that is likely independent of smoking status. Moreover, QUIN was found to correlate to both kynurenine and KYNA in patients but not in controls. Despite a correlation on the individual patient level, no difference in CSF QUIN levels between patients and controls was observed, suggesting that a putative enhancement of QUIN formation is substantially lower compared to the increase in kynurenine and KYNA in patients. The lack of correlation between CSF QUIN and CSF tryptophan might be attributed to the fact that no difference in the levels of neither tryptophan nor QUIN was observed.

Table 2. Demographics of participants.

PATIENT CONTROL	QUIN (nM)	AGE	GENDER	DIAGNOSIS	BODY HEIGHT (cm)	BMI	SMOKING	DOSE OLA (mg)	OLA (YEARS)	SERUM OLA (nM)	CSF OLA (nM)	BPRS	GAF
1	22.5	41	M	SZ	188	28.1	No	7.5	7.75	71	9.0	25	68
2	18.1	46	F	SZA	169.5	28.1	Yes	20	2	81	11.2	38	45
3	22.7	49.5	M	SZ	173.5	23.6	Yes	25	0.9	101	10.2	33	62
4	23.9	33	M	SZA	196	26.2	No	5	1.25	80	8.1	25	58
5	16.2	46	F	SZ	167	19.7	Yes	10	5.25	54	7.6	37	45
6	27.5	34	M	SZ	181.5	22.7	No	10	0.25	131	16.2	29	68
7	26.1	41	F	SZ	168	23.2	No	7.5	0.4	53	10.0	34	67
8	24.9	35	F	SZA	171	27.5	No	7.5	8.5	105	12.1	33	62
9	15.3	28	F	SZ	167	22.1	Yes	10	0.8	65	5.5	42	44
10	14.9	23	M	SZ	181.5	26.2	No	10	2	74	10.4	32	53
11	15.0	26	F	SZ	179.5	25.7	No	20	5	171	19.3	20	72
12	15.1	34	M	SZ	177.5	23.3	No	10	0.1	98	13.3	33	58
13	36.4	48	M	SZ	187.5	18.5	No	20	7.2	327	30.2	33	49
14	5.0	30	M	SZ	178	21.9	Yes	10	4.5	70	11.6	36	48
15	25.4	38	M	SZ	181	26.6	No	12.5	9.3	194	24.5	46	52
16	29.4	43.5	M	SZ	185.5	26.5	No	20	8.8	200	28.7	46	45
17	22.2	40	F	SZ	158.5	27.2	No	5	4.5	30	4.6	33	52
18	21.2	35.5	F	SZ	163	29.7	Yes	10	0.5	141	14.1	23	58
19	14.5	37.5	M	SZA	178	26.1	No	15	11	119	17.3	33	54
20	13.4	46.5	M	SZ	182	26.1	No	10	0.2	55	7.1	30	58
21	22.5	31	F	SZ	178	32.5	Yes	20	0.5	167	18.6	38	49
1	14.2	21	M	–	181.0	21.6	No	–	–	–	–	–	–
2	17.3	23	M	–	172.0	24.6	No	–	–	–	–	–	–
3	12.5	22	M	–	178.0	26.6	No	–	–	–	–	–	–
4	20.9	21	M	–	194.5	19.6	No	–	–	–	–	–	–
5	17.2	18	M	–	177.0	24.9	No	–	–	–	–	–	–
6	8.7	23	M	–	173.0	21.6	Yes	–	–	–	–	–	–
7	21.9	27	M	–	176.0	26.5	Yes	–	–	–	–	–	–
8	22.0	49	M	–	174.0	27.0	No	–	–	–	–	–	–
9	9.6	21	M	–	185.0	24.1	Yes	–	–	–	–	–	–
10	18.0	22	M	–	187.0	24.0	No	–	–	–	–	–	–
11	22.3	25	M	–	196.0	24.8	No	–	–	–	–	–	–

(*Continued*)

Table 2. (*Continued*)

PATIENT CONTROL	QUIN (nM)	AGE	GENDER	DIAGNOSIS	BODY HEIGHT (cm)	BMI	SMOKING	DOSE OLA (mg)	OLA (YEARS)	SERUM OLA (nM)	CSF OLA (nM)	BPRS	GAF
12	10.5	23	M	–	190.5	20.7	–	–	–	–	–	–	–
13	15.1	32	M	–	190.0	23.1	No	–	–	–	–	–	–
14	22.4	23	M	–	178.0	21.8	No	–	–	–	–	–	–
15	19.2	25	M	–	182.0	22.8	No	–	–	–	–	–	–
16	34.5	25	M	–	190.0	23.0	No	–	–	–	–	–	–
17	21.8	22	M	–	172.0	24.4	No	–	–	–	–	–	–
18	16.3	23	M	–	186.0	21.7	No	–	–	–	–	–	–
19	19.1	27.5	F	–	174.5	20.7	No	–	–	–	–	–	–
20	22.0	26.5	F	–	168.0	22.7	No	–	–	–	–	–	–
21	14.0	23.5	F	–	172.0	28.1	No	–	–	–	–	–	–
22	13.2	22	F	–	168.5	27.4	Yes	–	–	–	–	–	–
23	18.7	25	F	–	161.0	22.8	No	–	–	–	–	–	–
24	22.7	23	F	–	183.0	24.9	No	–	–	–	–	–	–
25	14.0	22	F	–	173.0	25.6	No	–	–	–	–	–	–
26	23.8	32	F	–	158.0	24.7	Yes	–	–	–	–	–	–

Abbreviations: BMI, Body mass index; OLA, Olanzapine; M, Male; F, Female; SZ, Schizophrenia; SZA, Schizoaffective disorder.

Table 3. QUIN levels in patients and controls and according to sex and smoking status.

	SMOKING		SEX		CASE	
	Yes	No	Male	Female	Patient	Control
N	12	34	30	17	21	26
QUIN (nM; mean ± SEM)	16.5 ± 1.8	20.5 ± 1.0	19.2 ± 1.3	19.4 ± 1.0	20.6 ± 1.5	18.2 ± 1.1
P-value	0.074		0.913		0.198	

The present findings indicate an over-activated but also an imbalanced kynurenine pathway in patients with schizophrenia, favoring the production of KYNA over QUIN.

The observed imbalance of the kynurenine pathway in schizophrenia is likely a result of a priority of L-kynurenine metabolism toward the KYNA branch. Several mechanisms, acting independently or in combination, may induce such a disparity. The increased activity in the kynurenine pathway is likely attributed to an upregulation of the initial and rate limiting enzyme TDO in patients with schizophrenia, tentatively induced by the increased secretion of the proinflammatory cytokine IL-1β.[10,26,35,36] Furthermore, the differential activation of the QUIN and KYNA branches of the kynurenine pathway is likely sought for in differences in activity of the enzymes guarding the entry of L-kynurenine into the different branches of the pathway. Thus, compared to the higher capacity of KAT enzymes, displaying K_m values in the low millimolar range,[37] the KMO enzyme gets saturated at relatively low concentrations ($K_m \approx 20$ μM),[38] and can therefore act as a rate limiting step in the synthesis of QUIN. In a situation where L-kynurenine is elevated (eg, by induction of TDO) such a limitation of KMO might guard against excessive production of QUIN. A suboptimal function of KMO could also present an explanatory mechanism of the observed increase in KYNA but not in QUIN.[7,8,39] Such a scenario would thus shunt the metabolism of L-kynurenine toward formation of KYNA, which indeed is observed in the present study. In agreement, experimental data show increased brain KYNA concentrations in *Kmo* knockout mice or following pharmacological blockade of KMO.[40–42] The importance of KMO in the kynurenine metabolism is supported by clinical data. A nonsynonymous single nucleotide polymorphism (SNP) in the KMO gene has been shown to affect the CSF KYNA levels in patients with schizophrenia[39] and bipolar disorder.[8] In addition, the activity[7] and expression[8] of the KMO enzyme was found to be reduced in prefrontal cortex in patients with schizophrenia and bipolar disorder patients with psychosis. Furthermore, a recent study suggests that the function of other enzymes in the pathway is worth investigating as well. Thus, examining rare mutations constituting a polygenic burden in schizophrenia, the lowest identified nominal *P*-value for disruptive mutations was for the enzyme kynureninase, converting 3-hydroxykynurenine to 3-hydroxyanthranilic acid.[43] Although these mutations did not reach statistical significance, it constitutes another piece of evidence for a downregulation of the QUIN branch of the kynurenine pathway in schizophrenia. The ratio between CSF levels of QUIN and KYNA is consequently telling us more than the individual measurements of QUIN and KYNA per se. Not only do these compounds represent different branches of the kynurenine pathway but also more importantly have opposing effects, with KYNA being an antagonist and QUIN an agonist of the NMDA receptor. A shift in the ratio between QUIN and KYNA is therefore likely to have an effect on behavioral domains.

The higher QUIN/KYNA ratio in controls is in line with the above-mentioned studies and renders support to the hypothesis that KYNA is elevated in patients with schizophrenia likely because of a lower input of kynurenine into the QUIN branch of the pathway. In comparison, we recently showed that this ratio (QUIN/KYNA) was elevated in suicide attempters compared to healthy volunteers.[22]

The kynurenine pathway is increasingly recognized as a pathophysiological promoter in several diseases. In this regard, the balance between the two oppositely acting metabolites, KYNA and QUIN, acting to antagonize and stimulate the NMDA receptor, respectively, is of major importance for glutamatergic neurotransmission. The present study, showing normal CSF QUIN levels concomitant with increased CSF kynurenine and CSF KYNA, resulting in a decreased QUIN/KYNA ratio in patients, offers support to the view of an imbalanced kynurenine pathway in schizophrenia. These data are thus in line with previous findings showing elevated levels of KYNA and a compromised function of enzymes involved in the synthesis of QUIN in patients with schizophrenia.

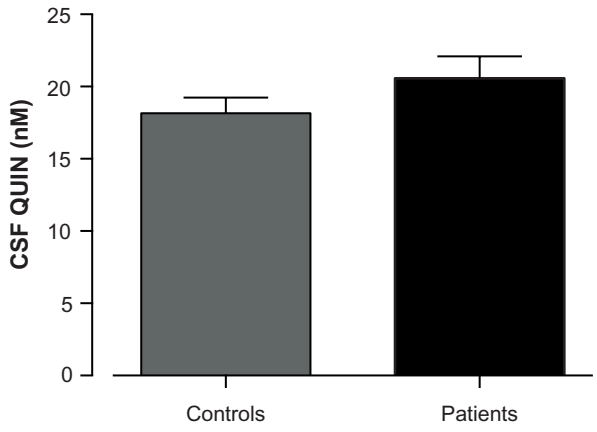

Figure 2. CSF QUIN levels, in healthy controls and in patients with schizophrenia. After adjustment for age and sex (OR = 0.93, 95% CI 0.80–1.10; *P* = 0.355).

Acknowledgments

We thank patients and healthy volunteers for their participation and express our gratitude toward health professionals who facilitated our work; in particular, we would like to thank the research nurses.

Author Contributions

Conceived and designed the experiments: MEK, MB, ES, MS, KL, MLD, LS, GE, ISK, SE. Analyzed the data: MEK, CS. Wrote the first draft of the manuscript: MEK. Contributed to the writing of the manuscript: MEK, MB, ES, MS, KL, MLD, CS, LS, GE, ISK, SE. Agree with manuscript results and conclusions: MEK, MB, ES, MS, KL, MLD, CS, LS, GE, ISK, SE. Jointly developed the structure and arguments for the paper: MEK, MB, ES, MS, KL, MLD, CS,

LS, GE, ISK, SE. Made critical revisions and approved final version: MEK, GE, SE. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenic acid – potential in the treatment of psychiatric disorders. *CNS Drugs*. 2009;23(2):91–101.
2. Schwarcz R, Bruno JP, Muchowski PJ, Wu H-Q. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012;13(7):465–77.
3. Erhardt S, Blennow K, Nordin C, Skogh E, Lindström LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci Lett*. 2001;313(1–2):96–8.
4. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry*. 2001;50(7):521–30.
5. Olsson S. Elevated levels of kynurenic acid in the cerebrospinal fluid of patients with bipolar disorder. *J Psychiatry Neurosci*. 2010;35(3):195–9.
6. Linderholm KR, Skogh E, Olsson SK, et al. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophr Bull*. 2012;38(3):426–32.
7. Sathyasaikumar KV, Stachowski EK, Wonodi I, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull*. 2011;37(6):1147–56.
8. Lavebratt C, Olsson S, Backlund L, et al. The KMO allele encoding Arg(452) is associated with psychotic features in bipolar disorder type 1, and with increased CSF KYNA level and reduced KMO expression. *Mol Psychiatry*. 2014;19(3):334–41.
9. Wonodi I, Stine OC, Sathyasaikumar KV, et al. Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Arch Gen Psychiatry*. 2011;68(7):665–74.
10. Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res*. 2006;107(3–1074):25–37.
11. Stone TW. Neuropharmacology of quinolinic and kynurenic acids. *Pharmacol Rev*. 1993;45(3):309–79.
12. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J Neurosci*. 2001;21(19):7463–73.
13. Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biol Psychiatry*. 2004;56(4):255–60.
14. Pocivavsek A, Wu H-Q, Elmer GI, Bruno JP, Schwarcz R. Pre- and postnatal exposure to kynurenine causes cognitive deficits in adulthood. *Eur J Neurosci*. 2012;35(10):1605–12.
15. Chess AC, Bucci DJ. Increased concentration of cerebral kynurenic acid alters stimulus processing and conditioned responding. *Behav Brain Res*. 2006;170(2):326–32.
16. Potter MC, Elmer GI, Bergeron R, et al. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. *Neuropsychopharmacology*. 2010;35(8):1734–42.
17. Chess AC, Simoni MK, Alling TE, Bucci DJ. Elevations of endogenous kynurenic acid produce spatial working memory deficits. *Schizophr Bull*. 2007;33(3):797–804.
18. Olsson SK, Larsson MK, Erhardt S. Subchronic elevation of brain kynurenic acid augments amphetamine-induced locomotor response in mice. *J Neural Transm*. 2012;119(2):155–63.
19. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J*. 2012;279(8):1356–65.
20. Guidetti P, Luthi-Carter RE, Augood SJ, Schwarcz R. Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiol Dis*. 2004;17(3):455–61.
21. Guillemin GJ, Smythe G, Takikawa O, Brew BJ. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia*. 2005;49(1):15–23.
22. Erhardt S, Lim CK, Linderholm KR, Janelidze S, Lindqvist D, Guillemin GJ. Connecting inflammation with glutamate agonism in suicidality. *Neuropsychopharmacology*. 2013;38:743–52.
23. Steiner J, Walter M, Gos T, et al. Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission? *J Neuroinflammation*. 2011;8:94.
24. Campbell BM, Charych E, Lee AW, Möller T. Kynurenines in CNS disease: regulation by inflammatory cytokines. *Front Neurosci*. 2014;8:12.
25. Mándi Y, Vécsei L. The kynurenine system and immunoregulation. *J Neural Transm*. 2012;119(2):197–209.
26. Kegel ME, Svensson CI, Erhardt S. IL-1 induces IDO and TDO transcription and provokes the release of kynurenic acid from human astrocytes in vitro. *Progr No* 24019/*E7 Neurosci Meet Planner* Washington, DC *Soc Neurosci Online*. 2011.
27. Schwarcz R, Tamminga CA, Kurlan R, Shoulson I. Cerebrospinal fluid levels of quinolinic acid in Huntington's disease and schizophrenia. *Ann Neurol*. 1988;24(4):580–2.
28. American Psychiatric Association X. *Diagnostic and Statistical Manual of Mental Disorders*. Fourth. Washington, DC: American Psychiatric Association; 1994.
29. Skogh E, Sjödin I, Josefsson M, Dahl M-L. High correlation between serum and cerebrospinal fluid olanzapine concentrations in patients with schizophrenia or schizoaffective disorder medicating with oral olanzapine as the only antipsychotic drug. *J Clin Psychopharmacol*. 2011;31(1):4–9.
30. Overall JE, Gorham DR. The brief psychiatric rating scale. *Psychol Rep*. 1962;10:799–812.
31. First M, Spitzer R, Gibbon M, Williams J. User's Guide for the Structured Clinical Interview for DMS-IV Axis I Disorders – Clinician Version (SCID-CV). Washington DC: American Psychiatry Press; 1997.
32. First M, Gibbon M, Spitzer R, Williams J, Benjamin L. *SCID-II Personality Questionnaire*. Washington DC: American Psychiatry Press; 1997.
33. Heyes M, Quearry B. Quantification of kynurenic acid in cerebrospinal fluid: effects of systemic and central L-kynurenine administration. *J Chromatogr*. 1990;530:108–15.
34. Josefsson M, Roman M, Skogh E, Dahl M-L. Liquid chromatography/tandem mass spectrometry method for determination of olanzapine and N-desmethylolanzapine in human serum and cerebrospinal fluid. *J Pharm Biomed Anal*. 2010;53(3):576–82.
35. Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol Dis*. 2004;15(3):618–29.
36. Söderlund J, Schröder J, Nordin C, et al. Activation of brain interleukin-1beta in schizophrenia. *Mol Psychiatry*. 2009;14(12):1069–71.
37. Okuno E, Nakamura M, Schwarcz R. Two kynurenine aminotransferases in human brain. *Brain Res*. 1991;542(2):307–12.
38. Bender DA, McCreanor GM. Kynurenine hydroxylase: a potential rate-limiting enzyme in tryptophan metabolism. *Biochem Soc Trans*. 1985;13(2):441–3.
39. Holtze M, Saetre P, Engberg G, et al. Kynurenine 3-monooxygenase polymorphisms: relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls. *J Psychiatry Neurosci*. 2012;37(1):53–7.
40. Speciale C, Wu H, Cini M, Marconi M, Varasi M, Schwarcz R. (R,S)-3,4-dichlorobenzoylalanine (FCE 28833 A) causes a large and persistent increase in brain kynurenic acid levels in rats. *Eur J Pharmacol*. 1996;315:263–7.
41. Erhardt S, Oberg H, Mathé JM, Engberg G. Pharmacological elevation of endogenous kynurenic acid levels activates nigral dopamine neurons. *Amino Acids*. 2001;20(4):353–62.
42. Giorgini F, Huang SY, Sathyasaikumar KV, et al. Targeted deletion of kynurenine 3-monooxygenase in mice: a new tool for studying kynurenine pathway metabolism in periphery and brain. *J Biol Chem*. 2013;288(51):36554–66.
43. Purcell SM, Moran JL, Fromer M, et al. A polygenic burden of rare disruptive mutations in schizophrenia. *Nature*. 2014;506(7487):185–90.