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Effects of inflammation modulation on tryptophan and kynurenine pathway regulation in treatment resistant bipolar depression

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ABSTRACT

Background: Adjunctive immune-modulation can be safe and effective for treatment-resistant bipolar depression (TRBDD), but molecular work is needed to further characterize the safety and efficacy involved in treatment response and the reversal of treatment resistance. Here we profiled the kynurenine pathway (KP) for biomarkers associated with TRBDD and treatment response to celecoxib (CBX)-augmentation.

Methods: 47 TRBDD patients with moderately severe HAMD-17 scores were randomized to receive either escitalopram (ESC) (10 mg twice daily) + CBX (200 mg twice daily), or ESC (10 mg twice daily) + placebo (PBO) (twice daily). Plasma kynurenine pathway (KP) metabolite levels were measured at baseline, week 4, and week 8, and in a healthy control (HC) group of subjects (N = 35) once.

Results: Patients receiving ESC + CBX had 4.278 greater odds of responding ($p = 0.021$) with NNT = 3, and 15.300 greater odds of remitting ($p < 0.001$) with NNT = 2, compared with ESC + PBO patients. Study patients exhibited elevated baseline tryptophan ($p < 0.001$), low kynurenine/tryptophan ($p < 0.001$), elevated 3-hydryoxykynurenine/kynurenine (adj-$p^* < 0.001$), low kynurenic acid/3-hydroxykynurenine, and low picolinic acid/quinolinic acid ($p < 0.001$) compared to healthy controls. Treatment responders exhibited tryptophan depletion ($p = 0.020$) without a concomitant change in kynurenine/tryptophan ratio by week 8 ($p = 0.163$).

Conclusion: Clinical response to CBX augmentation is not associated with altered neurotoxic or neuroprotective indices within the time frame of this study. TRBDD revealed alterations in neuroprotective and neurotoxic indices, in the context of low kynurenine/tryptophan and high tryptophan. Treatment responders revealed a depletion in tryptophan by week 8, without concomitant kynurenine pathway (KP) activation.

1. Introduction

Bipolar depression (BDD), the 6th leading cause of disability worldwide, is a lifelong condition with an alternating course of manic and depressive episodes, characterized by severe impairment in mood, cognition, sleep wakefulness, and psychosocial function (Sachs et al., 2007). A crucial challenge in the patient population diagnosed with bipolar disorder (BD) is treatment resistant depression (TRD). The Maudsley Staging Method (MSM) defines treatment resistance as failure to achieve remission from depressive symptoms after an adequately dosed antidepressant trial lasting at least six weeks (Fekadu, Donocik, & Cleare, 2018). As the MSM was originally intended for unipolar depression, similar consensus criteria were defined for treatment resistant bipolar depression (TRBDD): “failure to reach sustained symptomatic remission for 8 consecutive weeks after two different treatment trials, at adequate therapeutic doses, with at least two recommended monotherapy treatments or at least one monotherapy treatment and another combination treatment” (Hidalgo-Mazzei et al., 2019).

The biological basis of treatment-resistance appears to be related, at least in a subset of TRBDD subjects, to an underlying metabolic and immunologic dysregulation (Felger et al., 2018; Haroon et al., 2018). Over time, these disturbances contribute to the chronic, relapsing-remitting course of BD conceptualized as neuroprogression (Bauer, Soares, Selek, & Meyer, 2017; Berk et al., 2017; Boufidou & Halaris,

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2017). Considering the emerging metabolic-immune basis of depressive illness, investigators have hypothesized that modulation of inflammatory response may be helpful for pharmacologically induced arrest of neuroprogression (Faridhosseini, Sadeghi, Farid, & Pourgholami, 2014; Krause et al., 2017; Nery et al., 2008a).

To that end, adjunctive treatment with the cyclooxygenase-2 inhibitor, celecoxib (CBX), has been reported to lead to improved overall outcome in major depressive disorder (MDD) (Abbasi, Hosseini, Modabbernia, Ashrafi, & Akhondzadeh, 2012; Faridhosseini, Sadeghi, Farid, & Pourgholami, 2014; Muller et al., 2006). However, similar work in BDD is sparse (Ayorech, Tracy, Baumeister, & Giaroli, 2015; Husain et al., 2016; Nery et al., 2008b), and none to date has directly addressed TRBDD until the present study (Halaris, Alvi, Meresh, & Sharma, 2014). This study is part of a larger clinical study which was the first to show that adjunctive immune modulation with CBX is safe and effective for TRBDD (manuscript in preparation). Nevertheless, research at the molecular level should enable further characterization of the mechanism of action of COX-2 inhibition in effecting improved outcomes as well as the safety of such intervention.

The Kynurenine Pathway (KP) is an alternative pathway that has been implicated in depression, by which tryptophan (TRP) is catabolized towards multiple neuroactive KP metabolites, rather than towards serotonin (5-HT) and melatonin synthesis (Fig. 1) (Cervenka, Agudelo, & Ruas, 2017; Ogyu et al., 2018). The rationale for exploring the KP arises from its involvement in overlapping theories of depression: serotonergic neurotransmission, neurotoxicity, and inflammation (Anderson, Jacob, Bellivier, & Geoffroy, 2016; Miller, 2013; Myint, 2012). We postulated that the KP holds a crucial mechanistic link between the aforementioned domains: pro-inflammatory cytokines can induce the expression and activity of indoleamine 2,3-dioxygenase (IDO), an enzyme governing the first rate-limiting step of KP activation (Salazar, Gonzalez-Rivera, Redus, Parrott, & O'Connor, 2012; Taguchi et al., 2014). Prior studies have corroborated the concept that KP activation is a metabolic expression of elevated inflammatory burden, and that clinical response to adjunctive inflammatory modulation is predicted by baseline KP abnormalities (Krause et al., 2017).

We therefore hypothesized that the cumulative impact of chronic, high inflammatory burden in TRBDD (Bauer et al., 2017; Edberg et al., 2018a; Johansson et al., 2013; Tsai et al., 2014) is metabolically expressed, in part, through measurable shifts in KP metabolite ratios (Fig. 2). We further hypothesized the clinical efficacy of CBX augmentation is related to baseline abnormalities in KP, which are normalized in responders over the course of treatment. These predictions are expressed in the following four hypotheses:

- **Hypothesis 1**: *TRBDD subjects receiving ESC + CBX will show enhanced response rates compared to subjects receiving ESC + PBO*
- **Hypothesis 2:** *TRBDD will show different KP indices at baseline, compared to HC*
- **Hypothesis 3:** *Treatment responders will show distinct KP indices at baseline, compared to non-responders*
- **Hypothesis 4:** *Treatment responders will show KP index changes by treatment week 8*

2. Materials and methods

2.1. Study population

The study was approved by the Institutional Review Board of Loyola University Medical Center and the Review Board of the Medical Faculty of the LMU and conducted according to the principles of the Declaration of Helsinki. Potential candidates were screened to determine eligibility for the study by meeting inclusion criteria and their capability of understanding the study and giving informed consent. Males and females between the ages of 21 and 65 years who met DSM-IV criteria for bipolar disorder (BD I or II) depressed phase, without comorbid medical or psychiatric diagnoses, or substance abuse/dependence during the preceding 12 months, were considered for this study. Eligible BD depressed patients had to have previously failed to respond to two or more adequate trials with an antidepressant or experienced a breakthrough depressive episode despite being maintained on a mood stabilizer and/or atypical antipsychotic agent. Treatment resistance was established by the Maudsley scale (Fekadu et al., 2018).

A minimum score of 18 on the first 17 items of the 21-item Hamilton Depression Scale (HAM-D17) was required for admission into the study. Patients had to agree to undergo a washout from: Vitamin E, fish oils > 600 IU/day, non-aspirin NSAIDs, aspirin > 81 mg/day, H2 receptor antagonists, and Ginko biloba for at least two weeks, to refrain from caffeine on morning of blood drawing, and to institute lights-out at 23:00 h on nights before blood drawings.

Exclusion criteria included hypertension, anemia, liver disease, kidney disease, arthritis, diabetes, recurrent migraines, epilepsy, stroke, gum disease, and autoimmune disease. Additional exclusion criteria were: any abnormal findings on physical exam, ECG, or blood/urine

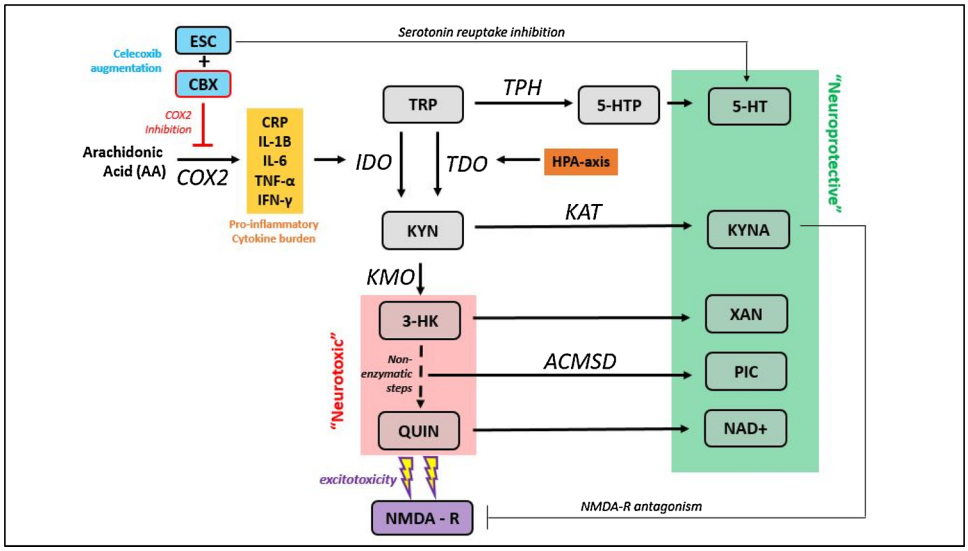

Fig. 1. Connection between the inflammatory and the kynurenine pathways. The kynurenine pathway (KP) is an alternate TRP breakdown pathway by which pro-inflammatory cytokines promote the catabolism of TRP away from 5-HT synthesis and towards neuroactive KP metabolites. Legend: TRP = tryptophan, KYN = kynurenine, 3 H K = 3-hydroxykynurenic acid, QUIN = quinolinic acid, NMDA-R = N-methyl-D-aspartate receptor, 5-HTP = 5-hydroxytryptophan, 5-HT = 5-hydroxytrypamine, KYNA = kynurenic acid, PIC = picolinic acid, NAD + = nicotinamide adenine dinucleotide, IDO = indoleamine 2,3-dioxygenase, TDO = tryptophan 2,3-dioxygenase, KMO = kynurenine monooxygenase, KAT II = kynurenine acyl transferase II, ACMSD = aminocarboxymuconate semialdehyde decarboxylase, TNF-α = tumor necrosis factor alpha, IFN-γ = interferon gamma, IL-1β = interleukin 1-beta, IL-6 = interleukin 6, CRP = C-reactive protein, COX2 = cyclooxygenase 2, CBX = celecoxib, ESC = escitalopram.

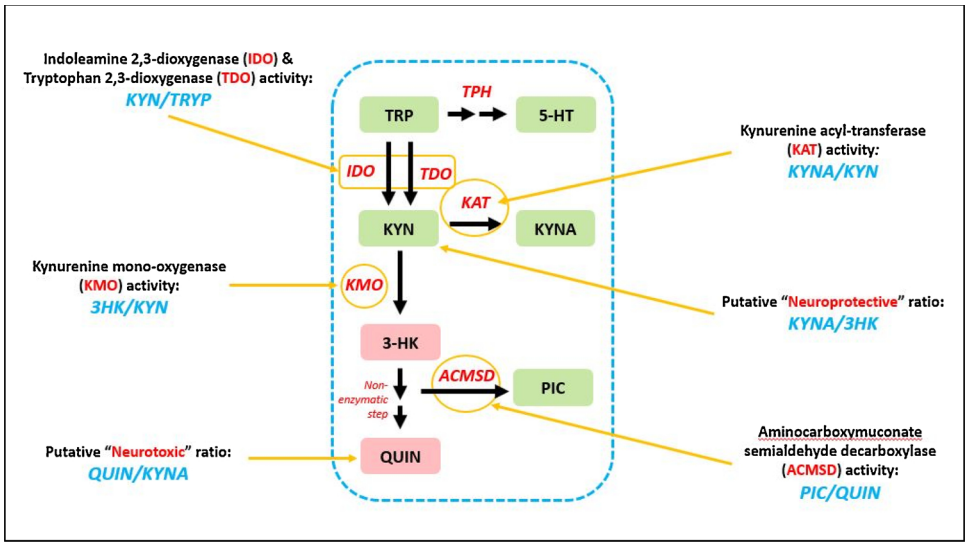

Fig. 2. KP metabolite ratios as indirect measures of enzymatic activity. Enzyme product-to-substrate ratios were constructed as indirect measures of KP enzyme activity. ACMSD activity is represented by PIC/QUIN because the substrate of ACMSD produces QUIN in non-enzymatic manner. The "putative" neuroactive ratios represent metabolic branch points between neurotoxic and neuroprotective arms in the KP. Legend: TRP = tryptophan, 5-HT = serotonin, KYN = kynurenine, KYNA = kynurenic acid, 3 H K = 3-hydroxykynurenine, PIC = picolinic acid, QUIN = quinolinic acid.

tests, any infections; any physical pain including fibromyalgia; history of peptic ulcer complicated by perforation, hemorrhage, or obstruction; and symptoms of peptic ulcer within 4 weeks of enrollment date. Other exclusion criteria included current use of lithium (due to the potential adverse drug reaction with celecoxib), stimulants, hormonal birth control and any corticosteroids (except for hormone replacement therapy), and anticoagulant agents. Subjects had to be smoke and nicotine-free for greater than 3 months prior to the assessment to be considered for the study. Sensitivity or allergy to study medications or a need to receive agents contraindicated in combination with celecoxib or escitalopram were additional exclusion criteria as was sleep disorder, except for insomnia or hypersomnia associated with BD.

2.2. Healthy control subjects

Healthy control (HC) subjects (total N = 43, reliable metabolites N = 26) were obtained from our database (Halaris et al., 2015). KP metabolites from HC and TRBDD groups were processed by separate labs in Australia and Germany, respectively, although both laboratories used the same assay procedures as previously described (Smythe et al., 2002). Blood samples for biomarker analyses were obtained from HC subjects only once because, based on our experience, measured values are stable barring intercurrent illnesses or stressful life events. All eligible HC subjects underwent the same screening assessments after providing written informed consent, as approved by the Institutional Review Board of the institution. Subjects were admitted into the study only if the screening test results fell within normal range. The exclusion criteria for HC subjects were presence of any kind of medical or mental illness, an inflammatory process, gum disease, substance use, mental illness or substance use amongst first degree relatives, pregnant or lactating females, and females taking oral contraceptives. The HAM-D and BDI scores had to be less than 5. A total of 26 HC subjects were included.

2.3. Study design

The study consisted of a screening visit, a minimum 2-week washout, a 1-week placebo run-in period, and 8 weeks of treatment. At the screening visit, subjects underwent a brief interview and were briefed about the study. If they passed the screening evaluation and agreed to participate, they were asked to sign the consent form. They then underwent comprehensive assessments that included quantifying depression and anxiety (HAM-D, HAM-A). If they were receiving an antidepressant agent, they were instructed to taper it over two weeks or longer. All prospective study participants had to be stable on mood stabilizing medication which included a mood stabilizer except lithium (due to its known adverse interaction with celecoxib) and/or an atypical antipsychotic. This stipulation was mandatory to prevent switch to mania or hypomania. Notably, of the 100 potential subjects screened for this study, 6 were already taking lithium and were willing to change to another mood stabilizer or atypical antipsychotic; only one subject was unwilling to switch to an alternate regimen than lithium and was therefore not accepted into the study. The remaining subjects were then placed on a 1-week single-blind placebo run-in phase until the next visit, which was the baseline visit. The purpose of the placebo run-in was to identify and exclude placebo responders. At the baseline visit study subjects were rated and if they continued to score at least 18 on the 17-item HAM-D scale, they were randomized to receive escitalopram (ESC) + celecoxib (CBX), or escitalopram (ESC) + placebo (PBO). CBX was dosed at 200 mg twice daily, while ESC was optimized according to each patient without exceeding 20 mg daily. The study pharmacist generated the randomization code, kept it in sealed envelopes, and dispensed the medication. At every visit thereafter, subjects were handed a medication installment to last until the following visit. Subjects were asked to return the used medication vials at the following visit so that a pill count could be made to ensure compliance. If at the baseline visit the subject scored HAM-D $\leq$17, they were designated placebo responders and were offered conventional care. The overall study was powered for 70 patients (35 in each arm of the study) to complete 8 weeks of active medication to be considered study completers. Eighty patients were enrolled, to allow for an anticipated 10% drop-out rate. 55 patients qualified as completers (20 in the ESC + PBO arm and 27 in the ESC + CBX arm). Of the 55 completers, 47 had complete biological data available for statistical analysis (Valid N = 47see Table 1).

2.4. Biochemical analyses

After an overnight fast, 20 ml of antecubital venous blood was

Table 1
Response and remission according to treatment arm.

	Drug Therapy	Valid N	Odds Ratio (95% CI)	*Exact p*
By Response	Celecoxib	27	4.278 (1.208–15.151)	0.021
	Placebo (*Ref*)	20	–	
By Remission	Celecoxib	27	15.300 (2.919–80.193)	< 0.001
	Placebo (*Ref*)	20	–	

drawn between 08:00 and 10:00 AM at baseline, week-4, and week-8 follow-up visits. For the HC subjects, blood was taken only upon completion of all assessments to confirm physical and mental health status. Blood samples were separated into plasma or serum, and samples were immediately stored at -80^{O} C until analyzed. TRBDD serum was separated and sent to the Institute of Laboratory Medicine of the University Hospital, LMU Munich for analyses in 2018. HC serum was analyzed in 2015 at the Department of Pharmacology, School of Medical Sciences, University of New South Wales, Australia. Both TRBDD and HC KP metabolites were measured by Ultra Performance Liquid Chromatography/Mass Spectrometry (UPLC-MS), using a Waters Acquity UPLC connected to a Xevo TQ MS triple-quadrupole mass spectrometer, equipped with a Z-spray ESI ion source (Waters, Milford, MA, USA). Separation was carried out using a Kinetex XBC18, 2.6 μm, 2.1 × 150 mm column (Phenomenex, Torrance, CA, USA).

2.5. Biological significance of KP metabolite ratios

Peripherally measured KP metabolites were used to construct product-to-substrate ratios (Fig. 2), which serve as indirect indices of KP enzymatic activity. Notably, the PIC/QUIN and KYNA/3HK ratios are not constructed using substrate-product pairs, but nonetheless are biologically significant:

- Indoleamine 2,3-dioxygenase (IDO) & tryptophan 2,3-dioxygenase (TDO) activity = KYN/TRP
- Kynurenine monooxygenase (KMO) activity = 3HK/KYN
- Kynurenine acyl transferase (KAT) activity = KYNA/KYN
- Aminocarboxymuconate semialdehyde decarboxylase (ACMSD) activity = PIC/QUIN
- Putative neuroprotective ratio = KYNA/3HK
- Putative neurotoxic ratio = QUIN/KYNA

2.6. Study endpoints

The primary endpoint was a significantly better-than-placebo mood response for ESC + CBX that was not related to altered pain perception which was assessed throughout the study. The HAM-D rating scale (17 items from the 21-item scale) was the main measure of clinical improvement. We defined onset of response as the day of treatment when a 30% decline in the initial (day 0) HAM-D score was recorded and maintained thereafter for at least two consecutive weeks. We defined treatment response time as the day of treatment when a 50% reduction in the initial HAMD score occurred. We defined symptom remission as a HAM-D score of ≤7 at the end of treatment. We established whether the combination of ESC + CBX showed improvement augmentation over the antidepressant efficacy of monotherapy with reference to (1) significantly earlier group response (earlier decline in HAM-D scores) and/or (2) a more robust group improvement in HAM-D scores at 8 weeks of treatment, and/or (3) more patients with symptom remission than in the ESC + PBO group at 8 weeks.

The secondary end points were a reduction in pro-inflammatory biomarkers and a normalization of KP metabolic indices (Fig. 2) at end of treatment. The baseline values were compared to age and sex matched controls from our existing normative dataset of healthy subjects. Notably, our secondary end points were exploratory in nature due to insufficient sample size to generate adequate statistical power. This paper's focus on KP metabolic indices is part of a larger clinical manuscript in preparation.

2.7. Statistical analyses

Number Needed to Treat (NNT) was derived by taking the inverse of the absolute risk reduction associated with patients at each level of drug therapy. First, the percentage of patients who did not respond in the CBX and placebo arms was recorded. The proportion of patients in the CBX cohort was then divided by the proportion in the placebo group and the inverse of that result is the NNT, assuming the rates observed in this sample of patients is representative of the population. This process was then repeated to derive similar estimates examining non-remission rates.

Non-parametric Mann-Whitney U tests were also used to assess for differences in baseline KP indices between study patients and HC subjects, then by treatment response. Univariable exact binary logistic regression models were then employed to examine the odds of treatment response and remission, respectively, as a function of drug therapy. A binomial distribution was specified for each response variable, while a logit link was used to estimate the odds ratio (OR) associated with treatment assignment. Supplementary linear mixed effects models compared KP metabolite index levels over time by drug therapy and response, adjusting for sex, age, and BMI. In these models, random intercepts were allowed for each patient to account for their multiple observations over the eight-week study period. An alpha error rate of $p \leq 0.05$ was considered statistically significant and all analyses were conducted using SAS 9.4 (Cary, NC).

3. Results

3.1. Treatment outcomes (addressing Hypothesis 1)

Patients receiving ESC + CBX had 4.278 (95% CI: 1.208–15.151) greater odds to respond to treatment compared to those randomized to ESC + PBO ($p = 0.021$) (**insert** Table 1). Further, the proportion of non-responders in the CBX and PBO groups was 22.2% and 55.0%, respectively. Thus, the *Number Needed to Treat* (NNT) is 3, meaning 3 patients would have to be treated with ESC + CBX to produce one responder.

Patients receiving CBX had 15.300 (95% CI: 2.919–80.193) greater odds of experiencing remission compared to those on PBO (exact $p < 0.001$) (Table 1). The observed proportion of patients receiving CBX who did not experience remission was 37.0%, while the proportion of individuals receiving the PBO combination who did not experience remission was 90.0%. Thus, the *Number Needed to Treat* (NNT) is 2, meaning 2 patients would have to be treated with ESC + CBX to produce 1 remission.

3.2. KP indices in TRBDD versus healthy controls (addressing Hypothesis 2)

In the group comparison adjusted for sex, age, and BMI (Table 2), *p*-values are reported accordingly with adjusted-p (*Adj-P**) values. TRBDD subjects revealed higher baseline TRP (*Adj-p** < 0.001), lower baseline KYN/TRP (*Adj-p** < 0.001), higher KYNA/KYN (*Adj-p** < 0.001), higher 3 H K/KYN (Adj-p* < 0.001), and lower PIC/QUIN (*Adj-p** = 0.034). There was no significant difference in KYNA/3 H K (*Adj-p** = 0.223) or QUIN/KYNA (*Adj-p** = 0.962) compared to HC.

3.3. KP index associations with treatment response (addressing Hypothesis 3)

In the intra-arm comparison of baseline KP indices by treatment response, responders revealed no statistically significant differences in baseline KP indices.

3.4. KP changes pre- and post-treatment (addressing Hypothesis 4)

The intra-arm comparison of KP index *change-scores* by treatment response revealed a significant difference in TRP change score ($p = 0.020$). The remaining KP ratio change-scores were statistically insignificant.

Table 2
Baseline KP indices by group.

KP Index	HC group Mean (std) Valid N	TRBDD group Mean (std) Valid N	*p*	*Adj-p**
TRP (μmol/L)	14,560.55 (6561.80) N = 26	77,978.12 (17241.93) N = 43	*P < 0.001*	*p* < 0.001*
KYN (μmol/L)	465.67 (286.11) N = 26	1,646.11 (587.13) N = 43	*P < 0.001*	*p* < 0.001*
KYNA (μmol/L)	0.013 (0.008) N = 26	42.351 (17.556) N = 43	*P < 0.001*	*p* < 0.001*
3HK (μmol/L)	0.019 (0.009) N = 25	81.929 (54.323) N = 43	*P < 0.001*	*p* < 0.001*
PIC (μmol/L)	0.072 (0.033) N = 24	179.059 (104.119) N = 43	*P < 0.001*	*p* < 0.001*
QUIN (μmol/L)	0.100 (0.056) N = 24	378.700 (229.659) N = 43	*P < 0.001*	*p* < 0.001*
KYN/TRP	0.032 (0.007) N = 26	0.022 (0.009) N = 43	*P < 0.001*	*p* < 0.001*
KYNA/KYN	3.10E-5 (1.60E-5) N = 26	0.027 (0.010) N = 43	*P < 0.001*	*p* < 0.001*
3HK/KYN	4.40E-5 (2.50E-5) N = 26	0.053 (0.041) N = 43	*P < 0.001*	*p* < 0.001*
PIC/QUIN	0.798 (0.368) N = 24	0.573 (0.430) N = 43	*P < 0.001*	*p* < 0.001*
KYNA/3HK	0.770 (0.325) N = 25	0.673 (0.364) N = 43	*p* = 0.152	*p** = 0.223
QUIN/KYNA	8.218 (3.790) N = 24	9.439 (5.105) N = 43	*p* = 0.320	*p** = 0.966

Note: Estimates reported as least-squared means with standard deviations in parentheses. *Adjusted for sex, age, and BMI.

4. Discussion

The purpose of this molecular profiling study was to characterize, using measurable changes in the KP, both the metabolomic endophenotype of TRBDD as well as the mechanisms underlying response to CBX augmentation. In order to maintain the conceptual structure of our driving hypotheses, we discuss the possible translational significance of our findings as follows:

Hypothesis 2: Section 4.1–4.3
Hypothesis 3: Section 4.4
Hypothesis 4: Section 4.5

4.1. High absolute KP metabolites at baseline, despite low KP activation in TRBDD

Interestingly, patients showed baseline elevations in all absolute KP metabolite levels compared to HC. This pattern may initially appear consistent with KP activation in patients. However, KYN/TRP was decreased in patients compared to HC, implying a state of low IDO and/or TDO activity and therefore low KP activation compared to HC. Because the KP is a dynamic system, it is possible that the excess of KP metabolites despite low KP activation may arise from shifts in enzymatic activity downstream of IDO and/or TDO. For instance, patients revealed a baseline elevation in KMO activity (3HK/KYN) which, similar to IDO, is induced by pro-inflammatory cytokines (Garrison et al., 2018).

Patients had higher TRP and lower KYN/TRP compared to HC's (Table 2). Since TRP is the substrate for enzymes IDO and TDO, the coincidence of high TRP with low KYN/TRP is consistent with an accumulation of TRP due to decreased IDO and/or TDO activity (Christmas, Potokar, & Davies, 2011; Taguchi et al., 2014). This interpretation is not straightforward to offer. In a previous study, we established an elevated inflammatory status of TRBDD at baseline by measuring high C-reactive protein (CRP) (Edberg et al., 2018b), an acute phase reactant inflammation biomarker, depression severity, and treatment resistance (Felger et al., 2018; Jha et al., 2017; Kohler-Forsberg et al., 2017). While the high baseline inflammation in TRBDD is consistent with a state of elevated IDO expression and activity (Salazar et al., 2012; Taguchi et al., 2014), this is difficult to reconcile with the observed decrease KYN/TRP. This discrepancy implies that pre-existing inflammation may not necessarily translate to KP activation. Additionally, it raises the question whether peripheral KYN/TRP is a reliable biomarker for IDO and TDO activity (Chen et al., 2017).

4.2. Patients show increased neurotoxicity in the upstream KP

Patients revealed elevated 3HK/KYN compared to HCs. The conversion of KYN to 3HK, a neurotoxic metabolite (Johansson et al., 2013; Okuda, Nishiyama, Saito, & Katsuki, 1998), is catalyzed by the microglial-expressed enzyme kynurenine mono-oxygenase (KMO) activity (represented by KYN/TRP) (Birner et al., 2017a; Parrott & O'Connor, 2015). The elevation in 3 H K/KYN is explained, at least in part, by the known ability of pro-inflammatory cytokines to induce the activity of KMO (Garrison et al., 2018), which is consistent with the previously established high pro-inflammatory status in TRBDD (Edberg et al., 2018b). Finally, no additional elevations in neurotoxic indices were observed, including QUIN/KYNA and 3HK/KYNA, which diverges from prior reports in BDD of reduced KYNA and increased 3HK/KYNA (Birner et al., 2017b).

We found that the increase in KMO activity is counterbalanced by elevated activity of astrocytic kynurenine acyltransferase (KAT) (represented by elevated KYNA/KYN) (Amori, Guidetti, Pellicciari, Kajii, & Schwarcz, 2009). The neuroprotective association of KAT is linked to the properties of its metabolite, KYNA, which has dual activities as an antagonist at the NMDA-R and a moderator at the α-7 nicotinic acetylcholine receptor (α7nAChR) (Foucault-Fruchard et al., 2017; Pershing et al., 2016; Rothhammer et al., 2018). Since KYNA and 3HK are alternate neuroprotective and neurotoxic metabolites in the upstream KP, respectively (Birner et al., 2017b), it is plausible that increased astrocytic KAT activity may serve to compensate for increased microglial KMO activity.

4.3. Patients showed decreased neuroprotection in the downstream KP

TRBDD patients revealed a decreased PIC/QUIN, an index of aminocarboxymuconate semialdehyde decarboxylase (ACMSD) activity. ACMSD catalyzes the downstream metabolites of 3HK to picolinic acid, a neuroprotective metabolite (Brundin et al., 2016a), thereby minimizing the alternate breakdown of 3HK towards QUIN, the NMDA-R excitotoxin (Brundin et al., 2016b; Feng et al., 2017; Poletti et al., 2016). As such, the biological meaning of the PIC/QUIN is analogous to KYNA/3HK, since both indices arise from metabolic branch points between neuroprotective and neurotoxic arms in the KP.

Clinically, decreased ACMSD activity moderates the link between suicidality and neurotoxic and pro-inflammatory burdens (Brundin et al., 2016a). Our observed decrease in PIC/QUIN in TRBDD is broadly consistent with the link between treatment resistance and suicide (Lex et al., 2019). In relation to one another, the decreases in both PIC/QUIN and QUIN imply a downregulation in ACMSD activity due to low substrate availability, rather than a *suppression* of ACMSD activity which would predict an increase in QUIN at the expense of PIC. These nuanced findings imply that even though ACMSD is associated with neuroprotection, a decrease ACMSD activity need not necessarily imply increased neurotoxicity, so long as the PIC/QUIN and QUIN indices are interpreted together as a coherent group.

4.4. Baseline KP indices do not predict treatment response

Treatment response did not vary by baseline KP indices, which implies that the mechanisms that predict treatment response do not involve the KP. In addition, here we also report our inability to replicate the positive correlation between high baseline KYN/TRP and treatment response—a predictive biomarker demonstrated by Krause et al. (2017) in conjunction with a CBX augmentation study in MDD (Krause et al., 2017). Krause's finding gave rise to the notion that clinical efficacy of adjunctive inflammatory modulation is dependent on the level of pre-existing immune activation and resultant KP activation (Krause et al., 2017). However, because our results showed no evidence of such an association, our findings challenge the assumption that treatment response is dependent on pre-existing KP activation.

4.5. Treatment response correlated with TRP depletion by Week 8

Within the entire KP panel, TRP was the only metabolite with a significant change score by week 8. Treatment responders showed a *decrease* in TRP by treatment week 8, with no accompanying change in KYN/TRP change-scores. TRP depletion may represent normalization from elevated baseline TRP compared to HC. When considered in relation to the non-change in KYN/TRP, TRP depletion may be explained by systemic metabolism through a separate pathway than KP. Possible explanations include restored global utilization of tryptophan in immune-vigilant tissues (Badawy, Namboodiri, & Moffett, 2016), bacterial degradation in the gut-brain axis (Martin-Subero, Anderson, Kanchanatawan, Berk, & Maes, 2016), or upregulation of 5-hydroxytryptophan (5-HTP) synthesis via tryptophan hydroxylase (TPH). Finally, it is worth noting that CBX-augmentation was efficacious despite the lack of restorative shifts in the KP. This suggests that the mechanisms underlying CBX augmentation need not involve the normalization of KP abnormalities seen at baseline, and further, that mechanisms responsible for treatment resistance may be different from those mediating reversal of resistance.

4.6. The confounding effects of prior or concurrent psychotropics

The confounding effects of prior or concurrent psychotropic agents are complex and lead in opposite directions. For example, while certain antipsychotic agents are associated with adverse metabolic effects (Singh, Bansal, Medhi, & Kuhad, 2019), it is increasingly clear that certain atypical antipsychotic and antidepressant drugs can correct immunologic and metabolic abnormalities (Muller, Myint, & Schwarz, 2009; Obuchowicz, Bielecka-Wajdman, Paul-Samojedny, & Nowacka, 2017; Stapel et al., 2018). Furthermore, TRBDD itself is associated with underlying molecular changes that persist independently of mood episodes (Reininghaus et al., 2014; Scifo et al., 2017). Other groups have avoided such confounding variables by studying psychotropic naïve patients (Breakspear et al., 2015; Kim et al., 2018). However, since these diverse confounders are by necessity incorporated into TRBDD treatment regimens, interpretation of metabolomic endophenotypes must incorporate the cumulative effects of both prior medications and BD neuroprogression, among other variables.

4.7. Limitations & future directions

While the inter-group comparison was adjusted for sex/age/BMI, we were unable to apply the adjustment to the intra-treatment arm comparison due to insufficient sample size (non-responders N = 6 vs. responders N = 21). Future studies might focus on measuring 5-HTP in order to construct 5-HTP/TRP (an indirect measure TPH1 activity). Additionally, future CBX augmentation studies might compare biomarkers both by treatment response and treatment arm. Provided that the KP metabolites were measured in plasma, we recommend interpretive caution when inferring impact on CNS. Because this is the first KP profiling study in TRBDD, and because the translational significance of our findings remain tentative, careful consideration of contextual parameters is warranted when comparing these results across diagnostic categories. Consideration should also be given to the rather short duration of treatment in our study design (8 weeks). A longer duration in a future study may clarify some of the above noted discrepancies.

5. Conclusion

An expanded therapeutic armamentarium is needed for TRBDD, and this study is the first to demonstrate clinical efficacy of CBX augmentation in this patient population. The immediate clinical significance arises from this study's confirmation CBX's safety profile, which revealed no evidence of adverse metabolic side effects such as increased neurotoxicity and decreased neuroprotective indices in the treatment arm, or adverse interaction in combianation with a SSRI agent. Our results are the first to reveal a metabolomic endophenotype for TRBDD as well as a candidate therapeutic biomarker for treatment. From the standpoint of public health, this work reveals the cumulative molecular impact of BDD—reinforcing the need for clinicians to strive for early recognition, treatment, and clinical remission to alter the neuroprogressive trajectory towards TRBDD.

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The authors have no financial disclosures to report.

Ethical statement

The study was approved by the Institutional Review Board of Loyola University Medical Center and the Review Board of the Medical Faculty of the LMU and conducted according to the principles of the Declaration of Helsinki.

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