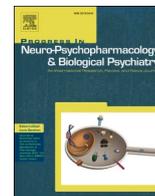




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## Activation and deactivation steps in the tryptophan breakdown pathway in major depressive disorder: A link to the monocyte inflammatory state of patients

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### ABSTRACT

It is unclear how the tryptophan (TRP) breakdown pathway relates to the activated inflammatory state of patients with major depressive disorder (MDD).

We determined in two different cohorts of patients with MDD ( $n = 281$ ) and healthy controls (HCs) ( $n = 206$ ) collected for the EU-MOODINFLAME project:

- the monocyte expression levels of 5 key pro-inflammatory cytokine/chemokine genes (ICCGs), 5 type I interferon stimulated genes (ISGs), and 4 kynurenine pathway (KP) enzyme genes (i.e. *IDO-1*, *KMO*, *CCBL1/KAT II* and *CCBL2/KAT III*) by standard q-PCR,
- serum levels of TRP, 5-HT<sub>trp</sub>, 5-HIAA, KYN, KYNA, 3-HK, XA, PIC, and QUIN by LC-MS/MS and/or HPLC, and calculated various TRP/KP metabolism ratios.

We then correlated outcomes to each other, and to the clinical characteristics of patients.

**Abbreviations:** MDD, major depressive disorder; ICCGs, pro-inflammatory cytokine/chemokine-related genes; *IL-1 $\beta$* , interleukin 1 beta gene; *CCL2*, chemokine C–C motif ligand 2 gene; 5-HT, 5-hydroxytryptamine; TRP, tryptophan; KP, kynurenine pathway; KYN, kynurenine; CSF, cerebrospinal fluid; IDO, indoleamine 2,3-dioxygenase enzyme; IFN, interferon; 3-HK, 3-hydroxykynurenine; QUIN, quinolinic acid; KYNA, kynurenic acid; *TNFAIP3*, tumor necrosis factor alpha-induced protein 3 gene; *CXCL2*, C-XC-chemokine ligand 2 gene; *IFI44*, interferon-induced protein 44 gene; *IFI44L*, interferon-induced protein 44 like; *IFIT3*, interferon-induced protein with tetratricopeptide repeats 3 gene; *LY6E*, E lymphocyte antigen 6 family member gene; *MX1*, interferon-regulated resistance GTP-binding protein MxA gene; HCs, healthy controls; ISGs, type I interferon-stimulated genes; *IDO-1*, indoleamine 2,3-dioxygenase gene; pSS, primary Sjögren Syndrome; *KMO*, kynurenine 3-monooxygenase gene; *CCBL/KAT*, cysteine conjugate beta lyase cytoplasmic/kynurenine aminotransferase gene; TDO, 2,3-dioxygenase enzyme; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders (4e), text revision; M.I.N.-I, Mini International Neuropsychiatric Interview; SCID-I, structured clinical interview for DSM-IV Axis I disorders; IDS-C, Inventory of Depressive Symptomatology, clinician-rated version; HAM-D 17, Hamilton Rating Scale for Depression, 17-item version; PBMCs, peripheral blood mononuclear cells; cDNA, complementary deoxyribonucleic acid; qPCR, quantitative polymerase chain reaction; RT, real time; *ABLI*, Abelson murine leukemia viral oncogene homolog 1; CT, comparative threshold cycle; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; TRB, tetramethylbenzidine; 5-HT<sub>trp</sub>, 5-hydroxytryptophan; 5-HIAA, 5-hydroxyindoleacetic acid; XA, xanthurenic acid; PIC, picolinic acid; LC-MS/MS, liquid chromatography-mass spectrometry; HPLC, high performance liquid chromatography; bmi, body mass index; ECT, electroconvulsive therapy; TPH-II, tryptophan hydroxylase enzyme; MAO, monoamine oxidase enzyme.

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Both cohorts of patients differed clinically; patients of the Munich cohort ( $n = 50$ ) were less overweight, less medicated, were less in the current episode and showed a higher HAM-D 17 score as compared with patients of the Muenster cohort ( $n = 231$ ).

An increased expression of ICCGs was found in the circulating monocytes of patients of both cohorts; this was in particular evident in the Munich cohort. In contrast, ISGs monocyte expression levels tended to be reduced (both cohorts).

TRP serum levels were linked to the pro-inflammatory (ICCGs) monocyte state of patients; a decrease in TRP serum levels was found in the Munich cohort; TRP levels correlated negatively to patient's HAM-D 17 score. Contrary to what expected, KYN serum levels were not increased in patients (both cohorts); and an increased KYN/TRP ratio was only found in the Munich patients (who showed the lowest TRP serum levels). *IDO-1* monocyte expression levels were decreased in patients (both cohorts) and negatively associated to their pro-inflammatory (ICCGs) monocyte state. Thus, a depletion of TRP via an ICCGs-inflammatory *IDO* activation is not likely in MDD.

Downstream from KYN, and regarding compounds influencing glutamate receptors (GR), reduced serum levels of KYNA (NMDA-R antagonist), 3-HK (NMDA-R agonist), and XA (mGlu2/3 agonist) were found in patients of both cohorts; PIC serum levels (NMDA-R antagonist) were increased in patients of both cohorts. Reduced QUIN serum levels (NMDA-R agonist) were found in patients of the Muenster cohort, only. 3-HK levels correlated to the monocyte inflammatory ICCG state of patients. The ultimate effect on brain glutamate receptor triggering of this altered equilibrium between peripheral agonists and antagonists remains to be elucidated.

## 1. Background

With a lifetime prevalence of up to 8–12% (Smith, 2014), major depressive disorder (MDD) is considered as a leading cause of disability worldwide (WHO, 2020). In addition, it is associated with numerous somatic diseases (e.g. rheumatoid arthritis, obesity) (Clarke and Currie, 2009; Preiss et al., 2013), increasing mortality rates. Importantly, MDD has been linked to an increased risk of suicidal behavior, acting as the major contributor to suicide deaths (Saxena and Fleischmann, 2014; WHO, 2020). However, understanding the exact pathogenesis of the disorder still remains challenging, making diagnosis, treatment stratification of patients and new drug discovery, difficult.

A growing body of evidence has suggested an involvement of an abnormal inflammatory response system in the pathogenesis of MDD. Altered immune cell functions and raised circulating concentrations of pro-inflammatory cytokines/chemokines have been repeatedly found in MDD (Müller et al., 1993; Connor and Leonard, 1998; Anisman and Merali, 2002; Mikova et al., 2001; Myint et al., 2005; Irwin and Miller, 2007; Dantzer et al., 2008; Wang et al., 2019). In line with this, we previously reported on the expression of a specific inflammation-related gene signature in the circulating monocytes of two cohorts of patients with MDD (Carvalho et al., 2014; Grosse et al., 2015; Grosse et al., 2016). In this signature, gene expression patterns formed two main clusters of strongly interrelated genes; one cluster was composed of a set of pro-inflammatory cytokine/chemokine-related genes (ICCGs), such as the interleukin (IL)1-beta gene (*IL-1 $\beta$* ), the *IL-6*, or the chemokine C–C motif ligand 2 gene (*CCL20*). In particular monocytes of the cohort of older patients with a severe and melancholic depression were characterized by an overexpression in this cluster of inflammatory genes. These patients were also characterized by elevated serum levels of pro-inflammatory cytokines/chemokines, such as *IL-6*, *IL-8* and/or *CCL2* (Weigelt et al., 2011; Carvalho et al., 2014; Grosse et al., 2015).

Whilst a functional deficit in the neurotransmitter 5-hydroxytryptamine (5-HT, also known as serotonin) has been classically mentioned as an important contributor to the pathogenesis of depression (Coopen and Doogan, 1988; Hamon and Blier, 2013), it is only in recent years that various theories have emerged to provide a mechanistic link between the altered inflammatory state of patients with MDD and neuronal functional abnormalities. One of these theories has focused on an inflammation-related enhanced breakdown of tryptophan (TRP) along the kynurenine pathway (KP) (Maes et al., 2011; Leonard and Maes, 2012; Arnone et al., 2018). TRP can be metabolized into 5-HT via the serotonin pathway, or into kynurenine (KYN) via the KP (Fig. 1) which in fact acts as the major catabolic pathway for TRP in the body (Stone et al., 2013; O'Farrell and Harkin, 2017) (Fig. 1).

An increased TRP-to-KYN breakdown ratio has repeatedly been found in the blood and cerebrospinal fluid (CSF) of individuals with depression (Maes et al., 1990; Quak et al., 2014; Baranyi et al., 2017), particularly in those at a high risk of suicide (Bradley et al., 2015; Bryleva and Brundin, 2017; Messaoud et al., 2019). Activation of the KP is mediated, –among other mechanisms–, through activation of one of its rate-limiting enzymes, indoleamine 2,3-dioxygenase (*IDO*) (Maes et al., 2011; Leonard and Maes, 2012; Schwarcz and Stone, 2017; Arnone et al., 2018). *IDO* is induced by pro-inflammatory stimuli such as interferon (IFN)-alpha and/or *IL-2* (Raison et al., 2010; Leonard and Maes, 2012); thus, *IDO* activation in pro-inflammatory monocytes and/or microglia has been suggested as a key pathogenic mechanism that could explain the link between pro-inflammatory immune cells, TRP depletion and characteristic brain 5-HT deficiencies in MDD (Myint et al., 2007; Schwarcz and Stone, 2017). KYN is further catabolized into various downstream neuroactive metabolites throughout two degrading arms, often referred to as the potentially neurotoxic and potentially neuroprotective arms of the KP (Fig. 1). An inflammation-related catabolic imbalance between these two breakdown arms, i.e. an enhanced production of the potentially neurotoxic catabolites 3-hydroxykynurenine (3-HK) and/or quinolinic acid (QUIN) at the expense of the potentially neuroprotective catabolites kynurenic acid (KYNA) and/or picolinic acid (PIC) has also been proposed in MDD (Myint and Kim, 2003; Myint et al., 2007). This hypothesis is supported by findings of decreased KYNA and increased 3-HK and QUIN concentrations in the serum/plasma of patients with MDD (Savitz et al., 2015; Doolin et al., 2018). However, recent studies have found normal or even reduced serum/plasma levels of KYN and 3-HK in individuals with depression (Hughes et al., 2012; Wurfel et al., 2017), questioning the idea of an *IDO* activation and overproduction of potentially neurotoxic downstream metabolites in MDD.

The aim of the present study was to investigate how the inflammatory monocyte state of patients with MDD correlates to the breakdown of TRP to KYN, and of KYN to its potentially neurotoxic and neuroprotective downstream products.

First, we determined the monocyte expression levels of five characteristic ICCGs (i.e. *IL-1B*, *CCL20*, *IL-6*, *TNFAIP3* and *CXCL2*) (Vogels et al., 2017) and of five genes driven by type I IFNs (i.e. *IFI44*, *IFI44L*, *IFIT3*, *LY6E* and *MX1*) (Brkic et al., 2013) in two cohorts of patients with MDD and healthy controls (HCs) participating in the EU-MOODINFLAME study (FP7-HEALTH:222963). These type I interferon-stimulated genes (ISGs) have been shown to be positively correlated to indoleamine 2,3-dioxygenase gene (*IDO-1*) expression and to a higher serum KYN/TRP in patients with primary Sjögren Syndrome (pSS) (Maria et al., 2016), supporting the theory of an inflammation-

induced activation of the KP via IDO.

We also determined the monocyte expression levels of four genes encoding important KP degrading enzymes (i.e. *IDO-1*, kynurenine 3-monooxygenase gene (*KMO*), cysteine conjugate beta lyase cytoplasmic/kynurenine aminotransferase genes (*CCBL1/KAT I* and *CCBL2/KAT III*), as well as serum levels of various important TRP/KP metabolites (TRP, 5-HT<sub>trp</sub>, 5-HIAA, KYN, KYNA, 3-HK, XA, PIC, QUIN).

In addition, the activity of the different KP enzymes was assessed by determining various KP metabolite ratios (e.g. the KYN/TRP ratio estimating IDO and/or tryptophan 2,3-dioxygenase (TDO) enzyme activity; the KYNA/KYN ratio estimating KATs enzyme activities, the 3-HK/KYN ratio estimating KMO enzyme activity) (Fig. 1).

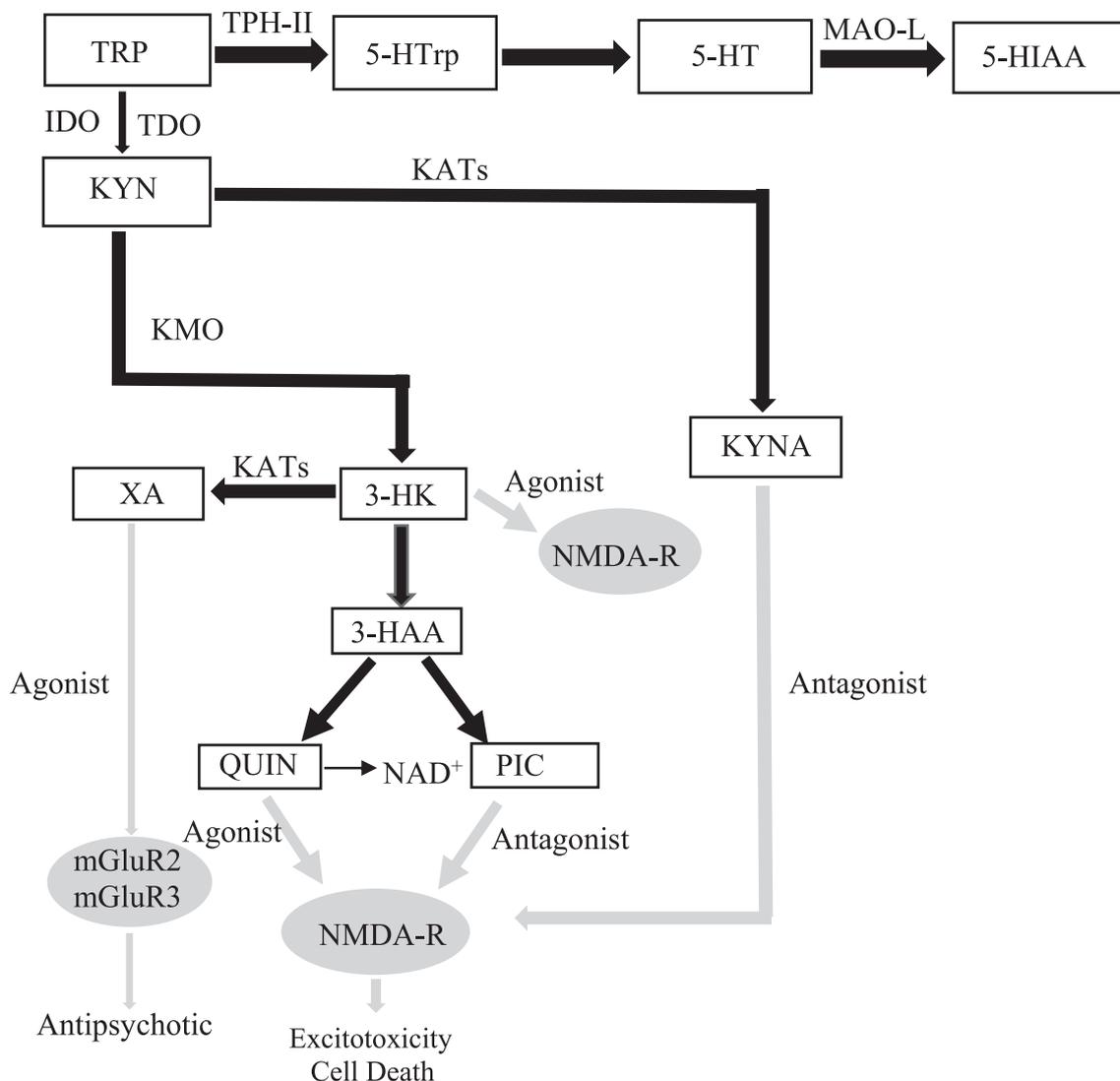
Finally, the serum levels of the different TRP/KP metabolites and the

different KP metabolite ratios were correlated to the monocyte ICCG and ISG inflammatory states, and to the monocyte expression levels of the four KP enzyme genes.

## 2. Methods

### 2.1. Study participants

From 2010 to 2013, a total of  $n = 281$  (aged 18–65 years) in- and outpatients with MDD were recruited from the Departments of Psychiatry at the University Hospital of Muenster, Germany ( $n = 231$ ) and the University Hospital of the Ludwig Maximilian University in Munich, Germany ( $n = 50$ ).



**Fig. 1.** The tryptophan (TRP) catabolic pathway. The main branches are towards the serotonin pathway (5-HT<sub>trp</sub>, 5-HT and 5-HIAA) and towards the potentially neuroprotective/neurotrophic (KYNA/PIC) and potentially neurotoxic (3-HK/QUIN) arms of the kynurenine pathway. Along the arrows, the main enzymes catalyzing the reactions are given (i.e. TPH-II, MAO-L, IDO, TDO, KMO and KATs).

**Abbreviations:** TRP: tryptophan; 5-HT<sub>trp</sub>: 5-hydroxytryptophan; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid; KYN: kynurenine; KYNA: kynurenic acid; 3-HK: 3-hydroxykynurenine; XA: xanthurenic acid; 3-HAA: 3-hydroxyanthranilic acid; QUIN: quinolinic acid; PIC: picolinic acid; TPH-II: tryptophan hydroxylase; MAO-L: monoamine oxidase; TDO: tryptophan 2,3-dioxygenase enzyme; IDO: indoleamine 2,3-dioxygenase enzyme; KMO: kynurenine 3-monooxygenase enzyme; KATs: kynurenine aminotransferase enzymes; NMDA-R: *N*-methyl-D-aspartate receptor; mGlu-R: metabotropic glutamate receptors; NAD<sup>+</sup>: nicotinamide adenine dinucleotide coenzyme. TRP breaks down to either 5-HT or KYN depending on IDO and/or TDO enzyme activity. KYN then breaks down towards KYNA (NMDA-R antagonist) or 3-HK (NMDA-R agonist) depending on KATs or KMO enzyme activities, respectively. 3-HK break down to 3-HAA, which further breaks down to the potentially neuroprotective PIC, or to the potentially neurotoxic QUIN. PIC acts as an NMDA-R antagonist, while QUIN acts as an NMDA-R agonist, inducing excitotoxicity and neuronal death. QUIN (as 3-HK) is in addition a source of NAD<sup>+</sup>, playing a role in provision of energy. Interestingly, 3-HK is also able to break down to XA, which acts as an mGluR2/mGluR3 agonist, blocking excitotoxicity and neuronal death.

In the EU-MOODINFLAME study, all patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (4e), Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000).

In Muenster cohort, the diagnosis was confirmed by the Mini-International Neuropsychiatric Interview (M.I.N.I) (Sheehan et al., 1998), while in the Munich cohort, the diagnosis was confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First and Pincus, 1999).

Severity of depression was assessed with the Inventory of Depressive Symptomatology, clinician-rated version (IDS-C) (Rush et al., 1986) (in the case of the Muenster cohort), and with the Hamilton Rating Scale for Depression, 17-item-version (HAM-D 17) (Hamilton, 1960) (in the case of the Munich cohort). All assessments were performed by two independent experienced clinical psychiatrists.

Excluded were patients who were unable to write or give informed consent, patients at immediate risk for suicidal behavior, and those who had another Axis I and II disorder. With the aim of homogenizing the sample, we decided not to include patients with MDD and psychotic symptoms, since it is discussed that psychotic depression has a different form of nosology than non-psychotic depression (Park et al., 2015).

Patients had to be free of any obvious major medical illness in their medical history (i.e. cardiovascular, gastrointestinal, respiratory, neurologic, hepatic, renal, neurologic, infectious, immune or inflammatory diseases as well as untreated metabolic disorders) and also of any minor medical illness, including allergic reactions or infections, in the 4 weeks before blood withdrawal. Excluded were also patients with any clinically significant physical findings (e.g. uncontrolled high blood pressure, claudication, heart murmurs, abnormal reflexes, etc.) or laboratory results (e.g. abnormal glucose, liver and kidney values, etc.), as were women who were pregnant or breastfeeding. Recent ( $\leq 4$  weeks) vaccinations were not allowed and patients taking immune-modulatory medication were excluded, too.

Only in the case of the Munich cohort, and since this cohort was used for a study on the antidepressants effects of cyclooxygenase-2 (COX-2) inhibitors in patients with MDD (Arteaga-Henriquez et al., 2019), the

**Table 1**  
Clinical and demographic data of patients with MDD of the Muenster and Munich cohorts.

	Muenster n = 231	Munich n = 50	Muenster vs Munich
	M(SD)	M(SD)	p
Number of previous depressive episodes	3.62(6.01)	1.96(1.37)	0.058
Duration of current episode (weeks)	42.98(52.38)	18.90 (15.89)	<b>0.001</b>
HAM-D 17 score	18.00(5.39)	24.46(2.48)	<b>&lt;0.001</b>
Age (years)	40.14(12.62)	39.64 (11.61)	0.795
bmi (kg/m <sup>2</sup> )	26.28(4.60)	23.40(3.16)	<b>&lt;0.001</b>
	n (%)	n (%)	P
Sex			0.274
Males	96(41.60)	25(50.0)	
Females	135(58.40)	25(50.0)	
Smoking			0.638
Yes	105(45.70)	21(42.00)	
No	125(54.30)	29(58.00)	
Medication			<b>&lt;0.001</b>
Yes	223(96.50)	38(76.00)	
No	8(3.50)	12(24.00)	
ECT			<b>0.020</b>
Yes	23(10.0)	0(0.00)	
No	208(90.0)	50(100.00)	

Values marked in bold indicate a p-value  $\leq 0.05$ . Abbreviations: M: mean; SD: standard deviation; HAM-D 17: Hamilton Rating Scale for Depression, 17-item version; bmi: body mass index; ECT: electroconvulsive therapy.

use of antidepressant drugs (apart from benzodiazepines/analogues) was prohibited; patients treated with monoamine-oxidase inhibitors during the last 14 days or with fluoxetine during the last 6 weeks were also excluded, as were patients currently taking warfarin or pain medications within 72 h prior to study entry. The Muenster patients used a variety of antidepressant and psychotropic drugs (Table 1).

HCs (n = 206) were recruited from the same communities as the patients. The inclusion criteria for HCs were the absence of major Axis I disorders according to DSM-IV-TR criteria; no use of antidepressants or other psychotropic drugs was allowed. Furthermore, HCs had to be in self-proclaimed good health and free of any obvious medical illness, including infections and allergic reactions, for at least 4 weeks before blood withdrawal.

The study was approved by the ethics committee of the Medical Association Westphalia-Lippe, Germany (2009-019-f-S) and the ethics committee of the medical faculty at the Ludwig Maximilian University of Munich, Germany (234-09). All participants provided written informed consent.

## 2.2. Laboratory assessments

All laboratory assays were centralized in the EU-MOODINFLAME study. Monocyte gene expression levels were measured by the Department of Immunology, Erasmus Medical Center (Rotterdam, the Netherlands), TRP/KP metabolites by the Institute of Laboratory Medicine, Medical Center of the Ludwig-Maximilian-University (Munich, Germany).

### 2.2.1. Determination of the inflammatory activation state of circulating monocytes

Details have been given in previous publications (Carvalho et al., 2014; Grosse et al., 2015; Vogels et al., 2017), and only a synopsis of the methodology is given here. Blood was collected in sodium heparin tubes (36 ml) for immune cell preparation. From the heparinized blood, we prepared peripheral blood mononuclear cells (PBMCs) suspensions by low-density gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) within 8 h to avoid erythrophagy-related activation of monocytes, as described previously in detail (Drexhage et al., 2010). PBMCs were then frozen in 10% dimethylsulfoxide and stored in liquid nitrogen. This enabled us to test the immune cells of patients and HCs together at a later stage at the Erasmus MC. CD14<sup>+</sup> monocytes were isolated from aliquots of frozen PBMCs (from approximately 20 ml of blood) by a magnetic cell sorting system (auto MACS Pro; Milteny Biotec, B.V., Bergisch Gladbach, Germany). The mean viability was  $86.3 \pm 10.4$  (Trypan blue staining); purity of monocytes,  $95.1 \pm 3.0\%$  (flow cytometry). mRNA was isolated from the purified CD14<sup>+</sup> monocytes with an RNA easy mini-kit in accordance with manufacturer's instructions (Qiagen, Hilden, Germany). The mean monocyte yield after isolation was  $2.0 \pm 1.6 \times 10^6$ /participant; the mean quantity of mRNA in monocytes was  $3.2 \pm 1.8 \mu\text{g}$ . One  $\mu\text{g}$  of mRNA was reverse transcribed by a high-capacity reverse transcription kit (Applied Biosystems, Foster City, CA, USA) to produce complementary deoxyribonucleic acid (cDNA) for quantitative polymerase chain reaction (qPCR) (Taqman Arrays, format 48, Applied Biosystems, Foster City, CA, USA). qPCR was performed according to the manufacturer's protocol and validated against the single real-time (RT)-qPCR method. Per fill port, 400 ng of cDNA was loaded. PCR amplification was performed with an Applied Biosystems Prism 7900HT sequence detection system with TaqMan Array block. Thermal cycler conditions were 2 min at 50 °C, 10 min at 94.5 °C, 30 s at 97 °C, and 1 min at 59.7 °C for 40 cycles. For our study, we determined five top ICCGs (i.e. *IL-1 $\beta$* , *CCL20*, *IL-6*, *TNFAIP3* and *CXCL2*) as determined in the study of Vogels et al. (2017) (Supplementary Fig. 1). We verified -by using principle component analysis-, that these genes were indeed also belonging to the top ICCGs in the present study (Supplementary Fig. 2). Also in the most recent study on monocyte gene expression levels in a large cohort patients with MDD

(Schiweck et al., 2020), these genes belonged to the ICCGs signature (Supplementary Fig. 3).

In addition, we determined five key type I ISGs (i.e. *IFI44*, *IFI44L*, *IFIT3*, *LY6E*, and *MX1*) of the study of Brkic et al. (2013) and Maria et al. (2016), and four important KP enzyme genes (i.e. *IDO-1*, *KMO*, *CCBL1/KAT I* and *CCBL2/KAT III*). The expression of these 14 genes was normalized by the value of the housekeeping gene *ABL1* and calculated by the comparative threshold cycle (CT) method. *ABL1* has been found as being superior to other housekeeping genes for leukocyte gene level determination (Beillard et al., 2003).

### 2.2.2. Determination of TRP/KP metabolite serum levels

All serum samples were collected from fasting, early morning (8 a.m.-11 a.m.) venous blood samples, and immediately centrifuged, aliquoted and stored at  $-80^{\circ}\text{C}$ . TRP, 5-hydroxytryptophan (5-HTp), 5-hydroxyindoleacetic acid (5-HIAA), KYN, KYNA, 3-HK, xanthurenic acid (XA), QUIN and picolinic acid (PIC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standards KYNA-D5, PIC-D4 and TRP-D5 were purchased from CDN Isotopes (Pointe-Claire, QC, Canada), and KYN-D4 was purchased from Buchem BV (Minden, the Netherlands). Reagents for protein precipitation, derivatization, and chromatography were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Biosolve (Valkenswaard, NL). Standards and a low- and high-quality control were established by adding defined amounts of each analyte to human serum samples obtained from a blood bank. The human serum was necessary because we had to take matrix effects into account to cover concentrations below the analyte concentrations present in healthy humans. The serum was diluted 1 + 1 with liquid chromatography-mass spectrometry (LC-MS/MS)-grade water and used as the lowest calibrator. The values of this calibrator were calculated by standard addition. Because serum is not available without these analytes, there was no blank sample. A total volume of 300  $\mu\text{l}$  serum samples, calibrators, and controls was used for sample preparation.

**2.2.2.1. HPLC method.** In the Muenster cohort, TRP, 5-HIAA, KYN, 3-HK and KYNA ( $n = 207$  MDD, 134 HC) serum levels were measured in a first set of determinations by a previously described high performance liquid chromatography (HPLC) method (Oades et al., 2010). In short, analytes were extracted from samples and calibrators/controls by using Waters Oasis MCX extraction cartridges (Waters Corporation, Milford, MA, USA). The eluent was then evaporated to dryness and reconstituted with 0.1 M phosphate buffered saline (PBS) for injection into the HPLC system. Analyses were carried out on a Waters 2695 chromatograph (Waters Corporation, Milford, MA, USA) with a 250 mm  $\times$  4 mm Supersphere 60 RP-select B, C8 column (Merck, Darmstadt, Germany), connected to a Waters 2487 dual- $\lambda$  UV detector and a 2475 fluorescence detector. TRP ( $\lambda_{\text{ex}}$ : 300 nm;  $\lambda_{\text{em}}$ : 350 nm) and 5-HIAA ( $\lambda_{\text{ex}}$ : 300 nm;  $\lambda_{\text{em}}$ : 340 nm) were measured by fluorescence detection; KYN (365 nm), KYNA (330 nm) and 3-HK (365 nm) were measured by UV detection.

**2.2.2.2. LC-MS/MS method.** In a second and third round of determinations, serum levels of 5-HTp, XA, QUIN and PIC ( $n = 157$  MDD,  $n = 73$  HC; Muenster cohort) and of TRP, 5-HTp, 5-HIAA, KYN, 3-HK, KYNA, XA, QUIN and PIC ( $n = 47$  MDD, 13 HC; Munich cohort) were analyzed by LC-MS/MS. The chromatographic system was composed on a Waters Acquity UPLC separation module connected to a Xevo TQ MS triple-quadrupole mass spectrometer with a Z-spray ESI ion source (Waters Corporation, Milford, MA, USA). Separation was performed on a Kinetex XB-C18, 2.6  $\mu\text{m}$ , 2.1  $\times$  150 mm column (Phenomenex, Torrance, CA, USA). Analytes were extracted by adding 50  $\mu\text{l}$  of 2.0 M urea and 50  $\mu\text{l}$  of an internal standard solution containing KYN-D4, KYNA-D5, PIC-D4, and TRP-D5. Two precipitation steps were performed by adding 200  $\mu\text{l}$  methanol/ethanol (2/1 v: v) and then 800  $\mu\text{l}$  acetonitrile. The supernatant was separated into two portions, which were evaporated separately. One of these portions was directly reconstituted in the

mobile phase. The other portion was derivatized with 200  $\mu\text{l}$  HCl/butanol at  $90^{\circ}\text{C}$  for 60 min and then, after evaporation, also reconstituted in the mobile phase. For chromatography, 7.5  $\mu\text{l}$  of the reconstituted samples, calibrators, and controls were loaded onto the LC-MS/MS system. QUIN and PIC were analyzed in the derivatized sample, while all other analytes were analyzed in the underivatized sample. For both derivatized and underivatized samples, gradient methods with a total duration of 7.5 min each were used for chromatographic separation. Mobile phase A was composed of 0.1% formic acid and 0.01% heptafluorobutyric acid in water; mobile phase B was methanol. The flow rate was set at 0.25 ml/min, and the column temperature was set at  $30.0^{\circ}\text{C}$ . Retention times for the analytes were between 3.1 and 6.0 min. The Xevo TQ MS was operating at atmospheric pressure, and electrospray ionization was in positive mode (ESI+). Ion source settings were as follows: capillary voltage, 1.00 kV; desolvation temperature,  $650^{\circ}\text{C}$ ; source temperature,  $150^{\circ}\text{C}$ . Nitrogen was used as the desolvation gas, at an API gas flow rate of 1200 l/h, and argon was used as the collision gas, at a flow rate of 0.15 ml/min. The analytes and internal standards were detected by multiple reaction monitoring (MRM) technique. System operation, data acquisition, and data processing were controlled with MassLynx V4.1 software (Waters Corporation, Milford, MA, USA). The lower limit of quantification (LLOQ) and lower limit of detection (LLOD) of the method described above were calculated according to DIN 32645 guidelines. The method was further validated based on the European Medicines Agency (EMA) guidelines at the Institute of Laboratory Medicine, Medical Center of the Ludwig Maximilian University, Munich.

### 2.3. Statistics

Statistical analyses were performed with IBM SPSS v.21 and Microsoft Excel v.15.30 (170107) for Mac. Continuous sample characteristics are reported as mean (M)  $\pm$  standard deviation (SD). Data were tested for normal distribution by the Shapiro-Wilk test ( $n < 30$ ) and by the Kolmogorov-Smirnov test ( $n \geq 30$ ). For group comparisons of sample characteristics (e.g. MDD vs HCs), continuous data were analyzed with Mann-Whitney  $U$  tests; categorical data were analyzed with Pearson's chi-square ( $\chi^2$ ) tests.

Since considerable differences were found between the Muenster and Munich patients regarding their clinical characteristics (disease acuteness, severity of current episode), as well as regarding their medication/ECT state, we decided to do to two different analyses: First, we compared patients of the Muenster and Munich cohorts with their respective HCs, and then, we compared patients of both cohorts between themselves. Second, comparisons between the entire group of patients with MDD and HCs, and between the small subgroup of medication/ECT-naïve patients, and their respective HCs were also made.

Group differences were tested by univariate analyses of covariance (ANCOVA), correcting for age, sex, bmi and smoking (patients vs. HCs), and for bmi, HAM-D 17 score, number of previous depressive episodes, durations of current episode, medication (yes/no), ECT (yes/no) (Muenster patients vs. Munich patients). In the case of medication/ECT-naïve subgroup of patients, we corrected only for smoking (yes/no). Bonferroni's adjustments for multiple comparisons were additionally applied in all analyses. Correlations between were determined by Spearman's-rank correlation coefficient ( $\rho$ ). All hypotheses were tested with  $\alpha \leq 0.05$  (two-sided).

Due to methodological differences, and in order to correct for site-dependent differences regarding monocyte gene expression levels and serum TRP/KP catabolite levels, we derived a fold change by dividing individual values of immune parameter or metabolite by the HC average value of each item per site (e.g. MDD individual value of site Muenster divided by HC average of site Muenster). This enabled us to pool the data of the two sites for the supplementary data (all patients together and all non-medicated patients of the two sites). The calculations of the values normalized to the HC values did not alter the fold differences (and statistical significances) between patients and HCs using the original data

per site (data not shown).

### 3. Results

#### 3.1. Sample characteristics

The clinical and demographic data of the study participants are shown in Table 1. In this table, all data are given per recruiting clinic (Muenster or Munich).

##### 3.1.1. Muenster cohort

The MDD group included 96 men and 135 women with a mean age of 40 years and a mean bmi of 26 kg/m<sup>2</sup>, indicating overweight. The IDS-C score was recorded in the 231 Muenster patients with MDD (see Section 2.1); we calculated a HAM-D 17 correlate score from this IDS-C score as described in the literature (Rush et al., 1986; Trivedi et al., 2004). Table 1 shows that this resulted in a mean HAM-D 17 score of 18, indicating -in general-, cases with a moderate depression. Patients reported a history of 4 previous depressive episodes on average; and being in the current episode since about 43 weeks. The vast majority of patients, i.e. 216 (93.5%) were on different regimens of antidepressants, 139 (60.2%) were treated with benzodiazepines/analogues, 135 (58.4%) were taking antipsychotic agents and/or mood stabilizers (15 (6.5%)); 23(10%) patients were undergoing electroconvulsive therapy (ECT).

##### 3.1.2. Munich cohort

The MDD group included 25 men and 25 women with a mean age of 40 years and a mean bmi of 23 kg/m<sup>2</sup>, indicating a normal weight. The mean HAM-D 17 score was 25, indicating -in general-, cases with a severe episode. Patients reported a history of 2 previous depressive episodes, and being in the current episode since 19 weeks; 38 (76%) were treated with benzodiazepines/analogues, the use of other psychotropic drugs/ECT was not allowed in the Munich patients (see Section 2.1).

Taken together, patients of the Munich cohort were less overweight

and less medicated as compared with patients of the Muenster cohort. They were also characterized by a more acute and severe depressive episode (Table 1). The Muenster cohort was characterized by a longer history of MDD, were also longer in the present episode; they were using extensively more medication, and had at the time of testing less severe depression (though still being depressed), they also had a significantly higher bmi.

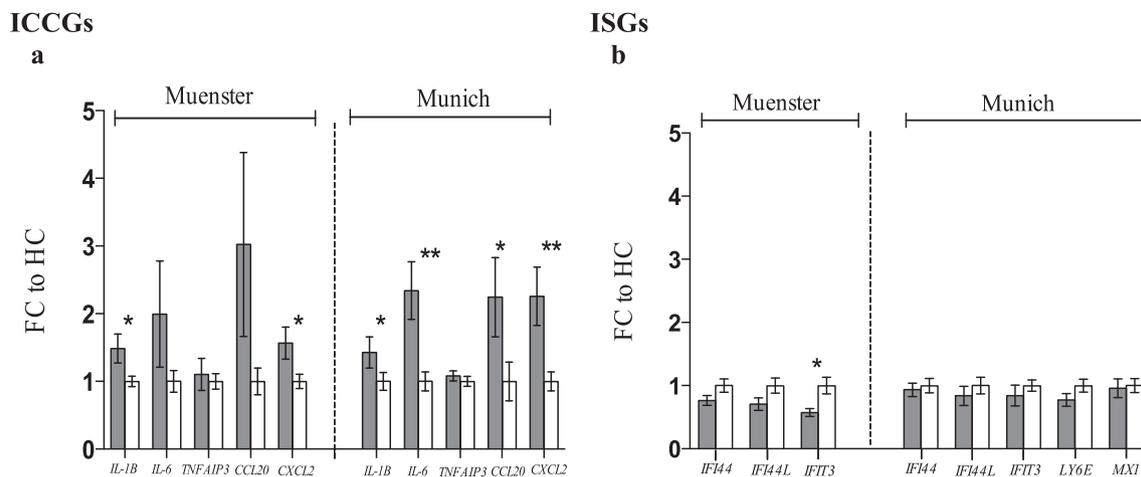
In Supplementary Table 3a, data are given for the entire group of patients with MDD (Muenster and Munich together,  $n = 281$ ); in Supplementary Table 3b, data are given separately for the patients without any form of medication ( $n = 20$ ) (also not benzodiazepines/analogues, as was allowed in the less medicated Munich group). From the total group of HCs, we selected 20 age, sex and bmi-matched HCs to be compared to the subgroup of 20 medication/ECT-free patients (Supplementary Table 3b). Where appropriate, we give immune and KP data for this subgroup underneath and in the supplementary material.

#### 3.2. Monocyte inflammatory state of patients with MDD and HCs

Relative mRNA expression levels of ICCGs and ISGs of study participants are shown in Fig. 2a,b. In these figures, the essential data are given per recruiting clinic. Supplementary Fig. 2a,c show data of the entire group of study participants, data of the subgroup of medication/ECT-naïve patients and their respective HCs are given in Supplementary Fig. 2b,d.

##### 3.2.1. Relative mRNA expression levels of ICCGs in circulating monocytes

The expression levels of five key ICCGs (i.e. *IL-1 $\beta$* , *IL-6*, *TNFAIP3*, *CCL20* and *CXCL2*) were determined in the circulating monocytes of 121 patients with MDD (Muenster cohort ( $n = 81$ ), Munich cohort ( $n = 40$ )), and in 124 HCs (Muenster cohort ( $n = 82$ ), Munich cohort ( $n = 42$ )) (Fig. 2a). Fig. 2a shows a higher expression of ICCGs in patients with MDD of both the Muenster and the Munich cohorts as compared to their respective HCs. The same trend was found in the subgroup of medication/ECT-naïve patients (yet here, a statistical significance was however



**Fig. 2.** a,b The relative mRNA expression levels of the indicated inflammatory genes in the circulating monocytes of patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . Abbreviations: ICCGs: pro-inflammatory cytokine/chemokine genes; ISGs: type I interferon-stimulated genes; *IL-1 $\beta$* : interleukin 1 $\beta$  gene; *IL-6*: interleukin 6 gene; *TNFAIP3*: tumor necrosis factor, alpha-induced protein 3 gene; *CCL20*: C-C-motif chemokine ligand 20 gene; *CXCL2*: C-X-X-chemokine ligand 2 gene; *IFI44*: interferon-induced protein 44 gene; *IFI44L*: interferon-induced protein 44 Like gene; *IFIT3*: interferon-induced protein with tetratricopeptide Repeats 3 gene; *LY6E*: lymphocyte antigen 6 family member E gene; *MX1*: interferon-regulated resistance GTP-binding protein MxA gene.

Compared to HCs, an increased expression of all ICCGs was found in patients of the Muenster cohort, values being statistically significant for *IL-1 $\beta$*  ( $F(1,157) = 4.022$ ,  $p = 0.047$ ,  $\eta^2 = 0.025$ ) and for *CXCL2* ( $F(1,157) = 4.104$ ,  $p = 0.044$ ,  $\eta^2 = 0.025$ ). The same increase was found in patients of the Munich cohort, values being in this case significant for *IL-1 $\beta$*  ( $F(1,76) = 4.411$ ,  $p = 0.039$ ,  $\eta^2 = 0.055$ ), *IL-6* ( $F(1,76) = 9.571$ ,  $p = 0.003$ ,  $\eta^2 = 0.112$ ), *CCL2* ( $F(1,76) = 5.299$ ,  $p = 0.024$ ,  $\eta^2 = 0.065$ ), and for *CXCL2* ( $F(1,76) = 8.375$ ,  $p = 0.005$ ,  $\eta^2 = 0.099$ ). On the contrary, patients were characterized by decreased in ISGs monocyte expression levels, a statistical significance was only reached for *IFIT3* in the case of the Muenster cohort ( $F(1,103) = 6.598$ ,  $p = 0.012$ ,  $\eta^2 = 0.060$ ). No significant differences were found between patients of the Muenster and Munich cohorts in relation to their ICCGs and ISGs monocyte expression levels.

only reached for *IL-6* (Supplementary Fig. 2b). The small number of subjects in this group probably plays a role here.

Both the Muenster and Munich patients showed almost equally increased monocyte expression levels of the ICCGs (Fig. 2a). However, the increase was a little more outspoken in patients of the Munich cohort, showing statistical significant values in 4 of the 5 genes against 2 of the 5 of the Muenster cohort when compared with HCs. Interestingly, a positive correlation was found between the monocyte expression levels of all top five ICCGs and patient's HAM-D 17 score (being values statistically significant only for *IL-6* ( $rho = 0.246$ ,  $p = 0.006$ )). Significant correlations were not found between ICCGs monocyte expression levels and neither medication/ECT state, nor disease acuteness (data not shown).

### 3.2.2. Relative mRNA expression levels of type I ISGs in circulating monocytes

We also determined the expression levels of five key type I ISGs (i.e. *IFI44*, *IFI44L*, *IFIT3*, *LY6E* and *MX1*) in the circulating monocytes of 92 patients with MDD (Muenster cohort ( $n = 52$ ), Munich cohort ( $n = 40$ )), and 99 HCs (Muenster cohort ( $n = 57$ ), Munich cohort ( $n = 42$ )) (Fig. 2b). Compared with HCs, patients with MDD of the two cohorts showed a reduced expression of all ISGs, yet a statistical significance was however only reached for *IFIT3* in the subgroup of patients of the Muenster cohort (Fig. 2b). Also in the entire group of patients, ISGs were underexpressed compared to HCs (Supplementary Fig. 2c), the same trend was found in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 2b).

Supplementary Fig. 2b also shows that treatment state had no obvious effect on this slightly reduced expression of the ISGs in monocytes. Significant correlations were not found between the monocyte expression levels of all ISGs and any of the clinical characteristics of patients (data not shown).

### 3.3. Serum levels of TRP/KP metabolites, KP metabolism ratios and monocyte expression levels of KP enzymes genes in patients with MDD and HCs

The TRP/KP catabolic data of study participants are shown in Figs. 3–7. In these figures, the essential data are given per recruiting clinic. Data of the entire group of patients and HCs, and the subgroup of medication/ECT-naïve patients and HCs are given in Supplementary

Figs. 3–7.

#### 3.3.1. TRP to 5-HTp and 5-HIAA breakdown pathway

Compared with HCs, significantly decreased TRP and 5-HTp serum levels were only found in the Munich patients (Fig. 3a,b); the same was found when compared with patients of the Muenster cohort. Considering the difference in treatment status between the Muenster and Munich patients, it is worthy to note that an effect of medication/ECT state was found for TRP and 5-HTp in patients (i.e. not taking medication was significantly associated with lower TRP and 5-HTp serum levels in patients ( $rho = 0.140$ ,  $p = 0.026$  and  $rho = 0.289$ ,  $p < 0.001$ , respectively)). In support of this, significantly reduced TRP and 5-HTp serum levels were found in the subgroup of medication/ECT-naïve patients as compared to HCs (Supplementary Fig. 3b,d). In addition, a significant negative correlation was also found between TRP and 5-HTp serum levels, and patient's HAM-D 17 score ( $rho = -0.279$ ,  $p < 0.001$  and  $rho = -0.363$ ,  $p < 0.001$ , respectively).

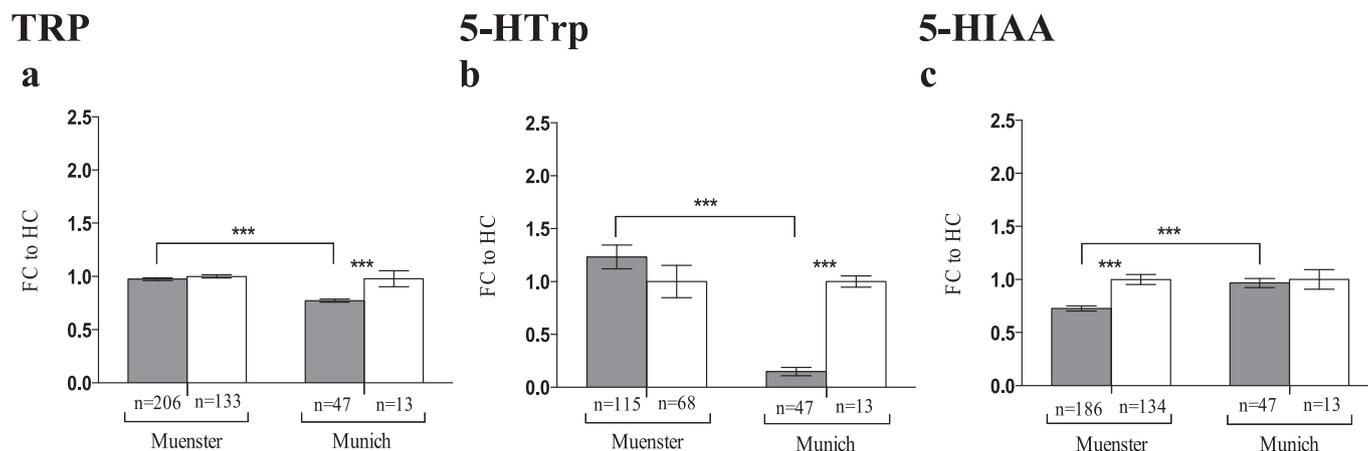
Muenster patients with MDD showed lower 5-HIAA serum levels compared to HCs and to patients of the Munich cohort (Fig. 3c). In this case, significant correlations were however not found between 5-HIAA serum levels in patients, medication/ECT state and diseases severity/acuteness (data not shown).

#### 3.3.2. TRP to KYN breakdown pathway

Patients of both the Muenster and Munich cohorts showed reduced KYN serum levels towards HCs, though a statistical significance was not reached (Fig. 4a); a statistical significance was reached for the combined entire group of patients with MDD and HCs (Supplementary Fig. 4a). Both the Muenster and Munich patients equally contributed to the reduced KYN serum levels in patients (Fig. 4a). Accordingly, statistical significant correlations were not found between KYN serum levels and neither medication/ECT state, nor disease severity/acuteness (data not shown).

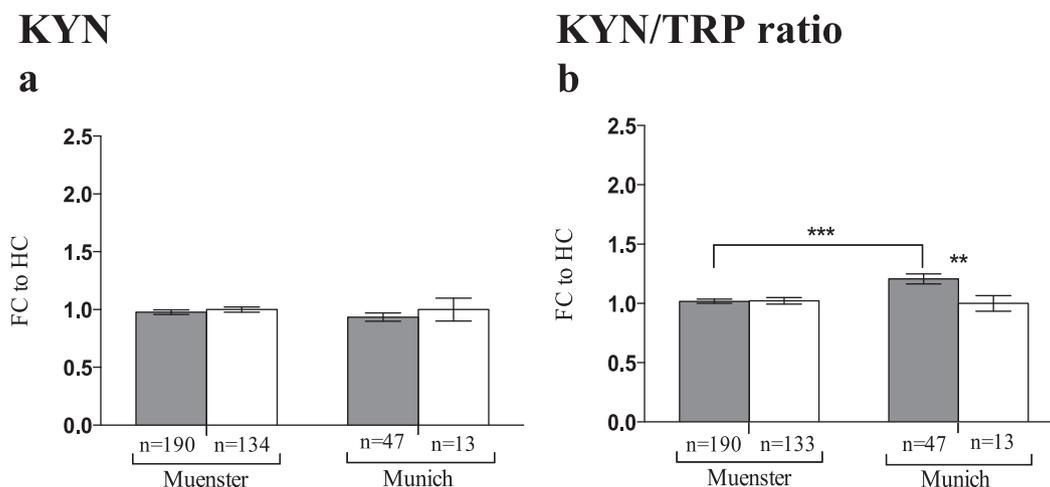
Interestingly, a significantly higher KYN/TRP ratio was found only in patients of the Munich cohort when compared with their respective HCs, and with patients of the Muenster cohort (Fig. 4b), although this did not result in higher KYN serum levels (since the precursor TRP was very low, see Section 3.3.1). A significant positive correlation was found between the KYN/TRP ratio and HAM-D17 score in MDD patients ( $rho = 0.141$ ,  $p = 0.029$ ).

Since IDO is an important inflammation-induced enzyme for the



**Fig. 3.** a–c. Serum levels of TRP, 5-HTp and 5-HIAA in patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . Abbreviations: TRP: tryptophan; 5-HTp: 5-hydroxytryptophan; 5-HIAA: 5-hydroxyindoleacetic acid.

Patients of the Munich cohort were characterized by decreased TRP and 5-HTp serum levels as compared with HCs ( $F(1,54) = 20.105$ ,  $p < 0.001$ ,  $\eta^2 = 0.271$  and  $F(1,54) = 122.455$ ,  $p < 0.001$ ,  $\eta^2 = 0.694$ , respectively), and with patients of the Muenster cohort ( $F(1,246) = 32.480$ ,  $p < 0.001$ ,  $\eta^2 = 0.117$  and  $F(1,155) = 34.202$ ,  $p < 0.001$ ,  $\eta^2 = 0.181$ , respectively). Patients of the Muenster cohort were characterized by decreased 5-HIAA serum levels as compared with HCs ( $F(1,314) = 25.589$ ,  $p < 0.001$ ,  $\eta^2 = 0.083$ ) and with patients of the Munich cohort ( $F(1,226) = 12.166$ ,  $p = 0.001$ ,  $\eta^2 = 0.051$ ).



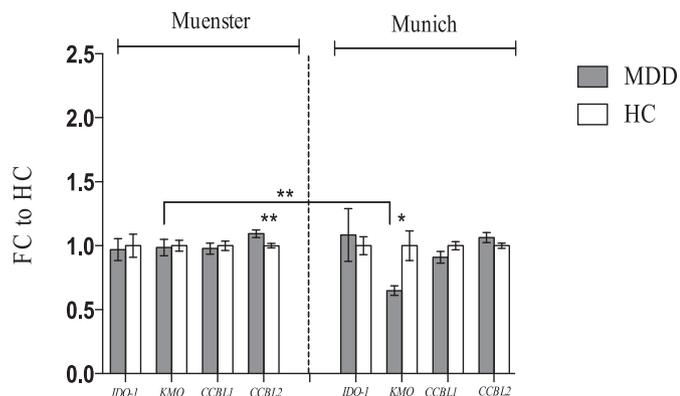
**Fig. 4.** a,b. Serum levels of KYN and the KYN/TRP ratio in patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . **Abbreviations:** KYN: kynurenine; TRP: tryptophan.

Significant differences were not found between patients and HCs of both cohorts regarding KYN serum levels. Interestingly, patients of the Munich cohort were characterized by an increased KYN/TRP ratio as compared with their respective HCs ( $F(1,54)=7.940, p = 0.007, \eta^2 = 0.128$ ) and with patients of the Muenster cohort ( $F(1,228) = 19.059, p < 0.001, \eta^2 = 0.077$ ).

conversion of TRP into KYN, we also determined the expression levels of *IDO-1* in the circulating monocytes of 68 patients with MDD (Muenster cohort ( $n = 29$ ), Munich cohort ( $n = 39$ ) and 67 HCs (Muenster cohort ( $n = 25$ ), Munich cohort ( $n = 43$ )) (Fig. 5).

*IDO-1* monocyte expression levels did not differ between patients and HCs of both cohorts (Fig. 5); a statistical significance was in this case also not reached for the combined entire group of patients with MDD and HCs (Supplementary Fig. 5a). Significant differences were also not found between the Muenster and Munich patients in relation to *IDO-1* monocyte expression levels (Fig. 5).

Accordingly, significant correlations were not found between



**Fig. 5.** The relative mRNA expression levels of the indicated inflammatory KP enzyme genes in the circulating monocytes of patients with MDD and HCs. Gray bars represent the group of patients with MDD, white bars represent the group of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . **Abbreviations:** *IDO-1*: indoleamine 2,3-dioxygenase gene; *KMO*: kynurenine 3-monooxygenase gene; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic 1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase III gene. Significantly decreased *KMO* monocyte expression levels were found in patients of the Munich cohort as compared with HCs ( $F(1,75)=6.865, p = 0.011, \eta^2 = 0.084$ ), and with patients of the Muenster cohort ( $F(1,60)=9.420, p = 0.003, \eta^2 = 0.136$ ). Significantly increased expression levels of *CCBL2/KAT III* were found in the circulating monocytes of patients of the Muenster cohort as compared with HCs ( $F(1,109) = 7.414, p = 0.008, \eta^2 = 0.064$ ).

medication/ECT state, illness severity/acuteness and monocyte expression levels of *IDO-1* in patients (data not shown).

### 3.3.3. KYN to KYNA breakdown arm

Significantly reduced serum levels of the NMDA-R antagonist KYNA, and KYNA/KYN ratios were found in both cohorts of patients with MDD (Fig. 6a,b). This was in particular evident in patients of the Muenster cohort (Fig. 6a,b). However, significant correlations were not found between these metabolite serum levels and neither medication/ECT state, nor disease severity/acuteness in patients (data not shown).

Since KAT enzymes are involved in the KYN to KYNA breakdown, we also determined the expression levels of *CCBL1/KAT I* and *CCBL2/KAT III* in the circulating monocytes of 94 patients with MDD (Muenster cohort ( $n = 55$ ), Munich cohort ( $n = 39$ )), and 103 HCs (Muenster cohort ( $n = 61$ ), Munich cohort ( $n = 42$ )) (Fig. 5).

Fig. 5 shows that – in accord with the reduced KYNA serum levels-, a reduced *CCBL1/KAT I* monocyte expression was found in patients with MDD of both cohorts, however, values did not reach statistical significance. A statistical significance was only reached in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 5b).

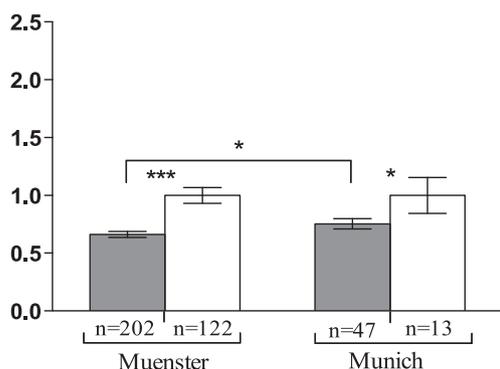
In contrast, an increased expression of *CCBL2/KAT III* was found in both cohorts of patients with MDD as compared to HCs; a statistical significance was however only found in the subgroup of patients of the Muenster cohort (Fig. 5) and in the entire group of patients with MDD (Supplementary Fig. 5a), in the latter irrespective of medication/ECT state (Supplementary Fig. 5b). Significant correlations were not found between *CCBL1/KAT I* and *CCBL2/KAT III* monocyte expression levels and neither medication/ECT state, nor disease severity in patients.

### 3.3.4. KYN to 3-HK breakdown arm

Compared to HCs, reduced serum levels of the NMDA-R agonist 3-HK (Fig. 6c), and a reduced 3-HK/KYN ratio were found in patients of both the Muenster and Munich cohorts (Fig. 6d). This was in particular evident in patients of the Munich cohort (Fig. 6c,d). Medication/ECT-naïve patients were also characterized by a decrease in serum levels of 3-HK (Supplementary Fig. 6d) and by a reduced 3-HK/KYN ratio (Supplementary Fig. 6f) as compared to their respective HCs. Accordingly, a significant negative correlation was found between 3-HK serum levels, the 3-HK/KYN ratio, and patient's HAM-D 17 score ( $\rho = -0.144, p = 0.028$  and  $\rho = -0.142, p = 0.030$ , respectively). Not taking medication was also associated with lower 3-HK serum levels ( $\rho = 0.179, p =$

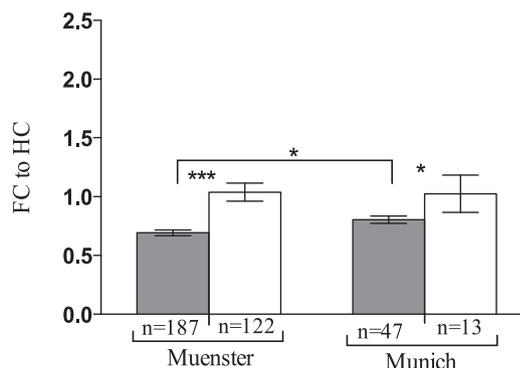
### KYNA

a



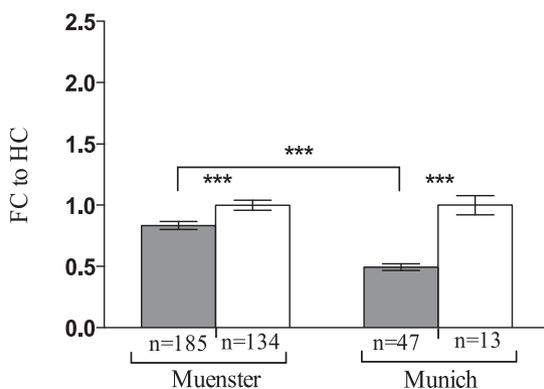
### KYNA/KYN ratio

b



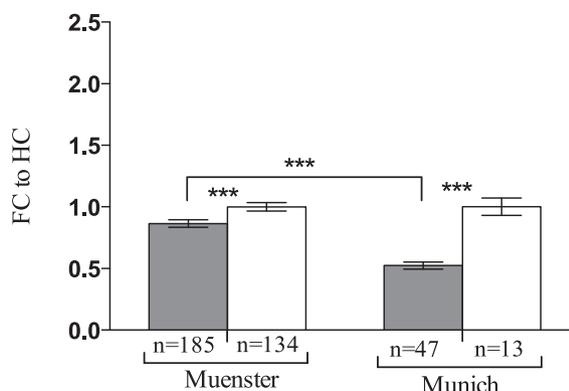
### 3-HK

c



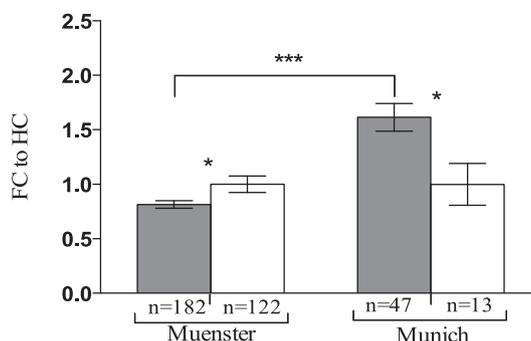
### 3-HK/KYN

d



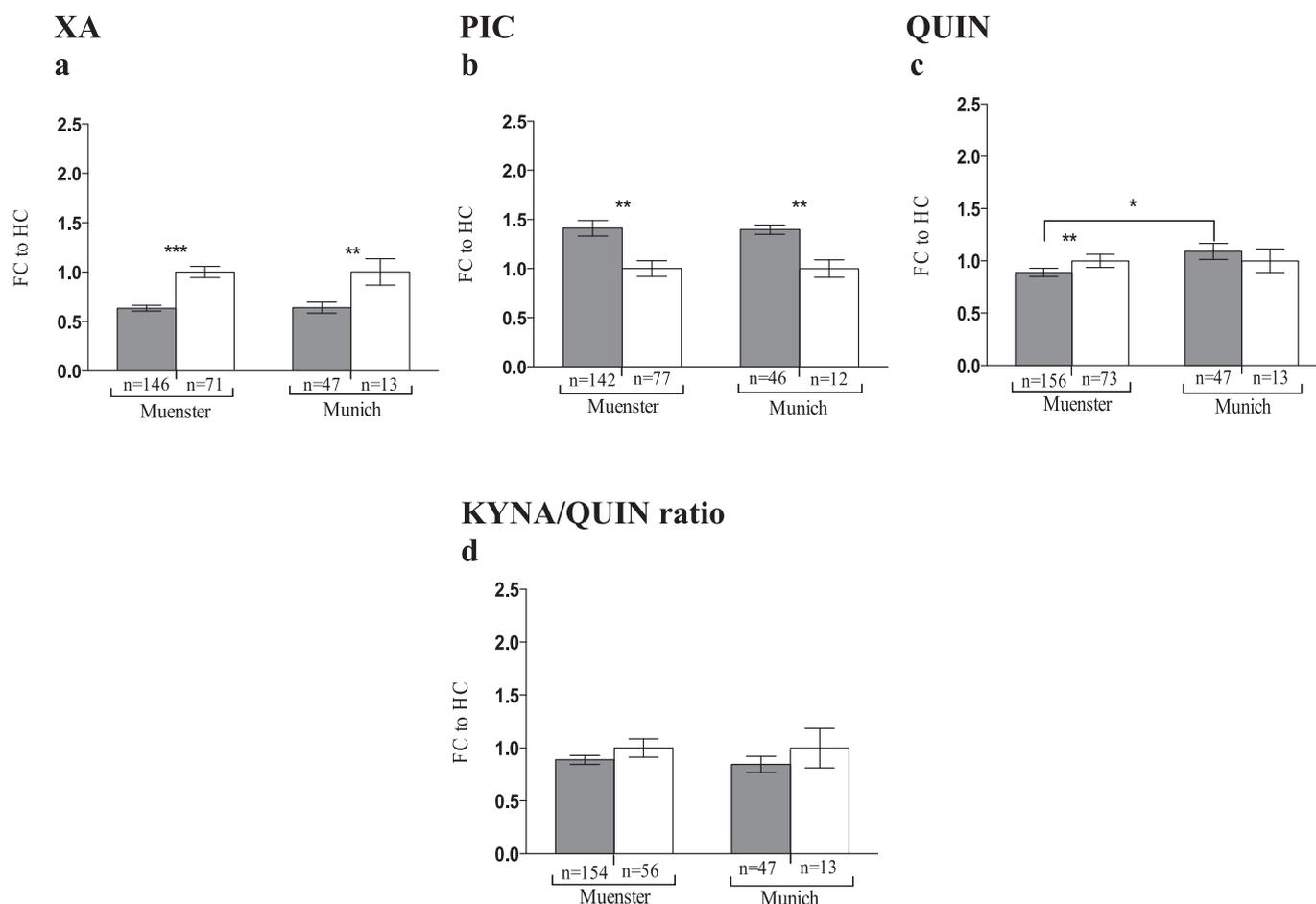
### KYNA/3-HK ratio

e



**Fig. 6.** a–e. Serum levels of the indicated KP metabolites and KP metabolism ratios in patients with MDD and HCs of both the Muenster and Munich cohorts. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . Abbreviations: KYNA: kynurenic acid; KYN: kynurenine.

Decreased KYNA serum levels and a decreased KYNA/KYN ratio were found in both cohorts of patients with MDD as compared with HCs. This was in particular evident in patients of the Muenster cohort, showing decreased KYNA serum levels ( $F(1,240) = 5.333, p = 0.022, \eta^2 = 0.022$ ), and a decreased KYNA/KYN ratio as compared with patients of the Muenster cohort ( $F(1,225) = 5.900, p = 0.016, \eta^2 = 0.026$ ). Decreased 3-HK serum levels, and a reduced 3-HK/KYN ratio were also found in both cohorts of patients with MDD. In this case, this was in particular evident in patients of the Munich cohort, showing significantly decreased 3-HK serum levels ( $F(1,223) = 14.235, p < 0.001, \eta^2 = 0.060$ ) and a decreased 3-HK/KYN ratio ( $F(1,223) = 22.845, p < 0.001, \eta^2 = 0.093$ ) as compared with patients of the Muenster cohort.



**Fig. 7.** a–d. Serum levels of the indicated KP downstream catabolites and the KYNA/QUIN ratio in patients with MDD and HCs. Gray bars represent the group of patients with MDD, white bars represent the group of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). \* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ . Abbreviations: MDD: major depressive disorder; HC: healthy controls; XA: xanthurenic acid; QUIN: quinolinic acid; PIC: picolinic acid; KYNA: kynurenic acid. Compared with HCs, patients with MDD of both cohorts showed reduced serum levels of the mGlu2/3 agonist XA ( $F(1,211) = 41.437, p < 0.001, \eta^2 = 0.164$  (Muenster cohort) and  $F(1,54) = 8.408, p = 0.005, \eta^2 = 0.135$  (Munich cohort)). Significant differences were not found between patients of both the Muenster and Munich cohorts in relation to their XA serum levels. On the contrary, patients with MDD of both cohorts were characterized by increased serum levels of the potentially NMDA-R antagonist PIC ( $F(1,201) = 8.593, p = 0.004, \eta^2 = 0.041$  (Muenster cohort) and  $F(1,52) = 10.926, p = 0.002, \eta^2 = 0.174$  (Munich cohort)). Both the Muenster and Munich patients equally contributed to the reduced XA, and increased PIC serum levels in patients. Interestingly, patients of the Muenster cohort were characterized by significantly decreased serum levels of the NMDA-R agonist QUIN as compared with HCs ( $F(1,223) = 7.647, p = 0.006, \eta^2 = 0.033$ ) and with patients of the Munich cohort ( $F(1,194) = 6.860, p = 0.010, \eta^2 = 0.034$ ).

0.006) and with a reduced 3-HK/KYN ratio in patients ( $\rho = 0.165, p = 0.012$ ).

Since KMO is the enzyme involved in the KYN to 3-HK transition (Fig. 1), KMO expression levels were also determined in the circulating monocytes of 68 patients with MDD (Muenster cohort ( $n = 29$ ), Munich cohort ( $n = 25$ )) and 67 HCs (Muenster cohort ( $n = 39$ ), Munich cohort ( $n = 42$ )) (Fig. 5). Compared to HCs, KMO was significantly underexpressed in the circulating monocytes of the Munich patients, but not of the Muenster patients (Fig. 5). The same trend was found in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 5b). We also found a significant negative correlation between KMO monocyte expression levels and patient's HAM-D 17 score ( $\rho = -0.357, p = 0.003$ ).

Since both 3-HK and KYNA serum levels were reduced in both cohorts of patients with MDD, we also determined the KYNA/3-HK ratio, in order to get an idea of the relative overweight of the potential neuroprotective over the potential neurotoxic arms of the KP. Fig. 6e interestingly shows a difference between Muenster and Munich patients regarding the KYNA/3-HK ratio; while in Muenster patients the potential neurotoxic arm was favored in comparison to HCs, the Munich patients showed an overweight of the potential neuroprotective arm. It

must again be stressed here that in both cohorts, levels of 3-HK and KYNA were actually reduced.

### 3.3.5. 3-HK to XA breakdown arm

Compared to HCs, significantly decreased XA serum levels were found in patients of both the Muenster and Munich cohorts (Fig. 7a). Also in the entire group, XA serum levels were significantly decreased in patients as compared to HCs (Supplementary Fig. 7a), this irrespective of the medication/ECT state (Supplementary Fig. 7b). Accordingly, significant correlations were neither found between XA serum levels and medication/ECT status, nor with disease severity/acuteness in patients (data not shown).

### 3.3.6. 3-HK to PIC breakdown arm

Significantly increased PIC serum levels were found in patients of both the Muenster and Munich cohorts as compared to their respective HCs (Fig. 7b). Also in the entire group, PIC serum levels were significantly increased as compared to HCs (Supplementary Fig. 7c), the same trend was found in the subgroup of medicated/ECT patients (Supplementary Fig. 7d,  $p = 0.053$ ). Accordingly, significant correlations were not found between PIC serum levels and neither medication/ECT status,

nor disease severity/acuteness (data not shown).

### 3.3.7. 3-HK to QUIN breakdown arm

Interestingly, patients of the Muenster cohort were characterized by significantly reduced QUIN serum levels as compared to their respective HCs, and to patients of the Munich cohort (Fig. 7c). However, significant correlations were not found between QUIN serum levels and neither medication/ECT status, nor with disease severity/acuteness (data not shown).

To investigate in more detail the apportioning of the so-called potentially neuroprotective versus potentially neurotoxic KP catabolites, we also analyzed the KYNA/QUIN ratio (Fig. 7d). Compared to HCs, this neuroprotective over neurotoxic ratio was found decreased in patients of both the Muenster and Munich cohorts, however, a statistical significance was not reached (Fig. 7d). A statistical significance was only reached if all patients were taken together (Supplementary Fig. 7g).

### 3.4. Correlations between the monocyte expression levels of pro-inflammatory genes and the monocyte expression levels of KP enzyme genes in patients with MDD

First, it is worthy to note that significant and negative correlations were found between the increased expression of ICCGs in patient's monocytes, and the monocyte expression levels of the 4 KP enzyme genes in patients (Table 2). This was in particular evident for *KMO* (catalyzing the transition of KYN to 3-HK), *CCBL1/KAT I* and *CCBL2/KAT III* (catalyzing the transition of KYN to KYNA). In contrast, a significant positive correlation was found between the monocyte expression levels of *IDO-1* and the monocyte expression levels of 4/5 ISGs in patients (Table 2), confirming the correlation data of Maria et al. (2016) in Sjögren's disease.

### 3.5. Correlations between the monocyte expression levels of pro-inflammatory genes and serum levels of TRP/KP metabolites and KP metabolite ratios in patients with MDD

Table 3 shows that the higher the expression of ICCGs in patient's monocytes, the lower the TRP serum levels in patients. In addition, a significant positive correlation was found between the monocyte expression levels of ICCGs and the KYN/TRP ratio.

Table 3 additionally shows that the expression of ICCGs in patient's monocytes significantly and negatively correlated to 3-HK serum levels

**Table 2**

Correlations between the monocyte expression levels of ICCGs/ISGs and the monocyte expression levels of KP enzyme genes in patients with MDD.

	<i>IDO-1</i>	<i>KMO</i>	<i>CCBL1/KATI</i>	<i>CCBL2/KATIII</i>
<i>IL-1β</i>	-0.137	0.016	-0.303**	-0.369***
<i>IL-6</i>	-0.175	-0.410**	-0.319**	-0.236*
<i>TNFAIP3</i>	0.061	-0.221	-0.207*	-0.010
<i>CCL20</i>	-0.309*	-0.372**	-0.396***	-0.233*
<i>CXCL2</i>	-0.337**	-0.317**	-0.290**	-0.250*
<i>IFI44</i>	0.404*	0.158	-0.014	-0.165
<i>IFI44L</i>	0.430**	0.317*	0.071	0.124
<i>IFIT3</i>	0.458**	0.306	0.161	-0.028
<i>LY6E</i>	0.229	0.088	-0.304	0.148
<i>MX1</i>	0.362*	0.187	0.174	0.010

Significant correlations are marked with an asterisk (\* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ ) and highlighted in bold. Abbreviations: *IDO-1*: indoleamine 2,3-dioxygenase gene; *KMO*: kynurenine 3-monooxygenase gene; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase III gene; *IL*: interleukin; *TNFAIP3*: tumor necrosis factor, alpha-induced protein 3 gene; *CCL20*: C—C motif chemokine ligand 20 gene; *CXCL2*: C-X-X-chemokine ligand 2 gene; *IFI*: interferon-induced protein gene; *LY6E*: lymphocyte antigen 6 family member E gene; *MX1*: interferon-regulated resistance GTP-binding protein MxA gene.

**Table 3**

Correlations between the monocyte expression levels of ICCGs/ISGs, serum levels of TRP/KP metabolites and KP metabolite ratios in patients with MDD.

	TRP	KYN/TRP	3-HK/KYN	3-HK
<i>IL-1β</i>	-0.018	0.070	-0.084	-0.111
<i>IL-6</i>	-0.220*	0.305**	-0.420***	-0.319**
<i>TNFAIP3</i>	-0.264**	0.314***	-0.442***	-0.282**
<i>CCL20</i>	-0.164	0.282**	-0.334**	-0.245*
<i>CXCL2</i>	-0.179	0.197*	-0.140	-0.170
<i>IFI44</i>	-0.124	-0.068	0.008	-0.173
<i>IFI44L</i>	-0.019	-0.218	0.070	-0.098
<i>IFIT3</i>	-0.055	-0.167	0.179	-0.006
<i>LY6E</i>	0.082	0.031	-0.286	-0.222
<i>MX1</i>	-0.043	-0.287	0.219	0.015

Significant correlations are marked with an asterisk (\* $p \leq 0.050$ , \*\* $p \leq 0.005$ , \*\*\* $p \leq 0.001$ ) and highlighted in bold. Abbreviations: TRP: tryptophan; KYN: kynurenine; 3-HK: 3-hydroxykynurenine; IL: interleukin; *TNFAIP3*: tumor necrosis factor, alpha-induced protein 3 gene; *CCL20*: C—C motif chemokine ligand 20 gene; *CXCL2*: C-X-X-chemokine ligand 2 gene; *IFI*: interferon-induced protein gene; *LY6E*: lymphocyte antigen 6 family member E gene; *MX1*: interferon-regulated resistance GTP-binding protein MxA gene.

and to the 3-HK/KYN ratio in patients.

Noteworthy significant correlations were not found between the expression levels of ICCGs in patient's monocytes and serum levels of KYN, KYNA, the KYNA/KYN ratio, XA, QUIN, PIC, and the KYNA/QUIN ratio in patients (data not shown). A consistent and noteworthy picture of correlations could also not be found for the monocyte expression levels of ISGs and any of the serum TRP/KP catabolites in patients.

## 4. Discussion

The TRP catabolic pathway in patients with MDD shows various activation and deactivation steps which are linked to their pro-inflammatory state. Table 4 gives an overview of the main data, we refer to Fig. 1 for the different TRP/KP catabolic pathways, and for the effects of TRP/KP metabolites on neuronal cell functions.

### 4.1. Tryptophan to 5-HT<sub>trp</sub> and 5-HIAA breakdown pathway

Consistent with previous findings (Maes et al., 1990; Charney, 1998; Hughes et al., 2012; Ogawa et al., 2014; Cowen and Browning, 2015; Kuwano et al., 2018), this study found reduced TRP and 5-HT<sub>trp</sub> serum levels in patients with MDD as compared to HCs, particularly in the more acute, severely affected and less medicated Munich patients with a relatively high monocyte ICCG expression. Indeed, the reduced TRP and 5-HT<sub>trp</sub> serum levels were associated with higher HAM-D 17 scores, and a higher monocyte ICCG expression (only TRP serum levels). They were also evident in the medication/ECT-naïve patients of the combined cohorts (Supplementary Fig. 3b,d).

Reduced TRP serum levels are a well-known feature of MDD and are thought to play a role in at least, part of the depressive syndrome, since TRP is – via 5-HT<sub>trp</sub> –, the ground substance for 5-hydroxytryptamine (5-HT, also known as serotonin), an essential neurotransmitter in mood regulation (Lapin and Oxenkrug, 1969; Toker et al., 2010).

Our data thus show a shortage of this ground substance particularly in non-medicated, severe and recent cases of MDD with a relatively high monocyte ICCG inflammatory state (the Munich cases), while this is was not the case in the medicated, longstanding and less severe cases of the Muenster cohort. Our observations thus support the important role of TRP and 5-HT deficiency in MDD, and a correcting effect of medication for this pathway.

### 4.2. TRP to KYN breakdown pathway

TRP is not only metabolized into 5-HT via the serotonin pathway, but also into KYN via the KP, which in fact acts as the major catabolic

**Table 4**  
Synopsis of the findings.

Pro-inflammatory genes/agents	MDD Munich Early episode	MDD Muenster Late episode	Correlation to demographic characteristics	Correlation to disease severity (HAM-D 17 score)
ICCGs (monocytes)	↑	↑	Positive to age	Positive
ISGs (monocytes)	=	=	No correlation	No correlation
TRP/KP catabolites, KP catabolite's ratios and KP enzyme genes	MDD Munich Early episode	MDD Muenster Late episode	Correlation to monocyte inflammatory state	Correlation to disease severity (HAMD-17 score)
TRP	↓	=	Negative to ICCGs	Negative
5-HT <sub>trp</sub>	↓	=	No correlation	Negative
KYN	=	=	No correlation	No correlation
KYN/TRP ratio	↑	=	Positive to ICCGs	Positive
<i>IDO-1</i> (monocytes)	=	=	Negative to ICCGs Positive to ISGs	No correlation
KYNA	↓	↓	No correlation	No correlation
KYNA/KYN ratio	↓	↓	No correlation	No correlation
<i>CCBL1/KAT I</i> (monocytes)	=	=	Negative to ICCGs	No correlation
<i>CCBL2/KAT III</i> (monocytes)	=	↑	Negative to ICCGs	No correlation
3-HK	↓	↓	Negative to ICCGs	Negative
3-HK/KYN ratio	↓	↓	Negative to ICCGs	Negative
<i>KMO</i> (monocytes)	↓	=	Negative to ICCGs	Negative
KYNA/3-HK ratio	↑	↓	Negative to ICCGs	Positive
XA	↓	↓	No correlation	No correlation
PIC	↑	↑	No correlation	No correlation
QUIN	=	↓	No correlation	No correlation
KYNA/QUIN ratio	=	=	No correlation	No correlation

↑: statistically significantly elevated compared to HCs; =: no statistically significant differences between patients and HCs; ↓: statistically significantly decreased compared to HCs. *Abbreviations:* MDD: major depressive disorder; HAM-D 17: Hamilton Rating scale for depression, 17-item version; ICCGs: inflammatory cytokine/chemokine genes; ISGs: interferon stimulated genes; TRP: tryptophan; 5-HT<sub>trp</sub>: 5-Hydroxytryptophan; KYN: kynurenine; *IDO-1*: indoleamine 2,3-dioxygenase gene; KYNA: kynurenic acid; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase II gene; 3-HK: 3-hydroxykynurenine; *KMO*: kynurenine 3-monooxygenase gene; XA: xanthurenic acid; PIC: picolinic acid; QUIN: quinolinic acid.

pathway for TRP in the body (Stone et al., 2013; O'Farrell and Harkin, 2017) (Fig. 1).

In recent years, various theories have emerged to provide a mechanistic link between the existence of an altered inflammatory state in patients with MDD and the decreased TRP availability for 5-HT synthesis in MDD. One of these theories has focused on an increased breakdown of TRP down the KP by activation of KP breakdown enzymes under the influence of inflammatory stimuli (Maes et al., 2011; Leonard and Maes, 2012; Arnone et al., 2018).

In support of such view, our study found an increased KYN/TRP ratio, but only in the subgroup of patients of the Munich cohort (characterized by a more severe episode as compared with patients of the Muenster cohort). The increased KYN/TRP ratio was found positively associated with the pro-inflammatory (ICCGs) state of patient's monocytes. This is in accord with a higher entrance of TRP into the KP in the

Munich patients with a high monocyte pro-inflammatory state, supporting a view of TRP depletion down the KP at the expense of the serotonin pathway.

However, KYN serum levels (and many of the KYN downstream products) were not raised in the Munich patients. We therefore assume that it is not likely that there is a considerable drain of TRP down the KP in the Munich patients and that, initially reduced TRP levels (perhaps reduced by low uptake via the gut) may play a more important role in acting as factor for the raised KYN/TRP ratio.

#### 4.2.1. A prominent role for *IDO* in the TRP to KYN breakdown pathway in patients with MDD?

*IDO* is one of the most well-known enzymes for the transition of TRP into KYN. It is specifically expressed in pro-inflammatory immune cells; therefore, a prominent role has been suggested for *IDO* in the phenomenon of inflammation-induced increased TRP to KYN degradation.

However, and against such a view of a prime role of *IDO* in depleting TRP levels in MDD, *IDO-1* was not overexpressed in the otherwise pro-inflammatory activated monocytes of patients. In fact, a negative correlation was found between the monocyte expression of ICCGs and *IDO-1* in patient's monocytes. Supporting our findings, Hughes et al. (2012) also found a strong TRP depletion, a high KYN/TRP ratio and normal KYN levels in the presence of a normal *IDO-1* expression in subjects with MDD, also raising doubts about an activation of *IDO* in MDD. Other investigators have pointed to liver *TDO*, activated by stress, as playing a more prominent role in the TRP to KYN transition in patients with MDD (Dantzer et al., 2011; Maes et al., 2011; Badawy, 2013; Godoy et al., 2018; Qin et al., 2018). However, as an increase in KYN serum levels was not observed in our study, it is unlikely that the depletion of TRP resulted from a stress-related increase in *TDO* activity.

Interestingly, a positive correlation was found between the monocyte expression levels of *IDO-1* and type I ISGs in patients, although the ISG expression was not raised (it was earlier suppressed). This positive correlation reinforces the idea that *IDO* is under the control of inflammation, but of another type than the ICCG expression. Since ISG inflammatory overexpression is a characteristic of patients with systemic autoimmune conditions such as pSS (Maria et al., 2016), while ICCG inflammatory over expression is a characteristic of patients with mood disorders, our present and previous findings suggest that an increased *IDO* mediated TRP to KYN breakdown is particularly relevant for the immune dysregulation in systemic autoimmune conditions and not for that in mood disorders. This may be of relevance since it may distinguish the MDD state from the state of depression that occurs during the course of other somatic diseases such as pSS or from depression induced by administration of exogenous cytokines such as IFN- $\alpha$  (Capuron et al., 2012; Raison et al., 2010).

#### 4.3. KYN to 3-HK and KYNA breakdown arms

In the present study, and contrary to what expected (see Background section), significantly reduced 3-HK serum levels, and a significantly reduced 3-HK/KYN ratio (reflecting *KMO* enzyme activity) were found in both cohorts of patients with MDD as compared to HCs, although these phenomena were in particular pronounced in patients of the Munich cohort. The more reduced *KMO* activity in the Munich patients was reflected in a significantly reduced monocyte expression level of *KMO*. Since the expression of the ICCGs in patient's monocytes were negatively associated with the monocyte expression level of *KMO*, the 3-HK/KYN ratio, and with the 3-HK serum levels, our findings suggest that the higher the pro-inflammatory ICCGs state of patient's monocytes, the lower the potential of monocytes to catabolize KYN into 3-HK. Importantly, this decrease in the KYN to 3-HK transition was also negatively associated with a higher HAM-D 17 score in patients.

In support of our findings, Wurfel et al., 2017 also found lower 3-HK serum levels in a cohort of patients with affective disorders (i.e. MDD, bipolar disorder and schizoaffective disorder), with 3-HK serum levels

being particularly reduced in affective psychosis. In addition, the study of Clark et al., 2016 also found a reduced KP metabolism, be it not in the blood, but in the ventrolateral prefrontal cortex of individuals with depression. Hughes et al. (2012) on the other hand did neither find a deactivation nor an activation of the KP in depressed patients.

With regard to the KYN to KYNA breakdown arm, reduced serum levels of KYNA, and a reduced KYNA/KYN ratio (reflecting a reduced activity of the KAT enzyme system) were found in patients of both cohorts. With regard to the KAT enzymes involved in the breakdown of KYN to KYNA, a contrasting picture was found. In accord with a reduced activity of the KAT enzyme system, we found a tendency for a reduced expression of the *CCBL1/KAT I* in the circulating monocytes of patients of both cohorts (but only in the unmediated patients). However, there also was an overexpression of *CCBL2/KAT III* in the monocytes of the Muenster patients. We explain this inconsistency by assuming that monocyte *CCBL2/KAT III* must play a minor role in the systemic transition of KYN to KYNA in patients with MDD. Clearly more investigations are needed on the role of the three KAT enzymes in KYN-to-KYNA transition in different tissues.

Collectively, our data suggest a deactivation of both the potentially neuroprotective KYN to KYNA, and the potentially neurotoxic KYN to 3-HK breakdown arms in MDD. However, consistent signs of an involvement of the monocyte pro-inflammatory (ICCGs) state of patients was only found for the deactivation of the KYN to 3-HK breakdown arm.

#### 4.4. 3-HK to XA breakdown arm

XA has been implicated in various central nervous system disorders such as chronic pain and/or epilepsy (Melnikova, 2003; Curto et al., 2015). In addition, it has also been shown to exert antipsychotic effects and, interestingly, it has been reported as substantially reduced in patients with schizophrenia (Curto et al., 2019). However, only few studies have measured XA serum levels in MDD (Colle et al., 2020; Ryan et al., 2020). Consistent with previous reports, reduced XA serum levels were found in both cohorts of patients with MDD as compared with HCs. This was irrespective of disease severity/acuteness, and of medication/ECT status.

XA production is also under the influence of KAT enzymes. As discussed in 4.3, a reduced monocyte expression of *CCBL1/KAT I*, accompanied by an increased monocyte expression of *CCBL2/KAT III* were found in the circulating monocytes of –especially–, patients of the Muenster cohort (and the subgroup of medication/ECT naïve patients, see Supplementary Fig. 5b). We reiterate the need of further studies to understand the relationship between the chronic low grade inflammatory state of patients with MDD, KATs expression, and XA production from 3-HK in MDD.

Our results could be however of relevance due to XA's capacity to activate metabotropic glutamate 2 and 3 (mGlu2/3) receptors and thereby, inhibit excitatory synaptic transmission (Fazio et al., 2015). In the last years, glutamatergic pathway disturbances have emerged as an important key factor in, at least, a subgroup of patients with MDD, and increasingly literature doubts about (a generalized) validity of the “serotonergic theory of MDD”, pointing in the direction that low serotonin levels may be an important factor for preserving recovery from depression rather than having a primary effect on mood lowering in vulnerable people (Cowen, 2008; Cowen and Browning, 2015). Therefore, modulation of glutamate receptors has been suggested as an important new target for the treatment of MDD (Matrisciano et al., 2007; Park et al., 2015). In line with this, recent evidence has showed antidepressant-like activity of mGlu2/3 receptor agonists in animal models of depression (Feinberg et al., 2002; Matrisciano et al., 2007).

#### 4.5. 3-HK to QUIN and PIC levels

Increased serum levels of the NMDA-R antagonist PIC were found in patients of both cohorts as compared to their respective HCs; this

increase was irrespective of disease severity/acuteness and of medication/ECT status.

On the opposite, and contrary to what expected, significantly decreased serum levels of the NMDA-R agonist QUIN were found in patients of the Muenster cohort as compared with both HCs, and with patients of the Munich cohort. However, significant correlations were neither found between QUIN serum levels nor with disease severity/acuteness or medication/ECT status in patients.

#### 4.6. Limitations of the study

The results of the present study should be interpreted in light of several important limitations.

First, the used parameters were determined in blood, and outcomes might differ from measurements in the brain or in the CSF. Of interest in this context is that McGuinness et al. (2016) showed that in the non-obese diabetic mouse model of anxiety and depression, inflammatory stimulation with lipopolysaccharides (LPS) did induce the expression of ISGs and *IDO-1* in the brain, but that it did not induce ISG expression in circulating monocytes (while it did induce ICCG expression in these cells, thus giving a monocyte profile similar to the pattern found here in patients). This shows that a different inflammatory reaction of the brain versus the periphery is possible and this thus also makes a local brain depletion of TRP due to a local *IDO-1* overexpression possible in the absence of a similar reaction in the periphery.

Furthermore, the inflammatory parameter used was the expression of inflammatory genes in circulating monocytes. We were only able to measure the circulating inflammatory compounds hsCRP and IL-6 in the Muenster patients and controls (Supplementary Table 4). Of note, we did not find significant elevations of these inflammatory compounds in patients, there did also not exist a positive correlation of these compounds with the monocyte inflammatory gene state. The latter suggests other sources of these serum inflammatory compounds than the monocytes (e.g. liver and adipose tissue). A similar discrepancy between monocyte inflammatory state and serum inflammatory serum compounds has been described for the metabolic syndrome too, where adiposity and blood lipid levels play an important role in the discrepancy (Baldeon-Rojas et al., 2016). Interestingly, KYN and QUIN serum levels did correlate to the serum levels of hsCRP and IL-6 (see legend Supplementary Table 4). All this is of relevance since various reports showed increased blood/CSF IL-6, KYN and QUIN levels in patients with MDD and suicidal behavior (Lindqvist et al., 2009; Sublette et al., 2011; Erhardt et al., 2013); high QUIN levels have also been detected in the microglia of suicide victims (Busse et al., 2015). Thus, the positive correlation between blood/CSF inflammatory compounds, QUIN, and KYN could highlight a detection possibility for an important subgroup of patients with MDD. Unfortunately, our information on patient's risk for suicidal behavior was not uniformly recorded in the patients under study here, while psychotic patients were excluded from recruitment in the MOODINFLAME study. Therefore, we could not perform studies on the effects of these variables in this sample of patients with MDD and HCs.

Also, and although we investigated a larger panel of TRP and KP metabolites than most of the previous studies, our panel is still limited. Future studies should consider other KP metabolites, such as 3-HAA and/or AA, as well as the genes for tryptophan hydroxylase (TPH)-II, monoamine oxidase (MAO), kynureninase and/or TDO.

Another limitation was the relatively small sample size of the Munich cohort compared to the Muenster cohort, yet we have tried to take this into account in our supplementary material and pooled the data of the two cohorts to be able to draw more generalized conclusions for MDD, irrespective of the phase of the episode. We additionally analyzed in this total cohort medicated and medication-free patients, separately.

Furthermore, methodologies for TRP metabolite determinations differed between both cohorts and unfortunately, various runs of assays were carried out on limited numbers of patients over time, resulting in the condition that not all variables were tested in all patients. To be able

to pool the data we harmonized data of a metabolite or immune parameter to the means of the HC value of that parameter, this harmonization had no effects on actual fold differences (and the significance level) between patients and control values of that parameter. Nevertheless, future studies on larger cohorts should try to standardize as much as possible their detection assays and include larger groups of antidepressant-treated and antidepressant-naïve patients with MDD in better defined phases of the disease to obtain more precise data on TRP metabolism.

## 5. Conclusions

The systemic TRP/KYN catabolic pathway shows various activation and deactivation steps in MDD which are linked to the inflammatory state of patients.

TRP serum levels were reduced in patients and linked to an increased pro-inflammatory (ICCGs) monocyte state. This was in particular evident in the subgroup of patients with the highest HAM-D 17 score (Munich patients). Contrary to what expected, KYN serum levels were not increased in patients; an increased KYN/TRP ratio was only found in the subgroup of patients with the lowest TRP serum levels (Munich patients). *IDO-1* monocyte expression levels were decreased in patients and negatively related to their pro-inflammatory (ICCGs) monocyte state. Thus, a decrease in TRP serum levels via an ICCGs-inflammatory activation of the KP is unlikely in MDD.

Downstream from KYN, various other activation and deactivation steps were detected, and linked to the inflammatory state of patients. This resulted, regarding compound capable of influencing glutamate receptors, in reduced serum levels of 3-HK (NMDA-R agonist), KYNA (NMDA-R antagonist), and XA (mGlu2/3 agonist). PIC (NMDA-R antagonist) was increased in patients, QUIN (NMDA-R agonist) was decreased only in the Muenster patients. Only 3-HK serum levels were related to the pro-inflammatory (ICCGs) monocyte state of patients; the deactivation of the KYN to 3-HK breakdown pathway was in particular evident in the cohort of patients with the highest HAM-D 17 score (Munich patients). The ultimate effect on brain glutamate receptor triggering of this altered equilibrium between peripheral agonists and antagonists needs further exploration.

## Ethical statement

This study has been conducted in compliance with standards for Good Clinical Practice (GCP), assuring that the rights, safety and well-being of patients were protected in accordance with the principles that have their origin in the Declaration of Helsinki (June 1964, last amendment Tokyo 2004). Additionally, for the conduct of the study, the relevant national and European regulations were adhered to. After study procedures had been fully explained, all subjects provided written informed consent prior to performance of any screening phase evaluations. Only patients who had the cognitive abilities for the informed consent of the study participation were included. The study was approved by the ethics committee of the Medical Association Westphalia-Lippe, Germany (2009-019-f-S) and the ethics committee of the medical faculty at the Ludwig-Maximilian-University of Munich, Germany (234-09).

## Declaration of competing interest

HAD has received grants from the Netherlands Organization for Health Research and Development, the European Union, the Stanley Medical Research Institute, the Dutch Diabetic Foundation and the JDRF; he has received speaker's fees from Astra Zenica and he serves/has served in advisory boards of the Netherlands Organization for Health Research and Development, the European Union and the JDRF. NM has given presentations for Janssen-Cilag during the last 6 months and was supported by the foundation 'Immunität und Seele'. GAH was

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## Authors contributions

BB, EW and LG collected the data, GAH analyzed the data. GAH and HAD drafted the manuscript. NM, GS, MS, NM, LG, HAD critically reviewed the manuscript. All other authors read and approved the final manuscript.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pnpbp.2020.110226>.

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### Further Reading

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