POLYMORPHISMS IN KYNURENINE PATHWAY GENES AND PSYCHOLOGICAL DISTRESS IN HIV PATIENTS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOMEDICAL SCIENCES

AUGUST 2015

By
Naomi Tanizaki

Thesis Committee:

Linda Chang, Chairperson
Vanessa Douet
Rosanne Harrigan

Keywords: aminoadipate transferase, kynurenine aminitransferase II, kynurenine 3-monooxygenase, Kynurenic Acid, Psycholopathology, HIV

ACKNOWLEDGEMENTS

I thank all the research participants for their contribution to the study, the community providers who refer the participants, and the clinical research staff of the University of Hawaii MR Research Program at Queen's Medical Center, who recruited and evaluated subjects. Thank you to Caroline Jiang, M.S., for statistical analyses support and revisions. Thank you to Dr. Adrien Franke and Dr. Xingnan Li who collaborated with us on the HPLC CSF KYNA measurements.Thank you to my committee chair, Dr. Linda Chang, and committee members Dr. Rosanne Harrigan, Ph.D., Vanessa Douet, Ph.D. This study was supported by NIH grant funding: R01 MH 61427, K24 DA016170, R01 DA035659, U54 NS56883, P30CA071789.

ABSTRACT

Background: HIV infection, neuroinflammation and psychopathology are each associated with imbalances in the kynurenic pathway (KP), which includes kynurenine-3-monooxygenase (KMO) and kynurenine aminotransferase II (KATII). Both KMO and KATII enzymes mediate levels of kynurenic acid (KYNA), which increases with HIV infection, and may decrease with psychopathological symptoms such as depression. Since people with HIV are more vulnerable to neuroinflammation and comorbid depression, variations in the genes of these enzymes may influence psychopathological symptoms in HIV.

Methods: 72 HIV seronegative (SN) and 72 HIV positive participants were evaluated using the Center for Epidemiologic Studies Depression and Symptom Checklist-90-Revised scales, and were genotyped at *KATII* rs1480544 at *KMO* rs1053230. Cerebrospinal fluid kynurenic acid concentration [KYNA] was measured in 51 SN and 49 HIV participants. T-test, and one-way and two-way AN(C)OVA were used to compare effects of genetic variation and HIV status on psychological symptoms or KYNA levels. Pearson or Spearman analyses was used to find correlation between psychopathological symptom scores and KYNA levels.

Results: For psychopathological symptoms, overall, HIV participants had higher scores than SN, and SN *KATII* C-carriers tended to have lower scores than SN TT homozygotes. Older age correlated with higher CSF [KYNA] in *KATII* C-carriers and *KMO* CC-homozygotes independently of serostatus. HIV participant psychopathological scores did not differ between *KMO* genotypes.

Conclusions: *KATII* genotypes and HIV serostatus were associated with psychopathological symptoms. Furthermore, CSF [KYNA] varies with age depending on both *KMO* and *KATII* genotypes. Together, this supports a functional role of these variations in the dysregulation of the KP that may moderate psychopathological symptoms in HIV and SN individuals. Understanding the KP mechanisms in HIV may lead to novel treatments for psychopathological symptoms.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HIV - human immunodeficiency virus

AIDS - acquired immune deficiency syndrome

HAART - highly active antiretroviral therapy

TRP - tryptophan

5-HT - 5-hydroxytryptamine; serotonin

KP - kynurenine pathway

KYN - kynurenine

KYNA - kynurenic acid

KMO - kynurenine 3-monooxygenase

3-HK - 3-hydroxykynurenine

KAT - kynurenine aminotransferase

CSF - cerebrospinal fluid

CES-D - Center for epidemiologic studies - depression scale

SCL-90-R - Symptom Checklist 90 - Revised

DEP - Depression

ANX - Anxiety

SOM - Somatization

O-C - Obsessive Compulsiveness

HOS - Hostility

I-S - Interpersonal Senstivity

PHB - Phobic Anxiety

PAR - Paranoid Ideation

PSY Psychoticism

GSI - General Symptom Index

PSDI - Positive Symptom Distress Index

PST - Positive Symptom Total

INTRODUCTION

More than 34 million people worldwide have died from HIV, making HIV/AIDS a major public health issue across the globe (WHO, 2015). Nearly 37 million people were living with HIV, and approximately two million people became newly infected with HIV, at the end of 2014 (WHO, 2015). In the United States, the majority of people living with HIV/AIDS are Black/African American (41%), followed by Caucasian (34%) and Hispanic/Latino individuals (20%; WHO, 2015). In Hawaii, the racial profile differs as most of the people with HIV/AIDS infection are Caucasian (57%), followed by Asian (15%), and Native Hawaiian/Pacific Islander (11%), with Black/African American (15%) and Latinos (11%) representing a minority of the HIV/AIDS population (Hawai`i State Department of Health, 2014). HIV afflicts people from all races (CDC, 2015a).

Since the advent of highly active antiretroviral therapy (HAART) in the mid 90's, HIV/AIDS patients live longer and have fewer opportunistic infections (CDC, 2015b). However, relative to the general population, they have a higher incidence of comorbid psychopathologies, including depression, anxiety, and somatization (Owe-Larsson et al. 2009; Jin et al. 2010; Rezaei et al. 2013). Furthermore, rates of current depression in the U. S. are higher in HIV patients at approximately 36% (Atkinson et al. 1994; Perkins et al. 1994; Dew et al. 1997; Bing et al. 2001; Asch et al. 2003; Rabkin 2008) compared to the general population of 6.8% (Collingridge et al. 1979). These symptoms have been linked to poor treatment adherence (Gonzalez et al. 2011), poor neurological and physiological clinical outcomes, increased sexual risk-taking behaviors (Owe-Larsson et al. 2009), and ultimately higher mortality rates (DeLorenze et al. 2010). Moreover, treatments for psychopathological symptoms in HIV patients may be ineffective, or have untoward side effects or drug-drug interactions with HIV treatment regimens (Sherr et al. 2011; Hill et al. 2013). Further investigation of the molecular mechanisms involved in the development of psychopathological symptoms in HIV patients is needed to find safer and more

efficacious treatment appropriate for psychopathological symptoms and psychopathological co-morbidities in HIV populations.

Tryptophan (TRP) is the precursor for serotonin or 5-hydroxytryptamine (5-HT), which is a neurotransmitter linked to feelings of well-being and happiness (Myint 2012), and is the major target for antidepressant therapy (Morrissette et al. 2014). A pro-inflammatory environment results in a net increase in the breakdown of TRP, initiating the start of kynurenine pathway (KP) activity in the central nervous system (CNS; Oxenkrug 2007; Maes 2011). An increase in KP activity is identified by the increased activation of indolamine 2,3-dioxygenase (IDO), the first enzyme of the KP that metabolizes TRP to kynurenine (KYN; Baran et al. 2012; Myint et al. 2012; Schwarcz et al. 2012). However, inflammation affects later steps in the KP differently. In the brain, KYN is inactive by itself, but can be further degraded (Figure 1; Schwarcz 2004; Guillemin et al. 2007; Ting et al. 2007) in microglia by kynurenine monooxygenase (KMO) into 3-hydroxykynurenine (3-HK), a neurotoxic metabolite in the glutamatergic excitatory pathway associated with increased oxidative stress (Schwarz et al. 2002; Stone et al. 2002). As with IDO, KMO activity increases with proinflammatory stimuli (Alberati-Giani et al. 1996; Mellor et al. 1999; Connor et al. 2008; Zunszain et al. 2012; Molteni et al. 2013). In astrocytes, kynurenine is converted primarily by kynurenine aminotransferase II (KAT II) into kynurenic acid (KYNA) (Ting et al., 2007), posited to be neuroprotective (Davies et al. 2010; Han et al. 2010; Schwarcz et al. 2012). Unlike IDO and KMO, KAT activity either decreases or is unaffected by proinflammatory stimuli (Alberati-Giani et al. 1996; Connor et al. 2008; Zunszain et al. 2012; Molteni et al. 2013). KP activity varies in neuroinflammatory diseases such as multiple sclerosis (Baran et al. 2010) and HIV (Fuchs et al. 1990; Heyes et al. 1992). KP dysregulation thus plays a critical role in neuroinflammatory and psychopathological disorders, but the molecular mechanisms are still unclear.

Individuals with more psychopathological symptoms such as melancholy (Gabbay et al. 2010), history of suicide attempts (Sublette et al. 2011), anhedonia (Gabbay et al. 2012), and

major depressive disorder (Myint et al. 2007) also have more KP activity. However, relationships between psychopathological symptoms and KYNA are less clear: Myint and colleagues (2014) proposed that imbalances in the glial-neuronal network were linked to reduction in KYNA formation, and indicated a predominance of microglial KP activity (i.e. 3-HK production), which could lead to recurrent and chronic major depression. Studies have also shown depressive symptoms are associated with lower levels of peripheral KYNA in patients with somatization, (Maes et al. 2012), and major depression (Myint et al. 2007; Maes et al. 2012), but others have shown that there is no relationship between KYNA and depressive symptoms (Hughes et al. 2012). In contrast, patients with schizophrenia (Linderholm et al. 2012) and bipolar I or II disorders (Olsson et al. 2010), particularly those with manic and psychotic features of bipolar I (Olsson et al. 2012) have higher CSF KYNA (i.e. astrocyte KP activity) compared to healthy controls. Regardless of the directionality, imbalance in KP activity may reflect the severity of psychopathological symptoms in the context of inflammatory-associated disorders.

Pre-HAART HIV-infected individuals had higher KP activity in the periphery and CSF compared to healthy controls (Fuchs et al. 1990; Heyes et al. 1992). Nowadays, HAART-treated HIV individuals have a reduced incidence of opportunistic infections and HIV-associated dementia (Matinella et al. 2015; Watkins et al. 2015), but still have persistent HIV-associated neuroinflammation, with reservoirs of inflammatory activity in microglia cells including monocytes/macrophages (Hong et al. 2015; Rappaport et al. 2015). Such ongoing brain inflammation likely contributes to cognitive deficits and psychopathological symptoms which may be mediated through KYNA levels since even today, HIV infected individuals still have higher KYNA in the CSF (Heyes et al. 1992; Atlas et al. 2007) and postmortem brain tissue (Baran et al. 2000), compared to controls. Furthermore, HIV-infected individuals with more psychopathological symptoms (i.e., depression) also have higher KP activity in plasma

(Schroecksnadel et al. 2008; Martinez et al. 2014). Table 1 conceptually summarizes the relationships depression and HIV have on metabolite and enzyme activity in the KP.

In the current study, I examined relationships between psychopathological symptoms and putative indicators of kynurenine pathway activity in post-HAART HIV seropositive participants (HIV), and HIV seronegative participants (SN) by analyzing serostatus, KP enzyme genotypes, CSF KYNA, and measures of psychopathological symptoms, in HIV and SN participants grouped by genetic variation in KP enzyme genes. Two functional genetic variants, *KMO*-rs1053230 and *KATII*-rs1480544, located in genes encoding for enzymes of the neurotoxic and neuroprotective KP branches and that can affect CSF [KYNA] levels were examined.

KMO rs1053230 variations has a small but growing body of research reflecting conflicting evidence in regards to the C allele or CC homozygous status as a marker for risk for psychopathological symptoms. The *KMO* C-allele showed a borderline association with presence of a current depressive episode in patients with Major Depression (Claes et al. 2011), with increased psychotic features in bipolar I patients (Lavebratt et al. 2014), and within schizophrenics, CC homozygotes had poorer global cognitive scores compared to T carriers (Wonodi et al. 2014). *KMO* C-carriers also had lower CSF KYNA than TT homozygotes in schizophrenics and controls (Holtze et al. 2012), which as described earlier, may be a risk marker for psychopathological symptoms (Myint et al. 2007; Maes et al. 2012).
Evidence for a protective effect of the C allele or CC homozygosity includes elevated CSF KYNA in bipolar I (Johansson et al. 2013; Lavebratt et al. 2014) and schizophrenia patients (Johansson et al. 2013), an increased association with control status, compared to bipolar and schizophrenia patients (Johansson et al. 2013), and decreased *KMO* expression in lymphoblasto cell lines and hippocampal biopsies (Lavebratt et al. 2014).

KATII rs148054 variation findings also reflect a small but growing body of literature showing conflicting findings supporting possible protective effect from psychopathological symptoms. In one study, suspected BM patients that were CC homozygotes had lower CSF KYNA than CT,

who in turn had lower CSF KYNA than T-carriers. (Coutinho et al. 2014), suggesting C-carriers would have the least neuroprotection from KYNA (Davies et al. 2010; Han et al. 2010; Schwarcz et al. 2012). In another study, *KATII* C-carriers had higher inflammatory cytokines compared to TT homozygotes (de Souza et al. 2011), which again upregulates KP activity (Oxenkrug 2007; Maes 2011) but can downregulate *KATII* activity (Alberati-Giani et al. 1996; Connor et al. 2008; Zunszain et al. 2012; Molteni et al. 2013), again leading to less neuroprotective KYNA. The same study, however, found that the *KATII* C-carriers were more likely to be controls, compared to bacterial meningitis patients, suggesting a protective link to this allele.

AIMS AND HYPOTHESES

Specific Aim 1: Determine if the genetic variation in kynurenine 3-monooxygenase (*KMO*; rs1053230) is associated with the levels of depressive symptoms in both seronegative controls and HIV patients.

Hypothesis 1A: Based on prior studies that showed the C allele or CC homozygosity was associated with psychotic features in Bipolar I patients (Lavebratt et al. 2014), a borderline association with Major depressive episodes (Claes et al. 2011), and poorer global cognition (Wonodi et al. 2014) I expected that all participants homozygous for the C allele will have more depressive symptoms compared to those who are T-carriers**.**

Hypothesis 1B: Since *KMO* gene is up-regulated by neuroinflammation (Alberati-Giani et al. 1996; Mellor et al. 1999; Connor et al. 2008; Zunszain et al. 2012; Molteni et al. 2013), and HIV subjects have more inflammation (McArthur et al. 2005; Brabers et al. 2006), I expected that HIV positive participants homozygous for the C allele will have even more depressive symptoms than HIV participants with the T allele and subjects who are HIV negative.

Specific Aim 2: Determine if the genetic variation in kynurenine aminotransferase II (*KAT II*; rs1480544) is associated with the levels of depressive symptoms in both seronegative controls and HIV patients.

Hypothesis 2A: Since the *KATII* C-allele was found to be more frequent in controls, compared to patients with bacterial meningitis (de Souza et al. 2011), I expected that in healthy individuals, the C-allele would have less psychopathological symptoms than those without the C allele.

Hypothesis 2B: Since *KATII* C-carriers had elevated inflammatory cytokines in the CSF, but this was shown only in bacterial meningitis patients (de Souza et al. 2011), and since elevated neuroinflammation is found in HIV patients (Hong et al. 2015; Rappaport et al.

2015) and in individuals with depression (Campbell et al. 2014), I also expected that in HIV participants, the C-allele would not be protective.

Specific Aim 3: Determine if CSF kynurenic acid (KYNA) level is a biomarker for depressive symptoms in HIV subjects (Table1).

Hypothesis 3A: As would be expected from prior literature (Heyes et al. 1992; Atlas et al. 2007), I expect higher CSF KYNA level in HIV positive participants compared to HIV negative.

Hypothesis 3B: Furthermore, since lower levels of peripheral KYNA have been associated with major depression (Myint et al. 2007; Maes et al. 2012), I expected that HIV participants with greater depressive symptoms will have lower CSF KYNA, compared to HIV participants with less depressive symptoms.

MATERIALS AND METHODS

Participants.144 participants (72 seronegative controls [SN] and 72 seropositive participants [HIV]) were evaluated. The investigation had approval from the joint UH Cooperative Institutional Review Boards for the University of Hawaii and the Queen's Medical Center. The inclusion criteria were: 1) Men or women of any ethnicity, >18 years and able to provide informed consent;2) Confirmed HIV-serostatus with documentation from medical records for HIV+, or a negative Clearview HIV test for the SN participants; 3) HIV participants were stable on an antiretroviral regimen for >6 months and have a nadir CD4 count <500/mm3; 4) All participants provided blood sample and agreed to the genetic analyses. 5) A subgroup (n=100) also consented to and underwent lumbar punctures for cerebrospinal fluid (CSF) sample.

The exclusion criteria for the current study were: 1) History of co-morbid psychiatric illness not related to the HIV-infection. 2) Significantly abnormal screening laboratory tests (>2 SD) that might indicate a chronic medical condition (e.g. diabetes, severe cardiac, renal or liver disorders) that might influence the outcome measures. 3) History of moderate to severe substance use disorders, except for tobacco and cannabis use within 2 years of the study or a positive urine toxicology for methamphetamines, cocaine, and opiates. 4) An estimated intelligence quotient < 80, verified by the Wechsler Test of Adult Reading.

Psychopathology measures.

Depressive symptoms screening scale - All participants completed the Center for Epidemiologic Studies Depression Scale (CES-D), a 20-item questionnaire that assessed how often symptoms associated with depression were experienced by the participant during the week prior to the evaluation. Item responses range from 0 (Rarely or None of the Time) to 3 (Most or Almost all the Time). A score $\geq$16 was used to identify those at risk for clinical depression (Radloff 1977; Lewinsohn et al. 1997).

Psychopathology symptoms - The Symptom Checklist-90-Revised (SCL-90-R) is a 90-item test that evaluates how often a participant felt distressed by a range of symptoms within the past week (Pearson Education). Responses range from 0 to 4 (0=Not at all, 1=A little bit, 2=Moderately, 3=Quite a bit, 4=Extremely). These questions are grouped into nine subscales,with T score conversion, for the following dimensions: Depression (DEP), Anxiety (ANX), Somatization (SOM), Obsessive Compulsiveness (O-C), Hostility (HOS), Interpersonal Senstivity (I-S), Phobic Anxiety (PHB), Paranoid Ideation (PAR), and Psychoticism (PSY). These subscales are also summarized into three global indices: General Symptom Index (GSI) measures overall psychological distress, and is calculated by the mean of all subscale scores (Derogatis 1994); Positive Symptom Total (PST) provides the number of self- reported symptoms; Positive Symptom Distress Index (PSDI) measures symptom intensity (Pearson Education). A T-score of 63 or higher on the GSI or on two of the dimensional subscales indicate possible clinical significance (Derogatis 1994).

Genotyping. Genomic DNA was extracted from whole blood samples using DNeasy Blood and Tissue kits (catalog number 69506, Qiagen Inc, Valencia, CA) for 139 (70 SN and 69 HIV) participants. Custom TaqMan® SNP Genotyping Assays for the *KATII* SNP rs1480544 and *KMO* SNPrs1053230 (catalog# 4351379 and #4324018) and TaqMan® Universal PCR Master Mix, no AmpErase® UNG (catalog #4351379 and #4324018, Life Technologies, Grand Island, NY) for Polymerase Chain Reaction (PCR) Endpoint genotyping were used. Replication and quality control filters (i.e., call rates >95%) were used. For the *KMO*-rs1053230, there were 106 CC, 28 TC, and 5 TT participants. In subsequent analyses, I combined the TC group with the TT group (*KMO* T-carrier) due to the rarity of the allele. For *KATII*-rs1480544, there were 20 TT, 65 TC, and 54 CC participants. In subsequent analyses, I combined the heterozygotic group (TC) with the homozygotic CC-carriers (*KATII* C-carriers), based on previous literature showing associations with C allele status differed from TT homozygotes (de Souza et al. 2011; Coutinho

et al. 2014). Genotype frequencies for the two examined variants did not differ from Hardy-Weinberg equilibrium (*KATII*-rs1480544: χ^2=0.004,p =0.95;*KMO*-rs1053230: χ^2=2.98, p=0.08).

Kynurenic Acid (KYNA) Concentration Measurement. CSF KYNA concentration was determined for 100 (51 SN and 49 HIV) participants by high performance liquid chromatography (HPLC, Surveyor, Thermo Electron) coupled with a fluorescence detector (Jasco FP-1520, Easton MD). In brief, 100 ul of CSF sample was treated with 10 ul of perchloric acid (3M), the mixture was vortexed vigorously and then centrifuged at 13,500 g for 5 min. The supernatant was transferred to HPLC inserts for analysis. Twenty-five ul of the supernatant was injected onto an Ascentis C18 column (150 x 3 mm, 2.7 um, Supelco, St. Louis MO) with a pre-column filter (0.2 um, Therm Electron). The analyte was eluted out using a mobile phase containing 250 mM of zinc acetate, 50 mM sodium acetate with 7% acetonitrile at a flow rate of 300 ul/min. Fluorescence was detected using an excitation wavelength of 344 nm and an emission wavelength of 398 nm. The gain value was set at 100 and the attenuation value at 16. Data were acquired and processed using an ss420 data converter from analog to digital signal and Chromoquest software. The limit of detection was 50 pg/ml.

Statistical Analysis. Statistical analyses were performed using SAS Enterprise Guide 7.1 software. Mann-Whitney-U, Chi Square, or Fisher's Exact tests were used to compare subject characteristics between groups. CSF [KYNA] was log transformed due to the skewness of the data distribution. Age was included as a covariate in all statistical models with CSF KYNA and mood questionnaires, since older age correlates with higher CSF KYNA (Kepplinger et al. 2005; Oxenkrug 2013). Pearson's correlations were used to examine the associations between age, CSF [KYNA] levels, CES-D scores, and SCL-90-R subscales. To analyze relationships between CES-D and CSF log[KYNA] for the 100 subjects with CSF [KYNA] measurements, SN and HIV participants were grouped according the risk cutoff score of 16 resulting in four groups: "SN CES-D<16" (n=43), "SN CES-D$\geq$16" (n=8), "HIV CES-D<16" (n=24), and "HIV CES-D<16"

(n=25). Logistic Regression was used to predict CES-D group membership with CSF log [KYNA] and serostatus as predictor variables. Two-way analyses of covariance (ANCOVA) with serostatus and genotype (*KAT II* group or *KMO* group) as factors and age as a covariate was used to find effects on mood questionnaires and CSF log [KYNA]. Two way ANCOVA with serostatus and log [KYNA] as predictor variables were also used to find association with mood questionnaires. One-way ANCOVA was used to examine the effects of serostatus on log[KYNA], and genotype main effects on mood questionnaires when no two-way interaction was found. All values are presented as means ± standard error unless otherwise stated.

RESULTS

Participant Characteristics (Table 2)

Across all participants, as well as the subgroup with CSF KYNA measurements, SN and HIV participants had similar age, sex proportion, education, race distribution, and Hollingshead Index of Social Position score (ISP), but a trend for differences between the three study groups was found for genotype distributions of the *KMO*-rs1053230 (*KMO)* and *KATII*-rs1480544 (*KATII)* variations (Table 2). As previously described (Appleman et al. 1988; Manji et al. 2004), HIV individuals had non-significantly higher total CSF protein levels but similar glucose concentrations compared to SN participants. Most HIV participants in this cohort were on HAART therapy and had undetectable viral loads. Although HIV participants were not clinically depressed relative to SN participants, they had higher Center for Epidemiologic Studies Depression scale (CES-D) scores (+62%; Figure 2A), and were 7.5 times more likely than SN participants to have CES-D scores $\geq$ 16 ($p=0.02$; Figure 2B). They also had higher scores on all Symptom Checklist-90-R (SCL-90-R) dimensional subscales (repeated measures ANCOVA=$p<0.0001$), confirmed by post-hoc analyses (Figure 2C), and higher scores on the three SCL-90-R summary scales (Figure 2C, box). Across all SCL-90-R subscales, compared to SN, HIV subjects showed largest group differences, with higher anxiety (ANX, +21%) and depression (DEP, +15%) T-scores, and smallest group difference in Phobic Anxiety (PHB, +9%) T-scores.

Genetic variations in the KP-related genes and psychological distress.

To evaluate the role of KP activity on psychological distress symptoms, I examined the associations of *KMO* and *KATII* genetic variations on CES-D and SCL-90-R scores.

KMO-by-Serostatus Group Differences- SN and HIV participants grouped by different *KMO* genotypes showed group differences on SCL-90-R T-scores for all dimensional and summary scales, even after Bonferroni-Holm correction (Figure 3A; Table 3). These group

differences were due to the HIV-serostatus (two-way ANCOVA-p=0.01 to p<0.0001). Post-hoc analysis showed that SN CC-homozygotes had fewer symptoms, with significantly lower SCL-90-R T-scores, than both HIV groups (CC-homozygotes and T-carriers) on all scales (Figure 3A; Table 4). Furthermore, SN T-carriers had lower T-scores than HIV CC participants for HOS and ANX subscales, as well as lower T-scores than HIV T-carriers for PAR, HOS, I-S, ANX, and GSI. Finally, HIV CC subjects also had lower PAR T-scores than HIV T-carriers.

KATII-by-Serostatus Group Differences - Significant group differences based on *KATII* genotypes and serostatus were also found on SCL-90-R scores across all dimensional and summary scales, even after Bonferroni-Holm correction (Figure 3B; Table 3). Post-hoc comparisons showed that amongst the C-carriers, SN had lower T-scores than HIV subjects on all measures (Figure 3B; Table 5). Furthermore, across the SN subjects, C-carriers had lower scores than TT participants for I-S, ANX, PSY, PST, and GSI. Finally, SN C-carriers had lower scores than HIV TT participants on DEP, SOM, HOS, ANX, PSY, PST and GSI.
Overall, I found trends for serostatus-by-*KATII* interactions on PAR, O-C, I-S, and ANX, as well as the summary (PST, and GSI) T-scores (Figures 4 A-F). *Post hoc* analysis showed the distinct pattern of fewer symptoms, with lower T-scores, in the SN *KATII* C-carriers compared to the other groups (Figures 4C-F),

CSF KYNA Levels in Relation to Serostatus, Genotype and psychological distress.

Log CSF [KYNA] levels were non-significantly higher in HIV individuals than SN participants (6.30±0.6 KYNA [pg/ml] vs. 5.57±0.5 KYNA [pg/ml], p=0.17). Additionally, Log CSF [KYNA] did not differ between genotype groups for each KP-related genes' variation for *KMO* (CC N=70, 5.83±0.41 pg/ml; T-carriers N=25, 6.16±0.95pg/ml, p=0.9) and *KATII* (TT N=18, 5.59±0.6pg/ml, C-carriers N=77, 5.99±0.46, p=0.9). I found no serostatus-by-*KMO* nor serostatus-by-*KATII*

interactions on CSF log[KYNA], which may be due to the small sample size in the *KATII* TT group (with only 7 SN and 13 HIV subjects).

I further evaluated whether CSF [KYNA] predicted psychological distress. I found no correlations between CSF log[KYNA] and any psychological distress scores, and no serostatus-by-CSF log[KYNA] interactions on CES-D scores and SCL-90-R subscales (interaction p-values ranged: 0.19 to 0.99, Table 6).

***Age, genetic variations and CSF* [*KYNA*].**

Consistent with earlier studies (Kepplinger et al. 2005; Oxenkrug 2013), CSF log[KYNA] showed age-dependent increases across all subjects (Figure 5A). However, across all *KMO* genotype groups, only CC-homozygotes showed significant age-dependent increases in log CSF [KYNA], independent of serostatus (Figure 5B). Similarly, across all *KATII* genotype groups, only the C-carriers had higher log CSF [KYNA] with older age, independent of serostatus (Figure 5C). No age-by-serostatus interactions, nor age-by-serostatus-by-genotype interactions, were found on log CSF [KYNA].

DISCUSSION

To my knowledge, this is the first study to investigate the effects of *KMO* and *KATII* genotypes, which encode two of the major KP enzymes, on psychopathological symptoms in HIV positive compared to SN individuals. The major findings are that: 1) For the *KMO* rs1053230 variants, genotype did not appear to be associated with psychopathological scores among SN and HIV participants; higher scores amongst HIV participants compared to SN participants accounted for group differences. This suggests that *KMO* variantion on psychopathological symptoms is not sufficient to mediate HIV-associated psychological stress. 2) Independent of HIV-serostatus, CSF [KYNA] from *KMO* CC-homozygotes showed age-dependent increases, which may result from less KMO enzyme older age, thus leaving more KYN to be catabolized into KYNA (Figure 1; Schwarcz 2004; Guillemin et al. 2007; Ting et al. 2007), specifically in *KMO* CC-homozygotes compared to *KMO* T-carriers. 3) For the *KATII* rs1480544 variants, HIV C-carriers had similar levels of psychological scores compared to HIV TT homozygotes, while SN C-carriers had lower scores for psychological distress than SN TT homozygotes, suggesting that C-carrier status protection from psychological stress occurs only in SN participants, and not for HIV participants. 4) Lastly, *KATII* C-carriers, regardless of HIV-serostatus, also showed an age-dependent increase in CSF [KYNA] which may be may due to greater *KATII* activity with older age only in the C-carriers, and therefore greater conversion of KYN to KYNA (Figure 1; Schwarcz 2004; Guillemin et al. 2007; Ting et al. 2007). Together, these findings indicate that both *KMO* CC and *KATII* C-carrier variants may prevent psychopathological symptoms in healthy individuals; however, HIV infection and its associated neuroinflammation may alter the expression of, or partly obliterate the protective effects. Additionally, our findings showed that aging may modulate enzyme production resulting from *KMO* and *KATII* genetic variations.

Consistent with previous studies (Jin et al. 2010; Rezaei et al. 2013), HIV participants had more psychopathological symptoms than SN controls overall. SN *KMO* CC-homozygotes had

less distress than HIV participants of either genotype, but had similar levels of psychological symptoms compared to SN T-carriers. This suggests that *KMO* genotype does not affect levels of psychological distress, and confirms that differences are due to HIV serostatus. This finding is consistent with the work of Claes (2011), who found the *KMO* C-allele had only a borderline association with current depressive episode in patients with Major Depression and Bipolar Disorder. It is also interesting to note that the *KMO* C-allele was found to be associated with not having a diagnosis of bipolar I in one study (Johansson et al. 2013), but with psychotic features in bipolar I patients in another study (Lavebratt et al. 2014), supporting that the association of *KMO* variations at rs1053230 with psychiatric symptoms is not direct. With the exception of Paranoid Ideation, *KMO* genotype groups among HIV participants had similar scores. This suggests *KMO* TT homozygous status may be protective for paranoid symptoms in HIV participants. However, for all other measures, any *KMO* genetic influences were insufficient to overcome the HIV-mediated psychological stress, possibly due to overwhelming enhanced and ongoing HIV-associated neuroinflammation (Hong et al. 2015; Rappaport et al. 2015), which also has been linked to psychopathological symptoms (Pavon et al. 2006).

At the molecular level, I showed for the first time that independent of HIV serostatus, *KMO* rs1053230 CC-homozygotes, but not T-carriers, showed age-dependent increases in CSF [KYNA]. Kepplinger (2005) found age-dependent increases in CSF [KYNA] in patients with acute headaches, but *KMO* genotype was not examined in this study. Lavebratt (2014), also found that CSF [KYNA] increase with age differently depending on the *KMO* genotype in bipolar I patients. However, their analyses did not include healthy controls. Furthermore, lower numbers of *KMO* C-alleles (i.e., CC to CT to TT) was previously associated with greater expression of *KMO* mRNA in lymphoblastoid cell lines and lower CSF [KYNA] levels from bipolar I disorder patients, in a dose dependent manner (Lavebratt et al. 2014). Another study also found that *KMO* genotype variations at this same allele did not lead to a difference in response to cytokine stimulus in fibroblasts from bipolar I or schizophrenia patients (Johansson

et al. 2013), but this was an ex vivo experiment and may not represent in vivo conditions such as aging. Thus, higher levels of CSF [KYNA] with older age in the HIV and SN *KMO* CC homozygotes may be a consequence of less *KMO* mRNA expression with aging, and hence less *KMO* to act on KYN, leaving more KYN for conversion to KYNA (Figure 1; Schwarcz 2004; Guillemin et al. 2007; Ting et al. 2007).

Examination of the rs1480544 *KATII* variants showed that amongst all genotype groups, SN C-carriers had the lowest scores for psychopathological symptoms for all measures, suggesting that the C-allele is protective against psychological symptoms in healthy controls. However, this C-allele associated protection was not always found in HIV, most likely due to the influence of HIV-mediated neuroinflammation (Hong et al. 2015; Rappaport et al. 2015) on psychological stress. CSF inflammatory cytokines of TNF-α, IL-1β, IL-6, MIP-1a/CCL3 and MIP-1b/CCL4, were reported to be higher in *KATII* C-carriers than in TT-homozygote patients with meningitis (de Souza et al. 2011). Exposure to HIV infection is associated with similar elevation of neuroinflammatory cytokines TNF-α (Chen et al. 1997; Kumar et al. 2013), IL-1β (Nath et al. 1999; Guo et al. 2014), IL-6 (Nath et al. 1999), CCL3/MIP-1α (Canque et al. 1996; Si et al. 2002; Kamat et al. 2012), and MIP-1b/CCL4 (Si et al. 2002; Carrol et al. 2007), suggesting *KATII* C-carrier status may exacerbate the effects of HIV infection. Furthermore, depressed patients had at least modest serum elevations of TNF-α (Mikova et al. 2001), IL-1β (Song et al. 2009), IL-6 (Maes et al. 1995), and CCL3/MIP-1α (Merendino et al. 2004), supporting that depression in C-carrier status may also be associated with depression. While SN participants with presumably normal physiological levels of neuroinflammation may be able to benefit from *KATII* C-carrier status, HIV patients carrying the *KATII* C-alleles may have even higher neuroinflammation that render them more vulnerable to psychopathological symptoms. Thus, my initial hypothesis that *KATII* C-carriers would have more psychopathological symptoms than TT homozygotes is contradictory to my findings for SN participants, but may be consistent with my findings regarding the HIV participants, possibly due to the neuroinflammation. However,

since past studies show that some cytokine elevations associated with *KATII* genotype (de Souza et al. 2011) and HIV (Si et al. 2002) differ from elevations associated with depressive symptoms (Lehto et al. 2010; Lindqvist et al. 2013), further research regarding the mediating role of neuroinflammation is warranted.

Similar to the *KMO* variants, I found that only *KATII* rs1480544 C-carriers, and not TT homozygotes, showed age-dependent increases in CSF [KYNA], independent of HIV serostatus. *KATII* rs1480544 is posited to be located in an exonic splicing silencer (Kralovicova et al. 2007), which might increase the mRNA expression and protein production of *KATII* (Coutinho et al. 2014). Greater *KATII* mRNA expression and/or KATII production could then lead to greater conversion of KYN to KYNA in the CSF of the participants. However, the definite functionality of the *KATII* gene at rs1480544 has not been determined.

Limitations - Although the HIV subjects had higher than normal levels of psychopathological symptoms scores, none of these HIV subjects had active clinical diagnoses of psychological disorders, and the relatively small and racially diverse sample may be insufficient to evaluate for group differences due to genetic associations. A larger sample size may have resulted in clearer differentiation between the CC and T-carrier *KMO* genotypes within SN and HIV sub-cohorts. Furthermore, I only investigated one kynurenine pathway catabolite, KYNA, in relationship to the KP enzymes KATII and KMO. A future study including a more systematic dissection of the kynurenine pathway, such as metabolite ratios to indicate enzyme activity, may further elucidate the mechanisms underlying the psychopathological phenotypes. Additionally, despite their higher scores for many psychological domains, the HIV subjects did not show abnormal levels of CSF KYNA, which suggests that the KP activation accompanying HIV infection may not be the same type of neuroinflammation linked to psychopathological symptoms in HIV patients. Since neuroinflammation is linked to both HIV-associated neurocognitive disorders (HAND) (For review, Glass et al. 1995; Chang et al. 2013; Yuan et al. 2013) and to HIV-associated depressive symptoms (Ciesla et al. 2001; Robertson et al. 2014),

future studies could evaluate the possible mediating relationships between inflammatory cytokines, CSF KYNA and other metabolites in the KP on psychological symptoms and cognitive disorders on of HIV individuals.

Conclusions - This study is the first to show that genotype variation at *KMO*-rs1053230 may not associate with psychological symptoms in SN and HIV groups, that C-carrier status at *KATII*-rs1480544 may confer protection from psychological stress, and *KMO* C-carriers and *KATII* CC homozygotes have an HIV status-independent, age-dependent increase in CSF [KYNA]. Although both HIV (Fuchs et al. 1990; Heyes et al. 1992) and depression (Myint et al. 2007), for example, are associated with a net increase in KP activity, a recent study found that differences between patients with major depression compared to healthy controls appeared not in overall activation of the KP (KYN/TRP), but in a reduced KYNA/QUIN ratio in the serum (Savitz et al. 2015). Therefore, KMO and KATII enzymes that mediate these ratios may be possible targets for intervention. For example, LPS (Connor et al. 2008; Molteni et al. 2013), interferon-gamma (Alberati-Giani et al. 1996), and interleukin 1-beta (Zunszain et al. 2012) induce expression or production for KMO, but not KATII, and antidepressant use was associated with reductions in IL 1-beta (Hannestad et al. 2011). Thus, antidepressants that reduce IL 1-beta may reduce KMO production, and may be especially suitable for treatment of depression in HIV patients, especially in younger *KMO* CC homozygotes. On the neuroprotective branch of the KP, KATII activity can be at least partially inhibited by aminoadipate, asparagine, glutamate, histidine, cysteine, lysine, 3-HK, and phenylalanine (Han et al. 2008). Inhibition of KATII would result in lower neuroprotective KYNA concentrations, but such activity may be combined with novel pharmaceutical interventions such as KYNA analogs already investigated for the treatment of migraine, neurodegeneration, and seizures to ameliorate depressive symptoms associated with HIV. Thus, further investigation of these processes may lead to novel pharmaceutical targets to that are especially relevant not only in treating HIV populations, but possibly in treating psychopathological symptoms comorbid with

other illnesses associated with heightened inflammatory and neuroinflammatory activity, and upregulation of the KP

APPENDIX A: TABLES

Table 1. Kynurenic Pathway, depression, and HIV

Summary of kynurenine, KMO, KATII, Neurotoxicity, and CSF KYNA relationships for depression and HIV.

	Kynurenine	KMO	KATII	Neurotoxicity	CSF KYNA
Depression	↑	↑	↓	↑	↓
HIV (Inflammation)	↑↑	↑↑	↓	↑↑	↑
HIV with depression	↑↑	↑↑↑?	↓?	↑↑↑?	↓?

Table 2. Demographic and clinical characteristics of the participants

Presented are means and standard errors of demographic data for participants overall, and for the sub-cohort of participants with CSF [KYNA] measurements. [a]Mann-Whitney, ChiSq, or Fisher's exact p value. [b]Higher scores indicate lower social position. [c]Distribution of the alleles in the entire cohort fulfilled the criteria for Hardy-Weinberg equilibrium for *KATII* SNP rs1480544 (p= 0.95), and the *KMO* SNP rs1053230 (p=0.08).

	All participants (n=144)			Participants w/CSF KYNA (n=100)		
	SN n=72	HIV n=72	p-value[a]	SN n=51	HIV n=49	p-value[a]
Age (years)	47.4±1.7	50.3±1.3	0.21	46.3±2.04	50.8±1.7	0.11
Sex (M%)	88%	96%	0.07	88%	96%	0.27
Education (years)	14.5±0.3	14.7±0.3	0.30	14.6±0.5	14.7±0.4	0.44
Index of Social Position[b] (range: 14-77)	40.4±2.0	39.3±2.0	0.52	41.2±2.6	37.5±2.3	0.17
Race						
American Indian/Alaska Native	1(1%)	0(0%)		1(2%)	0(0%)	
Asian	18(25%)	11(15%)		11(22%)	8(16%)	
Black or African American	1(1%)	7(10%)	0.14	1(2%)	4(8%)	0.38
More Than One Race	9(13%)	11(15%)		4(8%)	8(16%)	
Native Hawaiian/Pacific Islander	5(7%)	3(4%)		4(8%)	2(4%)	
White	38(53%)	40(56%)		30(58%)	27(56%)	
% on HAART	-	87.5%	-	-	87.8%	-
HIV duration (months)	-	160.8±11.3	-	-	154.2±13.2	-
CD4 (#/mm^3)	-	502.1±29.2	-	-	481.7±34.3	-
Nadir CD4 (#/mm^3)	-	182.3±17.1	-	-	181.7±19.3	-
Detectable Viral Load (>75 copy/mL), %	-	19.4%	-	-	22.4%	-
Log Viral Load (log copy/mL)	-	3.1±0.24	-	-	3.0±0.25	-
CSF Protein mg/dL	41.9±2.0	45.9±2.1	0.14	40.5±1.9	45.8±2.5	0.17
CSF Glucose mg/dL	62.0±0.7	64.8±1.5	0.44	62.8±0.8	64.9±1.8	0.73
KATII rs1480544 C-carrier %[c]	90%	81%	0.14	88%	74%	0.09
KMO rs1053230 CC %[c]	80%	72%	0.30	82%	65%	0.07

Table 3. Psychological Distress Differences between groups, and genotype and serostatus effects

One-way and two-way ANCOVA p-values for all psychological distress scores for the CES-D and all SCL-90-R scales in two-by-two designs for each KP-related gene variation. Mean scores differ between participants for all four groups defined by serostatus and kynurenine 3-monooxygenase (*KMO*) genotype (SN *KMO* CC, n=56; SN *KMO* T-Carrier, n=14, HIV *KMO* CC, n=50; and HIV *KMO* T-Carrier, n=19) or serostatus and kynurenine aminotransferase II (*KATII*) genotype (SN *KATII* C-carrier, n=63; SN *KATII* TT, n=7; HIV *KATII* C-carrier, n=56; and HIV *KATII* TT, n=13). [a] Covaried with age. [b] Significant after Bonferroni-Holm correction.

	***KMO* ANCOVA p-values[a]**				***KATII* ANCOVA p-values[a]**			
	One-way across four groups (SN or HIV)	**Two-way ANCOVA, Serostatus x *KMO***			**One-way across four groups (SN or HIV)**	**Two-way ANCOVA, Serostatus x *KATII***		
	***KMO* T-carrier vs CC**	**HIV**	***KMO* T-carrier vs. CC**	**HIVx*KMO* (T-carrier vs. CC)**	***KATII* C-carrier vs. TT**	**HIV**	***KATII* C-carrier vs. TT**	**HIVx*KATII* (C-carrier vs. TT)**
CES-D Raw Score (0-60)								
	0.02[b]	0.009[b]	0.99	0.95	0.02[b]	0.07	0.89	0.73
SCL-90-R Dimensional Subscale T-Scores								
DEP	0.0006[b]	0.01[b]	0.27	0.21	0.0007[b]	0.14	0.24	0.19
PAR	0.002[b]	0.002[b]	0.06	0.31	0.004[b]	0.41	0.93	0.08
SOM	0.006[b]	0.01[b]	0.29	0.74	0.003[b]	0.20	0.13	0.27
O-C	0.005[b]	0.01[b]	0.17	0.94	0.001[b]	0.59	0.46	0.03
HOS	0.002[b]	0.001[b]	0.73	0.79	0.0007[b]	0.14	0.23	0.19
I-S	0.001[b]	0.001[b]	0.11	0.42	0.0003[b]	0.60	0.11	0.02
PHB	0.01[b]	0.005[b]	0.26	0.58	0.02[b]	0.07	0.59	0.84
ANX	<.0001[b]	<.0001[b]	0.09	0.50	<.0001[b]	0.09	0.02	0.03
PSY	0.02[b]	0.006[b]	0.73	0.78	0.003[b]	0.43	0.09	0.12
SCL-90-R Summary Scale T-Scores								
PSDI	0.01[b]	0.02[b]	0.78	0.58	0.01[b]	0.20	0.47	0.29
PST	<.0001[b]	0.0003[b]	0.30	0.65	<.0001[b]	0.09	0.14	0.07
GSI	<.0001[b]	0.0002[b]	0.21	0.77	<.0001[b]	0.07	0.13	0.08

Table 4: *Post-hoc* p-values for differences psychopathological symptoms between four *KMO* groups (SN *KMO*-CC, SN *KMO* T-carriers, HIV *KMO*-CC, and HIV *KMO*-T carriers)

Presented are *post-hoc* p-values for comparisons of CES-D scores and SCL-90-R dimensional subscale and summary T-scores between groups defined by serostatus and *KMO* genotype.

	SN *KMO* CC, SN *KMO* T-Carrier	SN *KMO* CC, HIV *KMO* CC	SN *KMO* CC, HIV *KMO* T-Carrier	SN *KMO* T-Carrier, HIV *KMO* CC	SN *KMO* T-Carrier, HIV *KMO* T-Carrier	HIV *KMO* CC, HIV *KMO* T-Carrier
CES-D	1.0	0.008	0.05	0.08	0.1	1.0
SCL-90-R Dimensional Subscale T-Scores						
DEP	0.1	<.0001	0.006	0.3	0.5	0.9
PAR	0.6	0.03	0.0003	0.4	0.03	0.04
SOM	0.4	0.002	0.006	0.3	0.2	0.6
O-C	0.4	0.004	0.002	0.3	0.1	0.3
HOS	1.0	0.001	0.009	0.04	0.05	0.8
I-S	0.6	0.007	0.0002	0.2	0.02	0.08
PHB	0.7	0.02	0.004	0.2	0.06	0.3
ANX	0.5	<.0001	<.0001	0.05	0.003	0.08
PSY	1.0	0.009	0.02	0.1	0.09	0.7
SCL-90-R Summary Scale T-Scores						
PSDI	0.6	0.002	0.05	0.2	0.3	0.8
PST	0.3	<.0001	0.0004	0.08	0.07	0.7
GSI	0.3	<.0001	0.0002	0.08	0.05	0.5

Table 5: *Post-hoc* p-values for differences psychological distress between four *KATII* groups (SN *KATII* TT, SN *KATII* C-carriers, HIV *KATII* TT, and HIV *KATII* C-carriers)

Presented are *post-hoc* p-values for comparisons of CES-D scores and SCL-90-R T-scores between groups defined by serostatus and *KATII* genotype. [a] Serostatus-by-*KATII* interaction significance or trend found, described in Supplemental Table 1 and Figure 4.

	SN *KATII* C-carrier, SN *KATII* TT	SN *KATII* C-carrier, HIV *KATII* C-carrier	SN *KATII* C-carrier, HIV *KATII* TT	SN *KATII* TT, HIV *KATII* C-carrier	SN *KATII* TT, HIV *KATII* TT	HIV *KATII* C-carrier, HIV *KATII* TT
CES-D	0.7	0.003	0.1	0.3	0.4	0.8
SCL-90-R Dimensional Subscale T-Scores						
DEP	0.1	<.0001	0.02	0.8	0.9	0.9
PAR[a]	0.3	0.0004	0.6	0.6	0.5	0.09
SOM	0.1	0.0008	0.01	0.9	0.9	0.7
O-C[a]	0.06	<.0001	0.3	0.9	0.4	0.2
HOS	0.1	<.0001	0.04	0.9	0.9	0.8
I-S[a]	0.01	<.0001	0.07	0.5	0.3	0.5
PHB	0.6	0.004	0.06	0.4	0.4	0.9
ANX[a]	0.005	<.0001	0.0005	0.7	0.8	0.9
PSY	0.04	0.0008	0.03	0.6	0.6	0.9
SCL-90-R Summary Scale T-Scores						
PSDI	0.3	0.001	0.1	0.8	1.0	0.7
PST[a]	0.04	<.0001	0.005	0.9	0.9	0.8
GSI[a]	0.04	<.0001	0.004	0.9	1.0	0.8

Table 6: Differences in psychological distress depending on serostatus and CSF log[KYNA]

CES-D and SCL-90-R subscale T-scores two-way ANCOVA p-values with serostatus and CSF log[KYNA] as factors while covarying for age.

	Log[KYNA]	Serostatus	Log[KYNA] x Serostatus
CES-D	0.07	0.58	0.25
SCL-90-R Dimentional Subscale T-score			
DEP T	0.16	0.09	0.84
PAR T	0.87	0.39	0.66
SOM T	0.56	0.12	0.95
O-C T	0.28	0.20	0.98
HOS T	0.73	0.27	0.19
I-S T	0.88	0.16	0.87
PHB T	0.81	0.39	0.31
ANX T	1.00	0.02	0.99
PSY T	0.50	0.22	0.75
SCL-90-R Summary Scale T-Scores			
PSDI T	0.35	0.38	0.40
PST T	0.41	0.04	0.92
GSI T	0.34	0.07	0.75

APPENDIX B: FIGURES

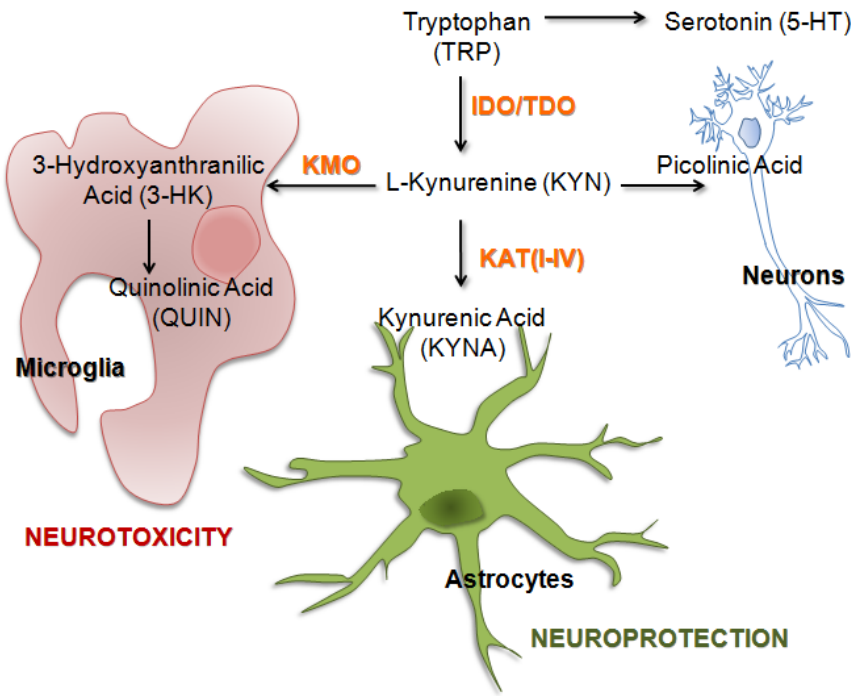

Figure 1: The Kynurenine Pathway Of Tryptophan Degradation.

Tryptophan, the precursor for serotonin, is catabolized into L-kynurenine by indoleamine 2,3-dioxygenase and tryptophan 2,3- dioxygenase. In the brain, L-kynurenine is mainly converted to 3-hydroxykynurenine by kynurenine 3-monooxygenase in microglial cells, to kynurenic acid by kynurenine aminoatransferase II in astrocytes, and to picolinic acid in neurons. 3-HK is subsequently converted to quinolinic acid.

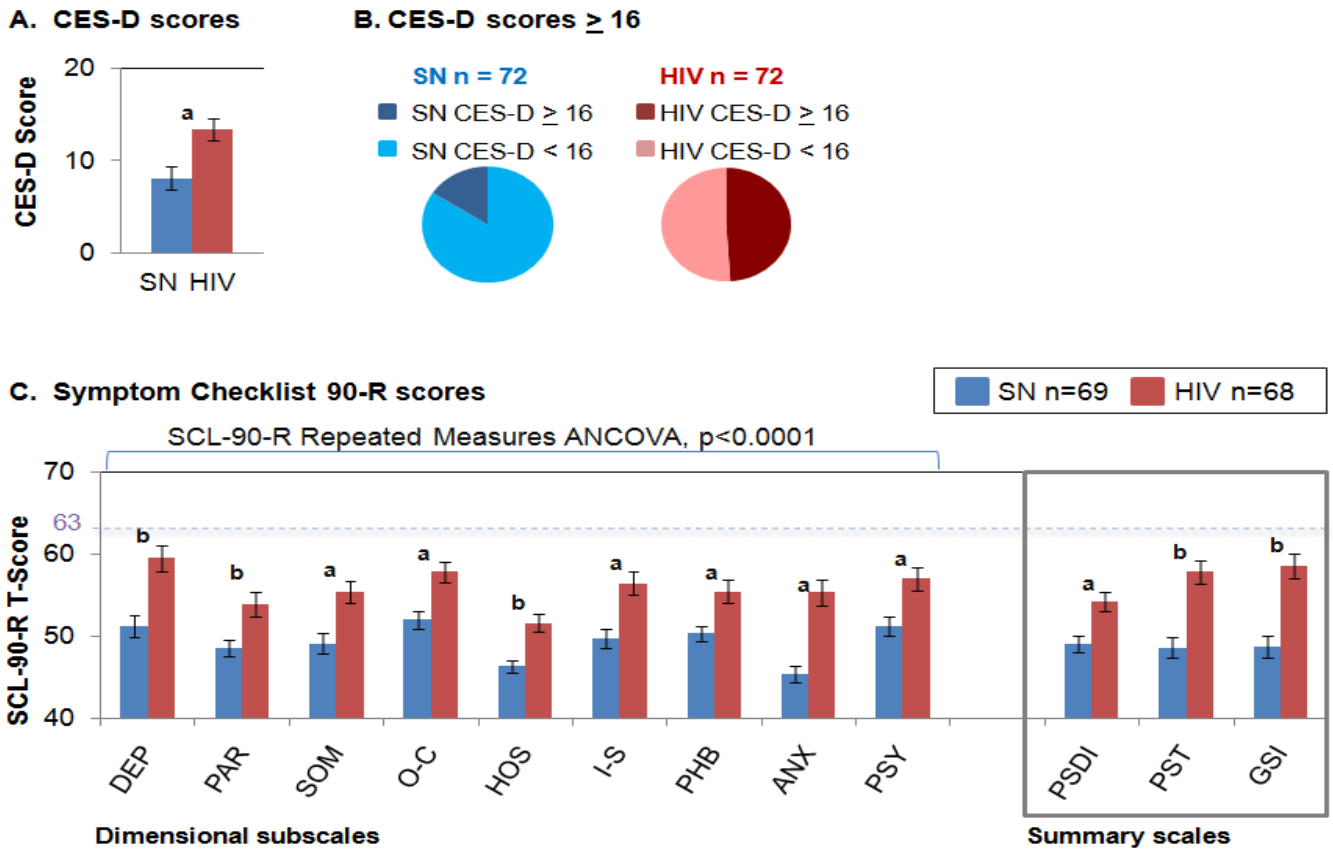

Figure 2: Psychological distress in HIV participants

SN and HIV participant means and standard errors for A) Center for Epidemiologic Studies Depression scale (CES-D), B) SN and HIV CES-D group membership and C) Symptom Checklist 90-R (SCL-90-R) dimensional T-scores. [a] One-way ANCOVA $p \leq 0.005$ [b] One-way ANCOVA $p \leq 0.0001$

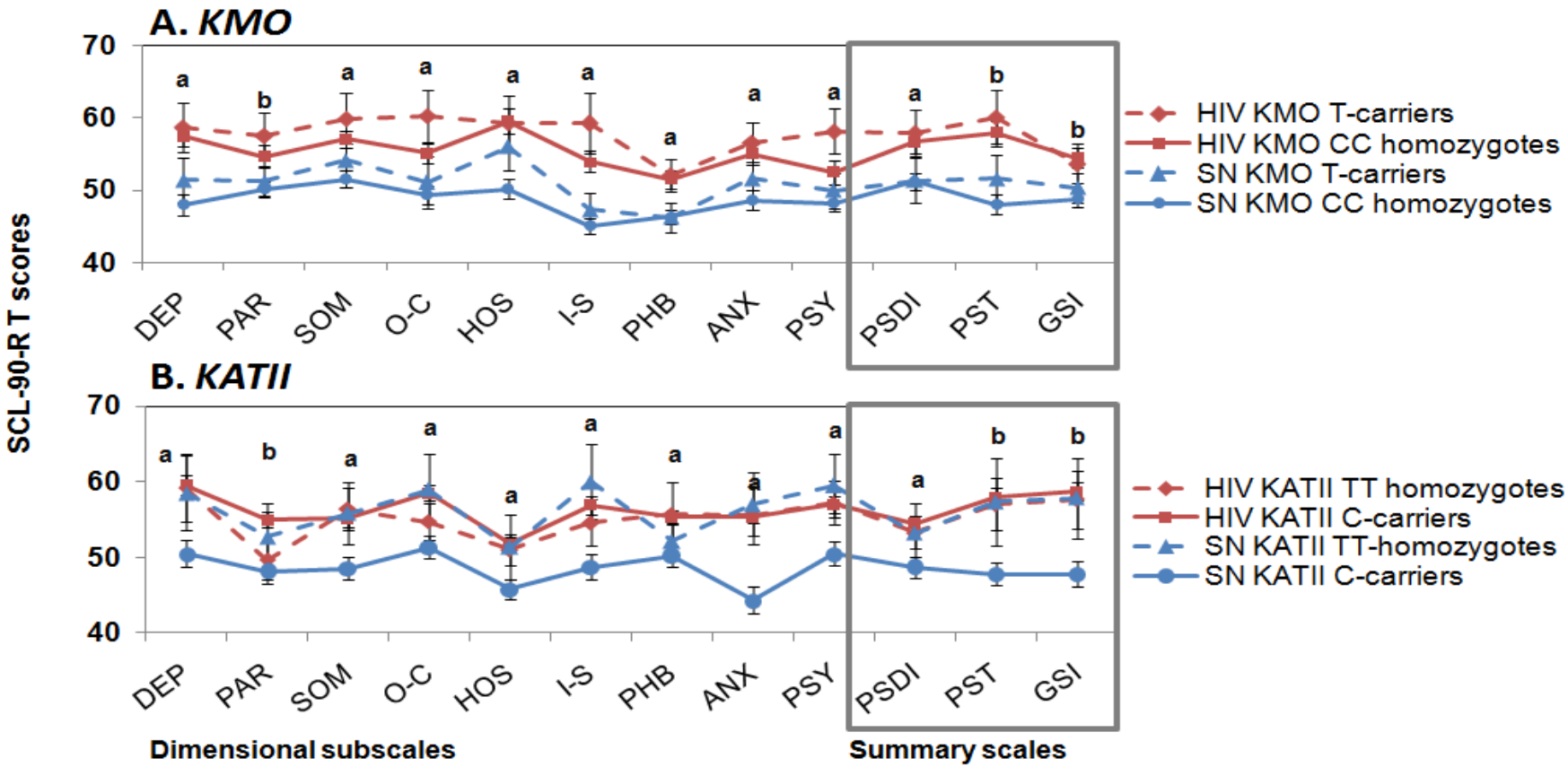

Figure 3: Genetic variations in KP-related genes on psychological distress

SN and HIV participants divided by *KMO* or *KATII* genotype have different levels of psychological distress for all Symptom Checklist 90-R (SCL-90-R) T-scores. [a] One-way ANCOVA across four groups $p \leq 0.05$. [b] One-way ANCOVA across four groups $p \leq 0.0001$.

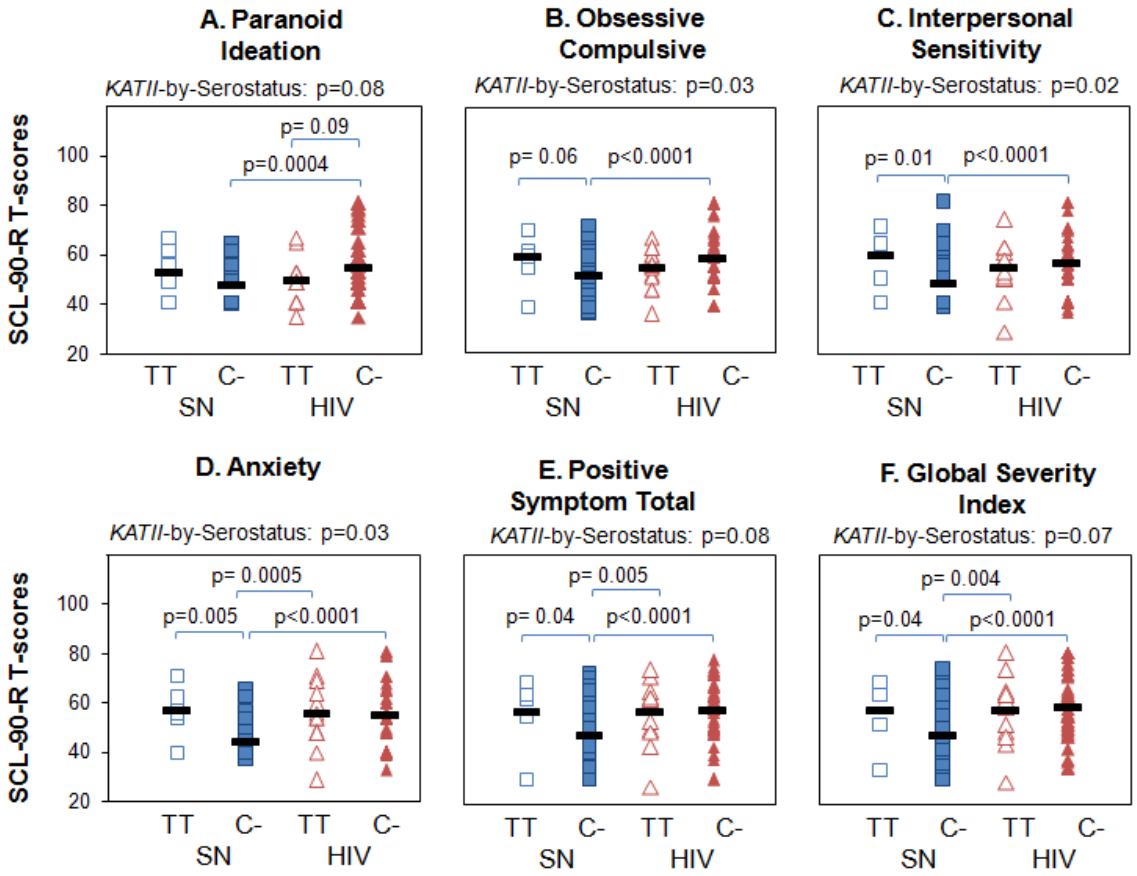

Figure 4: Serostatus-by-*KATII* interactions on SCL-90-R T-scores.

SCL-90-R: A) paranoid ideation (PAR), B) obsessive compulsiveness (O-C), C) interpersonal sensitivity (I-S), D) anxiety (ANX), E) positive symptom total (PST), F) global severity index (GSI) T-scores were evaluated with *KATII* variations (C-carriers [C-] or TT-homozygotes [TT]) and serostatus (SN or HIV) as factors.

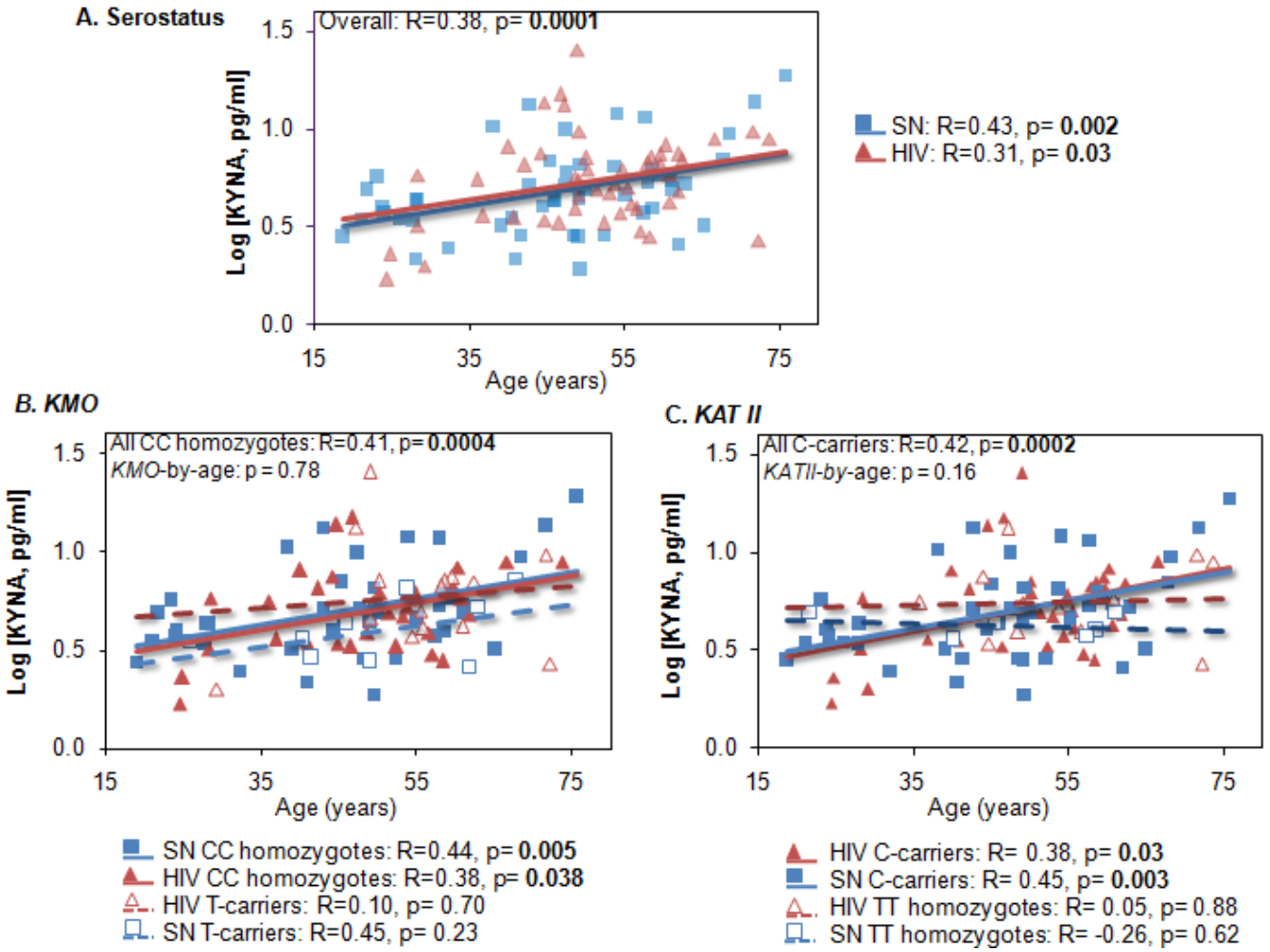
A. Serostatus
Overall: R=0.38, p= 0.0001
Log [KYNA, pg/ml]
Age (years)
SN: R=0.43, p= 0.002
HIV: R=0.31, p= 0.03
B. KMO
All CC homozygotes: R=0.41, p= 0.0004
KMO-by-age: p = 0.78
SN CC homozygotes: R=0.44, p= 0.005
HIV CC homozygotes: R=0.38, p= 0.038
HIV T-carriers: R=0.10, p= 0.70
SN T-carriers: R=0.45, p= 0.23
C. KAT II
All C-carriers: R=0.42, p= 0.0002
KATII-by-age: p = 0.16
HIV C-carriers: R= 0.38, p= 0.03
SN C-carriers: R= 0.45, p= 0.003
HIV TT homozygotes: R= 0.05, p= 0.88
SN TT homozygotes: R= -0.26, p= 0.62

Figure 5: CSF KYNA increases with age depending on genotype.

A) Correlations of CSF log[KYNA] with age in SN (n=51) and HIV (n=49) for all participants; no age-by-serostatus interaction was observed on CSF logKYNA (p=0.88). B) Independent of HIV serostatus, *KMO* CC-homozygotes (solid lines) had higher CSF log[KYNA] with older age, contrast to T-carriers (dashed lines), but no genotype-by-age interaction nor genotype-by-age-by-serostatus interaction was observed on CSF log[KYNA] (p=0.77). C) Similarly, older age in *KATII* C-carriers (solid lines) correlated with higher CSF log[KYNA], contrast to TT-homozygotes (dashed lines). No *KATII*-by-age-by-serostatus interaction was observed on CSF log[KYNA] (p=0.92).

BIBLIOGRAPHY

Alberati-Giani, D., P. Ricciardi-Castagnoli, et al. (1996). "Regulation of the kynurenine metabolic pathway by interferon-gamma in murine cloned macrophages and microglial cells." J Neurochem **66**(3): 996-1004.

Appleman, M. E., D. W. Marshall, et al. (1988). "Cerebrospinal fluid abnormalities in patients without AIDS who are seropositive for the human immunodeficiency virus." J Infect Dis **158**(1): 193-199.

Asch, S. M., A. M. Kilbourne, et al. (2003). "Underdiagnosis of depression in HIV: who are we missing?" J Gen Intern Med **18**(6): 450-460.

Atkinson, J. H. and I. Grant (1994). "Natural history of neuropsychiatric manifestations of HIV disease." Psychiatr Clin North Am **17**(1): 17-33.

Atlas, A., M. Gisslen, et al. (2007). "Acute psychotic symptoms in HIV-1 infected patients are associated with increased levels of kynurenic acid in cerebrospinal fluid." Brain Behav Immun **21**(1): 86-91.

Baran, H., J. A. Hainfellner, et al. (2012). "Kynurenic Acid Metabolism in Various Types of Brain Pathology in HIV-1 Infected Patients." Int J Tryptophan Res **5**: 49-64.

Baran, H., J. A. Hainfellner, et al. (2000). "Kynurenic acid metabolism in the brain of HIV-1 infected patients." J Neural Transm **107**(10): 1127-1138.

Baran, H., B. Kepplinger, et al. (2010). "Endogenous Kynurenine Aminotransferases Inhibitor is Proposed to Act as "Glia Depressing Factor" (GDF)." Int J Tryptophan Res **3**: 13-22.

Bing, E. G., M. A. Burnam, et al. (2001). "Psychiatric disorders and drug use among human immunodeficiency virus-infected adults in the United States." Arch Gen Psychiatry **58**(8): 721-728.

Brabers, N. A. and H. S. Nottet (2006). "Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia." Eur J Clin Invest **36**(7): 447-458.

Campbell, B. M., E. Charych, et al. (2014). "Kynurenines in CNS disease: regulation by inflammatory cytokines." Front Neurosci **8**: 12.

Canque, B., M. Rosenzwajg, et al. (1996). "Macrophage inflammatory protein-1alpha is induced by human immunodeficiency virus infection of monocyte-derived macrophages." Blood **87**(5): 2011-2019.

Carrol, E. D., L. A. Mankhambo, et al. (2007). "Chemokine responses are increased in HIV-infected Malawian children with invasive pneumococcal disease." J Acquir Immune Defic Syndr **44**(4): 443-450.

Centers for Disease Control and Prevention. (2014). "HIV in the United States: At A Glance." from http://www.cdc.gov/hiv/statistics/basics/ataglance.html (accessed August 13, 2014).

Chang, L., S. M. Munsaka, et al. (2013). "Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain." J Neuroimmune Pharmacol **8**(3): 576-593.

Chen, P., M. Mayne, et al. (1997). "The Tat protein of HIV-1 induces tumor necrosis factor-alpha production. Implications for HIV-1-associated neurological diseases." J Biol Chem **272**(36): 22385-22388.

Ciesla, J. A. and J. E. Roberts (2001). "Meta-analysis of the relationship between HIV infection and risk for depressive disorders." Am J Psychiatry **158**(5): 725-730.

Claes, S., A. M. Myint, et al. (2011). "The kynurenine pathway in major depression: haplotype analysis of three related functional candidate genes." Psychiatry Res **188**(3): 355-360.

Collingridge, G. L. and J. Davies (1979). "An evaluation of D-alpha-aminoadipate and D-(and DL-)alpha-aminosuberate as selective antagonists of excitatory amino acids in the substantia nigra and mesencephalic reticular formation of the rat." Neuropharmacology **18**(2): 193-199.

Connor, T. J., N. Starr, et al. (2008). "Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma?" Neurosci Lett **441**(1): 29-34.

Coutinho, L. G., S. Christen, et al. (2014). "The kynurenine pathway is involved in bacterial meningitis." J Neuroinflammation **11**: 169.

Davies, N. W., G. Guillemin, et al. (2010). "Tryptophan, Neurodegeneration and HIV-Associated Neurocognitive Disorder." Int J Tryptophan Res **3**: 121-140.

de Souza, F. R., F. L. Fontes, et al. (2011). "Association of kynurenine aminotransferase II gene C401T polymorphism with immune response in patients with meningitis." BMC Med Genet **12**: 51.

DeLorenze, G. N., D. D. Satre, et al. (2010). "Mortality after diagnosis of psychiatric disorders and co-occurring substance use disorders among HIV-infected patients." AIDS Patient Care STDS **24**(11): 705-712.

Derogatis, L. R. (1994). SCL-90-R Administration, Scoring, and Procedures Manual - Third Edition, NCS Pearson, Inc. .

Dew, M. A., J. T. Becker, et al. (1997). "Prevalence and predictors of depressive, anxiety and substance use disorders in HIV-infected and uninfected men: a longitudinal evaluation." Psychol Med **27**(2): 395-409.

Division of HIV/AIDS Prevention, N. C. f. H. A., Viral Hepatitis, Sexual Transmitted Diseases and Tuberculosis Prevention, Centers for Disease Control and Prevention. (2015). "HIV Mortality (through 2010)." Retrieved April 24, 2015, 2015, from http://www.cdc.gov/hiv/pdf/statistics_surveillance_hiv_mortality.pdf.

Fuchs, D., A. Forsman, et al. (1990). "Immune activation and decreased tryptophan in patients with HIV-1 infection." J Interferon Res **10**(6): 599-603.

Gabbay, V., B. A. Ely, et al. (2012). "The possible role of the kynurenine pathway in anhedonia in adolescents." J Neural Transm **119**(2): 253-260.

Gabbay, V., R. G. Klein, et al. (2010). "The possible role of the kynurenine pathway in adolescent depression with melancholic features." J Child Psychol Psychiatry **51**(8): 935-943.

Glass, J. D., H. Fedor, et al. (1995). "Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia." Ann Neurol **38**(5): 755-762.

Gonzalez, J. S., A. W. Batchelder, et al. (2011). "Depression and HIV/AIDS treatment nonadherence: a review and meta-analysis." J Acquir Immune Defic Syndr **58**(2): 181-187.

Guillemin, G. J., K. M. Cullen, et al. (2007). "Characterization of the kynurenine pathway in human neurons." J Neurosci **27**(47): 12884-12892.

Guo, H., J. Gao, et al. (2014). "HIV-1 infection induces interleukin-1beta production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes." J Biol Chem **289**(31): 21716-21726.

Han, Q., T. Cai, et al. (2010). "Structure, expression, and function of kynurenine aminotransferases in human and rodent brains." Cell Mol Life Sci **67**(3): 353-368.

Han, Q., T. Cai, et al. (2008). "Substrate specificity and structure of human aminoadipate aminotransferase/kynurenine aminotransferase II." Biosci Rep **28**(4): 205-215.

Hannestad, J., N. DellaGioia, et al. (2011). "The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis." Neuropsychopharmacology **36**(12): 2452-2459.

Hawai`i State Department of Health. (2014). "Hawai`i HIV Surveillance Report, October 2014." from http://health.hawaii.gov/std-aids/files/2013/05/2013-surveillance-report.pdf (accessed August 13, 2015).

Heyes, M. P., B. J. Brew, et al. (1992). "Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and beta 2-microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients." J Neuroimmunol **40**(1): 71-80.

Hill, L. and K. C. Lee (2013). "Pharmacotherapy considerations in patients with HIV and psychiatric disorders: focus on antidepressants and antipsychotics." Ann Pharmacother **47**(1): 75-89.

Holtze, M., P. Saetre, et al. (2012). "Kynurenine 3-monooxygenase polymorphisms: relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls." J Psychiatry Neurosci **37**(1): 53-57.

Hong, S. and W. A. Banks (2015). "Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications." Brain Behav Immun **45**: 1-12.

Hughes, M. M., A. Carballedo, et al. (2012). "Tryptophan depletion in depressed patients occurs independent of kynurenine pathway activation." Brain Behav Immun **26**(6): 979-987.

Jin, C., G. Zhao, et al. (2010). "The psychological status of HIV-positive people and their psychosocial experiences in eastern China." HIV Med **11**(4): 253-259.

Johansson, A. S., B. Owe-Larsson, et al. (2013). "Activation of kynurenine pathway in ex vivo fibroblasts from patients with bipolar disorder or schizophrenia: cytokine challenge increases production of 3-hydroxykynurenine." J Psychiatr Res **47**(11): 1815-1823.

Kamat, A., J. L. Lyons, et al. (2012). "Monocyte activation markers in cerebrospinal fluid associated with impaired neurocognitive testing in advanced HIV infection." J Acquir Immune Defic Syndr **60**(3): 234-243.

Kepplinger, B., H. Baran, et al. (2005). "Age-related increase of kynurenic acid in human cerebrospinal fluid - IgG and beta2-microglobulin changes." Neurosignals **14**(3): 126-135.

Kralovicova, J. and I. Vorechovsky (2007). "Global control of aberrant splice-site activation by auxiliary splicing sequences: evidence for a gradient in exon and intron definition." Nucleic Acids Res **35**(19): 6399-6413.

Kumar, A., W. Abbas, et al. (2013). "TNF and TNF receptor superfamily members in HIV infection: new cellular targets for therapy?" Mediators Inflamm **2013**: 484378.

Lavebratt, C., S. Olsson, et al. (2014). "The KMO allele encoding Arg452 is associated with psychotic features in bipolar disorder type 1, and with increased CSF KYNA level and reduced KMO expression." Mol Psychiatry **19**(3): 334-341.

Lehto, S. M., L. Niskanen, et al. (2010). "Serum chemokine levels in major depressive disorder." Psychoneuroendocrinology **35**(2): 226-232.

Lewinsohn, P. M., J. R. Seeley, et al. (1997). "Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults." Psychol Aging **12**(2): 277-287.

Linderholm, K. R., E. Skogh, et al. (2012). "Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia." Schizophr Bull **38**(3): 426-432.

Lindqvist, D., S. Hall, et al. (2013). "Cerebrospinal fluid inflammatory markers in Parkinson's disease--associations with depression, fatigue, and cognitive impairment." Brain Behav Immun **33**: 183-189.

Maes, M. (2011). "Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression." Prog Neuropsychopharmacol Biol Psychiatry **35**(3): 664-675.

Maes, M., H. Y. Meltzer, et al. (1995). "Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression." J Affect Disord **34**(4): 301-309.

Maes, M. and W. Rief (2012). "Diagnostic classifications in depression and somatization should include biomarkers, such as disorders in the tryptophan catabolite (TRYCAT) pathway." Psychiatry Res **196**(2-3): 243-249.

Manji, H. and R. Miller (2004). "The neurology of HIV infection." J Neurol Neurosurg Psychiatry **75 Suppl 1**: i29-35.

Martinez, P., A. C. Tsai, et al. (2014). "Reversal of the Kynurenine pathway of tryptophan catabolism may improve depression in ART-treated HIV-infected Ugandans." J Acquir Immune Defic Syndr **65**(4): 456-462.

Matinella, A., M. Lanzafame, et al. (2015). "Neurological complications of HIV infection in pre-HAART and HAART era: a retrospective study." J Neurol.

McArthur, J. C., B. J. Brew, et al. (2005). "Neurological complications of HIV infection." Lancet Neurol **4**(9): 543-555.

Mellor, A. L. and D. H. Munn (1999). "Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation?" Immunol Today **20**(10): 469-473.

Merendino, R. A., G. Di Pasquale, et al. (2004). "Involvement of fractalkine and macrophage inflammatory protein-1 alpha in moderate-severe depression." Mediators Inflamm **13**(3): 205-207.

Mikova, O., R. Yakimova, et al. (2001). "Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis." Eur Neuropsychopharmacol **11**(3): 203-208.

Molteni, R., F. Macchi, et al. (2013). "Modulation of the inflammatory response in rats chronically treated with the antidepressant agomelatine." Eur Neuropsychopharmacol **23**(11): 1645-1655.

Morrissette, D. A. and S. M. Stahl (2014). "Modulating the serotonin system in the treatment of major depressive disorder." CNS Spectr **19 Suppl 1**: 57-67; quiz 54-57, 68.

Myint, A. M. (2012). "Kynurenines: from the perspective of major psychiatric disorders." FEBS J **279**(8): 1375-1385.

Myint, A. M. and Y. K. Kim (2014). "Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis." Prog Neuropsychopharmacol Biol Psychiatry **48**: 304-313.

Myint, A. M., Y. K. Kim, et al. (2007). "Kynurenine pathway in major depression: evidence of impaired neuroprotection." J Affect Disord **98**(1-2): 143-151.

Myint, A. M., M. J. Schwarz, et al. (2012). "The role of the kynurenine metabolism in major depression." J Neural Transm **119**(2): 245-251.

Nath, A., K. Conant, et al. (1999). "Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon." J Biol Chem **274**(24): 17098-17102.

Olsson, S. K., M. Samuelsson, et al. (2010). "Elevated levels of kynurenic acid in the cerebrospinal fluid of patients with bipolar disorder." J Psychiatry Neurosci **35**(3): 195-199.

Olsson, S. K., C. Sellgren, et al. (2012). "Cerebrospinal fluid kynurenic acid is associated with manic and psychotic features in patients with bipolar I disorder." Bipolar Disord **14**(7): 719-726.

Owe-Larsson, B., L. Sall, et al. (2009). "HIV infection and psychiatric illness." Afr J Psychiatry (Johannesbg) **12**(2): 115-128.

Oxenkrug, G. (2013). "Serotonin-kynurenine hypothesis of depression: historical overview and recent developments." Curr Drug Targets **14**(5): 514-521.

Oxenkrug, G. F. (2007). "Genetic and hormonal regulation of tryptophan kynurenine metabolism: implications for vascular cognitive impairment, major depressive disorder, and aging." Ann N Y Acad Sci **1122**: 35-49.

Pavon, L., G. Sandoval-Lopez, et al. (2006). "Th2 cytokine response in Major Depressive Disorder patients before treatment." J Neuroimmunol **172**(1-2): 156-165.

Pearson Education, I. "Symptom Checklist-90-Revised (SCL-90-R®) ", 2014, from http://www.pearsonclinical.com/psychology/products/100000645/symptom-checklist-90-revised-scl-90-r.html#tab-details.

Perkins, D. O., R. A. Stern, et al. (1994). "Mood disorders in HIV infection: prevalence and risk factors in a nonepicenter of the AIDS epidemic." Am J Psychiatry **151**(2): 233-236.

Rabkin, J. G. (2008). "HIV and depression: 2008 review and update." Curr HIV/AIDS Rep **5**(4): 163-171.
Radloff, L. S. (1977). Center for Epidemiologic Studies Depression Scale.
Rappaport, J. and D. J. Volsky (2015). "Role of the macrophage in HIV-associated neurocognitive disorders and other comorbidities in patients on effective antiretroviral treatment." J Neurovirol.
Rezaei, S., S. Taramian, et al. (2013). "Psychopathological Dimensions in Substance Abusers with and without HIV/AIDS and Healthy Matched Group." Addict Health **5**(3-4): 115-125.
Robertson, K., C. Bayon, et al. (2014). "Screening for neurocognitive impairment, depression, and anxiety in HIV-infected patients in Western Europe and Canada." AIDS Care **26**(12): 1555-1561.
Savitz, J., W. C. Drevets, et al. (2015). "Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder." Brain Behav Immun **46**: 55-59.
Schroecksnadel, K., M. Sarcletti, et al. (2008). "Quality of life and immune activation in patients with HIV-infection." Brain Behav Immun **22**(6): 881-889.
Schwarcz, R. (2004). "The kynurenine pathway of tryptophan degradation as a drug target." Curr Opin Pharmacol **4**(1): 12-17.
Schwarcz, R., J. P. Bruno, et al. (2012). "Kynurenines in the mammalian brain: when physiology meets pathology." Nat Rev Neurosci **13**(7): 465-477.
Schwarz, M. J., M. Offenbaecher, et al. (2002). "Evidence for an altered tryptophan metabolism in fibromyalgia." Neurobiol Dis **11**(3): 434-442.
Sherr, L., C. Clucas, et al. (2011). "HIV and depression--a systematic review of interventions." Psychol Health Med **16**(5): 493-527.
Si, Q., M. Cosenza, et al. (2002). "GM-CSF and M-CSF modulate beta-chemokine and HIV-1 expression in microglia." Glia **39**(2): 174-183.
Song, C., U. Halbreich, et al. (2009). "Imbalance between pro- and anti-inflammatory cytokines, and between Th1 and Th2 cytokines in depressed patients: the effect of electroacupuncture or fluoxetine treatment." Pharmacopsychiatry **42**(5): 182-188.
Stone, T. W. and L. G. Darlington (2002). "Endogenous kynurenines as targets for drug discovery and development." Nat Rev Drug Discov **1**(8): 609-620.
Sublette, M. E., H. C. Galfalvy, et al. (2011). "Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder." Brain Behav Immun **25**(6): 1272-1278.
Ting, K. K., B. Brew, et al. (2007). "The involvement of astrocytes and kynurenine pathway in Alzheimer's disease." Neurotox Res **12**(4): 247-262.
Watkins, C. C. and G. J. Treisman (2015). "Cognitive impairment in patients with AIDS - prevalence and severity." HIV AIDS (Auckl) **7**: 35-47.
Wonodi, I., R. P. McMahon, et al. (2014). "Influence of kynurenine 3-monooxygenase (KMO) gene polymorphism on cognitive function in schizophrenia." Schizophr Res **160**(1-3): 80-87.
World Health Organization. "HIV/AIDS (Fact sheet No. 360), July 2015." from http://www.who.int/mediacentre/factsheets/fs360/en/ (accessed August 13, 2015).
Yuan, L., L. Qiao, et al. (2013). "Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-HAART era in China." J Neurovirol **19**(2): 144-149.
Zunszain, P. A., C. Anacker, et al. (2012). "Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis." Neuropsychopharmacology **37**(4): 939-949.