Altered monoamine and acylcarnitine metabolites in HIV patients with depressive symptoms

Edana Cassol[1-3], Vikas Misra[1], Susan Morgello[4], Gregory Kirk[5], Shruti Mehta[5] and Dana Gabuzda[1,2]

[1]Dana-Farber Cancer Institute and [2]Harvard Medical School, Boston, MA; [3]Carleton University, Ottawa, ON, [4]The Mount Sinai Medical Center, New York, NY, Johns Hopkins University, Baltimore, MD

Current Address:
Department of Health Sciences
Carleton University, Ottawa, Canada
E-mail: edana.cassol@carleton.ca

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Abstract

Background: Depression is a frequent comorbidity in HIV infection and an important predictor of poor treatment outcomes and increased mortality. Accumulating evidence suggests that inflammation and increased tryptophan catabolism are associated with increased risk of developing major depressive disorder in HIV infection, but the mechanisms leading to depression remain poorly understood.

Methods: The severity of depressive symptoms was assessed by Beck Depression Inventory or Center for Epidemiological Studies Depression Scale. Untargeted metabolomic profiling of plasma from 104 subjects across three independent cohorts (32 HIV+ subjects on ART from NNTC and CHARTER [median CD4 288 cells/ul, median plasma VL 50 copies/ml; n=15 and n=17 with and without depressive symptoms], 36 HIV+ [median CD4 350 cells/ul, median plasma VL 630 copies/ml; n=13 and n=23 with and without depressive symptoms] and 36 HIV- [n=12 and n=24 with and without depressive symptoms] subjects from ALIVE) was performed using liquid or gas chromatography followed by mass spectrometry to detect > 400 metabolites. Cytokine profiling was performed by Bioplex array. Bioinformatic analysis were performed in Metaboanalyst and R.

Results: Decreased monoamine metabolites of phenylalanine/tyrosine catabolism (phenylacetate and 4-hydroxyphenylacetate) and acylcarnitines (propionylcarnitine, isobutyrylcarnitine, isovalerylcarnitine, and 2-methylbutyrylcarnitine) in plasma distinguished between subjects with and without depressive symptoms in both HIV+ and HIV- cohorts (FC>1.2, p<0.05, FDR<10%), and these alterations correlated with the severity of depression. In HIV+ subjects, depressive symptoms were associated with augmented IFN responses (IFN-gamma, CXCL9, CXCL10) and tryptophan catabolism (increased kynurenine to tryptophan ratio), and these changes correlated with metabolite alterations associated with depression (p<0.05, FDR<10%). Altered metabolites mapped to pathways involved in monoamine metabolism, mitochondrial function, and inflammation, suggesting a model in which complex inter-relationships between monoamine metabolism and mitochondrial bioenergetics contribute to biological mechanisms involved in depression that may be augmented by inflammation during HIV infection.

Conclusions: Integrated approaches targeting inflammation, monoamine metabolism (tryptophan [serotonin], phenylalanine [dopamine], and trace amines), and mitochondrial pathways may be important for prevention and treatment of depression in people with and without HIV.

Methods

• Plasma samples (n=104; 68 HIV-positive subjects and 36 HIV-negative subjects) were collected between 2002-2012. HIV-positive test cohort was from the National NeuroAIDS Tissue Consortium (NNTC) and the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study. The HIV-positive validation and HIV-negative cohorts were from the AIDS Linked to the Intravenous Experience (ALIVE) Study, a longitudinal cohort of current or former injection drug users

• Depressive symptoms were assessed by the **Beck Depression Inventory (BDI)** in the NNTC and CHARTER studies and by the **Center for Epidemiological Studies Depression Scale (CES-D)** in the ALIVE study

•Cytokines and chemokines were measured using a Bio-Plex suspension Array System (Bio-Plex Human Cytokine 27-plex Assay plus IL-2RA, IFN-α, and CXCL9 Singleplex assays)

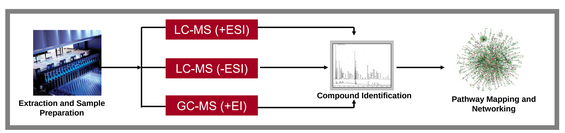


Figure 1. Liquid-and-gas-chromatography-based mass spectrometry platform used in global metabolite profiling. Untargeted metabolite profiling of plasma from HIV and control subjects detected a total of 445 metabolites (excluding xenobiotics).

• Untargeted metabolomic profiling was performed by Metabolon (Durham, NC) using ultra high performance liquid chromatography and tandem mass spectrometry (UHLC/MS2/MS) optimized for the detection of acidic or basic metabolites, and gas chromatography (GC)/MS (**Figure 1**)

Clinical and demographic characteristics

	HIV-positive test cohort (n=32)	HIV-positive validation cohort (n=36)	HIV-negative cohort (n=36)
Age (years)*	44 (33-59)	48 (35-57)	49 (36-62)
Gender (male)	67%	53%	53%
Race (African American)	60%	86%	78%
BMI*	23.6 (21.9-30.1)	25.3 (18.6-34.6)	26.1 (19.4-39.9)
HCV seropositive	73%	67%	64%
Smokers	NA	81%	78%
Alcohol use	40%	53%	50%
Cocaine use	47%	53%	56%
% on ART	100%	58%	NA
% on PI	77%	68%	NA
Current CD4 (cells/ul)*	288 (16-1396)	350 (64-992)	NA
Nadir CD4 (cells/ul)*	54 (2-267)	227 (6-856)	NA
Plasma HIV viral load (log copies/ml)*	1.7 (1.7-3.2)	2.8 (1.6-4.0)	NA
BDI*	9 (0-35)	NA	NA
% with BDI ≥ 14	47%	NA	NA
CES-D*	NA	11 (0-43)	12 (0-36)
% with CES-D ≥ 16	NA	36%	33%
Antidepressant use	30%	27%	16%

Depression is associated with alterations in monoamine metabolites and decreased acylcarnitines in HIV-positive and HIV-negative cohorts

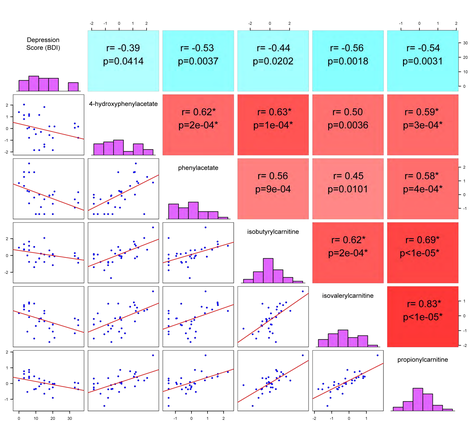


Figure 2 (Above). Inter-relationships between plasma metabolites and depression scores in HIV patients on ART. Spearman correlation matrix visualization reveals inter-relationships between a set of 5 plasma metabolites and BDI scores in the HIV-positive test cohort (n=30). Comparisons were performed in a pair-wise manner and the correlation matrix was constructed in R. Diagonals display the histogram for each individual variable. Panels to the right of the histogram show Spearman correlation coefficients and p-values. Red and blue represent positive and negative correlations, respectively.

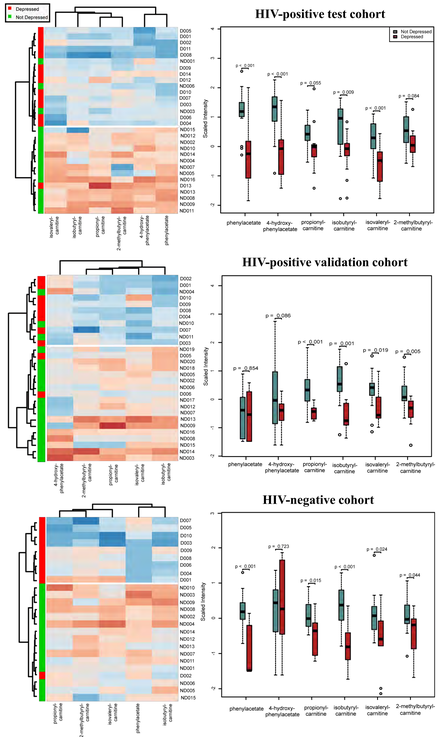


Figure 3 (Right). Plasma metabolites distinguishing between depressed and not depressed subjects in HIV-positive and HIV-negative cohorts. Unsupervised hierarchical clustering of signature metabolites (n=6; FC>1.2, p<0.01, FDR <10%) altered across independent cohorts of HIV-positive and HIV-negative subjects with compared to without depression. Red and blue indicate increased and decreased metabolite levels, respectively. The groups were matched for age, gender, race, HIV status, and other covariates as described in the Results section. On the right, box plots of metabolites altered in depressed compared to not depressed subjects. Medians are represented by horizontal bars, boxes span the interquartile range (IQR), and whiskers extend to extreme data points within 1.5 times IQR. Outliers plotted as open circles lie outside 1.5 times the IQR. Blue and red represent not depressed and depressed subjects, respectively. P-values were calculated using Welch's t-tests.

Depression in HIV-positive subjects is associated with augmented IFN responses and tryptophan catabolism

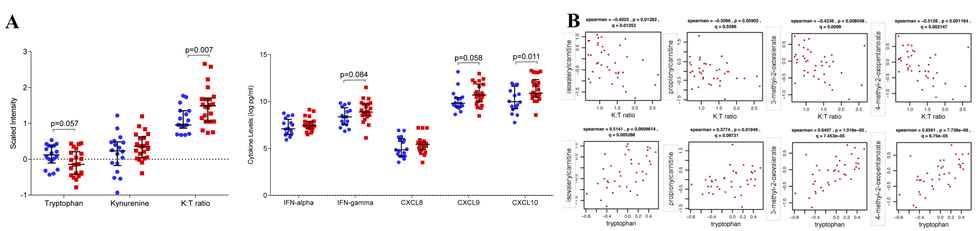


Figure 4. Depression is associated with augmented IFN responses and increased tryptophan catabolism in HIV patients. A) Beeswarm plots of tryptophan catabolism metabolites and inflammation markers altered in HIV patients with (n=20, red) vs. without depression (n=20, blue) from the merged HIV dataset (n=40, age<50 years old). Groups were matched for age, gender, race, HIV status, and other clinical covariates as described in the Results. Medians are represented by horizontal bars and error bars represent IQR. P-values were calculated using Mann Whitney U test. B) Correlation plots of markers of tryptophan catabolism and metabolites associated with depression in the merged HIV dataset (n=40). Spearman correlations were used to examine relationships between IFN-gamma-induced chemokines and metabolite levels. The correlation coefficient R, and p-value are shown above each plot.

Inter-relationships between plasma metabolites, depression scores, and tryptophan catabolism in HIV-positive cohorts

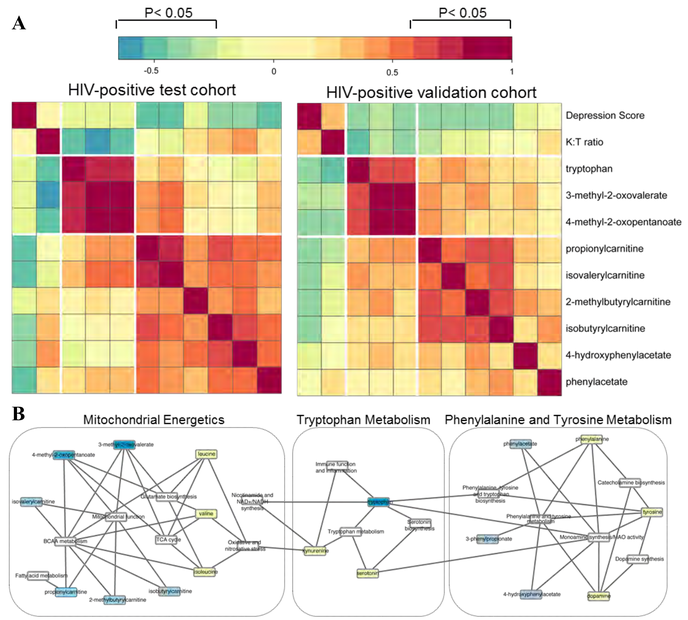


Figure 5. Inter-relationships between plasma metabolites, depression scores, and markers of tryptophan catabolism in two independent HIV-positive cohorts. A) Pearson correlations were used to evaluate relationships between metabolites.Spearman correlations were used to examine relationships between metabolites, depression scores, and K:T ratio. Significant correlations had a −0.32> r >0.32, p<0.05, and FDR<10%. B) Network model of metabolites and metabolic pathways altered in HIV patients with depression. Metabolites were mapped to metabolite pathways, and interaction networks were generated in Cytoscape. Light blue nodes represent signature metabolites associated with depression across independent cohorts. Dark blue nodes represent inter-related metabolites identified by correlation analysis. Yellow and white nodes represent other related metabolites and metabolic pathways, respectively.

Conclusion

• Untargeted metabolomics and identified altered monoamine and acylcarnitine metabolites, along with increased inflammatory markers in HIV-positive and HIV-negative subjects with depression compared to non-depressed controls

• Consistent with previous studies, depression was also associated with decreased tryptophan and increased K:T ratio in two independent cohorts of HIV-infected individuals. Increased tryptophan catabolism via the kynurenine pathway may contribute to depression not only by effects on serotonin biosynthesis, but also by production of neurotoxic metabolites

• These results suggest inter-relationships between inflammation, altered monoamine metabolism (serotonin, dopamine, trace amines) and mitochondrial energetics may be involved in the biological mechanisms underlying depression

• Integrated approaches targeting inflammation, specific steps in monoamine metabolism (tryptophan [serotonin], phenylalanine [dopamine], and trace amines), and mitochondrial pathways may be beneficial for prevention and treatment of depressive symptoms affecting quality of life and clinical outcomes in both HIV-positive and HIV-negative populations suffering from major depressive disorder (MDD)