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Transcriptome analysis of HIV-infected peripheral blood monocytes: gene transcripts and networks associated with neurocognitive functioning

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Abstract

Immunologic dysfunction, mediated via monocyte activity, has been implicated in the development of HIV-associated neurocognitive disorder (HAND). We hypothesized that transcriptome changes in peripheral blood monocytes relate to neurocognitive functioning in HIV + individuals, and that such alterations could be useful as biomarkers of worsening HAND.

METHODS—mRNA was isolated from the monocytes of 86 HIV+ adults and analyzed with the Illumina HT-12 v4 Expression BeadChip. Neurocognitive functioning, HAND diagnosis, and other clinical and virologic variables were determined. Data were analyzed using standard expression analysis and weighted gene co-expression network analysis (WGCNA).

RESULTS—Neurocognitive functioning was correlated with multiple gene transcripts in the standard expression analysis. WGCNA identified two nominally significant co-expression modules associated with neurocognitive functioning, which were enriched with genes involved in mitotic processes and translational elongation.

CONCLUSIONS—Multiple modified gene transcripts involved in inflammation, cytoprotection, and neurodegeneration were correlated with neurocognitive functioning. The associations were not strong enough to justify their use as biomarkers of HAND; however, the associations of two co-expression modules with neurocognitive functioning warrants further exploration.

Keywords

HIV-associated neurocognitive disorder; NeuroAIDS; monocyte; IL6R; KEAP1; LRP12; CSNK1A1; WGCNA

INTRODUCTION

Over 32 million individuals are currently infected with HIV-1 worldwide, and 1.2 million within the United States. In most developed countries, the availability of a combination of effective antiretroviral medicines has significantly increased life expectancy. As a chronic disease, HIV has lead to a host of new challenges. Perhaps the most common is HIV-associated neurocognitive disorder (Antinori et al., 2007), which may affect between 40%–70% of infected individuals (McArthur et al., 2005). Prior to the availability of combination anti-retroviral therapy (cART), neurological illness in HIV+ individuals often presented as dementia (i.e., HIV-associated dementia, or HAD), with an acute encephalitis as the frequent underlying cause. A key aspect of the neuropathogenic process of HAD is the phenotypic modification of peripheral blood monocytes and their subsequent migration across the blood brain barrier (Pulliam et al., 1997). CD16+ monocytes are among the first cells to become infected with HIV. This subset of cells expand in HIV and Simian immunodeficiency virus (SIV) infection (Thiebaut et al., 1995, Otani et al., 1998), and also appears to be associated with the pathogenesis of milder HIV-Associated Neurocognitive Disorder (HAND) (Pulliam et al., 1997, Ellery et al., 2007). As the virus gains momentum and the immune system weakens, the subset of CD16+ monocytes increases and cross the blood brain barrier (BBB) as “Trojan Horse” cells with increased frequency. This migration is driven by enhanced chemokine release in the CNS as well as the peripheral immune response (Peluso et al., 1985, Ancuta et al., 2004, Kraft-Terry et al., 2009). Increased traffic of monocytes into the CNS, which is often followed by differentiation into perivascular macrophages (Cosenza et al., 2002), further increases the expression of chemokines such as monocyte chemoattractant protein (MCP-1), leading to recruitment of even more monocytes in a feed-forward manner (Persidsky et al., 1999, Persidsky et al., 2000). These processes have also been described in terms of a “push” (peripheral immune activation) and “pull” (a chemokine gradient released from cells within the CNS) mechanisms (Kraft-Terry et al., 2009). In addition to the release of pro-inflammatory cytokines and chemokines, infected

monocytes also release viral proteins that are harmful to nearby neurons and other cells (Kedzierska and Crowe, 2002, Glass et al., 1995, Adle-Biassette et al., 1999, Lindl et al., 2007, Kaul and Lipton, 2006, Kraft-Terry et al., 2009). In summary, the crosstalk between the CNS and circulating blood monocytes is thought to be a key mechanism underlying HAND neuropathogenesis. As such, delineating cellular dynamics that occur within monocytes at the early stages of this process could lead to biomarker identification and pharmaceutical intervention.

To date, investigations into the molecular biology of HAND have generally utilized models or tissue representing advanced disease states (e.g., HAD or HIV-encephalitis (HIVE)). Such studies have included transcriptomic and proteomic changes in monocytes, macrophages, brain cells and brain tissue in conjunction with advanced immunosuppression (Pulliam et al., 1997, Pulliam et al., 2004, Gendelman et al., 1997, Carlson et al., 2004, Luo et al., 2003, Wojna et al., 2004, Roberts et al., 2003, Buckner et al., 2011). Gene expression studies have also been widely used to investigate and discover cellular mechanism involved in the pathogenesis advanced HAND and HIVE. Many of these have involved the use of homogenized samples of post-mortem brain tissue (Masliah et al., 2004, Roberts et al., 2003, Roberts et al., 2004, Shapshak et al., 2004a, Gelman et al., 2012), while others have examined specific brain cells in vitro, such as astrocytes or neurons (Shapshak et al., 2004b, Kim et al., 2004, Kolson et al., 2004, Borjabad et al., Shapshak P, 2011), the results of which have led to further insights regarding the neuropathogenesis of HAD and HIVE. However, while studies using brain tissue have been useful in describing cellular alterations associated with HAD, they are of limited utility for defining clinically practical biomarkers. In addition, because most *in vivo* gene expression studies have examined advanced states of disease, such findings may not generalize to the more pervasive mild HAND. (Brew, 2004, Cysique et al., 2005, Cysique et al., 2004, Robertson et al., 2007, Dore et al., 2003, Sacktor et al., 2002). Currently, in the context of combined antiretroviral therapy (cART), it is thought that HAND develops in a chronic manner, with persistent neuroinflammation and low grade viral replication driven in part by monocytes-derived perivascular macrophages in the CNS (Langford et al., 2003, Yadav and Collman, 2009). Not surprisingly, research targeting monocyte gene expression that utilizes both genome-wide and targeted approaches has yielded important results, implicating an evolving monocyte phenotype characterized by increased chemotactic properties and chronic inflammatory response (Pulliam et al., 2004, Buckner et al., 2011). Despite this, studies thus far have not found an association between monocyte gene transcription and behavioral HAND phenotypes (Sun et al., 2010). However, such studies have had very limited power to detect differences, considering the vast number of comparisons characteristic of transcriptome association studies.

In order to arrive at a biologically meaningful reduction of high dimensional transcriptomic data and to integrate such data with orthogonal genetic and behavioral data, systems biologic data analysis methods such as weighted gene co-expression network analysis (WGCNA) have been developed (Zhang and Horvath, 2005b). Here we used WGCNA to integrate transcriptomic and behavioral data derived from the largest clinical sample to date in an effort to investigate of the neuropathogenesis of mild HAND. We focused our investigation on circulating blood monocytes, as these cells are a primary and early component of this pathogenesis. Our hypothesis was that the WGCNA would identify biological pathways associated with increasing HAND severity.

METHODS

Participants

This study was conducted in accordance with the University of California, Los Angeles Medical Institutional Review Board. Eighty-four (86) participants were recruited from the Multicenter AIDS Cohort Study (MACS) in Los Angeles. MACS participants are seen on a semi-annual basis during which they provide blood and complete comprehensive self-report questionnaires assessing drug use, medication use, and medical co-morbidities. In addition, all participants undergo comprehensive neuropsychological testing and assessment of activities of daily living every two years, from which their HAND status is determined. Forty-nine (57%) participants in the current study were Caucasian/non-Hispanic, 14 (16%) were Caucasian/Hispanic, 8 (9%) were African American/non-Hispanic, and the remaining 15 (17%) considered themselves Other/Hispanic. Additional group characteristics are shown in Tables 1 & 2.

Blood processing, Monocyte Isolation, mRNA extraction, and microarray analysis

24ml of fresh blood was collected from participants. Blood was drawn into three 8 mL Cell Preparation Tubes (CPT) containing sodium citrate. Peripheral blood mononuclear cells (PBMCs) were then isolated through centrifugation within 6 hours of collection (Salazar-Gonzalez et al., 1997). PBMCs were washed with phosphate buffered saline, and then monocytes were isolated through Rosette separation (RosetteSep[®]; Stem Cell Technologies) according to the manufacturer instructions. Monocytes were then pelleted, lysed, and RNA extracted using the Qiagen RNeasy kit including a DNase treatment to eliminate any potentially confounding genomic DNA contamination (Shay et al., 2003). RNA was stored at -80°C and sent in batches to the Southern California Genotyping Consortium (SCGC) for microarray analysis, which was performed with the Illumina Human HT-12 v4 gene expression BeadChip. The expression data and sample characteristics, including all information required by the MIAME standard, are available from our web site <http://labs.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/HIVMonocyteExpression/as> well as from the NCBI Gene Expression Omnibus (GEO).

Variables Included in the Gene Expression Analysis

Neurocognitive functioning—Participants completed a comprehensive battery of neuropsychological tests as part of the standard MACS protocol. This includes measures of working memory, learning, memory, executive functioning, motor functioning, and information processing speed. T-scores were calculated using normative data derived from the HIV-seronegative MACS cohort, with demographic corrections for age, education, and ethnicity. For this study, we calculated a Global Neurocognitive Functioning score based on the average of the six domain T-scores.

HAND Severity—HAND status was determined via an algorithm developed by MACS investigators. The algorithm is based on neurocognitive test performance and self-reported deficits in activities of daily living (Lawton and Brody, 1969) in accordance with current research criteria (Antinori et al., 2007). Participants are rated as normal, mild (i.e. Asymptomatic Neurocognitive Impairment-ANI), moderate (Minor Neurocognitive Disorder-MND), or severely impaired (HIV-Associated Dementia-HAD).

CNS Penetration Effectiveness (CPE)—The effects of antiretroviral medications on neurocognitive functioning remains unclear (Tozzi et al., 2007, Cysique et al., 2009, Marra et al., 2003). We sought to determine the impact of antiretroviral regimen on monocyte gene expression. We employed the CPE, a score that is based on the pharmacokinetic and pharmacodynamic characteristics of antiretroviral medications (Letendre et al., 2008). CPE

scores for the regimen reported at the time of neurocognitive testing were calculated. Higher scores indicate a regimen with increased penetration of the blood brain barrier.

Substance Use—In this study we considered the effects of alcohol, marijuana, and stimulant (cocaine and/or methamphetamine) use on gene expression. MACS participants complete a substance use questionnaire every six months. Drug and alcohol use is recorded in terms of frequency during the last six months and average amount used each time. Participants were considered *active* users of alcohol, stimulants, or marijuana if they report daily or weekly use, and *non-users* if they report monthly or less use in the six months preceding the visit.

Depression—Depression was determined with the Center for Epidemiologic Studies Depression Scale (CES-D)(Radloff, 1977). Scores on the CES-D are entered as a continuous variable, with higher scores indicating greater degree of depression.

Virologic measures—The percentage of lymphocytes that were CD4+ T-cells was determined by flow cytometry. HIV viral load was determined via either the COBAS TaqMan HIV-1 Test, Version 2.0 or Roche Amplicor HIV-1 MONITOR Test, Version 1.5. Both tests quantify HIV-1 RNA based on in vitro amplification of the highly conserved HIV-1 gag gene. Nadir CD4 was obtained either by self-reports or, for those who seroconverted during the course of the study, their lowest CD4 according to study records.

Statistical Analysis

Gene array data was processed in Illumina BeadStudio software and log₂-transformed to stabilize variance. The normalized data were batch-corrected using the ComBat R function (Johnson et al., 2007). Potential outliers were removed both within each batch and, after batch correction, in the combined data. Outlier identification was based on scaled connectivity of each sample in a sample network (Oldham et al., 2012) based on Euclidean distance. An apparent technical sample-to-sample variation that remained was then removed by quantile normalization (Bolstad et al., 2003). Low-expressed probe sets were removed. The expression data were then adjusted for age by regressing gene expression on age using a linear model and retaining the residuals as age-adjusted expression values.

Differential expression analysis—The relationship between each gene and continuous or ordinal clinical variables (neurocognitive domain and global scores, HAND rating, CPE, CES-D, and substance use variables) and virologic variables (nadir CD4, current CD4, and current viral load) were assessed using Pearson correlation. The Pearson correlation test is appropriate since it only assumes that one of the quantitative variables (here gene expression) follows a normal distribution. For the single dichotomous clinical variable (HAND diagnosis) we used the Mann-Whitney U test (a special case of the Kruskal-Wallis test). To adjust for multiple testing we report the local False Discovery Rate (FDR) q-value estimates calculated using code from the qvalue R package(Storey et al., 2004). Results of these differential expression analyses were used in two ways. First, sets of top identified genes for each phenotype (i.e., clinical and virologic variable) were tested for enrichment in known gene ontology (GO) terms(Ashburner et al., 2000). Second, the clinical/virologic variable - gene relationship values (i.e., correlations) for all genes were retained and converted into a color vectors for visual assessment of disease-gene relationships in the network analysis.

Weighted Gene Co-Expression Network Analysis (WGCNA)—Here we employ for the first time WGCNA in the analysis of HIV monocyte transcriptome data. The WGCNA starts out from the over 36,000 assayed probe set, identifies modules of co-expressed genes,

and relates these modules to clinical variables and gene ontology information (Zhang and Horvath, 2005b, Langfelder and Horvath, 2008, Horvath et al., 2006). Because gene modules may correspond to biological pathways, focusing the analysis on modules (and their highly connected intramodular hub genes) amounts to a biologically meaningful data reduction scheme. Highly correlated module genes are represented and summarized by the module eigengene, or ME (Langfelder and Horvath, 2007), as described below. By correlating each gene with the ME we defined a measure of module membership which quantifies how close (i.e., correlated) a gene is to a given module. Module membership measures allow one to annotate all genes on the array and to screen for highly connected intramodular hub genes, which may have particular relevance to disease (Voineagu et al., 2011, Horvath et al., 2006, Ghazalpour et al., 2006). As described below, we use functional enrichment analysis with regard to known gene ontologies to understand the biological significance of the ME and to identify putative disease pathways.

In the following, we provide more details on the weighted correlation network construction. The network construction starts by calculating robust correlations between all genes across all relevant samples. A signed “hybrid” network is constructed by setting adjacency equal to the 5th power of the robust correlation when the correlation is positive, and zero otherwise. Thus, the adjacency takes on values between 0 and 1 and is 0 whenever the corresponding correlation is negative. The power 5 was chosen using the Scale-Free Topology Criterion (Zhang and Horvath, 2005a). We chose the signed network because it keeps tracks of the sign of the correlation and results in modules comprised of positively correlated genes. Recent studies have shown that signed co-expression networks can yield modules with more coherent functional annotation and lead to better identification of small modules (Mason et al., 2009). Topological overlap (TO), a more biologically meaningful measure of node interconnectedness (similarity), was then calculated as described previously (Zhang and Horvath, 2005b, Oldham et al., 2006, Ravasz et al., 2002, Yip and Horvath, 2007). Next, genes were clustered using average-linkage hierarchical clustering with pairwise dissimilarity equal $1 - TO$. Modules correspond to branches of the resulting hierarchical clustering tree and were identified using the Dynamic Tree Cut algorithm (Langfelder et al., 2008). This procedure results in modules of highly correlated genes for which a summary expression profile can be defined. We use the module eigengene (ME), defined as the first right-singular vector of the standardized expression matrix (Zhang and Horvath, 2005a). The ME is the mathematically optimal summary of the module expression profiles since it captures the maximum amount of variation (Langfelder and Horvath, 2007, Horvath and Dong, 2008). The MEs were then correlated to the clinical and virologic variables in order to identify modules related to the clinical and virologic variables. To further enhance data interpretation, we used functional enrichment in GO terms and the function *userListEnrichment* from the WGCNA package to find enrichment for cell type markers and other brain-related categories (Miller et al., 2011).

All data, analysis code, and additional details can be found at our webpage: <http://labs.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/HIVMonocyteExpression/>.

RESULTS

Standard differential gene expression analysis of clinical variables

The relationship between individual gene expression and the clinical and virologic variables was examined via correlation analysis. Table 3 lists associations that remained statistically significant after correcting for multiple comparisons using False Discovery Rate (Benjamini et al., 2001). We used a q-value cutoff of 0.1, as suggested by Benjamini and Yekutieli (Benjamini and Yekutieli, 2005). Only Global Neurocognitive Functioning showed

significant associations with gene transcripts. The full list of associations between genes and variables is provided in Supplemental File A.

Detailed enrichment analysis of top genes related to clinical variables

To further understand monocyte gene expression pathways related to HAND and the other variables, we examined enriched GO categories based on the top gene associations identified in the differential expression analysis. After applying corrections for multiple comparisons, no gene ontology categories were found to be significantly associated with the variables.

Weighted Gene Co-Expression Network Analysis

We next applied WGCNA. This approach differs from the standard differential expression analysis described above because it identifies biological meaningful networks before examining their association with the variables of interest. As such, WGCNA makes significantly fewer comparisons, thereby reducing the chance of Type I error. The network analysis (see Methods) identified twenty-four co-expression modules. The gene clustering dendrogram, modules, and a heatmap of gene-variable associations are presented in Figure 1. Multiple modules are highly enriched in existing GO terms (Table 4), which suggests their functional interpretation.

Correlations between Module Eigengenes and Clinical Variables

Clinical and virologic variables were correlated to module eigengenes. Figure 2 shows in grid format the correlations and adjusted p-values for the module/variable associations. Gene ontology analysis was conducted for each module, reflected in their respective labels. Thirteen correlations reached the nominal .05 threshold; however, after applying FDR correction only two associations reached a suggestive 1q-value of $<.20$. Module 13 (Mitotic Cell Cycle) was positively correlated with Global Neurocognitive Functioning ($R=.34$; $p=.001$, $q=.16$). This module largely consisted of GO categories involved in mitosis. Module 11 was negatively correlated with Global Neurocognitive Functioning ($R= -.32$; $p=.003$, $q=.16$).

Association of HAND and Global Neurocognitive Functioning with Transcripts Implicated in Previous Studies

As a supplemental analysis, we examined expression levels of genes implicated in HAND in previous studies (Table 5). None of the genes was found to be significantly correlated with HAND or Global Neurocognitive Functioning.

Power considerations

While our sample size ($n=84$) is unprecedented in regards to the study of monocyte expression in relation to HAND, it only allows us to detect moderate to large correlations as can be seen by the following power calculations. At an uncorrected significance threshold of 0.05, a sample size of $n=80$ achieves 78% power or 96% power if the true underlying correlation is 0.30 or 0.40, respectively. We caution the reader that the uncorrected significance threshold of $\alpha=0.05$ is not appropriate in the context of high dimensional gene expression data. If we had found only 10 co-expression modules, then one could justify a Bonferroni correction that adjusts for 10 comparisons resulting in a significance threshold of $\alpha=0.005$. At this significance level, $n=80$ subjects would lead to a power of 82% to detect an absolute correlation of 0.40 or higher. Given that we found 24 coexpression modules, these considerations indicate that our study was not sufficiently powered to detect correlations lower than 0.40 between modules and a quantitative clinical outcome.

DISCUSSION

HAND pathogenesis in the current era of widespread cART use and relative virologic control is likely due in part to chronic immunologic over-reactivity and neuroinflammation fueled by an influx of monocytes into the CNS. Delineating the molecular changes that occur in monocytes in relation to neurobehavioral dysfunction may lead to the identification of clinically relevant biomarkers, and further illuminate the biological pathways involved in HAND. While an altered monocyte phenotype was previously identified as being associated with HAD, studies of monocyte transcription changes associated with more mild HIV-related neurocognitive impairment has thus far yielded negative results (Sun et al., 2010). In the current study, we utilized a fairly large sample, a monocyte extraction method with high purity, and a statistical approach allowing for a systems-wide understanding of transcription changes while minimizing the risk of type-I error inherent in gene expression studies.

A standard differential expression analysis, in which we correlated mRNA levels with the clinical variables, identified Global Neurocognitive Functioning as the sole variable with significant associations following correction for multiple comparisons. Interestingly, many of the genes associated with Global Neurocognitive Functioning have either previously been implicated in NeuroAIDS or other neurodegenerative disease, or are involved in pathways relevant to HAND neuropathogenesis. The unexpected finding is that most of those previous studies used brain tissue/cells. The current study found that interleukin-6 receptor (IL-6R) expression was negatively correlated with Global Neurocognitive Functioning. That is, better neurocognitive functioning was associated with lower IL-6R mRNA levels. Increased levels of IL-6R have long been implicated in central nervous system pathology in general, including multiple sclerosis (Padberg et al., 1999) and head trauma (Hans et al., 1999), consistent with our findings. However, it is this receptor's ligand, IL-6, that has primarily been associated with NeuroAIDS. Yoshioka et al (1994) reported increased expression of IL-6 in endothelial cells in lumbosacral sensory dorsal root ganglia from patients who died with AIDS vs. HIV- controls, leading to the conclusion that IL-6 expression was associated with neuronal degeneration (Yoshioka et al., 1994). More recently, plasma levels of IL-6 mRNA were found to be weakly correlated with memory and learning ability in HIV+ adults (Pedersen et al., 2013). Others have reported associations between depression and IL-6 mRNA in HIV+ cases, but not neurocognitive functioning, suggesting that depression may be a moderating variable (Warriner et al., 2010). However, in our analysis, IL-6 and IL-6R mRNA had very weak correlations with depression, memory, and learning. Further, Pulliam et al found that PBMC-derived soluble IL-6 levels were significantly higher in a very small group of patients with HIV-associated dementia (Pulliam et al., 1996). As such, it is plausible that the decreased expression of IL-6 receptors associated with better neurocognitive functioning reflects decreased IL-6 activity in these monocytes. It should be noted that an early study implicated a possibly neuroprotective and regenerative role for IL-6 that may explain the variable findings over the years for this cytokine (Yoshioka et al., 1995). This is supported by a recent study showing that IL-6 protected against NMDA-induced neurotoxicity when a functioning IL-6 receptor was present (Fang et al., 2012).

Additional genes found to be associated with Global Neurocognitive Functioning warrant attention. Casein kinase 1-alpha-1 (CSNK1A1) is one of a family of casein kinases previously implicated in neurodegenerative disease (Perez et al., 2011). Expression of this gene was negatively correlated with Global Neurocognitive Functioning. Hypoxia up-regulated-1 (HYOU1) has been shown in numerous studies to play a protective role in various cells types, including astrocytes, neurons, and macrophages (Janeczko, 1991, Kuwabara et al., 1996, Ozawa et al., 2001, Tamatani et al., 2001, Zhao et al., 2010). Consistent with this, expression of this gene was positively correlated with Global Neurocognitive Functioning. Low density lipoprotein receptor-related protein-12 (LRP12),

an apolipoprotein-E receptor that mediates the endocytosis and degradation of amyloid precursor protein(Kounnas et al., 1995), was negatively correlated with Global Neurocognitive Functioning. Activation of this receptor by Tat has been shown to mediate HIV-induced neuropathology(Liu et al., 2000). This gene has also been linked to an increased risk of Alzheimer's disease(Kang et al., 1997, Bian et al., 2005). Kelch-like ECH-Associated protein-1 (KEAP1) was negatively correlated with Global Neurocognitive Functioning. The transcription factor Nrf2, which enhances cytoprotective gene expression following pathological insults, is sequestered by KEAP1 in cytoplasm, where it is subsequently degraded by proteasomes(Itoh et al., 1999). KEAP1 is inactivated under oxidative stress, leading to Nrf2 activation of antioxidant and detoxifying genes(Motohashi and Yamamoto, 2004). Consistent with this, KEAP1 antibodies have been immunostained in the lesions of numerous neurodegenerative diseases, including Dementia with Lewy bodies, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis(Tanji et al., 2013). As such, some have begun examining molecular agents that inhibit the KEAP1/Nrf2 interaction as a treatment for neurodegenerative diseases(Zhao et al., 2011); our findings provide initial support for the inclusion of HAND.

The module-centric analysis afforded by WGCNA allowed us to greatly reduce the number of statistical tests: only 216 hypothesis tests resulted from relating 24 modules to 9 clinical variables. Two of these 216 tests resulted in statistically significant findings following FDR correction. GO analysis of the significant modules indicates that *mitotic cell cycle* was positively correlated with Global Neurocognitive Functioning, whereas *translational elongation* was negatively correlated. Interestingly, *mitotic cell cycle* was negatively correlated with pre-mortem neurocognitive functioning in our examination of frontal grey matter from deceased cases in the National NeuroAIDS Tissue Consortium(Morgello et al., 2001, Levine et al., 2013). Those cases died with advanced illness, some including HIV-encephalitis. With regards to *translational elongation*, this GO category shares genes with *viral transcription* and *viral infectious cycle* GO categories. One possible explanation is that greater transcription activity and viral replication within monocytes is associated with neurocognitive impairment.

In relation to previous studies of monocyte gene expression in HIV+ individuals, our findings provided little if any validation. One possible reason for this is that these past studies used monocytes of a specific CD-receptor phenotype. Pulliam et al (2004) isolated CD14+ monocytes from 23 HIV+ individuals with high or low viral loads, as well as HIV-seronegative controls, and used DNA microarrays to characterize differentially regulated genes in the cells(Pulliam et al., 2004). Monocytes from individuals with high viral loads (>10,000 copies) increased expression of CD16, CCR5, MCP-1, and sialoadhesin, suggesting an inflammatory phenotype. However, they noted that the monocytes are not activated in the "classical sense"; that is, they did not produce a number of proinflammatory cytokines common in the pre-cART era (e.g., IL-6, TNF-alpha), and they showed a transcription profile consistent with chemotactic properties. Specifically, among individuals with high viral loads, they found evidence of increased propensity for chemotaxis characterized by an increase in CCR5 and MCP-1, leading the authors to conclude that the circulating monocyte has evolved in the cART era to a chronic inflammatory state with additional chemotactic features, essentially a mature monocyte/macrophage hybrid. In our analysis, we did not observe any associations between gene expression and viral load; however, the range of viral loads within our sample was more restricted than that of Pulliam et al (2004)(Pulliam et al., 2004), and, as already mentioned, we did not focus our analysis on CD14+ monocytes. In a second study, Buckner et al (2011) examined dynamic transcription changes in monocytes using an *in vitro* culture model(Buckner et al., 2011). Focusing on a specific monocyte subpopulation (CD14+CD16+CD11b+Mac387+, derived by infecting healthy donor cells with HIV), these investigators found these monocytes to be

adept at crossing a laboratory model of the blood brain barrier. Gene expression analysis revealed upregulation of chemotactic and metastasis-related genes, as opposed to inflammatory genes. In addition, Buckner et al. described dynamic changes as the monocytes matured into macrophages, including an increase in the expression of enolase 2, followed by a decrease once the cell was fully differentiated. Osteopontin was also observed to have increased expression in the maturing monocytes. This important *in vitro* study demonstrated that the dynamic changes in monocyte transcription may provide clues concerning biological processes necessary for neuropathogenesis. While our *in vivo* analysis did not identify any of the genes observed by Buckner et al., we did not isolate this particular subpopulation of monocytes.

Finally, in an attempt to link global monocyte transcription changes to HAND, Sun et al examined whether or not CD14+ monocyte gene expression and other peripheral factors (CD4, ApoE genotype, viral load, lipopolysaccharide and soluble CD14) were associated with neurocognitive impairment in a group of 44 HIV+ individuals on cART, as well as 11 HIV-seronegative controls (Sun et al., 2010). Monocyte gene expression was not correlated with neurocognitive impairment. More recently, the same group focused their analysis on neurophysiological measures rather than HAND (Pulliam et al., 2011). Specifically, they examined whether peripheral immune activation was associated with brain metabolite concentrations, as measured by magnetic resonance spectroscopy. Thirty-five HIV+ persons on cART and 8 HIV-seronegative adults were examined. Approximately half of the HIV+ sample was considered to have mild neurocognitive impairment. Absolute concentrations of brain metabolites in the frontal white matter, anterior cingulate cortex, and basal ganglia were determined and then examined in relation to the monocyte transcriptome and a global neurocognitive measure. Among the HIV+ participants, an interferon-alpha induced activation transcriptome phenotype was strongly correlated with N-acetyl-aspartate in the frontal white matter. Notably, Interferon-gamma inducible protein-10 (IP-10) was strongly correlated with plasma protein levels, and plasma IP-10 was inversely correlated with N-acetyl-aspartate in the anterior cingulate cortex. This study is particularly notable as it is the first to connect transcription changes with putative HIV-related neurophysiological changes. This tactic of focusing on more intermediate phenotypes, which may be more reliably measured as compared to the clinical syndrome of HAND, may hold the greatest promise for elucidating the neuropathogenesis of NeuroAIDS. While our findings did not indicate an association between IP-10 and HAND, we again point out that we did not isolate monocytes of a specific phenotype.

A number of methodological factors limit the extent to which our findings may be generalized. Firstly, the sample was largely homogeneous concerning disease severity. As such, the variation in neurocognitive functioning was small. We are currently recruiting participants from the National NeuroAIDS Tissue Consortium, a cohort with considerable variation with regards to HAND spectrum diagnoses and neurocognitive functioning (Morgello et al., 2001). Secondly, while ours is the largest sample thus far in HAND-related transcriptome studies, it is still relatively underpowered to detect true differences in gene expression (as can be seen from our power studies). Finally, we did not employ an HIV-seronegative comparison group since our main interest was to study HAND development among HIV+ individuals. However, since Global Neurocognitive Functioning was the sole variable with significant findings, the significant associations described here will need to be re-examined in a seronegative sample to ensure that any associations are indeed HIV-related, as opposed to being a general biological association regardless of infection.

In summary, our analysis of global monocyte gene expression revealed interesting associations of a number of highly relevant genes with neurocognitive functioning, but did

not reveal significant associations with the diagnosis of HAND. Our finding of elevated levels of IL6R, CSNK1A1, LRP12, and KEAP1 mRNA in association with greater neurocognitive impairment is consistent with past studies and may provide justification for exploring these proteins and their related pathways further; however, the associations were not adequately robust to justify their use as biomarkers of HAND. A modest association between two co-expression modules and neurocognitive functioning may also be worthy of further exploration.

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References

- ADLE-BIASSETTE H, CHRETIEN F, WINGERTSMANN L, HERY C, EREAU T, SCARAVILLI F, TARDIEU M, GRAY F. Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. *Neuropathol Appl Neurobiol.* 1999; 25:123–33. [PubMed: 10216000]
- ANCUTA P, MOSES A, GABUZDA D. Transendothelial migration of CD16+ monocytes in response to fractalkine under constitutive and inflammatory conditions. *Immunobiology.* 2004; 209:11–20. [PubMed: 15481136]
- ANTINORI A, ARENDT G, BECKER JT, BREW BJ, BYRD DA, CHERNER M, CLIFFORD DB, CINQUE P, EPSTEIN LG, GOODKIN K, GISSLEN M, GRANT I, HEATON RK, JOSEPH J, MARDER K, MARRA CM, MCARTHUR JC, NUNN M, PRICE RW, PULLIAM L, ROBERTSON KR, SACKTOR N, VALCOUR V, WOJNA VE. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology.* 2007; 69:1789–99. [PubMed: 17914061]
- ASHBURNER M, BALL CA, BLAKE JA, BOTSTEIN D, BUTLER H, CHERRY JM, DAVIS AP, DOLINSKI K, DWIGHT SS, EPPIG JT, HARRIS MA, HILL DP, ISSEL-TARVER L, KASARSKIS A, LEWIS S, MATESE JC, RICHARDSON JE, RINGWALD M, RUBIN GM, SHERLOCK G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* 2000; 25:25n9. [PubMed: 10802651]
- BENJAMINI Y, DRAI D, ELMER G, KAFKAFI N, GOLANI I. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res.* 2001; 125:279–84. [PubMed: 11682119]
- BENJAMINI Y, YEKUTIELI D. Quantitative trait Loci analysis using the false discovery rate. *Genetics.* 2005; 171:783–90. [PubMed: 15956674]
- BIAN L, YANG JD, GUO TW, DUAN Y, QIN W, SUN Y, FENG GY, HE L. Association study of the A2M and LRP1 Genes with Alzheimer disease in the Han Chinese. *Biol Psychiatry.* 2005; 58:731–7. [PubMed: 16040006]
- BOLSTAD BM, IRIZARRY RA, ASTRAND M, SPEED TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics.* 2003; 19:185–93. [PubMed: 12538238]
- BORJABAD A, BROOKS AI, VOLSKY DJ. Gene expression profiles of HIV-1-infected glia and brain: toward better understanding of the role of astrocytes in HIV-1-associated neurocognitive disorders. *J Neuroimmune Pharmacol.* 5:44–62. [PubMed: 19697136]
- BREW BJ. Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. *Aids.* 2004; 18(Suppl 1):S75–8. [PubMed: 15075501]
- BUCKNER CM, CALDERON TM, WILLAMS DW, BELBIN TJ, BERMAN JW. Characterization of monocyte maturation/differentiation that facilitates their transmigration across the blood-brain

barrier and infection by HIV: implications for NeuroAIDS. *Cell Immunol.* 2011; 267:109–23. [PubMed: 21292246]

- CARLSON KA, CIBOROWSKI P, SCHELLPEPER CN, BISKUP TM, SHEN RF, LUO X, DESTACHE CJ, GENDELMAN HE. Proteomic fingerprinting of HIV-1-infected human monocyte-derived macrophages: a preliminary report. *J Neuroimmunol.* 2004; 147:35–42. [PubMed: 14741425]
- COSENZA MA, ZHAO ML, SI Q, LEE SC. Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. *Brain Pathol.* 2002; 12:442–55. [PubMed: 12408230]
- CYSIQUE LA, BREW BJ, HALMAN M, CATALAN J, SACKTOR N, PRICE RW, BROWN S, ATKINSON JH, CLIFFORD DB, SIMPSON D, TORRES G, HALL C, POWER C, MARDER K, MCARTHUR JC, SYMONDS W, ROMERO C. Undetectable cerebrospinal fluid HIV RNA and beta-2 microglobulin do not indicate inactive AIDS dementia complex in highly active antiretroviral therapy-treated patients. *J Acquir Immune Defic Syndr.* 2005; 39:426–9. [PubMed: 16010165]
- CYSIQUE LA, MARUFF P, BREW BJ. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. *J Neurovirol.* 2004; 10:350–7. [PubMed: 15765806]
- CYSIQUE LA, VAIDA F, LETENDRE S, GIBSON S, CHERNER M, WOODS SP, MCCUTCHAN JA, HEATON RK, ELLIS RJ. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. *Neurology.* 2009; 73:342–8. [PubMed: 19474412]
- DORE GJ, MCDONALD A, LI Y, KALDOR JM, BREW BJ. Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. *Aids.* 2003; 17:1539–45. [PubMed: 12824792]
- ELLERY PJ, TIPPETT E, CHIU YL, PAUKOVICS G, CAMERON PU, SOLOMON A, LEWIN SR, GORRY PR, JAWOROWSKI A, GREENE WC, SONZA S, CROWE SM. The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. *J Immunol.* 2007; 178:6581–9. [PubMed: 17475889]
- FANG XX, JIANG XL, HAN XH, PENG YP, QIU YH. Neuroprotection of Interleukin-6 Against NMDA-induced Neurotoxicity is Mediated by JAK/STAT3, MAPK/ERK, and PI3K/AKT Signaling Pathways. *Cell Mol Neurobiol.* 2012
- GELMAN BB, CHEN T, LISINICCHIA JG, SOUKUP VM, CARMICAL JR, STARKEY JM, MASLIAH E, COMMINS DL, BRANDT D, GRANT I, SINGER EJ, LEVINE AJ, MILLER J, WINKLER JM, FOX HS, LUXON BA. The National NeuroAIDS Tissue Consortium Brain Gene Array: Two Types of HIV-Associated Neurocognitive Impairment. *PLoS One.* 2012; 7:e46178. [PubMed: 23049970]
- GENDELMAN HE, PERSIDSKY Y, GHORPADE A, LIMOGES J, STINS M, FIALA M, MORRISETT R. The neuropathogenesis of the AIDS dementia complex. *Aids.* 1997; 11(Suppl A):S35–45. [PubMed: 9451964]
- GHAZALPOUR A, DOSS S, ZHANG B, WANG S, PLAISIER C, CASTELLANOS R, BROZELL A, SCHATZ EE, DRAKE TA, LUSIS AJ, HORVATH S. Integrating genetic and network analysis to characterize genes related to mouse weight. *PLoS Genet.* 2006; 2:e130. [PubMed: 16934000]
- GLASS JD, FEDOR H, WESSELINGH SL, MCARTHUR JC. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol.* 1995; 38:755–62. [PubMed: 7486867]
- HANS VH, KOSSMANN T, JOLLER H, OTTO V, MORGANTI-KOSSMANN MC. Interleukin-6 and its soluble receptor in serum and cerebrospinal fluid after cerebral trauma. *Neuroreport.* 1999; 10:409–12. [PubMed: 10203344]
- HORVATH S, DONG J. Geometric interpretation of gene coexpression network analysis. *PLoS Comput Biol.* 2008; 4:e1000117. [PubMed: 18704157]
- HORVATH S, ZHANG B, CARLSON M, LU KV, ZHU S, FELCIANO RM, LAURANCE MF, ZHAO W, QI S, CHEN Z, LEE Y, SCHECK AC, LIAU LM, WU H, GESCHWIND DH, FEBBO PG, KORNBLUM HI, CLOUGHESY TF, NELSON SF, MISCHER PS. Analysis of oncogenic

- signaling networks in glioblastoma identifies ASPM as a molecular target. *Proc Natl Acad Sci U S A*. 2006; 103:17402–7. [PubMed: 17090670]
- ITO H, WAKABAYASHI N, KATO H, ISHII T, IGARASHI K, ENGEL JD, YAMAMOTO M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev*. 1999; 13:76–86. [PubMed: 9887101]
- JANECZKO K. The proliferative response of S-100 protein-positive glial cells to injury in the neonatal rat brain. *Brain Res*. 1991; 564:86–90. [PubMed: 1777824]
- JOHNSON WE, RABINOVIC A, LI C. Adjusting batch effects in microarray expression data using Empirical Bayes methods. *Biostatistics*. 2007; 8:118–127. [PubMed: 16632515]
- KANG DE, SAITOH T, CHEN X, XIA Y, MASLIAH E, HANSEN LA, THOMAS RG, THAL LJ, KATZMAN R. Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology*. 1997; 49:56–61. [PubMed: 9222170]
- KAUL M, LIPTON SA. Mechanisms of neuronal injury and death in HIV-1 associated dementia. *Curr HIV Res*. 2006; 4:307–18. [PubMed: 16842083]
- KEDZIERSKA K, CROWE SM. The role of monocytes and macrophages in the pathogenesis of HIV-1 infection. *Curr Med Chem*. 2002; 9:1893–903. [PubMed: 12369874]
- KIM SY, LI J, BENTSMAN G, BROOKS AI, VOLSKY DJ. Microarray analysis of changes in cellular gene expression induced by productive infection of primary human astrocytes: implications for HAD. *J Neuroimmunol*. 2004; 157:17–26. [PubMed: 15579276]
- KOLSON DL, SABNEKAR P, BAYBIS M, CRINO PB. Gene expression in TUNEL-positive neurons in human immunodeficiency virus-infected brain. *J Neurovirol*. 2004; 10(Suppl 1):102–7. [PubMed: 14982747]
- KOUNNAS MZ, MOIR RD, REBECK GW, BUSH AI, ARGRAVES WS, TANZI RE, HYMAN BT, STRICKLAND DK. LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation. *Cell*. 1995; 82:331–40. [PubMed: 7543026]
- KRAFT-TERRY SD, BUCH SJ, FOX HS, GENDELMAN HE. A coat of many colors: neuroimmune crosstalk in human immunodeficiency virus infection. *Neuron*. 2009; 64:133–45. [PubMed: 19840555]
- KUWABARA K, MATSUMOTO M, IKEDA J, HORI O, OGAWA S, MAEDA Y, KITAGAWA K, IMUTA N, KINOSHITA T, STERN DM, YANAGI H, KAMADA T. Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. *J Biol Chem*. 1996; 271:5025–32. [PubMed: 8617779]
- LANGFELDER P, HORVATH S. Eigengene networks for studying the relationships between co-expression modules. *BMC Syst Biol*. 2007; 1:54. [PubMed: 18031580]
- LANGFELDER P, HORVATH S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008; 9:559. [PubMed: 19114008]
- LANGFELDER P, ZHANG B, HORVATH S. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. *Bioinformatics*. 2008; 24:719–20. [PubMed: 18024473]
- LANGFORD TD, LETENDRE SL, LARREA GJ, MASLIAH E. Changing patterns in the neuropathogenesis of HIV during the HAART era. *Brain Pathol*. 2003; 13:195–210. [PubMed: 12744473]
- LAWTON MP, BRODY EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist*. 1969; 9:179–86. [PubMed: 5349366]
- LETENDRE S, MARQUIE-BECK J, CAPPARELLI E, BEST B, CLIFFORD D, COLLIER AC, GELMAN BB, MCARTHUR JC, MCCUTCHAN JA, MORGELLO S, SIMPSON D, GRANT I, ELLIS RJ. Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch Neurol*. 2008; 65:65–70. [PubMed: 18195140]
- LEVINE AJ, MILLER JA, SHAPSHAK P, GELMAN B, SINGER EJ, HINKIN CH, COMMINS D, MORGELLO S, GRANT I, HORVATH S. Systems analysis of human brain gene expression: mechanisms for HIV-associated neurocognitive impairment and common pathways with Alzheimer's disease. *BMC Med Genomics*. 2013; 6:4. [PubMed: 23406646]

- LINDL KA, AKAY C, WANG Y, WHITE MG, JORDAN-SCIUTTO KL. Expression of the endoplasmic reticulum stress response marker, BiP, in the central nervous system of HIV-positive individuals. *Neuropathol Appl Neurobiol.* 2007; 33:658–69. [PubMed: 17931354]
- LIU Y, JONES M, HINGTGEN CM, BU G, LARIBEE N, TANZI RE, MOIR RD, NATH A, HE JJ. Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med.* 2000; 6:1380–7. [PubMed: 11100124]
- LUO X, CARLSON KA, WOJNA V, MAYO R, BISKUP TM, STONER J, ANDERSON J, GENDELMAN HE, MELENDEZ LM. Macrophage proteomic fingerprinting predicts HIV-1-associated cognitive impairment. *Neurology.* 2003; 60:1931–7. [PubMed: 12821735]
- MARRA CM, LOCKHART D, ZUNT JR, PERRIN M, COOMBS RW, COLLIER AC. Changes in CSF and plasma HIV-1 RNA and cognition after starting potent antiretroviral therapy. *Neurology.* 2003; 60:1388–90. [PubMed: 12707454]
- MASLIAH E, ROBERTS ES, LANGFORD D, EVERALL I, CREWS L, ADAME A, ROCKENSTEIN E, FOX HS. Patterns of gene dysregulation in the frontal cortex of patients with HIV encephalitis. *J Neuroimmunol.* 2004; 157:163–75. [PubMed: 15579294]
- MASON MJ, FAN G, PLATH K, ZHOU Q, HORVATH S. Signed weighted gene co-expression network analysis of transcriptional regulation in murine embryonic stem cells. *BMC Genomics.* 2009; 10:327. [PubMed: 19619308]
- MCARTHUR JC, BREW BJ, NATH A. Neurological complications of HIV infection. *Lancet Neurol.* 2005; 4:543–55. [PubMed: 16109361]
- MILLER JA, CAI C, LANGFELDER P, GESCHWIND DH, KURIAN SM, SALOMON DR, HORVATH S. Strategies for aggregating gene expression data: The collapseRows R function. *BMC Bioinformatics.* 2011; 12:322. [PubMed: 21816037]
- MORGELLO S, GELMAN BB, KOZLOWSKI PB, VINTERS HV, MASLIAH E, CORNFORD M, CAVERT W, MARRA C, GRANT I, SINGER EJ. The National NeuroAIDS Tissue Consortium: a new paradigm in brain banking with an emphasis on infectious disease. *Neuropathol Appl Neurobiol.* 2001; 27:326–35. [PubMed: 11532163]
- MOTOHASHI H, YAMAMOTO M. Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol Med.* 2004; 10:549–57. [PubMed: 15519281]
- OLDHAM MC, HORVATH S, GESCHWIND DH. Conservation and evolution of gene coexpression networks in human and chimpanzee brains. *Proc Natl Acad Sci U S A.* 2006; 103:17973–8. [PubMed: 17101986]
- OLDHAM MC, LANGFELDER P, HORVATH S. Network methods for describing sample relationships in genomic datasets: application to Huntington's disease. *BMC Syst Biol.* 2012; 6:63. [PubMed: 22691535]
- OTANI I, AKARI H, NAM KH, MORI K, SUZUKI E, SHIBATA H, DOI K, TERAOKA K, YOSIKAWA Y. Phenotypic changes in peripheral blood monocytes of cynomolgus monkeys acutely infected with simian immunodeficiency virus. *AIDS Res Hum Retroviruses.* 1998; 14:1181–6. [PubMed: 9737589]
- OZAWA K, KONDO T, HORI O, KITAO Y, STERN DM, EISENMENGER W, OGAWA S, OHSHIMA T. Expression of the oxygen-regulated protein ORP150 accelerates wound healing by modulating intracellular VEGF transport. *J Clin Invest.* 2001; 108:41–50. [PubMed: 11435456]
- PADBERG F, FENEBERG W, SCHMIDT S, SCHWARZ MJ, KORSCHENHAUSEN D, GREENBERG BD, NOLDE T, MULLER N, TRAPMANN H, KONIG N, MOLLER HJ, HAMPEL H. CSF and serum levels of soluble interleukin-6 receptors (sIL-6R and sgp130), but not of interleukin-6 are altered in multiple sclerosis. *J Neuroimmunol.* 1999; 99:218–23. [PubMed: 10505978]
- PEDERSEN KK, PEDERSEN M, GAARDBO JC, RONIT A, HARTLING HJ, BRUUNSGAARD H, GERSTOFT J, ULLUM H, NIELSEN SD. Persisting Inflammation and Chronic Immune Activation but Intact Cognitive Function in HIV-infected patients After Long Term Treatment with Combination Antiretroviral Therapy. *J Acquir Immune Defic Syndr.* 2013
- PELUSO R, HAASE A, STOWRING L, EDWARDS M, VENTURA P. A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology.* 1985; 147:231–6. [PubMed: 2998068]

- PEREZ DI, GIL C, MARTINEZ A. Protein kinases CK1 and CK2 as new targets for neurodegenerative diseases. *Med Res Rev.* 2011; 31:924–54. [PubMed: 20577972]
- PERSIDSKY Y, GHORPADE A, RASMUSSEN J, LIMOGES J, LIU XJ, STINS M, FIALA M, WAY D, KIM KS, WITTE MH, WEINAND M, CARHART L, GENDELMAN HE. Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am J Pathol.* 1999; 155:1599–611. [PubMed: 10550317]
- PERSIDSKY Y, ZHENG J, MILLER D, GENDELMAN HE. Mononuclear phagocytes mediate blood-brain barrier compromise and neuronal injury during HIV-1-associated dementia. *J Leukoc Biol.* 2000; 68:413–22. [PubMed: 10985259]
- PULLIAM L, CLARKE JA, MCGRATH MS, MOORE D, MCGUIRE D. Monokine products as predictors of AIDS dementia. *AIDS.* 1996; 10:1495–500. [PubMed: 8931783]
- PULLIAM L, GASCON R, STUBBLEBINE M, MCGUIRE D, MCGRATH MS. Unique monocyte subset in patients with AIDS dementia. *Lancet.* 1997; 349:692–5. [PubMed: 9078201]
- PULLIAM L, REMPEL H, SUN B, ABADJIAN L, CALOSING C, MEYERHOFF DJ. A peripheral monocyte interferon phenotype in HIV infection correlates with a decrease in magnetic resonance spectroscopy metabolite concentrations. *AIDS.* 2011; 25:1721–6. [PubMed: 21750421]
- PULLIAM L, SUN B, REMPEL H. Invasive chronic inflammatory monocyte phenotype in subjects with high HIV-1 viral load. *J Neuroimmunol.* 2004; 157:93–8. [PubMed: 15579285]
- RADLOFF LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement.* 1977; 1:385–401.
- RAVASZ E, SOMERA AL, MONGRU DA, OLTVAI ZN, BARABASI AL. Hierarchical organization of modularity in metabolic networks. *Science.* 2002; 297:1551–5. [PubMed: 12202830]
- ROBERTS ES, BURUDI EM, FLYNN C, MADDEN LJ, ROINICK KL, WATRY DD, ZANDONATTI MA, TAFTE MA, FOX HS. Acute SIV infection of the brain leads to upregulation of IL6 and interferon-regulated genes: expression patterns throughout disease progression and impact on neuroAIDS. *J Neuroimmunol.* 2004; 157:81–92. [PubMed: 15579284]
- ROBERTS ES, ZANDONATTI MA, WATRY DD, MADDEN LJ, HENRIKSEN SJ, TAFTE MA, FOX HS. Induction of pathogenic sets of genes in macrophages and neurons in NeuroAIDS. *Am J Pathol.* 2003; 162:2041–57. [PubMed: 12759259]
- ROBERTSON KR, SMURZYNSKI M, PARSONS TD, WU K, BOSCH RJ, WU J, MCARTHUR JC, COLLIER AC, EVANS SR, ELLIS RJ. The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS.* 2007; 21:1915–21. [PubMed: 17721099]
- SACKTOR N, MCDERMOTT MP, MARDER K, SCHIFITTO G, SELNES OA, MCARTHUR JC, STERN Y, ALBERT S, PALUMBO D, KIEBURTZ K, DE MARCAIDA JA, COHEN B, EPSTEIN L. HIV-associated cognitive impairment before and after the advent of combination therapy. *J Neurovirol.* 2002; 8:136–42. [PubMed: 11935465]
- SALAZAR-GONZALEZ JF, MARTINEZ-MAZA O, AZIZ N, KOLBERG JA, YEGHIAZARIAN T, SHEN LP, FAHEY JL. Relationship of plasma HIV-RNA levels and levels of TNF-alpha and immune activation products in HIV infection. *Clin Immunol Immunopathol.* 1997; 84:36–45. [PubMed: 9191882]
- SHAPSHAK PDR, DURAN EM, FARINETTI F, MINAGAR A, COMMINS D, RODRIGUEZ H, CHIAPPELLI F, KANGUEANE P, LEVINE AJ, SINGER E, SOMBOONWIT C, SINNOTT JT. Gene expression for HIV-associated dementia and HIV encephalitis in microdissected neurons 1: preliminary analysis. *Neurobehavioral HIV Medicine.* 2011; 3:53–78.
- SHAPSHAK P, DUNCAN R, MINAGAR A, RODRIGUEZ DE LA VEGA P, STEWART RV, GOODKIN K. Elevated expression of IFN-gamma in the HIV-1 infected brain. *Front Biosci.* 2004a; 9:1073–81. [PubMed: 14977530]
- SHAPSHAK P, DUNCAN R, TORRES-MUNOZ JE, DURAN EM, MINAGAR A, PETITO CK. Analytic approaches to differential gene expression in AIDS versus control brains. *Front Biosci.* 2004b; 9:2935–46. [PubMed: 15353327]
- SHAY AH, CHOI R, WHITTAKER K, SALEHI K, KITCHEN CM, TASHKIN DP, ROTH MD, BALDWIN GC. Impairment of antimicrobial activity and nitric oxide production in alveolar

- macrophages from smokers of marijuana and cocaine. *J Infect Dis.* 2003; 187:700–4. [PubMed: 12599091]
- STOREY JD, TAYLOR JE, SIEGMUND D. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. *Journal of the Royal Statistical Society Series B-Statistical Methodology.* 2004; 66:187–205.
- SUN B, ABADJIAN L, REMPEL H, CALOSING C, ROTHLIND J, PULLIAM L. Peripheral biomarkers do not correlate with cognitive impairment in highly active antiretroviral therapy-treated subjects with human immunodeficiency virus type 1 infection. *J Neurovirol.* 2010; 16:115–24. [PubMed: 20307252]
- TAMATANI M, MATSUYAMA T, YAMAGUCHI A, MITSUDA N, TSUKAMOTO Y, TANIGUCHI M, CHE YH, OZAWA K, HORI O, NISHIMURA H, YAMASHITA A, OKABE M, YANAGI H, STERN DM, OGAWA S, TOHYAMA M. ORP150 protects against hypoxia/ischemia-induced neuronal death. *Nat Med.* 2001; 7:317–23. [PubMed: 11231630]
- TANJI K, MARUYAMA A, ODAGIRI S, MORI F, ITOH K, KAKITA A, TAKAHASHI H, WAKABAYASHI K. Keap1 is localized in neuronal and glial cytoplasmic inclusions in various neurodegenerative diseases. *J Neuropathol Exp Neurol.* 2013; 72:18–28. [PubMed: 23242280]
- THIEBLEMONT N, WEISS L, SADEGHI HM, ESTCOURT C, HAEFFNER-CAVAILLON N. CD14^{low}CD16^{high}: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. *Eur J Immunol.* 1995; 25:3418–24. [PubMed: 8566032]
- TOZZI V, BALESTRA P, BELLAGAMBA R, CORPOLONGO A, SALVATORI MF, VISCO-COMANDINI U, VLASSI C, GIULIANELLI M, GALGANI S, ANTINORI A, NARCISO P. Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: prevalence and risk factors. *J Acquir Immune Defic Syndr.* 2007; 45:174–82. [PubMed: 17356465]
- VOINEAGU I, WANG X, JOHNSTON P, LOWE JK, TIAN Y, HORVATH S, MILL J, CANTOR RM, BLENOWE BJ, GESCHWIND DH. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature.* 2011; 474:380–4. [PubMed: 21614001]
- WARRINER EM, ROURKE SB, ROURKE BP, RUBENSTEIN S, MILLIKIN C, BUCHANAN L, CONNELLY P, HYRCZA M, OSTROWSKI M, DER S, GOUGH K. Immune activation and neuropsychiatric symptoms in HIV infection. *J Neuropsychiatry Clin Neurosci.* 2010; 22:321–8. [PubMed: 20686139]
- WOJNA V, CARLSON KA, LUO X, MAYO R, MELENDEZ LM, KRAISELBURD E, GENDELMAN HE. Proteomic fingerprinting of human immunodeficiency virus type 1-associated dementia from patient monocyte-derived macrophages: A case study. *J Neurovirol.* 2004; 10(Suppl 1):74–81. [PubMed: 14982743]
- YADAV A, COLLMAN RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *J Neuroimmune Pharmacol.* 2009; 4:430–47. [PubMed: 19768553]
- YIP AM, HORVATH S. Gene network interconnectedness and the generalized topological overlap measure. *BMC Bioinformatics.* 2007; 8:22. [PubMed: 17250769]
- YOSHIOKA M, BRADLEY WG, SHAPSHAK P, NAGANO I, STEWART RV, XIN KQ, SRIVASTAVA AK, NAKAMURA S. Role of immune activation and cytokine expression in HIV-1-associated neurologic diseases. *Adv Neuroimmunol.* 1995; 5:335–58. [PubMed: 8748077]
- YOSHIOKA M, SHAPSHAK P, SRIVASTAVA AK, STEWART RV, NELSON SJ, BRADLEY WG, BERGER JR, RHODES RH, SUN NC, NAKAMURA S. Expression of HIV-1 and interleukin-6 in lumbosacral dorsal root ganglia of patients with AIDS. *Neurology.* 1994; 44:1120–30. [PubMed: 7516054]
- ZHANG B, HORVATH S. A general framework for weighted gene co-expression network analysis. *Statistical Applications in Genetics and Molecular Biology.* 2005a:4.
- ZHANG B, HORVATH S. A general framework for weighted gene co-expression network analysis. *Stat Appl Genet Mol Biol.* 2005b; 4:Article17. [PubMed: 16646834]
- ZHAO J, REDELL JB, MOORE AN, DASH PK. A novel strategy to activate cytoprotective genes in the injured brain. *Biochem Biophys Res Commun.* 2011; 407:501–6. [PubMed: 21414291]

ZHAO L, ROSALES C, SEBURN K, RON D, ACKERMAN SL. Alteration of the unfolded protein response modifies neurodegeneration in a mouse model of Marinesco-Sjogren syndrome. *Hum Mol Genet.* 2010; 19:25–35. [PubMed: 19801575]

Highlights

- This study used standard differential gene expression analysis and WGCNA
- Expression of several genes was genes associated with neurocognitive functioning
- Two biological processes were associated with neurocognitive functioning
- No associations were found with HAND diagnosis, CD4, viral load, or substance use

Figure 1.

The gene clustering dendrograms (top) shows that co-expressed genes tend to cluster into modules corresponding to major biological divisions (cell type, molecular function, etc), as well as disease-relevant changes. The dendrogram results from hierarchical clustering and groups genes into distinct modules. The y-axis corresponds to dissimilarity determined by the extent of topological overlap (1-TO). Top color band (Module label): dynamic tree cutting was used to identify highly parsimonious module definitions, generally dividing modules at significant branch points in the dendrogram. Labels below the module band indicate numeric module labels (1–24) and highest enriched GO terms that suggest the functional interpretation of the modules (modules without a GO term were not significantly enriched in any GO term). The remaining rows indicate the strength of associations between the clinical variables (listed on the left) and individual probes. Red indicates a positive correlation, blue indicates negative correlation.

Figure 2.

Tombstone chart showing correlations and p-values between modules and clinical variables. After correcting for multiple testing using FDR, module 13 (Mitotic Cell Cycle) was positively correlated with Global Neurocognitive Functioning and module 11 (No clear gene ontology) was negatively correlated with Global Neurocognitive Functioning at the suggestive level of $q=0.14$. Other associations with nominal p-values of less than 0.05 are shown, although these did not survive FDR correction.

Table 1

Sample characteristics

	Mean	Median	Standard Deviation	Range
AGE	52	52	9.6	30–67
GNF*	49	48	14	23–78
Nadir CD4	250	260	150	1–670
Current CD4	590	580	200	73–1100
Viral Load	0.38	0	1.2	0–5.2
CES-D	14	10	12	0–48
CPE	1.5	1.5	0.77	0–4.5

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Table 2

Substance use and HAND status

	N	Percent of Sample
Current HAND		
Diagnosis		
Normal	33	38
ANI	18	21
MND	26	30
HAD	9	10
Smoke Tobacco		
Never	21	25
Past	47	54
Current	18	21
Alcohol Use		
None/Rarely	45	51
Monthly	19	23
Weekly	10	12
Daily	12	14
Cannabis Use		
None/Rarely	62	71
Monthly	4	5
Weekly	8	10
Daily	12	14
Cocaine Use		
None/Rarely	84	98
Monthly	0	0
Weekly	2	2
Daily	0	0
Heroin Use		
None/Rarely	86	100

Table 3

Significant associations between gene expression and Global Neurocognitive Functioning

Gene Name (HGNC Approved Gene Symbol)	R	q-value
Chromosome 3 open reading frame 17 (C3orf17)	-.472	0.03
Cytoplasmic FMRP-Interacting Protein-2 (CYFIP2)	.475	0.03
Testis-Derived Transcript (TES)	.478	0.03
Prostate androgen-regulated mucin-like protein (PARM1)	.483	0.03
Inositol Hexaphosphate Kinase 1 (IHPK1)	-.474	0.03
GPI-Anchored Membrane Protein-1 (CAPRIN1)	-.468	0.03
Hypoxia Up-regulated-1 (HYOU1)	.451	0.05
Interleukin 6-Receptor (IL6R)	-.435	0.07
GPN-loop GTPase-2 (GPN2)	-.437	0.07
Pumilio, Drosophila, Homolog Of, 1 (Pum1)	-.436	0.07
Casein Kinase I, Alpha-1 (CSNK1A1)	-.437	0.07
Vibrio Polysaccharide (VPS)	.431	0.07
Low density lipoprotein receptor-related protein 12 (LRP12)	-.425	0.08
Mannose-1-phosphate guanyltransferase beta (GMPPB)	.425	0.08
Nodal Modulator-1 (NOMO1)	.426	0.08
Kelch-Like ECH-Associated Protein-1 (KEAP1)	-.419	0.09

Enrichment of gene modules in selected GO terms(Ashburner et al., 2000). For each module, the table lists its size (number of probe sets), GO term, enrichment p-value, Bonferroni-corrected enrichment p-value, and number of genes from the module that are included in the listed GO term. For each module we only list the top few terms. Modules not listed in this table were not significantly enriched in any GO terms (after Bonferroni correction)

Table 4

Module	Size	Term (Ontology)	p	Bonferroni	Genes in term
1	4812	cytoplasmic part (CC)	6.90E-43	8.50E-39	1450
1	4812	mitochondrion (CC)	2.90E-40	3.60E-36	464
1	4812	catalytic activity (MF)	7.40E-32	9.20E-28	1189
2	4018	regulation of cellular metabolic process (BP)	3.10E-29	3.80E-25	735
2	4018	nucleus (CC)	6.90E-27	8.50E-23	945
3	806	ribonucleoprotein complex (CC)	4.90E-15	6.20E-11	37
3	806	mRNA metabolic process (BP)	5.60E-15	7.00E-11	39
4	788	nucleus (CC)	1.70E-20	2.20E-16	258
4	788	RNA metabolic process (BP)	9.30E-15	1.20E-10	174
4	788	membrane-bounded organelle (CC)	2.80E-14	3.50E-10	334
5	770	cytoplasmic part (CC)	2.60E-09	3.30E-05	196
6	640	zinc ion binding (MF)	8.00E-05	0.99	44
7	631	platelet activation (BP)	2.00E-18	2.50E-14	32
7	631	blood coagulation (BP)	8.90E-17	1.10E-12	46
8	624	intracellular membrane-bounded organelle (CC)	2.30E-13	2.80E-09	281
8	624	nucleus (CC)	6.00E-11	7.40E-07	195
9	349	hemoglobin complex (CC)	1.70E-15	2.10E-11	9
10	271	immune response (BP)	1.40E-19	1.80E-15	44
11	250	translational elongation (BP)	2.50E-05	0.31	6
11	250	mRNA metabolic process (BP)	1.00E-04	1	12
12	159	mitochondrial respiratory chain (CC)	1.00E-05	0.12	5
12	159	respiratory chain (CC)	1.50E-05	0.19	5
13	150	mitotic cell cycle (BP)	7.90E-17	9.80E-13	28
14	119	RNA binding (MF)	8.40E-07	0.011	14
14	119	ribonucleoprotein complex (CC)	2.30E-05	0.29	10
15	102	type I interferon-mediated signaling pathway (BP)	2.50E-27	3.10E-23	17
15	102	innate immune response (BP)	4.40E-27	5.40E-23	29

Module	Size	Term (Ontology)	p	Bonferroni	Genes in term
15	102	cytokine-mediated signaling pathway (BP)	1.20E-23	1.50E-19	23
16	87	interleukin-5-mediated signaling pathway (BP)	8.60E-06	0.11	2
20	50	response to bacterium (BP)	6.40E-19	7.90E-15	15
20	50	extracellular region (CC)	2.00E-13	2.40E-09	21
20	50	defense response to fungus (BP)	1.20E-12	1.50E-08	6
23	28	response to external stimulus (BP)	1.10E-07	0.0014	8
24	28	maternal aggressive behavior (BP)	4.00E-06	0.049	2

CC (Cellular Compartment), MF (Molecular Function) and BP (Biological Process) are the three ontologies used to classify GO terms, as described in (Ashburner et al., 2000).

Table 5

Associations of previously implicated mRNA transcripts with Global Neurocognitive Functioning and HAND

Gene Product (HGNC Approved Gene Symbol)	Global Neurocognitive Functioning		HAND Diagnosis	
	R	q-value*	R	q-value*
Osteopontin (SPP1)(Buckner et al., 2011)	.051	.68	.011	.997
Monocyte Chemotactic Protein 1 (CCL2)(Pulliam et al., 2004)	-.036	.71	-.019	.997
Chemokine-CC Motif Receptor 5 (CCR5)(Pulliam et al., 2004)	-.078	.63	-.033	.997
Enolase-2 (ENO2)(Buckner et al., 2011)	-.143	.49	.132	.997
Ubiquitin-like Modifier ISG15 (ISG15)(Roberts et al., 2003)	.054	.62	.066	.997
Interferon-Gamma-Inducible Protein-10 (CXCL10)(Pulliam et al., 2011)	-.119	.54	.198	.997
Interleukin 6 (IL6)(Yang et al., 2009)	-.063	.66	.046	.997
Sialoadhesin (SIGLEC1)(Pulliam et al., 2004)	-.01	.92	.182	.997