[Frontiers in Bioscience 9, 2935-2946, September 1, 2004]

ANALYTIC APPROACHES TO DIFFERENTIAL GENE EXPRESSION IN AIDS vs. CONTROL BRAINS

Shapshak, P [1-5], Duncan, R [6], Torres-Munoz JE [3], Duran EM[1], Minagar A [7], and Petito CK [3]

[1]Department of Psychiatry & Behavioral Sciences, [2]Department of Neurology, [3]Department of Pathology (Neuropathology), 4Comprehensive Drug Research Center, [5]Pediatrics McDonald Foundation GeneTeam, [6]Department of Epidemiology, University of Miami School of Medicine, Miami, FL 33136, [7]Department of Neurology, Louisiana State University Health Sciences Center-Shreveport, Shreveport, LA 71130, United States of America

TABLE OF CONTENTS

1. ABSTRACT

We previously showed that specific strains of human immunodeficiency virus (HIV)-1 infect the brain and contribute to Neuropathology, Cognitive Distress, and Neuropsychiatric Disease (1). To study further brain disease that results from HIV-1 infection, we commenced analysis of changes in gene expression in brain (2, 3). We analyzed RNA purified from Frontal Cortex of 5 HIV-1 infected and 4 HIV-1 negative control subjects RNA was amplified and Affymetrix technology was used to analyze gene expression using the 12,585 gene Affymetrix Human Genome U95A chip. The expressed genes showed highly significant Pearson's correlations with each other within the two groups. Expression intensities were transferred to Microsoft Excel and Spotfire was used to analyze the results. Twenty-group K-means cluster analysis was done for HIV+ and HIV- subjects. Genes that were expressed in the same cluster numbers in the two groups were removed from further analysis. Analysis of Gene expression in the top 13 HIV+ clusters showed expression in the 40 gene categories designated in our prior studies. Genes from several categories occurred in more than one K-means cluster. Genes identified in these lists included several genes that have been previously studied: MBP, Myelin-PLP, NMDA receptor, MAG, astrocytic protein, Notch 3, APP, Senescence, proteasome, Ferritin, signaling, cell cycle, iNOS, Chemokine, splicing, synapse, protein tags, and ribosomal proteins. The first (primary significant) axis of both Principal Component Analyses ordered the genes in the same patient groups as the K-means cluster analysis for the respective patient groups. PCA was thus not more informative than K-Means cluster analysis. Ratios of HIV+ to HIV- intensities were calculated for all the averaged gene expression intensities. The ratio range was 0.14 to 9.26. The genes at the extremes (*ad extrema*) did not correspond to the gene order by K-means clustering (or PCA). The genes in the top 13 K-means clusters showed low-level changes by expression ratio. Genes *ad extrema* by ratio were in clusters with very large memberships. Mann-Whitney analysis confirmed expression ratio results. Several inferences result from our preliminary study. First, study design will be different in future studies involving additional replicates. Second, ratios inform us of the extent of changes in gene expression quantitatively. Third, Cluster methodology provides us with more subtle information, how bunches (clusters) of genes behave in terms of their centroids (attractors). Fourth, genes that change extensively by ratio tend to be in the larger k-Means clusters. We conclude that ranking gene expression with the use of expression ratio or by K-means clustering, yield different representations of the data.

2. INTRODUCTION

Recently, Microarray technology emerged as a technique to monitor rapidly and efficiently transcript abundance for all of any organism's genes. Global and select gene expression can be analyzed (4.). The systematic characterization of expression patterns by Microarray technology is the natural extension of differential display and cloning studies (5.). These methods have the advantage of generating data relating the activity of many gene families for which data is unavailable currently. Overall, Microarray technology potentially allows an integrated understanding of the process of transcription for an entire genome health and in disease. Microarrays act as gene probes for hybridization to labeled RNA or cDNA prepared from RNA samples. Microarrays can be constructed from sources such as specific cDNA clones of interest, a cDNA library, or oligonucleotide RNA or DNA specific for a select number of open reading frames (ORFs) from any sequenced genome database (www.Affymetrix.com, Santa Clara, CA). An important question is whether Bioinformatics data analysis tools can keep up with data production by Microarray technology (6, 7, 8, 9). Additionally, questions in ethics are raised by wide scale analysis of human gene expression (10).

2.1. AIDS and Drug Abuse (Intersecting Epidemics)

World-wide, drug addiction and AIDS are viewed as intersecting epidemics… the behavioral risks exhibited by each in initially supposedly separate venues are seen as overlapping and evolve to fuel each other (11). Drug addiction is a significant risk factor for human immunodeficiency virus (HIV-1) infection worldwide (12, 13). Intravenous injection of drugs accounts for as many as 50% of new cases of HIV-1 infection in the USA annually (14). Similar phenomena are demonstrated for drug abuse, sexual transmission, and risk of HIV-1 infection in Miami (15, 16, 17, 18).

2.2. HIV-1 Heterogeneity

HIV-1 associated brain disease involves interactions between host and virus. HIV-1 comprising nine genes is able to destroy its human host during the course of 9 years on the average or more (when treatment is limiting). Even the most advanced therapies have limitations. With an elevated replication capacity added to a high degree of mutation at a rate of $\geq$ 9 mutations per (provirus) genome of 9,000 base pairs, HIV-1 shows one of the highest mutation rates known for any organism. These features in combination with the persistence within a long-lived CD4-T cell compartment allow the infection to evolve eventually to resist therapy. The infecting virus populations of sequences are not considered a single species but swarms, quasispecies, or sequence clusters. In addition, HIV strains are divided between HIV-1 and HIV-2 with subdivisions including subtypes A, B, C, D, etc. HIV-1B predominates in the USA (19). HIV's extreme heterogeneity and high-paced evolution are major barriers to treatment especially to developing vaccines as well as other immune based therapies (20, 21).

2.3 HIV-1 Neurodegenerative disease

In chronically infected individuals, heterogeneous and distinct viral populations are found in different compartments of the human body (22). Furthermore, accumulating evidence shows that the structure of the HIV genome in the brain is even further sub compartmentalized (1, 23) and that HIV recombination events in the brain are frequent (24). These findings in combination with the characteristics of macrophage/microglia as a reservoir are relevant to understanding how the HIV infection pool is generated, maintained and hopefully, how it might be reduced or eliminated. The host response is also a crucial aspect of the pathogenesis of AIDS and HIV Associated Dementia. Many host genes are known to be involved due to prior studies over several decades. HIV-1 associated dementia is a neuroinflammatory brain disorder that is fueled by viral infection and immune activation of brain mononuclear phagocytes (MP-macrophages and microglia) (25). Prior studies over the past few decades have shown that many host genes involved in primary immune responses indeed are known to be involved and to facilitate the HIV viral life cycle, i.e. chemokine receptors. Schutzer et al (26) has recently proposed the possibility of associated autoimmune phenomena in addition to direct viral effects in HAD. With the advent of the new large-scale laboratory specimen processing (High Through-put) and information processing (Bioinformatics) methodologies, development of AIDS dementia models is progressing (3, 27, 28, 29; and reviewed by Minagar A, P Shapshak, EM Duran, AS Kablinger, JS Alexander, RE Kelley, & Toni Kazic, Gene expression in neurologic and psychiatric disorders: HIV-Associated Dementia, Alzheimer's disease, multiple sclerosis, and schizophrenia, submitted, [2004]) A few representative aspects of pathogenesis involving host-viral interactions are outlined as follows.

2.3.1. HIV-1 infection of the brain is associated with Neuropsychiatric disease

HIV-1 crosses the Blood-Brain Barrier (BBB) and infects the brain early in AIDS disease (30, 31, 32, 33, 34). HIV-1 infection of the brain causes cognitive motor disorders, disrupts host gene expression, and is compounded by abused drugs (35, 36, 37, 38, 39). Patients show brain damage at the time of seroconversion as well as later after post-infection. Thus, latent HIV-1 brain infection may be a component of pathogenic mechanisms. Not all HIV-1-infected patients show brain disease clinically. CSF studies and a few post-mortem analyses indicate brain-related inflammation early in disease and that possibly the brain itself may be infected (30, 31, 32, 33, 34, 40, 41, 42, 43; Shapshak P, Segal D, Srivastava AK, Stewart RV, Douyon A, Fujimura RK, and Goodkin K, Detection of HIV-1 using PCR techniques in brain tissue from HIV-1 infected patients: clinical neuropsychiatric and neuropathological correlation with HIV-1 Associated Dementia, in progress, 2004). This paradox has not been explained and several additional hypotheses have been advanced including ongoing infection of the brain due to carriage of HIV into the brain from bone marrow by recruited macrophage/monocytes (44 McArthur et al, 2003). . This is a productive reason as well as advantage for the use of wider data acquisition on brain gene expression through Microarray analysis.

2.3.2. HIV-1 infection of the brain is associated with pathology

Macrophage/microglia are the key cells exhibiting productive infection and serve as reservoirs for

persistent viral infection, vehicles for viral dissemination in the brain, and a major source of neurotoxic products that when produced in abundance (43, 45, 46). Furthermore, chemokine receptors including CCR3, CCR5, and CXCR4 are expressed on neural cells (microglia, astrocytes and neurons) and these are the cells that suffer damage during progressive HIV-1 infection of the central nervous system (23, 47). The ingress of HIV-1 through the BBB results in inflammation termed HIV encephalitis (HIVE). This process involves activation of brain cells including macrophage/microglia and astrocytes and consequent generation of several inflammatory host and viral factors (including cytokines, chemokines, quinolinic acid, tumor necrosis factor-α, platelet-activating factor, nitric oxide, peroxynitrite, neopterins, gp120, tat, and nef) (41, 48, 43, 49, 50, 51,) as well as neuronal destruction and apoptosis (45, 52).

2.4. Microarray Chips in Neurodegenerative Disease

Microarrays technology is a rapidly expanding field. Several brain studies utilized Affymetrix (Santa Clara, CA) as well as other formats for gene expression analysis (reviewed by Minagar A, P Shapshak, EM Duran, AS Kablinger, JS Alexander, RE Kelley, R. Seth, T. Kazic, Gene expression in neurologic and psychiatric disorders: HIV-Associated Dementia, Alzheimer's disease, Multiple Sclerosis, and Schizophrenia, *submitted*, 2004). Microarray technology has been employed to characterize the genomic features of several human diseases, including animal and cell culture models of brain HIV-1 infection, Multiple Sclerosis (53), Rheumatoid Arthritis (54), Alzheimer's disease (55, 56), and Schizophrenia (57, 58). Microarrays were used to analyze HIV-host interactions. Geiss et al (59) analyzed host cell gene expression during HIV-1 infection. They studied the expression of 1,506 genes during HIV-1 infection of the CD4+ T-cell line CEM-CCRF and identified 20 mRNAs from various cellular pathways, whose levels were changed due to HIV-1 infection. These genes were involved in a number of cellular processes including T-cell receptor-mediated signaling, subcellular trafficking, transcriptional regulation, and a variety of cellular metabolic pathways as well as other genes involved in the host cell defense mechanisms and facilitation of the viral life cycle.

Galey *et al.* (60) studied gene expression in astrocytes in the context of HIV infection. The investigators used Microarray analysis and ribonuclease protection assays (RPA) and showed that HIV infection and gp120 treatment effected gene expression. Of the 1153 oligonucleotides on the immune-based array, the expression of 108 genes (53 up regulated; 55 down regulated) and 82 genes (32 up regulated; 50 down regulated) were significantly changed by gp120 and HIV infection. Of the 1153 oligonucleotides on the neuro-based array, 58 genes (25 up regulated; 33 down regulated) and 47 genes (17 up regulated; 30 down regulated) were significantly altered by gp120 and HIV infection respectively.

Animal models serve as important arenas for developing and testing HIV hypotheses and models and 70-80% of the genes examined on the Affymetrix U95A GeneChip showed similar expression profiles in frontal cortex among human, chimpanzees, orangutans, and macaques (61). Recent work by Roberts et al (62) used SIVmac182 and Rhesus Macaques to characterize brain gene expression 70-110 days post-infection. All the infected monkeys showed CNS abnormalities and neuropathology. Genes that changed in expression were confirmed using immunohistochemistry and *in situ* hybridization in brain sections. Gene expression was quantified on human Affymetrix arrays. Genes that showed significant changes included 10 genes for monocyte migration, 17 for inflammation, 18 for antigen presentation, 7 for lysozymes, and 13 for interferon induction. Examples included CD163, GLUT5, ISG15, cyclin D3, and STAT1.

Gene expression changes were characterized in PBMNCs from Rhesus macaques infected with SIVmac251 (63). Animals with different rates of disease progression had distinct profiles in gene expression. Rapid, typical, and slow progressor groups showed elevated immune response genes over time. Immune response and cytoskeleton genes were elevated in the typical vs. rapid progressor groups.

Gene expression differences were most pronounced between rapid vs. slow progressors. These studies have predictive value concerning human AIDS disease and should be replicated for humans.

A papova virus, JC Virus, is sometimes associated with HIV infection and in the immune suppressed state causes progressive multifocal leukoencephalopathy (PML) a rapidly fatal demyelinating disease. Thus, PML is a relevant viral-caused Neuropsychiatric disease in studies in NeuroAIDS. Radhakrishnan et al (64) examined changes in gene expression at the mRNA using Microarrays and protein levels using Western blots in JC virus infected human astroctye cultures. Genes elevated as a results of infection included cyclins A and B1, signaling pathways including transforming growth factor beta receptor 1, platelet-derived growth factor receptor and fibroblast growth factor family receptor as well as inflammation such as cyclo-oxygenase-2 (Cox-2). Cyclins A, B1, E, and Cdk2 were detected in PML brain tissue sections using immunohistochemistry in PML brain tissue. This is one of the first papers in this area to attempt to correlate mRNA and protein expression. In this analysis, the two were not correlated.

2.5. Gene Expression in Drug Abuse

A major purpose of drug abuse research is to elucidate the fundamental molecular mechanisms responsible for the behavioral alterations caused by repeated (addictive) exposure to drugs including cocaine, opiates, tetrahydro-cannabinols, ethanol, and nicotine. For example, enduring behavioral alterations like behavioral sensitization can be induced in rodents by repeated cocaine administration. The neurobiological mechanisms responsible for this behavioral pattern are associated with the brain meso-cortico-limbic dopamine (DA) pathway. Additionally, DA D1 receptors are involved in mediating

the long-term behavioral effects of cocaine. The long-lasting behavioral effects of repeated cocaine exposure are highly likely to be associated with underlying changes in gene expression. Zhang and colleagues (65) combined the use of D1 receptor mutant mice with cDNA Microarrays to identify gene expression changes mediated through the D1 receptors induced by repeated cocaine administration. Their preliminary experiments centered on a target of the meso-cortico-limbic DA pathway, the nucleus accumbens (NAc) that is the primary neural substrate for mediating the long-term effects of cocaine. Their findings indicated that multiple genes were differentially expressed in wild type and D1 receptor mutant mice after chronic cocaine treatment.

A few studies of Drug Abuse have been performed using Differential Display (DD) technique. This method showed that both 16S and 12S mitochondrial rRNAs were increased in the nucleus accumbens of five alcoholic brains compared to five normal brains (66). A study of fetal alcohol syndrome in the mouse model demonstrated that of 1,080 genes, three genes showed increased expression in embryos exposed to ethanol () these were alpha-tropomyosin (31,000 d, most likely a brain specific isoform), heat shock protein 47, and an EST. DD showed that mitochondrial NADH dehydrogenase subunit four mRNA was increased in the hippocampi but not cortex from ethanol-treated vs. control Male Wistar rats (67). DD analysis of caudate putamen tissue from Sprague-Dawley rats post-cocaine treatment vs. controls showed an immediately decrease of 8G226 resembling a transcriptional regulatory protein, mouse zinc-finger protein (PZf). A few days later, its expression returned to normal. There was a cocaine-dependent increased expression of 8G247 and NGFI-A, another zinc-finger protein, and closely paralleled changes in c-fos. As with HIV-1 infection there is no model yet for the panoply of changes in cell gene expression on a wide scale that result from Drug Abuse (or HIV infection). Extensive study and analysis are required.

We previously found that in vitro HIV infection of macrophage/microglia and exposure to drugs (including cocaine, cocaethylene, and heroin), separately and in combination, results in changed macrophage surface marker expression (CD14, CD68, and HLA-DR, as well as CD4) (68). In post-mortem tissue, we examined Drug abuse and HIV infection and found changes in brain gene expression. Drug abuse and HIV infection are factors that differentially exert multiple macrophage-surface marker effects. Thus, since there may be several sub-types of macrophages in the brain, and it remains to be determined which macrophage sub-type(s) might be most pathognomic for pathology (27, 68)? In addition, we showed increased nitric oxide synthesis in dorsal root ganglia and in temporal gray matter associated with HIV infection and drug abuse (69, 70) and increased IFN-γ associated with HIV infection of temporal grey matter (71).

The current study utilizes Affymetrix chips and Bioinformatics methods for preliminary analysis of AIDS frontal cortex gene expression compared to HIV-negative brains.

3. MATERIALS AND METHODS

3.1. Brain Tissue

Brain tissue was obtained through Human Subjects University of Miami Internal Review Board and NIH approved methods. We used cryopreserved frontal cortex brain tissue with neuropathology analyzed from five AIDS cases and four controls. The subjects ranged from ages 29 to 62 years and post-mortem intervals ranged from 4 hours to 20 hours. There was one female subject. Two subjects were black and eight were Caucasian. Three cases had HIV encephalitis (HIVE), one of which also had toxoplasmosis in remote brain regions, and a fourth case had carcinomatous meningitis. Three HIV+ cases had HIV associated dementia. Four subjects received HAART therapy. All four control brains were normal.

3.2. RNA purification

RNA was purified from brain tissue using Qiagen Kits (Qiagen Inc., Valencia, CA) according to manufacturer's instructions (28, 29). Purified RNA was analyzed for integrity and did not show degradation on agarose gels and micro-isotacophoresis (Agilent RNA 600 Assay chips, Agilent Technologies, Palo Alto, CA). Detection of peaks representing 18S and 28S ribosomal fractions, were taken as an initial total RNA quality parameter. mRNA quality was determined by RT-PCR using human β-Tubulin and GFAP housekeeping genes set of primers.) (Data not shown).

3.3. RNA amplification and Microarray

3.3.1. Affymetrix Procedures

We used the Affymetrix DNA Chip: Human Genome U95A, Part # 510448 (lot # 9914805). This chip has 12,626 probe sets (oligonucleotides) (approx. 1/3rd of human genes) in an area 1.24 cm x 1.24 cm. Using procedures according to Affymetrix Protocols based on studies by Drs. Lockhart and Wodicka (72), RNA was linearly amplified with phage T7 RNA polymerase according to kit manufacturer instructions (Arcturus, City, State). The RNA was labeled with biotin during amplification and then further complexed with phyco-erythrin-avidin. The RNA was hybridized to the chips, washed, and then scanned using Affymetrix semi-automated fluorescence technology according to the manufacturer's instructions. Affymetrix chip methods allow for additional examination of RNA integrity since the gene oligonucleotides are representative of the 5', mid, and 3' exons. After the chip scan, several control and target genes are examined to ascertain if there is a lack of concordance among the 20-oligonucleotide probe sets across each gene as per the Affymetrix instruction manual procedures. Data is further analyzed only if there is a concordance across the exons. The laboratory of Dr. R. Polavarapu (Decatur VA Medical Center, GA) performed the Affymetrix Microarray analyses.

3.3.2. Spotfire Bioinformatics

We converted the Affymetrix files obtained from the scans to Microsoft Excel (XL) files. ESTs (estimated sequence tags) are updated and identified at NCBI/GenBank. For changes in gene expression, Spotfire

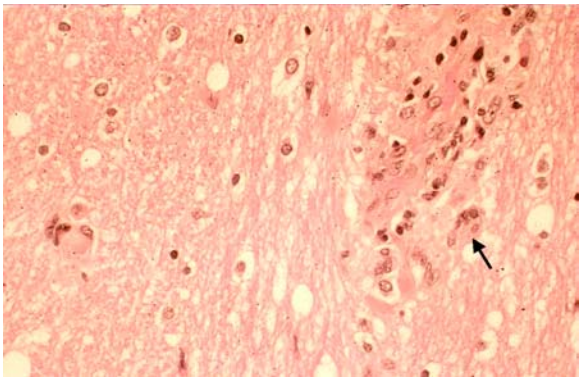

Figure 1. Neuropathology of HIV infected brain.

Cluster analysis and Principal Component (PCA) analyses were performed. In addition, intra-group and inter-group comparisons were made. Initially we had six AIDS cases and five HIV-negative controls. Scatter plots were done and one HIV+ and one HIV-negative subject were excluded from further analysis due to lack of correlation within and between groups). Thus, our analyses are based five AIDS and four HIV-negative controls.

3.3.2.1. K-Means Clustering

Spotfire Clustering method: UPGMA (unweighted average), similarity measure: Euclidean distance, Ordering function: average value. K-means clustering uses 'centroids' derived from the data and processed iteratively based on the number centroids selected (Spotfire Manual, 2002). Total records (cases) included G0010+ Signal, H0011+ Signal, G0017+ Signal, H0002+ Signal, G0036+ Signal, BTB 3455- Signal, BTB 3648- Signal, BTB 3749- Signal, and D97 2916- Signal. Empty values were replaced by row averages. There are 12,584 genes in the analyses.

3.3.2.2. Principal Component Analysis

Spotfire Principal Component Analysis was performed. Principal component scores of each projected record were calculated. Empty values were replaced by row averages. Preserved variability was also calculated for each analysis.

4. RESULTS

Figure 1 shows typical neuropathology for the HIV-positive cases (performed by Dr. CK Petito) (Hematoxylin/eosin stained section 400x magnification originally). This Figure shows myelinated fiber bundles, capillary with red blood cells and solitary white blood cell, as well as multinucleated giant cells (one within fiber bundle at top), and chronic inflammatory cells.

RNA was amplified and Affymetrix technology was used to analyze gene expression using Affymetrix Human Genome U95A chips. Expression intensities were transferred to Microsoft Excel and Spotfire was used to analyze the results. Forty-two control genes were removed from data analysis. Expression intensities of 12,585 genes showed highly significant Pearson's correlations with each other within each of the two groups (Table 1a and 1b). It should be noted that. Pearson coefficients for one AIDS case and one control case showed no correlations with the others cases within each respective group. Scatter plots of each of these cases with all the other cases within each group confirmed the lack of correlations. Therefore, these two cases were removed from further analysis and are not reported here. Figure 1 shows a representative scatter plot of two HIV+ cases with correlation coefficient, 0.955. The data points in this figure are "jittered" by the Spotfire program to separate them from each other slightly in order to bring more of them into view in the figure.

Spotfire was used to calculate 20-group K-means clusters for the two groups of HIV+ and HIV- subjects, separately. Genes that were expressed in the same cluster numbers in the two groups were removed from further analysis and genes in Table 2 showed a shift of cluster number (upward or downward) between the HIV+ and HIV- cases. It should be noted that initially 10- and 15-group K-means clusters were calculated (data not shown) and the numbers of genes in the clusters were too large to be utilized readily. 20-group K-means clusters resulted in feasible numbers of genes to examine in several of the clusters. This is exemplified in Figure 2 where numbers of genes per cluster are exhibited. Figure 3 show the gene expression profiles across subjects in the HIV- group for clusters 13-20. . The first few clusters (starting at cluster 1) for each group had the largest numbers of genes. (Not shown). Analysis of Gene expression in the top 13 HIV+ clusters showed expression in the 40 gene categories designated in our prior studies (3). For brevity, 26 categories are shown in Table 3. A summary of this analysis is shown in Table 4. Several gene categories are shown within each cluster.

As an additional analysis, PCA was performed on the HIV+ and HIV- groups (Figure 5a and 5b). These Figures show three-dimensional displays of the data. The first (primary or significant) axis of Principal Component Analysis ordered the genes in the same patient groups as the K-means cluster analysis in the respective HIV+ and HIV- groups (data not shown). .

Ratios of HIV+ to HIV- intensities were calculated for all the averaged gene expression intensities (for each HIV+ and HIV- groups) and ratios and the extremes (*ad extrema*) are noted in Table 5. The ratio range shown is 0.14 to 0.22 and from 6.03 to 9.26. . These genes ad *extrema* did not correspond to the gene order by K-means or PCA.

The genes in the top 13 K-means clusters corresponded to low-level changes in expression by ratio; genes *ad extrema* by ratio were in clusters composed of very large numbers of genes (data not shown).

5. DISCUSSION

In order to carry out a Microarray project involving the human brain, crucial aspects involve the tissue itself, the RNA produced, the cost of the experiments, and the Bioinformatics procedures used. It is more advantageous under these conditions and limitations

Table 1. **Pearson Correlations for 12584 Genes**

A. Between HIV + Subjects. Means (and Standard Deviations) on the Diagonal

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
Subject 1	198	0.9455	0.6861	0.7062	0.9292
	(1019)	<.0001	<.0001	<.0001	<.0001
Subject 2	0.9455	216	0.8031	0.8255	0.9589
	<.0001	(1090)	<.0001	<.0001	<.0001
Subject 3	0.6861	0.8031	230	0.9546	0.8384
	<.0001	<.0001	(853)	<.0001	<.0001
Subject 4	0.7062	0.8255	0.9546	209	0.8508
	<.0001	<.0001	<.0001	(686)	<.0001
Subject 5	0.9292	0.9589	0.8384	0.8508	215
	<.0001	<.0001	<.0001	<.0001	(1003)

B. Between Control Subjects Means (and Standard Deviations) on the Diagonal

	Subject 6	Subject 7	Subject 8	Subject 9
Subject 6	195	0.9431	0.9346	0.9022
	(536)	<.0001	<.0001	<.0001
Subject 7	0.9431	221	0.9516	0.9370
	<.0001	(830)	<.0001	<.0001
Subject 8	0.9346	0.9516	223	0.9222
	<.0001	<.0001	(826)	<.0001
Subject 9	0.9022	0.9370	0.9222	234
	<.0001	<.0001	<.0001	(848)

Table 2. Descriptions of (20) K-means clusters

Descriptions	**HIV+**	**HIV -**
M83738 protein-tyr phosphatase (PTPase MEG2)	18	17
AL031670:ferritin, light polypeptide-like 1	18	16
M13577: myelin basic protein (MBP)	18	16
D29675 inducible nitric oxide synthase gene	17	19
Serine/Threonine Protein Kinase Cdk3	17	18
M54927: myelin proteolipid protein	16	15
J04755: ferritin H processed pseudogene,	16	14
V-Erba Related Ear-3 Protein	15	17
H06628Soares infant brain1NIB TYR KIN PREC	14	16
M31667 cytochrome P450 (CYP1A2)	14	16
U12022: calmodulin (CALM1)	14	15
U48437: amyloid precursor-like protein 1	14	15
L20941: ferritin heavy chain	14	13
L13266: N-methyl-d-aspartate receptor (NR1-1)	14	12
M58378: synapsin I (SYN1) gene	13	16
L07807: dynamin	13	16
M33210: colony stimulating factor 1 receptor	13	16
M16660: 90-kDa heat-shock protein gene	13	15
M21121 T cell-specific protein (RANTES)	13	14
M29273myelin-associated glycoprotein (MAG)	11	13
X86809major astrocytic phosphoprotein PEA-15	11	13
U07364: inwardly rectifying potassium channel	11	12
L13268: N-methyl-d-aspartate receptor (NR1-3)	11	12
U28389: dematin 52 kDa subunit	11	12
U97669: Notch3 (NOTCH3)	11	12
X00129: retinol binding protein (RBP)	11	12
Calmodulin Type I	10	14
AF044253: potassium channel beta 2 subunit	10	13
U22970 interferon-inducible peptide (6-16) gene	10	13
D29012 / HUMPSY proteasome subunit Y	10	13
D31815 / senescence marker protein-30	10	13
M19311 HUMCAM calmodulin ,	10	13
AF039555: visinin-like protein 1 (VSNL1)	10	12
X04106:Ca dependent protease (small subunit)	10	12
AJ133534: prenylated Rab acceptor 1	9	12
AB015202: gene for hippocalcin	7	15
D88799 / D88799 cadherin, partial cds	7	13
AL022326:Synaptogyrin 1A	7	12
U31767: neuronatin alpha & beta genes,	5	13

of experimentation to use a larger number of cases than replicates since cases provide power to the results. Furthermore, in our study, tissue was limiting and future studies will utilize a larger sample size. Brain tissue must be obtained with the shortest autolysis time (post-mortem interval) possible to preserve mRNAs integrity. . Various analytic methods were performed to demonstrate the quality of the RNA. The vendor exercised quality control for the Affymetrix chips. In addition, mass-produced uniform quality-controlled chips provide an advantage of great importance in this type of study design. The clinical information that comes with tissue is crucial because only then can accurate disease states enter into the data analysis (73). In addition, Neuropathology is important because it defines the detailed pathogenic state of the tissue from which the RNA is purified. It is noted that in the post-mortem ranges we studied, and under the conditions of obtaining brain tissue, we previously demonstrated a lack of correlation between post-mortem interval time and RNA degradation (28, 29, 46, 74).

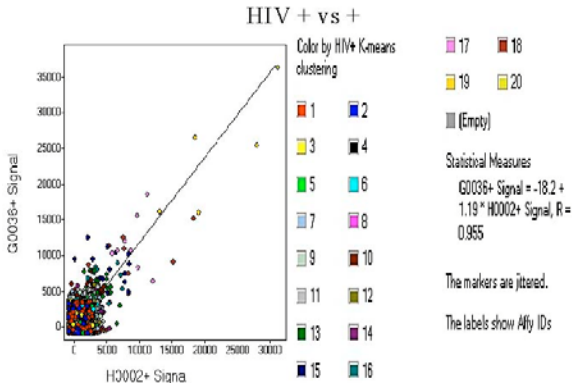

Figure 2. Scatter plot.

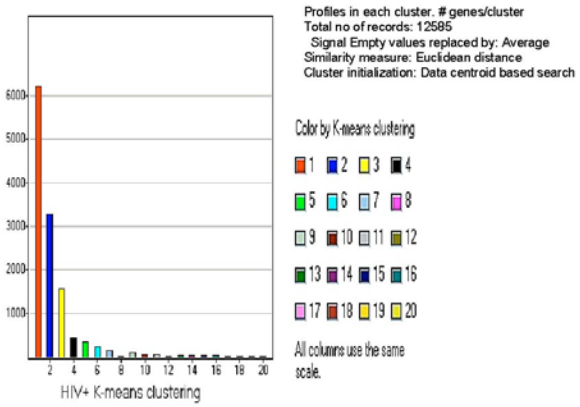

Figure 3. HIV+ K-means clustering

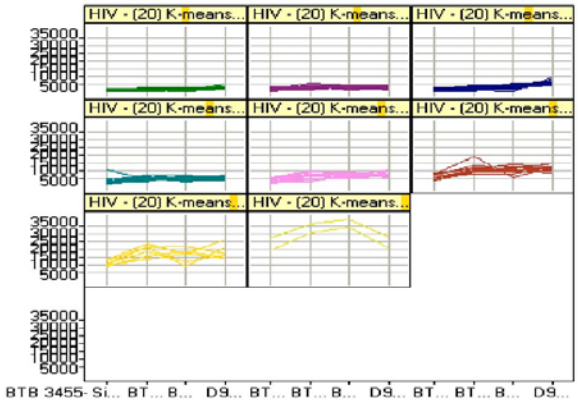

Figure 4. HIV -(20) K-means clustering. Left to right. Top row, clusters 13, 14, 15; Middle row, clusters 16, 17, 18; Bottom row, clusters 19 & 20.

We previously categorized human genes for MicroArray studies (3). In the current study, genes from several categories occurred in more than one K-means cluster. Genes identified in these lists included several genes that have been previously studied using classical molecular biological methods: MBP, Myelin-PLP, NMDA receptor, MAG, astrocytic protein, Notch 3, APP, Senescence, proteasome, Ferritin, signaling, cell cycle, iNOS, Chemokine, splicing, synapse, protein tags, and ribosomal proteins. These findings are consistent with prior studies implicating mechanisms including apoptosis, inflammation, nitric oxide, and ferritin in pathogenesis in the HIV-infected nervous system (2, 40, 45, 46, 52, 71, 74, 75, 76). Similarly, genes identified as significant using each of the means of analysis presented here also appear in other prior publications (59, 60, 63)

The studies presented here are a stage in our application of the new Microarray Technology and there are limitations. For example, some limitations in a study such as this are the number of cases, sources of tissues, Clinical information, and statistical methods. An additional possible limitation is having a method so that data obtained from different generations of chips and software will be compatible for cumulative future comparative analysis. . Future studies are being done with various improvements including the use of laser capture microdissection/microscopy (LCM) and additional statistical methods (6, 77, 78). In addition, we musts relate the genes expression not only in groups but discern pathways and relationships (8, 79, 80).

Radhakrishnan et al (64) examined changes in gene expression at the mRNA using Microarrays and protein levels using Western blots in JC virus infected human astroctye cultures. This is one of the first papers in this area to attempt to correlate mRNA and protein expression and in this analysis, the two were not correlated. We wish to point out that a perfect correlation of mRNA and protein gene expression may not be expected due to the following reasons: 1. mRNA is generally less stable than protein; 2. Proteins tend to accumulate over time. 3. Change in mRNA expression will not have neutral effects on cellular function. For example, there are many known and probably as yet unknown proteins that bind to precursor mRNAs and mRNAs, some of which are present in stoichiometric quantities. These proteins may be in a poised concentration balance overall in the cell and not present in excess compared to the different types of mRNAs. Changes in mRNA levels may result in disequilibrium of these proteins and it is not known if the proteins can respond to changes in mRNA levels due to processes relating to Dementia and to pathology. If they do respond over time, it is unknown at what rates they respond. Thus, it is yet also unknown as to the extent that Microarray for gene expression at the mRNA level and protein level will be commensurate.

6. CONCLUSIONS

We performed several types of data analysis on our data set and the findings were counter-intuitive in that different methods identified different genes. Spotfire's K-means clustering and PCA gave the same results. However, these results differed from ranking the genes by ratio. Two thousand genes identified by ratio in the low and high HIV+/HIV- ratio ordering were confirmed by Mann-Whitney analysis. (A larger number of cases would be required for confirmation of remaining genes ordered by ratio).

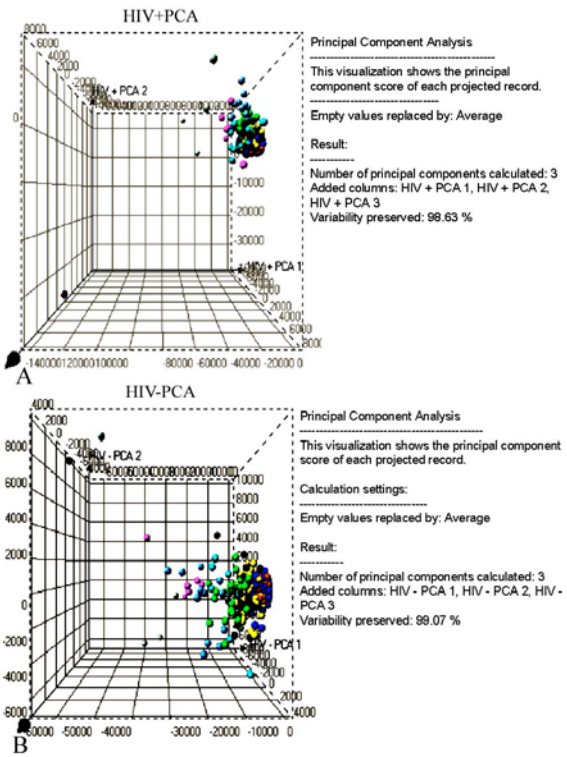

Figure 5. PCA analysis. A. HIV-positive cases. B. HIV-negative cases. PCA was performed using Spotfire. This visualization of the data shows the different arrangements of the gene expression data in 3-dimensional space for HIV-positive and HIV-negative cases.

Table 3. Gene types in the K-Means clusters. This is a compilation of a few genes and their identities in several clusters.

Cluster #	**18**	**17**	**16**	**15**	**14**	**13**	**11**	**10**	**9**	**7**
# genes Key genes	**4** MBP, Ferritin, signaling	**4** iNOS, splicing, signaling	**7** Myelin – PLP, Ferritin, signaling, cell cycle	**7** Brain and protein tags	**17** APP, NMDA receptor, signaling, synaptic protein, ion transport	**19** Chemokine, signaling, synapse, protein tags	**33** MAG, astrocytic protein, Notch 3, ribosomal proteins, transcription, NMDA receptor,	**33** Senescence, proteasome, cytoskeleton, signaling, protein tags, ion transport	**11** Signaling, protein tag, mitochondrial translation	**8** Signaling, ion transport

Table 4. Gene group codes Examples from top 146 genes from K-Means analysis categorized in previously designated gene group codes (3)

Gene groups	**# Genes**
Group 1: NMDA and GABA receptors	2
Group 10. Cytokines, Chemokines	3
Group 30. Nitric oxide synthase	1
Group 32. Myelin	3
Group 40. Synaptic & neuronal proteins	2
Group 60. Adhesion molecules/ligands	1
Group 70. Apoptosis	2
Group 100. DNA binding proteins	2
Group 110. Signal transduction pathways	10
Group 115. Kinases, phosphatases	5
Group 120. Transcription and RNA binding	3
Group 130. Cell cycle regulator	4
Group 160. Astrocyte	1
Group 180. Defense/Immunity	1
Group 210. Stress/Heat Shock, Chaperone	1
Group 220. Oncogenes, anti-oncogene	3
Group 230. Storage protein	6
Group 240. Differentiation/homeobox, Development, notch	1
Group 250. Protein tagging and modification	6
Group 260. Anti-oxidant	1
Group 300. Transporters, channels, and ion adhesion	9
Group 310. Hormones, receptors, induced proteins, and regulators, Neuroendocrines	3
Group 335. Ribosomal protein	19
Group 350. Small nuclear RNA and protein	2
Group 400. Dementia-Alzheimer's Disease, Mental Retardation	3
Group 500. Brain related (not in above categories)	3

Table 5. Genes ranked by ratios of HIV+/HIV- (*ad extrema*). This is a selection of highest and lowest ratios

Gene description	ratio	ratio_rank
AF062739: GSK-3 binding protein FRAT2	0.140941	1
M61156: activator protein 2B (AP-2B)	0.142538	2
D00096: prealbumin, (26,469)	0.143123	3
D78514 ubiquitin-conjugating enzyme	0.155713	4
J04131: gamma-glutamyl transpeptidase (GGT) protein	0.158651	5
AF030107: regulator of G protein signaling (RGS13)	0.165479	6
L23805 CATENIN alpha1(E)-catenin	0.168352	7
AF082558: truncated TRF1-interacting ankyrin-related ADP-ribose polymerase TT7	0.168513	8
X70944: PTB-associated splicing factor	0.180754	12
U18235: ATP-binding cassette protein (ABC2) HFBCD04	0.188074	14
M95167: dopamine transporter (SLC6A3)	0.193213	16
AL022727: DNA sequence from clone 80I19 on chromosome 6p21.31-22.2, olfactory receptor-like proteins	0.211588	19
U05589: ribosomal protein S1 homolog	0.212016	20
Y07827: put. B7,3 molecule of CD80-CD60	0.212927	21
M57423: phosphoribosylpyrophosphate synthetase subunit III	0.214216	22
L38928: 5,10-methenyltetrahydrofolate synthetase	0.215135	23
AF030196: stannin , (145,411)	0.215743	24
U79751: basic-leucine zipper nuclear factor (JEM-1)	0.216416	26
U45975: phosphatidylinositol (4,5)bisphosphate 5-phosphatase homolog	0.219459	27
X78338:Synthetic adenovirus transformed retina cell line	0.220969	28
U09414: zinc finger protein ZNF137	0.221424	29
U78556 HSU78556 cisplatin resistance associated alpha protein (hCRA alpha)	0.221552	30
J00123: enkephalin gene	0.221625	31
---	---	---
Y00062: T200 leukocyte common antigen (CD45, LC-A)	6.029358	12555
AJ000327: adrenoleukodystrophy related protein (ALDR)	6.315232	12558
U56816 kinase Myt1 (Myt1)	6.347074	12559
M89914 NF1B neurofibromin (NF1) gene	6.58292	12562
AJ010046: Rho guanine nucleotide-exchange factor, splice variant NET1A	6.661224	12563
AF055011: clone 24587	6.683544	12564
AF037989: STAT-induced STAT inhibitor-2	6.717382	12565
U40572: beta2-syntrophin (SNT B2)	7.2271	12569
X64877: serum protein	7.380571	12570
AL031652:dJ1119D9.1 (PAK1 LIKE Serine/Threonine-Protein Kinase)	7.595604	12571
M63438: Ig rearranged gamma chain , V-J-C region and (0,1049)	8.058278	12572
M27504 TOPIIX topoisomerase type II (Topo II)	8.585115	12574
U06632: p80-coilin	8.685422	12575
AJ223948: putative RNA helicase	9.021651	12576
D86864: acetyl LDL receptor	9.109537	12577
X97324: adipophilin	9.171688	12578
Y14737: immunoglobulin lambda heavy chain	9.224281	12579
L10717 TKTCS T cell-specific tyrosine kinase	9.260073	12581

Thus, ranking gene expression by Expression Ratio or by K-means Cluster yields different gene expression relations. Ratios exhibit the extent of changes in gene expression quantitatively. Cluster methodology exhibits how clusters of genes behave in terms of their centroids of change. Examination of specific gene lists showed that genes that change extensively by ratio tend to be embedded in the larger k-Means clusters and thus do not appear to be informative within those very large clusters. Perhaps they behave as “attractors" and thus are associated within larger aggregates/numbers of genes or gene pathways. These clusters contain hundreds of genes. However, the 10 top k-Means clusters have fewer members and their HIV+/HIV- gene expression ratios are around 1-2 and 1-0.5 and thus not significant according to Mann-Whitney analysis of these ratios. Thus, each method appears to yield information related to a different aspect of pathogenesis due to gene expression. The brute force calculation of large changes in gene expression may not provide the only purely significant insights into the disease process. Rather, the disease process may be governed over long durations by small changes in gene expression in coordinated groups (pathways) that we need to examine in greater detail. We need to ascertain the effects of these small changes over time. Finally, it is not known yet if any particular method developed to date is the method of choice for such studies. Confirmation of expression using independent means of gene expression analysis including real time reverse-transcriptase PCR and protein expression are in progress. Comparisons across several studies and production of databases (8, 80) will continue to be an important method to confirm the importance of identified genes.

7. ACKNOWLEDGMENTS

Brain tissue was obtained through the National NeuroAIDS consortium, University of Texas, Galveston (MH59656, Dr. B. Gelman, PI) and the NICHD Brain and Tissue bank for developmental disorders (HD 83284, Dr. C. K. Petito, PI). We thank Dr. Toni Kazic (University of Missouri) and Dr. Willy Valdivia Granda (North Dakota State University) for discussions. This work was supported

in part by NIH Grants: DA 14533, DA 12580, and NS39177).

8. REFERENCES

1. Shapshak P, Segal DM., Crandall KA, Fujimura RK, Zhang BT, Xin KQ, Okuda K, Petito C, Eisdorfer C, & Goodkin K: Independent evolution of HIV-1 in different brain regions. *AIDS Res & Hu Retro*, 15, 811-820 (1999)

2. Shapshak P, R.K. Fujimura, A. Srivastava, & K. Goodkin: Dementia and the Neurovirulence of HIV-1. CNS Spectrums (An International Psychiatry Journal), 5, 31-42 (2000)

3. Shapshak P, J. Torres-Munos, & C.K. Petito: Bioinformatics in Neurodegenerative Disease, 1st Proc. Virt. Conf. Genom. and Bioinf, www.ndsu/virtual-genomics, North Dakota State University (2001)

4. Brown P.O. & D. Botstein: Exploring the new world of the genome with DNA MicroArrays. *Nat Genet*,; 21, 33-37 (1999)

5. Liang P. & A.B. Pardee: Differential display of eukaryotic messenger RNA by means of PCR. Science, 257, 967-971 (1992)

6. Duncan RC. Exaptation of Statistical Methods for Genomic Research, 6th World Multiconference on Systemics, Cybernetics and Informatics. www.iiis.org/sci2002/ Orlando, FL, July 14-18 (2002)

7. Shapshak P & R. Stengel: Discovering Brain Mechanisms and the rules of Molecular Biology, 6th World Multiconference on Systemics, Cybernetics and Informatics, www.iiis.org/sci2002/ Orlando, FL, July 14-18 (2002)

8. Kazic T: Semiotes: a semantics for sharing, Bioinformatics, 16, 1129-1144 (2000)

9. Valdivia Granda W: Chapter 8. Strategies of clustering, classifying, integrating, standardizing, and visualizing MicroArray gene expression data. In A Beginner's guide to Microarrays, Editor: EM Blalock, Kluwer Academic Publishers (Boston), pp. 277-340 (2003)

10. Ridley M: Nature via Nurture; Genes, Experience, and what makes us human, Harper Collins Publishers (New York), pp. 1-326 (2003)

11. Edlin BR, K.L. Irwin, S. Faruque, C.B. McCoy, C. Word, Y. Serrano, & J.A. Inciardi: Intersecting epidemics – crack cocaine and HIV infection among inner-city young adults. Multicenter Crack Cocaine and HIV infection Study Team, *N Engl J of Med*, 332, 1233-1235 (1995)

12. Gibson DR, N.M. Flynn, & J.J. McCarthy: Effectiveness of methadone treatment in reducing HIV risk behavior and HIV seroconversion among injecting drug users. AIDS. 13(14), 1807-1818 (1999)

13. Sorensen JL & Copeland AL: Drug abuse treatment as an HIV prevention strategy: a review. *Drug Alcohol Depend*, 59(1), 17-31 (2001)

14. Holmberg SD: The estimated prevalence and incidence of HIV in 96 large US metropolitan areas. Am J Public Health. ; 86(5), 642-54 (1996)

15. McCoy CB & E. Khoury: Drug use and risk of AIDS. *Am Behav Sci*, 33, 419-31 (1990)

16. McCoy CB, L.R. Metsch, H.V. McCoy, & S. Lai: A gender comparison of HIV and drug use across the rural-urban continuum. *Popul Res and Policy Rev*, 18, 71-87 (1999)

17. McCoy CB, L.R. Metsch, D.D. Chitwood, J.E. Rivers, H.V. McCoy, & S. Messiah: Health Services for chronic drug users in an era of managed care. The University of Miami Community-based Health Services Research Center. *Adv in Med Sociol*, 7, 151-174 (2000)

18. Shapshak P, R.K. Fujimura, J.B. Page, D. Segal, S.M. Shah, G. Graham, J.E. Rivers, D.D. Chitwood, & C.B. McCoy: HIV-1 RNA load in needle/syringes from shooting galleries in Miami: a preliminary laboratory report. Drug and Alcohol Dependence, 58, 153-157 (2000)

19. McCutchan FE: Understanding the genetic diversity of HIV-1. AIDS, 14 (suppl 3), S31-S44 (2000)

20. Xin KQ, P. Shapshak, S. Kawamoto, I. Nagano, C.B. McCoy, & K. Okuda: Highly Divergent env Sequences of HIV-1 B Subtype with Two Novel V3 Loop Motifs Detected in an AIDS Patient in Miami, Florida. *AIDS Res Hum Retroviruses*. 11, 1139-1141 (1995)

21. Xin KQ, K. Hamajima, S. Sasaki, A. Honsho, T. Tsuji, N. Ishii, X.R. Cao, Y. Lu, J. Fukushima, P. Shapshak, S. Kawamoto, & K. Okuda: Intranasal administration of HIV-1 DNA vaccine with IL-2 expression plasmid enhances cell-mediated immunity against HIV. Immunology, 94, 438-444 (1998)

22. Zhang L, L. Rowe, T. He, C. Chung, J. Yu, W. Yu, A. Talal, M. Markowitz, & D.D. Ho: Compartmentalization of surface envelope glycoprotein of HIV-1 during acute and chronic infection. *J Virol*, Sep;76(18), 9465-73 (2002)

23. Ohagen A, A. Devitt, K.J. Krunstman, P.R. Gorry, P.P. Rose, B. Korber, J. Taylor, R. Levy, R.L. Murphy, S.M. Wolinsky, & D. Gabuzda: Genetic and functional analysis of full length HIV-1 env genes derived from brain and blood of patients with AIDS. *J Virol* Nov; 77(22), 12336-45 (2003)

24. Morris A, M. Marsden, K. Halcrow, E.S. Hughes, R.P. Brettle, J.E. Bell, P. Simmonds. Mosaic structure of the HIV-1 genome infectiong lymphoid cells and the brain: evidence for frequent in vivo recombination events in the evolution of region populations. *J Virol*. Oct;73(10):8720-31 (1999)

25. Persidsky Y & H. Gendelman: Mononuclear phagocyte immunity and the neuropathogenesis of HIV-1 infection. *J Leuk Biol.* Nov, 74, 691-701 (2003)

26. Schutzer SE, M. Brunner, H.M. Fillit, & J.R. Berger: Autoimmune markers in HIV-associated dementia. *J Neuroimmunol*, May;138(1-2), 156-61 (2003)

27. Shapshak P, R.V. Stewart, P. Rodriguez de la Vega, B. Dominguez. R. Fujimura, D.M. Segal, N.C.J. Sun, S. Delgado, T. Sly, & C.K. Petito: Brain macrophage surface marker expression with HIV-1 infection and drug abuse: a preliminary study. *J of NeuroAIDS*, 2, 37-50 (2002)

28. Torres-Muñoz J, P. Shapshak, R. Polavarapu, M. Bowman, M. Keegan, & C.K. Petito: RNA preservation in post-mortem cerebral cortex of AIDS and control brains. *J Neurovirol. In press*, 2004.

29. Torres-Muñoz J, P. Shapshak, G. Polavarapu, M. Bowman, & C.K. Petito: Gene expression in AIDS cerebral cortex, *Int. Neurovirol. Meeting*, Düsseldorf, Germany, June 11-19 (2002)

30. Resnick L, J.R. Berger, P. Shapshak, & W.W. Tourtellotte: Early penetration of the blood-brain-barrier by HTLV-III/LAV. Neurology, 38, 9-15 (1988)

31. Resnick L, F. DiMarzo-Veronese, J. Schopbach, W.W. Tourtellotte, D.D. Ho, F. Muller, P. Shapshak, & R.C. Gallo: Intra-blood-brain-barrier synthesis of HTLV-III specific IgG in patients with AIDS or AIDS-related complex. *New Engl J Med*, 313, 1498-1504 (1985)
32. Shapshak P, W.W. Tourtellotte, L. Resnick, M.A. Osborne, P. Schmid, M. Lee, G. Rubinshtein, D.T. Imagawa, R. Mitsuyasu, M. Gottlieb, & R.C. Gallo: HTLV-III can cross the blood brain barrier. The Fourth Colloquium in Biological Sciences, Blood Brain Transfer. *Annals of the NY Academy of Sci*, 529, 291-294 (1988)
33. Singer EJ, K. Syndulko, B.N. Fahy-Chandon, P. Shapshak, L. Resnick, P. Schmid, A.J. Conrad, & W.W Tourtellotte: Cerebrospinal fluid p24 antigen levels and intrathecal immunoglobulin G synthesis are associated with cognitive disease severity in HIV-1. AIDS, 8, 197-204 (1994)
34. Tourtellotte WW, Singer E, Syndulko K, Shapshak P, & Resnick L, Intra-blood-brain-barrier IgG synthesis in HIV, in Enzymes as Targets for Drug Design. Editors: MG Palfreyman, McCann, W Lovenberg, JG Temple, A Sjoerdsma. Merrell Dow Research Institute, Academic Press, San Diego, pp. 211-217 (1990)
35. Goodkin K, Baldewicz TT, Wilkie FL, Tyll MD, & Shapshak P: HIV-1 Infection of the Brain: A Region-Specific Approach to its Neuropathophysiology and Therapeutic Prospects. Psychiatry Annals, 31, 182-192 (2001)
36. Goodkin K, P. Shapshak, L. Metsch, C.B. McCoy, K.A. Crandall, M. Kumar, R.K. Fujimura, McCoy V, Zhang BT, Reyblat S, Xin KQ, & Kumar AM: Cocaine abuse and HIV-1: epidemiology and neuropathogenesis. Special Issue, J of Neuroimmunol, 83, 88-101 (1998)
37. Goodkin K, P. Shapshak, R.K. Fujimura, R.S. Tuttle, W.G. Bradley, M. Yoshioka, I. Nagano, K.Q. Xin, A. Kumar, M. Kumar, T.T. Baldewicz, K.J. Maher, D. Asthana, & M.A. Fletcher: Immune function, brain, and HIV-1 infection. In Psychoneuroimmunology: Stress, Mental Disorders and Health, Washington, DC. Editors: Goodkin K, and A Visser. American Psychiatric Association Press, Inc, pp. 243-316 (2000)
38. Shapshak P, I. Nagano, K.Q. Xin, W.B. Bradley, C.B. McCoy, N.C.J. Sun, R.V. Stewart, M. Yoshioka, C.K. Petito, K. Goodkin, R. Douyon, A.K. Srivastava, & K.A. Crandall: HIV-1 heterogeneity and cytokines: neuropathogenesis, in The Brain Immune Axis and Substance Abuse (*Adv in Exp Med and Biol*, 373), Editors. B. Sharp, T. Eisenstein, J. Madden, and H. Friedman: Chapter 31, Plenum Press, pp. 225-236 (1995)
39. Shapshak P, K.A. Crandall, K.Q. Xin, K. Goodkin, R.K. Fujimura, W.G. Bradley, & C.B. McCoy: HIV-1 Neuropathogenesis and abused drugs. Current views: problems and solutions, *Adv in Exp Med and Biol*, (*Adv in Exp Med and Biol*, 374), Editors. B. Sharp, T. Eisenstein, J. Madden, and H. Friedman: Chapter 23, Plenum Press, 171-186 (1996)
40. Bell JE, A. Busutil, & J.W. Ironside: Human immunodeficiency virus and the brain: Investigation of virus load and neuropathologic changes in Pre-AIDS subjects. *J Infect Dis*, 168, 818-824 (1993)
41. Brew BJ. AIDS dementia complex and its prevention. Antiviral Therapy. 2(suppl 3), 69-73 (1997)
42. Sinclair E, Gray F, & Giardi A*:* Immunohistochemical changes and PCR detection of HIV provirus DNA in brains of asymptomatic HIV positive patients. *J Neuropathol Exp Neurol*, 53, 43-50 (1994)
43. Price RW: The cellular basis of central nervous systemHIV-1 infection and AIDS dementia complex: Introduction. *J NeuroAIDS*, 1, 1-30 (1996)
44. McArthur JC, N. Haughey, S. Gartner, K. Conant, C. Pardo, A. Nath, & Sacktor: Human immunodeficiency virus-associated N dementia: an evolving disease. *J Neurovirol*, Apr; 9(2), 205-21 (2003)
45. Price RW & Perry SW: HIV, AIDS, and the Brain, Association for Research in Nervous and Mental Disease, vol. 72, Raven Press (NY, NY) (1994)
46. Yoshioka M, P. Shapshak, N.C.J. Sun, A Svenningsson, Nelson S, Resnick L, & Tate L: Ferritin immunoreactivity in microglial nodules in AIDS brain. *Acta Neuropathologica*, 84, 297-306 (1992)
47. Zheng J, A. Ghorpade, D. Niemann, R. Cotter, M. Thylin, L. Epstein, J. Swartz, R Shepard, X. Liu, A. Nukuna, & H. Gendelman: Lymphotropic virions affect signaling and apoptosis: Implications for HIV-1 associated dementia. *J Virol*, Oct:73(10), 8256-67 (1999)
48. Petito CK, E-S Cho, W Lemann, B.A Navia, & R. W Price. Neuropathology of Acquired Immunodeficiency Syndrome (AIDS): Autopsy Review. *J Neuropathol Exp Neurol*; 45: 635-646 (1986)
49. Adamson DC, Wildemann B, & Sasaki M: Immunologic NO synthase: Elevation in severe AIDS dementia and induction by HIV-1 gp41. Science, 274, 1917-1921 (1996)
50. Boven LA, L. Gomes, & C. Hery*:* Increased peroxynitrite activity in AIDS dementia complex: Implications for the neuropathogenesis of HIV-1 infection. *J Immunol*, 162, 4319-4327 (1999)
51. Vincent VAM, C.J.A. De Groot, & P.J. Lucassen*:* Nitric oxide synthase expression and apoptotic cell death in brains of AIDS and AIDS dementia patients. AIDS, 13, 317-326 (1999)
52. Petito C.K. & B. Roberts: Evidence of apoptotic death in HIV encephalitis. *Am J of Pathol*, 146, 1121-1130 (1995)
53. Whitney LW, Becker KG, Tresser NJ, Caballero-Ramos CI, Munson PJ, Prabhu VV, Trent JM, McFarland HF, & Biddison WE: Analysis of gene expression in multiple sclerosis lesions using cDNA MicroArrays. Ann Neurol, 46(3), 425-428 (1999)
54. Heller RA, M. Schena, A. Chai, D. Shalon, T. Bedilion, J. Gilmore, D.E. Woolley, & R.W. Davis: Discovery and analysis of inflammatory disease-related genes using cDNA MicroArrays. *Proc Natl Acad Sci USA*, 18, 94(6), 2150-2155 (1997)
55. Walker DG, L.F. Lue, & T.G. Beach: Gene expression profiling of amyloid beta peptide-stimulated human post-mortem brain microglia. Neurobiol Aging, 22(6), 957-966 (2001)
56. Pasinetti GM: Use of cDNA Microarray in the search for molecular markers involved in the onset of Alzheimer's disease dementia. *J Neurosci Res*, 65(6), 471-476 (2001)
57. Mirnics K, F.A. Middleton, A. Marquez, D.A. Lewis, & P. Levitt: Molecular characterization of schizophrenia viewed by Microarray analysis of gene expression in prefrontal cortex. Neuron, 28(1), 53-67 (2000)
58. Mirnics K, F.A. Middleton, G.D. Stanwood, D.A. Lewis, & P. Levitt: Disease-specific changes in regulator

of G-protein signaling 4 (RGS4) expression in schizophrenia. *Mol Psychiatry*, 6(3), 293-301 (2001)
59. Geiss GK, R.E. Bumgarner, & M.C. An: Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays. Virology, 266, 8-16 (2000)
60. Galey D, K. Becker, N. Haughey, A. Kalehua, D. Taub, J. Woodward, M.P. Mattson, & A. Nath. Differential transcriptional regulation by human immunodeficiency virus type 1 and gp120 in human astrocytes. *J Neurovirol*, 9(3), 358-371 (2003)
61. Marvanova M, J. Menager, E. Bezard, R.E. Bontrop, L. Pradier, & G. Wong: Microarray analysis of nonhuman primates: validation of experimental models in neurological disorders, *The FASEB Journal*, 17, 929-931 (2003)
62. Roberts ES, M.A. Zandonatti, D.D. Watry, L.J. Madden, M.A. Henriksen, & HS Fox: Induction of pathogenic sets of genes in macrophages and neurons in NeuroAIDS. *Am J of Pathol*, 162, 2041-2057 (2003).
63. Vahey MT, Nau ME, Taubman M, Yalley-Ogunro J, Silvera P, & Lewis MG: Patterns of gene expression in PBMNCs of Rhesus Macaques infected with SIVmac251 and exhibiting differential rates of disease progression, *AIDS Res and Hum Retrov*, 19, 369-387 (2003)
64. Radhakrishnan S, J. Otte, S. Enam, L. Del Valle, K. Khalili, & J. Gordon: JC Virus-induced changes in cellular gene expression in primary human astrocytes. *J of Virol*, 77, 10638-10644 (2003)
65. Zhang J, D. Zhang, & M. Xu: Identification of chronic cocaine-induced gene expression through Dopamine D1 receptors by using cDNA Microarrays. *Ann N Y Acad Sci*, 965, 1-9 (2002)
66. Fan L, M. van der Brug, W. Chen, P.R. Dodd, I. Matsumoto, S. Niwa, & P.A. Wilce: Increased expression of mitochondrial genes in human alcoholic brain revealed by differential display. *Alcohol Clin Exp Res*, Mar; 23(3), 408-13 (1999)
67. Chen W, Hardy P, & Wilce PA: Differential expression of mitochondrial NADPH dehydrogenase in ethanol-treated rat brain: revealed by differential display. *Alcohol Clin Exp Res*, Sep; 21(6), 1053-6 (1997)
68. Stewart RV, P. Shapshak, A. Matthews, N.C.J. Sun, R.A. Bitonte, M. Perez, S.M. Shah, M. Yoshioka, I. Nagano, K.S. Sundar, M. Fiala, T. Davis, & J.C. Arguello: Effects of cocaethylene on surface marker expression of HIV-1 infected macrophages isolated from human brain using antibody-coated magnetic beads: a preliminary study. Vision Research (A Journal of Morphology), 4, 66-72 (1997)
69. Kuljis RO, Shapshak P, P. Rodriguez de la Vega, R. Fujimura, & C. K. Petito: Increased density of Neurons containing NADPH diaphorase/nitric oxide synthase in the cerebral cortex of patients with drug abuse and HIV-1 infection. *J of NeuroAIDS*, 2, 19-36 (2002)
70. Nagano I, P. Shapshak, M. Yoshioka, Kq. Xin, Nakamura S, and Bradley WG: Increased NADPH-diaphorase reactivity and cytokine expression in dorsal root ganglia in AIDS. *J Neuro Sci*, 136, 117-128 (1996)
71. Shapshak P, R Duncan, A Minagar, P Rodriguez de la Vega, RV Stewart, & K Goodkin: Elevated Expression of IFN-γ in the HIV-1 Infected Brain. Frontiers in Bioscience, 9, 1073-1081 (2004)
72. Wodicka, L., H. Dong, M. Mittmann, & D.J. Lockhart: Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nature Biotechnol,* **15**, 1359–1367 (1997)
73. Sperber K & L. Shao: Neurologic consequences of HIV infection in the era of HAART. AIDS Patient Care Oct;17(10), 509-18 (2003)
74. Yoshioka M, P. Shapshak, A.K. Srivastava, S.J. Nelson, W.G. Bradley, J.R. Berger, R.H. Rhodes, N.C.J. Sun, R.V. Stewart, & S. Nakamura: Role of immune activation, interleukin-6 expression and HIV-1 in the pathology of dorsal root ganglia of patients with AIDS. Neurology, 44, 1120-1130 (1994)
75. Nagano I, Shapshak P, Yoshioka M, Xin Kq, Nakamura S, & Bradley WG: Parvalbumin and calbindin D-28k immunoreactivity in dorsal root ganglia in AIDS. *Neuropathology and Appl Neurobiol*, 22, 293-301 (1996)
76. 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. in Neuroimm*, 5, 335-358 (1995)
77. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, & Lander ES: Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science, Oct 15; 286(5439), 531-7 (1999)
78. Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, Lander ES, & Golub TR: Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. Proc Natl Acad Sci U S A, Mar 16, 96(6), 2907-12 (1999)
79. Biswas DK, T.R. Salas, F. Wang, C.H. Ahlers, B.J. Dezube, & A.B. Pardee: A Tat-induced auto-regulatory loop for superactivation of the HIV-1 promoter. *J of Virol*, 69, 7437-7444 (1995)
80. Mummaneni A & T. Kazic: The Architectural Dynamics of Cellular Biochemistry and molecular Biology, 6th World Multiconference on Systemics, Cybernetics and Informatics. www.iiis.org/sci2002/ Orlando, FL, July 14-18 (2002)

Key Words: Brain, HIV, AIDS, Microarray, Gene Expression, Encephalitis, HIV Associated Dementia (HAD), Affymetrix, Spotfire

Send correspondence to: Paul Shapshak, PhD, Department of Psychiatry & Behavioral Sciences, Elliot Bldg. 2013 (M849), 1800 NW 10th Ave, University of Miami School of Medicine, Miami, FL 33136, Tel: 305-243-3917, Fax: 305-243-5572, E-mail: pshapsha@med.miami.edu