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HIV associated dementia and HIV encephalitis II: Genes on chromosome 22 expressed in individually microdissected *Globus pallidus* neurons (Preliminary analysis)

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Abstract:
We analyzed RNA gene expression in neurons from 16 cases in four categories, HIV associated dementia with HIV encephalitis (HAD/HIVE), HAD alone, HIVE alone, and HIV-1-positive (HIV+)with neither HAD nor HIVE. We produced the neurons by laser capture microdissection (LCM) from cryopreserved *globus pallidus*. Of 55,000 gene fragments analyzed, expression of 197 genes was identified with significance ($p = 0.005$).We examined each gene for its position in the human genome and found a non-stochastic occurrence for only seven genes, on chromosome 22. Six of the seven genes were identified, CSNK1E (casein kinase 1 epsilon), DGCR8 (Di George syndrome critical region 8), GGA1 (Golgi associated gamma adaptin ear containing ARF binding protein 1), MAPK11 (mitogen activated protein kinase 11), SMCR7L (Smith-Magenis syndrome chromosome region candidate 7-like), andTBC1D22A (TBC1 domain family member 22A). Six genes (CSNK1E, DGCR8, GGA1, MAPK11, SMCR7L, and one unidentified gene) had similar expression profiles across HAD/HIVE, HAD, and HIVE vs. HIV+ whereas one gene (TBC1D22A) had a differing gene expression profile across these patient categories. There are several mental disease-related genes including miRNAs on chromosome 22 and two of the genes (DGCR8 and SMCR7L) identified here are mental disease-related. We speculate that dysregulation of gene expression may occur through mechanisms involving chromatin damage and remodeling. We conclude that the pathogenesis of NeuroAIDS involves dysregulation of expression of mental disease-related genes on chromosome 22 as well as additional genes on other chromosomes. The involvement of these genes as well as miRNA requires additional investigation since numerous genes appear to be involved.

Keywords: Chromosome 22, gene expression, network, pathway, miRNA, HAD, HIVE, LCM, *Globus pallidus*, neuron, brain.

Background:

NeuroAIDS and HAD:

The involvement of the CNS in HIV-related disease generally has been termed NeuroAIDS. HIV associated dementia (HAD) and minor cognitive motor disorder (MCMD) were terms used as well. The presence of infiltrating macrophages and activated microglial containing HIV-1, termed HIV encephalitis (HIVE) is part of the neuropathological substrate of this disease. In addition, neuronal damage is a key end-point in the disease process during and subsequent to HIV-1 brain infection **[1, 2, 3]**. Since the advent of HAART, a new term, HIV-associated neurocognitive disorders (HAND) has come to the fore. Due to HAART, the incidence of motor abnormalities and cognitive impairment of HAD and HIVE decreased although their prevalence has increased. Factors possibly associated with these changes may include viral mutations and patient longevity **[3, 4, 5]**. In the results reported here, the patients are from studies in an earlier time-period, prior to the implementation of HAND as a diagnostic entity **[6]**.

NeuroAIDS Gene expression and Neurogenetics:

The neurogenetics of NeuroAIDS has been under study for several years. A variety of genes, largely immune-related, but also those related to neurobiological functioning, have been found to be associated with neurocognitive outcomes and risk of HAD, although not all findings have been replicated **[7, 8, 9]**. This lack of consistency may be due to the difficulty in classifying NeuroAIDS neurocognitive disorders and the heterogeneous mechanisms leading to neurocognitive dysfunction. Gene expression in NeuroAIDS was studied for many years and has been previously reviewed **[3, 6, 10, 11, 12]**. In addition, a recent study produced gene expression data from *Globus pallidus* neurons purified by LCM from patients with HAD/HIVE,

HAD, HIVE, and HIV+ without HAD or HIVE **[6]**. A variety of inflammatory mediators and signaling pathway genes were implicated. These studies have yielded widely variable findings that will require additional efforts to integrate. The use of high throughput microarrays, for example, provides expression data for tens of thousands of probes. As such, methods for biologically relevant interpretation of these data have come into use, drawing largely from the burgeoning fields of systems biology and bioinformatics.

Materials & Methodology:

Brain Tissue, LCM, RNA Purification, and Gene Expression analysis:

Brain Tissue was obtained and LCM, RNA purification, and gene expression analysis were done as previously described **[6, 13]**. Autopsied cryopreserved brain tissue was obtained from several tissue banks in the NIH sponsored National NeuroAIDS Tissue Consortium (NNTC) **[14, 15]**. Neurons were microdissected, using LCM from *Globus pallidus* from HIV-positive individuals in four categories HAD/HIVE, HAD alone, HIVE alone, and HIV+ only. RNA was then purified from microdissected neurons, amplified, and gene expression analysis performed **[6, 13]**. Expression of 55,000 genes was analyzed using CodeLink Human Whole Genome Bioarrays **[16]**.

Statistical analysis:

CodeLink Expression Analysis software (GE Healthcare) was used,as previously described, to process the scanned images from the arrays **[6]**. GeneSpring software (Agilent Technologies) was then used to generate the gene expression data including quality control gene analyses **[6]**. Gene identification was based on urls **[17, 18]**. One-Way Analysis of Variance (ANOVA) was used to identify genes with expression that was statistically significant (at $p \leq 0.005$) across the four groups HAD/HIVE, HAD alone, HIVE alone, and HIV+ only, as previously described **[6]**. Pair-wise student t tests were then performed using the MSE (mean square error) term from the ANOVA, to test the simple effects of HAD/HIVE vs. control, HAD alone vs. control, and HAD alone vs. control for each selected gene **[6]**. In addition, the selected genes were identified as to their location on each of the 23 human chromosomes. (The Y chromosome was not considered). The expected number of genes per chromosome was calculated by multiplying the total of ascribed genes by the proportion of the total number of genes with known chromosome locations on that particular chromosome. The allocation of genes to each chromosome was then tested for randomness by a single degree of freedom Chi Square test and Bonferroni-adjusted ($p = .05/23 = 0.0023$).

Pathway analysis:

Gene Network Central PRO at was used to generate gene interaction and pathway figures **[19]**.

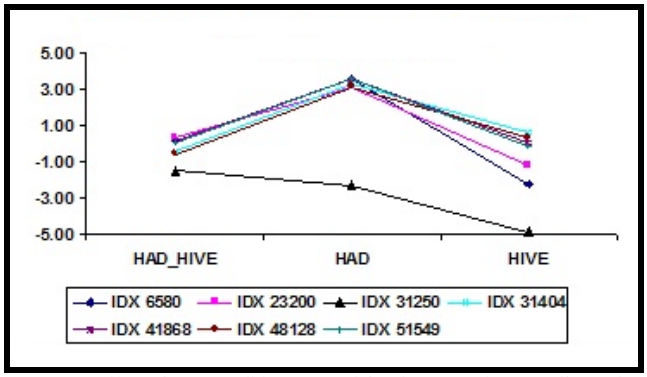

Figure 1: Effect Size [(Condition- control)/ Std. Dev.] of genes allocated to Chromosome 22.

Results:

190 genes, previously shown to be significantly expressed across HAD/HIVE, HAD alone, and HIVE alone vs. HIV+ only, had known locations on 23 human chromosomes. The result of gene allocation to chromosome is shown in **Table 1 (see Supplementary material)**. Only chromosome 22 demonstrated a non-stochastic number of ascribed genes ($p \leq 0.0001$).Six genes identified on chromosome 22 are CSNK1E (casein kinase 1 epsilon), DGCR8 (Di George syndrome critical region 8), GGA1 (Golgi associated gamma adapt in ear containing ARF binding protein 1), MAPK11 (mitogen activated protein kinase 11), SMCR7L (Smith-Magenis syndrome chromosome region candidate 7-like), andTBC1D22A (TBC1 domain family member 22A). One gene ascribed to chromosome 22 was unidentified. The array responses of the seven genes allocated to chromosome 22 across HAD/HIVE, HAD, and HIVE vs. HIV+, expressed as the effect size ratio (condition mean-control mean)/(standard deviation) are shown in **Figure 1**. Six of the genes show very similar responses, while the seventh shows a different response from the other six. The six genes with similar expression profiles were CSNK1E, DGCR8, GGA1, MAPK11, SMCR7L, and the single unidentified gene. One gene, TBC1D22A, had a differing gene expression profile across the patient categories compared to controls. **Figure 2** shows the molecular pathway network connections among the six identified genes located on chromosome 22. This pathway analysis shows the six genes interconnected with 69 additional nearest neighbor gene interactions. **Table 2 (see Supplementary material)** shows CodeLink and GE IDs, aliases, chromosome 22 band locations, and basic functions of the six identified genes as well as the interconnecting genes.

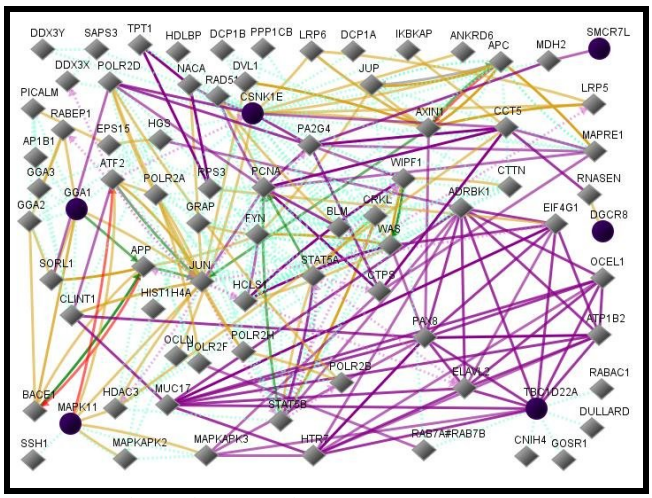

Figure 2: Pathways interconnecting 6 genes CSNK1E, DGCR8, GGA1, MAPK11, SMCR7L, and TBC1D22A as well as 69 additional nearest neighbor gene interactions.

Discussion:

A plethora of genes has already been implicated in the pathogenesis of neurological and neuropsychiatric disease that result from HIV-1 brain infection. Recent studies implicate additional genes with progress and use of newer techniques. Due to the complexity and prolonged course of HIV-1 associate brain disease, it should not be surprising that so many genes may be involved. We recently identified 150 genes involved in pathogenesis of HAD/HIVE, HAD alone, HIVE alone compared to only HIV-1 infection. The HAD/HIVE, HAD alone, and HIVE alone gene expression means for all of these genes are significantly different from the HIV+ control **[6]**. Here, we show that of seven genes that were associated with chromosome 22, two of the genes are mental disease-related. Moreover, there are additional mental disease-related genes on this chromosome.

Many additional genes interconnect the six genes that we identified on chromosome 22 and 69 such genes are shown in **Figure 2**. Four of the additional interconnection genes are also on chromosome 22, AP1B1, GRAP, CRKL, and POLR2D. AP1B1 (adapter-related protein complex 1, beta 1 subunit), forms complexes that mediate both the recruitment of clathrin to membranes and the recognition of sorting signals within the cytosolic tails of transmembrane cargo molecules. GRAP (GRB2-related adapter protein), couples signals from receptor and cytoplasmic tyrosine kinases to the Ras signaling pathway. CRKL (V-crk sarcoma virus CT10 oncogene homolog (avian)-like), participates in intra-cellular signal transduction. POLR2D (polymerase (RNA) II (DNA directed) polypeptide D), is a component of RNA polymerase. Thus, dysfunction and pathogenesis for the neurons caused by the dysregulation of the six genesis further exacerbated by the dysregulation of networks involving the four additional interconnection genes on this small chromosome.

Chromosome 22 is the second smallest chromosome and has several mental disease-related genes in addition to the two that we identified in our study that are on this chromosome. The *COMT* gene is located at 22q11.21-q11.23 and is associated with cognitive decline and schizophrenia as well as with HAND **[3, 10, 20, 21]**. CAG repeat loci on chromosome 22 are associated with schizophrenia and bipolar disorder at 22q11.2–q11.23 **[22]**. On chromosome 22, two miRNAs are associated with schizophrenia- microRNA 130b and microRNA 301b **[21]**. Children with 22q11 deletions exhibit cognitive, motor,

and other neurological problems **[23]**. Emanuel syndrome is consequent to chromosome imbalance of a derivative chromosome 22 [der(22)] as a supernumerary chromosome with the following karyotype: 47, XX, +der(22)t(11;22) (q23;q11) in females or 47, XY, +der(22)t(11;22)(q23;q11) in males or, rarely, as well as a balanced (11; 22) translocation with the supernumerary derivative chromosome**[24, 25]**. Seizures are associated with a gene, seizure related 6 homolog (mouse)-like at 22q12.1 **[21]**. Cerebral gyration disorders (polymicrogyria) result from deletions in the 22q11 region **[26]**. This disorder is probably due to neuronal migrational dysfunction during development. Moreover, there are several additional genes on chromosome 22 that involve processes already implicated in neuronal damage, to state a few, including aubiquitin pathway gene at 22q11.21, neurofilament heavy polypeptide at 22q12.2, and synaptosomal-associated protein at 22q11.21.**[21]**.

In a previous study, we developed a hypothesis that coerced gene expression caused by treatment of neurons in culture with gp120, tat, and/or cocaine was deleterious for the neurons and that this resulted in the breakdown of natural barriers or firewalls that prevent expression spillover that could occur **[27]**. Thus, uninterrupted stimulation of gene expression could result in contiguously expressed genes that could be deleterious. This hypothesis was supported in a recent study on the evolutionary significance of contiguous gene expression, even up to five megabases apart, indicating that this was disadvantageous for the organism. Moreover, on an evolutionary time-scale one gene of each such pair of genes was likely to migrate from where it was originally located, to another portion of the genome **[28]**. This indicates a driving force reacting to an otherwise harmful condition.

Conclusions and future directions:

Two of seven genes that show changes in expression on chromosome 22 are involved in mental disease and may be involved in the pathogenesis of HAD and HIVE (compared to HIV+ patients). In addition to disruption of molecular gene expression-networks in these neurons, disruption of these and additional genes on this small chromosome may occur through mechanisms involving chromatin damage, remodeling, and miRNA. Along these lines, we hypothesize that coerced gene expression associated with chronic disease and persistent HIV-1 infection may result in disruption of gene expression control of contiguous genes. Moreover, under optimal conditions, genes that are in proximity that are undergoing gene expression, should be as far from each other as possible. Concisely, hotspots of gene expression are potential sites of disease association.

The pathogenesis and treatment of neurological and neuropsychiatric diseases stemming from HIV-1 infection and subsequent invasion of the brain by HIV-1 are being extensively studied. New treatment paradigms are in progress; however, much depends on the identification of the molecular mechanisms involved. Clearly, total inhibition of HIV-1 replication and development of HIV-1 vaccines are desirable goals, but yet unaccomplished. Therefore, it is important to develop novel paradigms of molecular analysis that reveal new areas for treatment of neurological and neuropsychiatric disease that result from HIV-1 brain infestation.

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Supplementary material:

Table 1: Allocation of Selected Genes to Chromosomes

Chromosome	# of genes	# Allocated	# Expected	Chi Square	p
1	2968	23	18.38	1.28	0.2579
2	2288	15	14.17	0.05	0.8190
3	2032	9	12.58	1.09	0.2963
4	1297	7	8.03	0.14	0.7099
5	1643	7	10.18	1.04	0.3067
6	1963	6	12.16	3.32	0.0683
7	1443	12	8.94	1.10	0.2943
8	1127	9	6.98	0.61	0.4362
9	1299	5	8.04	1.20	0.2730
10	1440	13	8.92	1.96	0.1619
11	2093	9	12.96	1.30	0.2548
12	1652	12	10.23	0.32	0.5701
13	748	5	4.63	0.03	0.8628
14	1098	9	6.80	0.74	0.3906
15	1122	9	6.95	0.63	0.4282
16	1098	7	6.80	0.01	0.9378
17	1576	8	9.76	0.33	0.5633
18	766	3	4.74	0.66	0.4176
19	1454	9	9.00	0.00	0.9987
20	927	4	5.74	0.54	0.4608
21	303	2	1.88	0.01	0.9278
22	288	7	1.78	15.39	0.0001
X	1184	7	7.33	0.02	0.9003
Total	30625	190	197.00	-	-

Table 2: Seven additional genes on Chromosome 22. **[17, 18]**

CodeLink ID	GE ID	Alias	Chromosome 22 location	Function
IDX 6580	GE519998	-	22q11.21	XM_498870 Hs.434330
IDX 23200	GE61539	SMCR7L	22q13	Smith-Magenis syndrome chromosome region, candidate 7-like (SMCR7L)
IDX 31250	GE726916	TBC1D22A	22q13.3	GTPase activator
IDX 31404	GE729136	GGA1	22q13.31	protein binding and intracellular transporter,protein complex assembly, intra-Golgi transport, clathrin coat of trans-Golgi network vesicle, contains Alu repetitive element.
IDX 41868	GE82723	DGCR8	22q11.2	double-stranded RNA and protein binding, DiGeorge syndrome critical region gene 8.
IDX 48128	GE86394	CSNK1E	22q13.1	nucleotide binding, protein serine/threonine kinase activity, casein kinase I activity, protein-tyrosine kinase activity, ATP binding, transferase activity, DNA repair, protein amino acid phosphorylation, signal transduction, casein kinase 1-epsilon-transcript variant 1
IDX 51549	GE88364	MAPK11	22q13.33	nucleotide binding, protein serine/threonine kinase activity and cascade, MAP kinase activity, ATP binding, transferase activity, response to stress, signal transduction, antimicrobial humoral responsemitogen-activated protein kinase 11-transcript variant 1