INFLUENCE OF BEHAVIORAL EXPOSURES ON MICRORNA DYSREGULATION IN RELATION WITH HIV-1 ASSOCIATED NEUROCOGNITIVE DISORDER (HAND)

by

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INFLUENCE OF BEHAVIORAL EXPOSURES ON MICRORNA DYSREGULATION IN RELATION WITH HIV-1 ASSOCIATED NEUROCOGNITIVE DISORDER (HAND)

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Abstract

HIV-1 positive individuals demonstrate a wide variety of comorbidities based on their genetic makeup and their behavioral factors. Differentiation of gene expression caused by behavioral factors such as smoking of tobacco, consumption of alcohol and illicit drug use have been shown to occur through miRNA and can have additive and adverse effects on the co-morbidities associated with HIV-1 infection. Here, we proposed to identify the miRNA that are being differentially regulated by the above mentioned behavioral exposures and their possible effects on the progression to HIV-1 associated neurocognitive disorder (HAND) by performing a cross sectional study.

The Multi-center AIDS Cohort Study (MACS) provided information on all the subjects used in this study. The samples collected were divided into study groups based on their infection and cognitive status, resulting in three categories, uninfected controls, HIV-1 positive/dementia negative and HIV-1 positive/ dementia positive. The term dementia was used to identify an individual with any neurocognitive impairment regardless of the actual impairment. The individuals in each category were further divided based on their behavioral habits, smokers, non-smokers etc. The miRNA was obtained from PBMCs of the selected individuals and quantified

using TaqMan real time PCR assays. Computational analysis was used to find the miRNA that was significantly regulated by performing inter and intra comparisons within the established categories.

Comparison of miRNA's in controls vs. HIV-1 positive/dementia negative subjects resulted in a majority of miRNA's being up-regulated in all variables (smoking, alcohol consumption and pot use). Whereas, comparison of miRNA's in controls versus HIV-1 positive/dementia positive and HIV-1 positive/dementia negative vs. HIV-1 positive/dementia positive subjects resulted in a majority of miRNA's being down-regulated in all variables. This anomaly shows that there is an association between HAND and miRNA expression, mostly by down-regulation.

Public Health Significance: The major public health significance projected to come from this study include the possible identification of patients who are prone to develop cognitive disorders and identification of mechanism(s) underlying the development of HAND, which may lead to identification of new drug targets. In the long term, this knowledge may yield novel therapeutic interventions for the treatment of cognitive disorders and antivirals targeting CNS compartments.

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1.0 INTRODUCTION

There are approximately 35.0 million people living with HIV-1 infection globally (WHO, HIV/AIDS key facts). In the era of antiretroviral therapy prevalence of HIV-1 has been steadily increasing, though incidence has decreased from 3.3 million in 2002 to 2.3 million in 2012 (Maartens, Celum, & Lewin, 2014). As the population of HIV-1 positive individual's ages, we now see an increase in HIV-1 associated co-morbidities, such as HIV-1 associated neurocognitive disorder (HAND) (Hellmuth, Milanini, & Valcour, 2014). Highly active antiretroviral therapy (HAART) has greatly reduced the morbidity and mortality of HIV-1 infection, but high rates of HIV-1 associated neurocognitive disorders (HAND) continue to rise. What is more, HIV-1 positive individuals, who have received antiretroviral therapy, are still more prone to develop neurologic diseases and at a younger age then uninfected individuals (Chen, Gill, & Kolson, 2014).

HAND is observed in 30-60% of all identified HIV-1 positive individuals; though it does present in a milder form than in the pre-HAART era, it still disrupts an individual's quality of life (Lindl, Marks, Kolson, & Jordan-Sciutto, 2010). Behavioral habits, such as smoking of tobacco, consumption of alcohol and illicit drug use, have all been shown to have adverse health effects. Coupling these behavioral factors with HIV-1 infection has been shown to have an additive effect that adversely impacts neurocognitive functioning (Martin-Thormeyer & Paul, 2009). Literature on both HIV-1 and stimulant useage indicates that along with host genetic

variants in immunologic genes affect an individual's risk for neurocognitive impairments. These effects may occur at the transcriptional level by regulation of microRNA (need to find the source)

MicroRNAs (miRNA) are small non-coding RNA molecules that regulate translation, stabilization and degradation of messenger RNA at the posttranscriptional level. MicroRNAs have been indicated to have a roll in various normal cellular functions; therefore disruption of their expression can lead to pathological conditions. It has been shown that a single miRNA can control several genes, which in turn regulate numerous signaling pathways. This is one of the reasons that miRNAs are thought to make good biomarkers to indicate a diseased state (Rico-Rosillo, Vega-Robledo, & Oliva-Rico, 2014)

HIV-associated comorbidities such as HAND can be aggravated by an individual's behavioral habits. We believe that habits such as smoking, drinking, and illicit drug use may be affecting the individuals on a genetic level, which is they are affecting regulation of host cellular genes at the post-transcriptional level through miRNA's. If we identify the miRNA's being regulated and understand the pathways being regulated we may be able to use the miRNA's as potential biomarkers for HAND.

2.0 BACKGROUND

2.1 HIV DISEASE PROGRESSION AND ASSOCIATED CO-MORBIDITIES

2.1.1 Epidemiology of HIV-1 Infection

Human immunodeficiency virus-1, which causes Acquired immunodeficiency syndrome (AIDS) was first reported in the United States in 1981(Gottlieb et al., 1981), and since then has been responsible for nearly 75 million infections (UNAIDS, 2013). Today despite our vast knowledge of inter species cross-transmission of simian immunodeficiency virus (SIV); known to be the virus from which HIV-1 originated; to humans from primates we are still unable to fully understand the complexity that is HIV-1 and how it continually exists as a global epidemic (Faria et al., 2014). Currently there are approximately 35.0 million individuals living with HIV in the world, with only 12.9 million people receiving antiretroviral therapy (ART) globally (WHO, 2014).

HIV-1 causes AIDS by attacking the immune system by primarily infecting two types of cells, the CD4+ T cells and the macrophages. Disease progression with HIV-1 infection is usually determined by loss of CD4+ T lymphocytes (Post, Wood, & Maartens, 1996). HIV-1 infection can be categorized into stages based on the CD4 count, as viral load increases the CD4 count decreases until a viral set point is reached. After this point virus stabilization or viral set point occurs where the virus load levels off and the CD4 count increases slightly (Mehandru et

al., 2004). The next stage of HIV-1 infection usually lasts an average of ten years and is characterized as clinical latency, because though the virus is still active, very low levels of viral replication occur. At this stage an individual may exhibit healthy CD4 counts as well as undetectable amounts of virus (Wannamethee et al., 1998). The final stage of HIV-1 infection is termed AIDS and is represented by a CD4 count of less than 200 cells per cubic millimeter of blood. At this point the average survival is three years without treatment (Fevrier, Dorgham, & Rebollo, 2011). It is during this stage that co-infections termed opportunistic infections are more prevalent and most likely to be the cause of death.

2.1.2 Types of HIV

There are two types of human immunodeficiency viruses, HIV-1 and HIV-2, that cause acquired immunodeficiency syndrome, both of which originated in West Africa (Sharp & Hahn, 2011).HIV-1 infection in humans was caused by four cross-species transmissions of simian immunodeficiency viruses (SIVs) from various primates, whereas, HIV-2 virus is due to eight independent transmissions of SIVs; only one of these transmission, the HIV-1 group M from chimpanzees in southeastern Cameroon, resulted in the global epidemic we see today (Peeters, Jung, & Ayouba, 2013). The virus that causes HIV-2 appears to be confined mostly to the West African countries (Cohen, 2000), HIV-1 on the other hand is seen in almost every country. Whereas, SIVs most closely related to HIV-1, have been shown to cause CD4 T-cell depletion and increased mortality in wild chimpanzees, suggesting that evolutionarily, some form of acquired immunodeficiency syndrome originated long before HIV-1 crossed species (Sharp & Hahn, 2010).

2.1.3 Mode of Infection

HIV-1 spreads through direct contact of bodily fluids from someone with HIV. The main spread of the disease in the United States is through sexual contact or sharing infected needles (CDC, 2014). In many other countries HIV-1 is transmitted through blood transfusion (Lackritz, 1998). HIV can also be transferred vertically from infected mother to child during pregnancy, childbirth or breastfeeding (Nadal, Arlettaz, & Berger, 2014).

HIV-1 can infect various cells in the host, but it primarily infects two types of cells, CD4 T-cells and macrophages. After a CD4 cell is infected with HIV, the virus goes through multiple steps to reproduce itself and create many more virus particles (Kedzierska & Crowe, 2002)

Cell to cell transmission of HIV enables it to escape elimination by the immune system, by infecting cells that are not easily infected (Costiniuk & Jenabian, 2014). Cell to cell transmission is where the virus exploits and manipulates cell to cell contact which leads to increased spread and higher viral load in less time (Mothes, Sherer, Jin, & Zhong, 2010). Certain HIV-1 lineages are better adapted to enter cells that express low levels of CD4 such as cells in the CNS, and maintain sustained replication, which is associated with severe neurocognitive impairment (Joseph, Arrildt, Sturdevant, & Swanstrom, 2014).

2.1.4 Co-morbidities and Risk Factors Associated with HIV-1

HIV-1 infection can lead to an individual to become more susceptible to various other co-infections and co-morbidities, especially during the later stage defined as AIDS. Individuals with weakened immune systems, such as those with AIDS are prone to develop illness from co-infections called opportunistic infections which are infections caused by various pathogens that

do not usually cause illness in individuals with a healthy immune system. Some of the most common co-infections are candidiasis, which manifests as oral thrush, hepatitis, encephalopathy, herpes simplex and pneumonia (AIDS.gov, 2014).

There are several other co-morbidities associated with HIV-1 infection that are aggravated by the virus and therefore cause a more devastating outcome in those with HIV-1 when compared to those who do not have HIV-1. For example it has been shown that there is a greater risk of cardiovascular disease in HIV-1 positive individuals than in the general population (Tripathi et al., 2014). One of the least understood and therefore hardest to treat co-morbidities associated with HIV-1 infection is HIV-1-associated neurocognitive disorder or HAND.

The term HAND was coined by the National Institute of Mental Health in 2007, and it encompasses the entire spectrum of neurological disease associated with HIV infection except delirium. A few of the more commonly seen manifestations of HAND are asymptomatic neurocognitive impairment (ANI), minor neurocognitive disorder (MND) and HIV-associated dementia (HAD) (Rosca, Rosca, Simu, & Chirileanu, 2012). HAND occurs quite early in the timeline of HIV infection, it has most likely taken root before an individual has even started on an antiretroviral regime. Early signs of HAND include memory loss and decreased concentration, which are mistakenly associated with age as opposed to HIV-1 infection (Eggers & fur die Deutsche Neuro, 2014). Recently, due to advanced antiretroviral therapy started as soon as an individual has tested positive, there has been a decrease in the incidence of HAND, but as the population of HIV positive individual's ages the prevalence of HAND increases (Ances & Ellis, 2007).

MRI scanning of HIV-1 positive individuals using HAART, who have a history of substance abuse (alcohol, tobacco, etc.) shows increased degradation of the brain tissue, which can lead to HAND (Holt, Kraft-Terry, & Chang, 2012).

2.2 EPIGENETIC EXPLANATION FOR HIV-ASSOCIATED COMORBIDITY PROGRESSION

2.2.1 Introduction to microRNA

MicroRNAs or miRNAs are small nucleotide of highly conserved non-coding RNA molecules that in normal cells maintain various necessary cellular processes. Dysregulation of these miRNAs can therefore lead to dire consequences (Kasinski & Slack, 2011). More than three percent of all the genes in an individual codes for miRNA which in turn regulates the expression of thousands of target mRNAs (O'Driscoll, 2006). MicroRNAs, through regulation of mRNAs are involved in development, stem cell regulation and human diseases. The association between miRNAs and their target mRNA is extremely diverse and complex as a single miRNA has the potential to regulate multiple mRNA, and on the other had multiple miRNA can all regulate a single mRNA as well (Yi & Fuchs, 2011).

2.2.2 Function and Processing of microRNA

MicroRNAs were previously thought to be unnecessary bits of RNA floating around with no necessary function, but it was discovered that these small, about 18-25 nucleotides, clusters of

non-coding RNA actually play a major role in regulating gene expression (Ranganathan & Sivasankar, 2014). Mature miRNA form protein complexes called miRISC-miRNA-induced silencing complex, to bind to target mRNA to either inhibit translation of the mRNA or degrade the mRNA (Williams, 2008).

2.2.3 Relationship between miRNA and Disease

There is dysregulation seen in miRNA in cancer cells and various damaged tissues, therefore miRNA is believed to play a functional role in the development of diseases. It is believed that circulating miRNA, ("miRNA that is seen in cell-free serum") can be used to identify individuals that are diseased for those that are not (Weiland, Gao, Zhou, & Mi, 2012). Though there is still relatively little known about the actual function of miRNA, because of their roles in clinical

3.0 THESIS AIMS

Host cellular gene expression (transcriptome) profile in response to pathogen interaction is directly correlated with disease patterns. Gene expression in response to pathogens is regulated by host genetics, epigenetics and external factors. We hypothesize that behavioral factors, such as smoking of tobacco, drinking of alcohol, and illicit drug usage influence host cellular gene expression in part by microRNA regulation. This differential regulation in turn may accelerate HIV-1 disease progression and HIV-1 associated co-morbidities such as CVD (cardio-vascular disease), COPD (constrictive obstructive pulmonary disease) and HAND.

Aim #1 - To assess whether smoking, alcohol consumption and marijuana use have any role in miRNA expression in the context of HIV-1 infection and neurocognitive disorder/dementia

A) MicroRNA profiling in PBMCs of uninfected controls and HIV-1 infected subjects with and without dementia
B) Determine the effect of behavioral factors on miRNA expression and dysregulation

Aim #2- To identify the biological pathways associated with differentially regulated miRNAs in HIV-1 infected subjects with or without dementia

A) Computational analyses to predict the potential mRNA target genes
B) Identification of biological pathways regulated by the predicted mRNAs

4.0 MATERIALS AND METHODS

4.1 SAMPLE SELECTION

Samples used in this study were acquired from the Multicenter AIDS Cohort Study (MACS). The samples were divided, into three categories based on their HIV-1 status and the presence of dementia, in order to carry out a cross-sectional study. The behavioral habits and antiretroviral use information for each individuals was also provided by the MACS. The samples were categorized as uninfected seronegative group, 1A (N=32), HIV-1 positive dementia negative 2D, (N=20), and HIV-1 positive dementia positive 2E, (N=26). HIV-1 positive individuals were grouped into dementia negative and dementia positive based on a comprehensive exam performed in the MACS clinics. The individuals are placed in the dementia category if they consistently (more than 3 times) fail the cognitive exams as well as based on the brain pathology, if the samples were taken from deceased individuals. Peripheral blood mononuclear cells were obtained from each patient, from which RNA was extracted. The extracted RNA was then converted to cDNA, which then profiled for various miRNA using the TaqMan Human MicroRNA Array A and the TaqMan Array Human MicroRNA B card. The lists of miRNA obtained from these arrays were used in the computation analysis; to detect miRNA that could potentially altered by behavioral factors associated with the development of HAND. Details of the subjects including age, collection date, CD4 count, viral load, behavioral habits and antiviral

use are listed in Table 1, 2 and 3. In tables 1, 2 and 3, individuals are placed in the used smoking category if they have 30 or more pack years. A pack year is defined as twenty cigarettes smoked every day for one year according to the National Cancer Institute. Individuals are placed in the used alcohol category if they are defined as being alcoholics by the FDA, hat is if they have thirteen or more drinks per week. Individuals are placed in the marijuana category if they have ever used marijuana.

Table 1: Details of Study Subjects (Uninfected Control Group)

ID	Age	CD4 on collection day	Smoke	Alcohol	Marijuana
1A	50	790	2	1	1
2A	51	2194	2	2	2
3A	77	489	1	2	1
4A	72	530	1	1	1
5A	58	568	2	1	1
6A	52	993	2	1	1
7A	56	488	2	1	1
8A	52	733	2	1	1
9A	50	944	1	1	1
10A	64	879	2	1	1
11A	54	644	1	1	1
12A	55	584	2	1	1
13A	56	385	2	2	2
14A	55	889	2	2	2
15A	51	885	1	1	2
16A	53	702	1	2	2
17A	57	537	1	1	1
18A	60	1019	1	1	1
19A	49	589	1	1	1
20A	46	918	2	1	1
21A	49	685	2	2	2
22A	42	841	2	2	2
23A	38	1074	2	1	1
24A	42	1634	1	1	2
25A	38	545	1	1	1
26A	28	1178	2	2	2
27A	36	852	2	1	1
28A	36	789	2	1	1
29A	30	732	1	2	2
30A	28	857	2	2	2

Table 1 Continued

31A	43	1106	1	2	1
32A	33	886	2	2	1

Legend

1- Did not use

2- Used

The above table represents the number of individuals categorized as HIV-1 positive/dementia negative, their age, date of sample collection, CD 4 count, viral load, antiviral use and lists their behavioral exposures**.**

Table 2: Details of Study Subjects (HIV-1 Positive Dementia Negative Group)

MY ID	Age	Collection Date	CD4 Count	Viral Load	Smoke	Alcohol	Marijuana	Antiviral
1D	33	5/27/92	310	127376	2	1	2	1
2D		2/8/91	100	111314	2	1	1	2
3D	35	11/5/90	38	167261	2	1	1	1
4D	42	3/28/01	232	252692	1	1	1	2
5D	50	4/9/96	120	297672	1	1	1	2
6D		10/18/88	336	161325	2	2	2	2
7D	39	2/8/96	188	201158	1	2	1	1
8D		11/3/87	272	50530	1	1	1	2
9D		5/14/94	215	184411	1	2	1	2
10D		1/13/87	120	370999	2	2	1	1
11D	51	5/2/96	136	561627	1	2	1	2
12D	28	5/22/92	234	46053	1	2	1	1
13D	47	11/12/94	151	146580	2	2	1	1
14D	36	4/23/99	312	71580	2	1	1	2
15D	40	11/13/87	351	116293	1	1	1	1
16D	39	7/17/95	96	47310	1	1	2	2
17D	37	4/14/99	378	289528	2	1	1	2
18D	37	9/29/08	222	96534	2	1	2	2
19D	43	2/6/11	179	79454	1	1	1	2
20D		12/22/93	36		2	1	1	2

Legend

1- Did not use

2- Used

The above table represents the number of individuals categorized as HIV-1 positive/dementia negative, their age, date of sample collection, CD 4 count, viral load, antiviral use and lists their behavioral exposures.

Table 3: Details of Study Subjects (HIV-1 Positive Dementia Positive Group)

MY ID	Age	Collection date	CD4 count	Viral Load	Smoke	Alcohol	Marijuana	Antiviral
1E	45	09/18/03	636	261140	2	1	2	2
2E	37	05/07/89	601	11888	2	2	1	1
3E	33	01/04/95	39	470923	2	2	2	1
4E	36	05/15/95	205	.	2	2	2	1
5E	40	11/15/90	418	195000	2	2	2	1
6E	42	11/06/94	41	277132	2	2	2	1
7E	49	06/23/93	271	.	2	2	2	1
8E	43	08/01/93	696	18991	2	2	2	1
9E	46	10/03/93	22	288518	1	2	1	1
10E	42	08/03/92	33	28120	2	2	2	1
11E	50	02/13/92	209	103947	2	2	2	1
12E	48	05/20/96	416	313669	1	1	2	2
13E	52	06/20/02	179	63386	2	2	1	2
14E	40	02/09/93	416	.	1	2	2	1
15E		9/21/99	337	76521	2	2	2	2
16E	49	10/19/92	336	161325	2	2	2	1
17E		4/6/99	162	198196	2	1	2	2
18E	39	07/21/93	451	11595	2	2	1	1
19E		6/29/07	219	336300	2	1	1	2
20E		4/12/07	246	47083	2	1	1	2
21E	34	07/24/92	65	280987	1	1	1	2
22E	36	08/07/89	265	791689	2	2	2	1
23E	34	05/15/92	446	116040	2	2	1	1
24E	38	06/22/92	197	80600	1	2	1	1
25E	45	09/28/89	267	681894	2	1	1	1
26E	47	09/30/93	151	95883	2	1	1	2

Legend

1- **Did not use**
2- **Used**

The above table represents the number of individuals categorized as HIV-1 positive/dementia positive, their age, date of sample collection, CD 4 count, viral load, antiviral use and lists their behavioral exposures.

4.2 CATEGORIZATION OF SAMPLES

Once the individuals were separated based on their HIV-1 status, they were further grouped based on their behavioral habits. Subjects were placed in the tobacco smoking category, depending on how long an individual smoked and whether they were heavy smokes, or mild/non-smokers. In the alcohol consumption category, the patients were stratified based as alcoholic who had thirteen or more drinks a week, or non-alcoholic who had less than three drinks a week. In the illicit drug use, the individuals were grouped based on whether an individual used or did not use marijuana. The individuals were then finally grouped based on whether they were on antiretroviral therapy or not.

4.3 COMPUTATIONAL ANALYSIS

Statminer, a bio-informatics tool, was used to identify genes that showed significant differential expression/integration of biological pathways by comparing the miRNA's of controls vs. HIV-1 positive/dementia negative vs. HIV-1 positive/dementia positive.

After the individuals were separated based on their behavioral habits, the individuals' miRNA were first intra-compared (comparison within a group, heavy smokers in uninfected

controls vs mild/non- smokers in uninfected controls, etc.) to recognize the miRNA that was commonly regulated within each category. Then the miRNA was inter-compared, for example individuals who were heavy smokers in the uninfected controls were compared with the individuals that were heavy smokers in HIV-1 positive group with and without dementia, etc. to identify the miRNA that were commonly effected in heavy smokers, in all three categories.

Target Scan, an online software program provided by MIT, was used for predication of mRNA target genes. This software is effective in providing a list of predicated gene targets for a particular miRNA. These gene target lists were then analyzed using another software that predicts the most probable pathways affected by the target genes.

Integrated pathway analysis (IPA), an online-based tool used to perform functional analysis was used to construct canonical pathways that are possibly affected by the regulated miRNA. These pathways help us visualize the different functions that are potentially affected by the miRNA.

5.0 RESULTS

5.1 AIM #1 - TO ASSESS WHETHER SMOKING, ALCOHOL CONSUMPTION AND POT USE HAVE ANY ROLE IN MIRNA EXPRESSION IN THE CONTEXT OF HIV-1 ASSOCIATED NEUROCOGNITIVE DISORDER/DEMENTIA

5.1.1 MicroRNA profiling in PBMCs of uninfected controls and HIV-1 infected subjects with and without dementia

To examine the effects of the behavioral exposures on miRNA profiles in the uninfected controls, HIV-1+/dementia- and HIV-1+/dementia+, we isolated miRNA from PBMC's and profiled it using Taqman assay using four endogenous controls.

The miRNA profiling consisted of running two microfluidic cards per sample for the combined effect of the ability to detect 768 miRNA and 12 controls. The samples, where the endogenous controls exceeded Ct value of 25 were eliminated. Our endogenous control analyses indicate that the Ct values for all our samples range from 15-22 and none of them are more than 25.

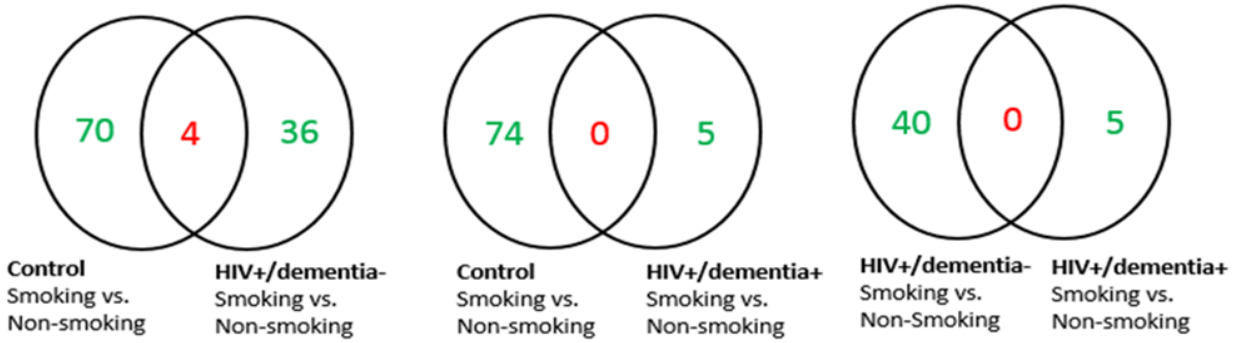
Initially miRNA within the groups that is the miRNA obtained for the uninfected control group in smoking was compared to the uninfected control group non-smoking. The same was assessed within the HIV-1 positive/dementia negatives groups and the HIV-1positive/dementia positive groups, using all three behavioral factors. By comparing the resulting miRNA lists from

the intra-comparisons, inter- comparison (between the categories) was done to find commonly regulated miRNA. Table 2 shows the number or subjects in each category.

Table 4: Number of Subjects in Each Category Separated by Behavioral Exposures

	Smoking		Alcohol		Marijuana		All three Substances	
	Yes	No	Yes	No	Yes	No	Yes	No
Uninfected Controls (n=32)	19	13	12	20	11	21	7	25
HIV-1 positive/dementia negative (n=20)	10	10	7	13	4	16	1	19
HIV-1 positive/dementia positive (n=26)	21	5	18	8	9	17	11	15

Differentially regulated miRNAs between these groups were analyzed using two different software packages using appropriate settings required for each program to maximize the confidence. The first program used was Realtime Statminer, a bioinformatics program which analyzes significantly differentially regulated miRNA within and between these groups. Results indicate that the behavioral exposures, differentially regulated the expression of several miRNAs, signified by p-value of less than 0.05 and fold change calculated based on normalization with endogenous control, U6 snRNA/MammU6. Figures 2, 3, 4 illustrate the number miRNA regulated in each category as well as the number miRNAs that were commonly regulated. Tables 3, 4, 5 exhibit the miRNA that were found to be commonly regulated in selected two categories.



The Venn diagram displays the number and overlap of significantly differentially expressed miRNA (no FDR adjustment, p<0.05) among the HIV-positive/dementia negative and HIV-positive/ dementia positive groups relative to the Uninfected controls and within the infected groups. MiRNAs represent those miRNAs that are found to be significantly differentially regulated.

Figure 1: Venn Diagrams Illustrating Number of Commonly Regulated MiRNA in Smoking

Venn diagrams illustrating the number of miRNA significantly regulated due to smoking in each category as well as the number of miRNA that are commonly regulated in both categories.

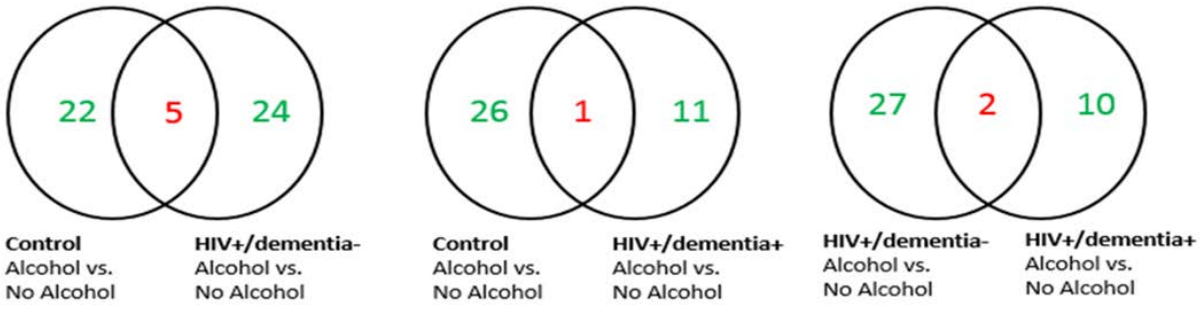
Table 5: MicroRNA Regulated by Smoking in Controls and HIV-1 Positive Dementia Negative

	Uninfected Controls		HIV-1 positive/dementia negative	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-152	7.5407	0.0122	2.6747	0.0193
hsa-miR-629	-2.3568	0.0330	4.1165	0.0103
hsa-miR-874	5.3286	0.0133	19.2739	0.0250
hsa-miR-148	3.4703	0.0328	2.9295	0.0183

The above table lists the miRNA that were found to be differentially regulated due to smoking in uninfected controls and HIV-1 positive/dementia negative individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on 0.05 significance)

The above table indicates that there is significant miRNA dysregulation due to smoking. For example, hsa-miR-629 is down regulated in the uninfected controls, whereas, it is significantly up-regulated in HIV-1 positive/dementia negative individuals. Hsa-miR-874 is up regulated in both categories, but in HIV-1 positive/dementia negative individuals this miRNA has a much

higher fold change, compared to control group. These dysregulations could be due to the smoking of tobacco, due to the HIV-1 infection or due to a combination of both.



The Venn diagram displays the number and overlap of significantly differentially expressed miRNA (no FDR adjustment, p<0.05) among the HIV-1 positive/dementia negative and HIV-1 positive/ dementia positive groups relative to the Uninfected controls and within the infected groups. MiRNAs represent those miRNAs that are found to be significantly differentially regulated.

Figure 2: Venn Diagrams Illustrating Number of Commonly Regulated MiRNA in Alcohol

Venn diagrams illustrating the number of miRNA significantly regulated due to alcohol in each category as well as the number of miRNA that are commonly regulated in both categories.

Table 6: MicroRNA Regulated by Alcohol in Controls and HIV-1 Positive Dementia Negative

	Uninfected Controls		HIV-1 positive/dementia negative	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-125a-3p	-4.4821	0.013	-8.2404	0.0311
hsa-miR-1304	26.9748	0.000	37.3787	0.0384
hsa-miR-455-3p	8.1547	0.0032	-13.5234	0.0444
hsa-miR-147	13.4689	0.0040	8.5758	0.0296
hsa-miR-556	-33.4100	0.0166	7.7604	0.0194

The above table lists the miRNA that are found to be differentially regulated due to alcohol in uninfected controls and HIV-1 positive/dementia negative individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on 0.05 significance)

In Table 6, the miRNA deregulated by alcohol are listed and here we observed differential regulation of these miRNA when comparing the miRNAs fold change and significant p-value in the controls vs HIV-1 positive/dementia negative subjects. Though all the miRNA listed have differences in their quantity and regulation, the two miRNA that are most significantly regulated are hsa-miR-455-3p and hsa-miR-556, both of them are inversely correlated in controls compared to HIV-1+/dementia- individuals. In this behavioral exposure as well we cannot explicitly say whether the alcohol or the virus is causing the disparities, though disparity is observed.

Table 7: MicroRNA Regulated by Alcohol in Controls and HIV-1 Positive Dementia Positive

	Uninfected Control		HIV-1 positive/dementia positive	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-518	28.2518	0.0039	-97.1187	0.0221

The above table lists the miRNA that are found to be differentially regulated due to alcohol in uninfected controls and HIV-1 positive/dementia positive individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on $p < 0.05$)

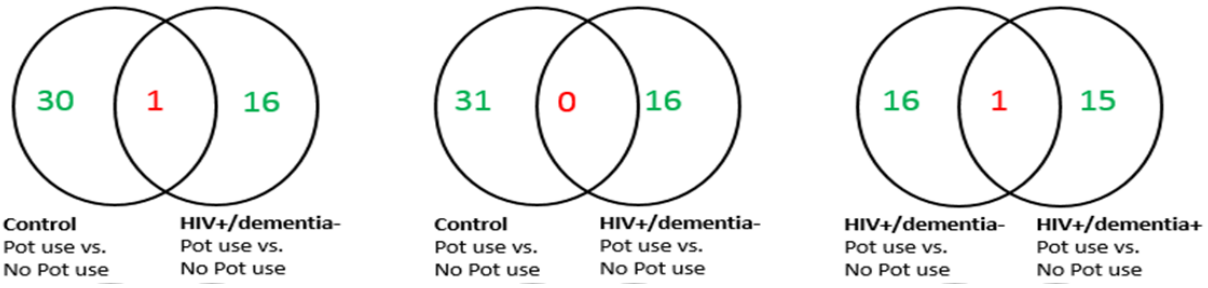
The above table highlights one of the largest disparities, the miRNA is up-regulated in the controls but is extremely down-regulated in HIV-1 positive/dementia positive individuals. This disparity could be caused by one of four conditions, it could be due to the virus; it could be due to the alcohol; it could be due to the dementia, or it could be due to any combination of the three.

Table 8: MicroRNA Regulated by Marijuana in HIV-1 Positive Dementia Negative and HIV-1 Positive Dementia Positive

	HIV-1 positive/dementia negative		HIV-1 positive/dementia positive	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-218-1#	12988.3322	0.0019	-6.2417	0.0213
hsa-miR-497	21.7973	0.0007	-20.1807	0.0009

The above table lists the miRNA that are found to be differentially regulated due to alcohol in HIV-1 positive/ dementia negative individuals and HIV-1 positive/dementia positive individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on 0.05 significance)

The above table indicates that miRNA that are dysregulated due to marijuana use in HIV-1 positive individuals with and without dementia. This table shows that hsa-miR-218-1# was present in extremely large quantities and up-regulated in HIV-1 positive/dementia negative individuals but was down regulated in HIV-1 positive/dementia positive individuals. This extremely large discrepancy could have been due to a technical error on part of the software program used, but it could also be that this miRNA is up-regulated and is present in large quantities in the HIV-1 positive/dementia negative individuals.



The Venn diagram displays the number and overlap of significantly differentially expressed miRNA (no FDR adjustment, $p<0.05$) among the HIV-positive/dementia negative and HIV-positive/ dementia positive groups relative to the Uninfected controls and within the infected groups. MiRNAs represent those miRNAs that are found to be significantly differentially regulated by RealTime StatMiner.

Figure 3: Venn Diagrams Illustrating Number of Commonly Regulated MiRNA in Marijuana

Venn diagrams illustrating the number of miRNAs significantly regulated due to pot use in each category as well as the number of miRNAs that are commonly regulated in both categories.

Table 9: MicroRNA Regulated by Marijuana in Controls and HIV-1 Positive Dementia Negative

	Uninfected Controls		HIV-1 positive/dementia negative	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-548	8.0224	0.0088	10.6886	0.0445

The above table lists the miRNA that are found to be differentially regulated due to pot use in uninfected controls and HIV-1 positive/dementia positive individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on 0.05 significance)

The above table shows that the miRNA hsa-miR-548 was up-regulated in both the uninfected controls as well as the HIV-1 positive/ dementia negative individuals, thought there was a slightly larger quantity of the miRNA in the latter.

Table 10: MicroRNA Regulated by Marijuana in HIV-1 Positive Dementia Negative and HIV-1 Positive Dementia Positive

	HIV-1 positive/dementia negative		HIV-1 positive/dementia positive	
MicroRNA	Fold Change	P-Value	Fold Change	P-Value
hsa-miR-558	22.1775	0.0043	-6.1437	0.0090

The above table lists the miRNA that are found to be differentially regulated due to pot use in HIV-1 positive/dementia negative and HIV-1 positive/dementia positive individuals. The fold change indicates the quantity changes of expression of the test group by expression of the control group, a positive number specifies up-regulation, and a negative number specifies down-regulation. The p-value indicates the significance of the fold change (based on 0.05 significance)

The above table indicates that hsa-miR-558 is differentially regulated in individuals with HIV-1 and with or without dementia. In HIV-1 positive/dementia negative individuals the miRNA is present 22 times higher fold than the endogenous miRNA and is being up-regulated, whereas, in HIV-1 positive/dementia positive individuals this miRNA has decreased in quantity by 6 fold and is being down-regulated.

5.2 AIM #2- IDENTIFY THE BIOLOGICAL PATHWAYS ASSOCIATED WITH DIFFERENTIALLY REGULATED MIRNAS IN HIV-1 INFECTED SUBJECTS WITH OR WITHOUT DEMENTIA

5.2.1 Computational analyses to predict possible mRNA target genes

Once we had identified the miRNA that were being differentially regulated due to the behavioral exposures, we next conducted an analysis to predict the most probable mRNA gene targets, and their downstream pathways. The program used to predict possible mRNA target genes was Target Scan. The miRNA (listed in Tables 3,4,5) obtained through the cross sectional study were input into an online database called Target Scan, which produced a list of possible target genes. These lists of target genes were then analyzed using Integrated Pathway Analysis (IPA), an analytical software program, which generates images of affected genes and their upstream and downstram pathways. It was also used to create canonical pathways, which show the downstream effects of the genes being regulated by the miRNAs. Figures 5, 6, and 7 represent the potential pathways affected due to several of the listed miRNA. Figures 8, 9, and 10 illustrate the network of genes predicted to be regulated by several of the listed miRNAs.

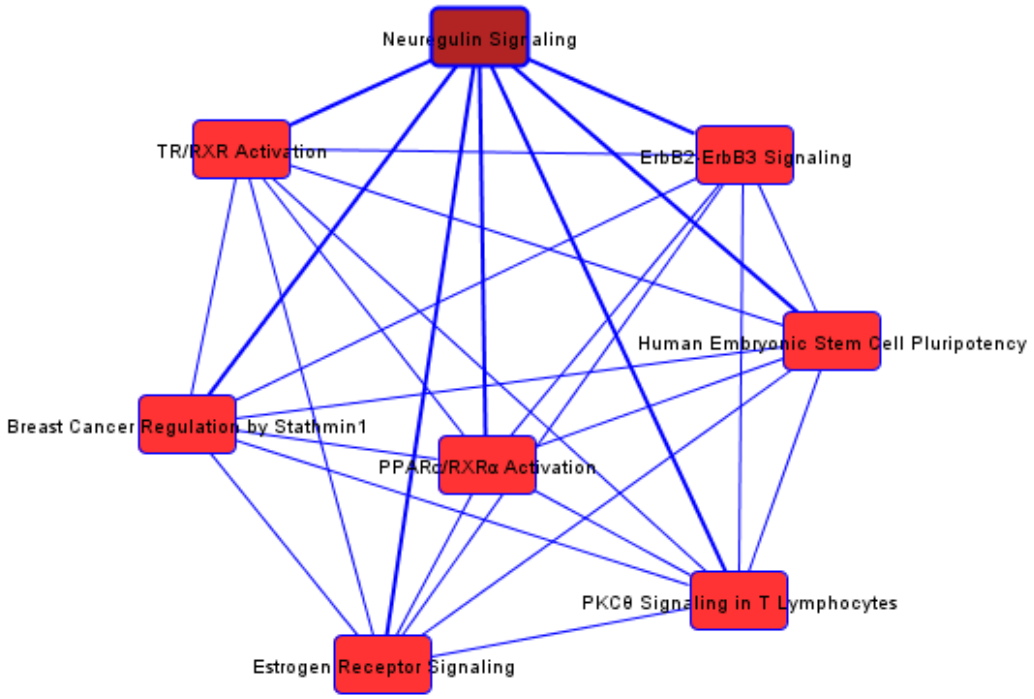


Figure 4: Network Showcasing Pathways Affected by hsa-miR-152

The above figure represents the top eight predicted pathways that miR-152 is most likely affecting according to Integrated Pathway Analysis. Has-miR-152 was found to be commonly regulated due to smoking in both uninfected controls and HIV-1 positive/dementia negative individuals. The Neuregulin Signaling is responsible for many various functions including development of the nervous system, Schwann cell and oligodendrocyte differentiation, and formation of neuromuscular synapses.

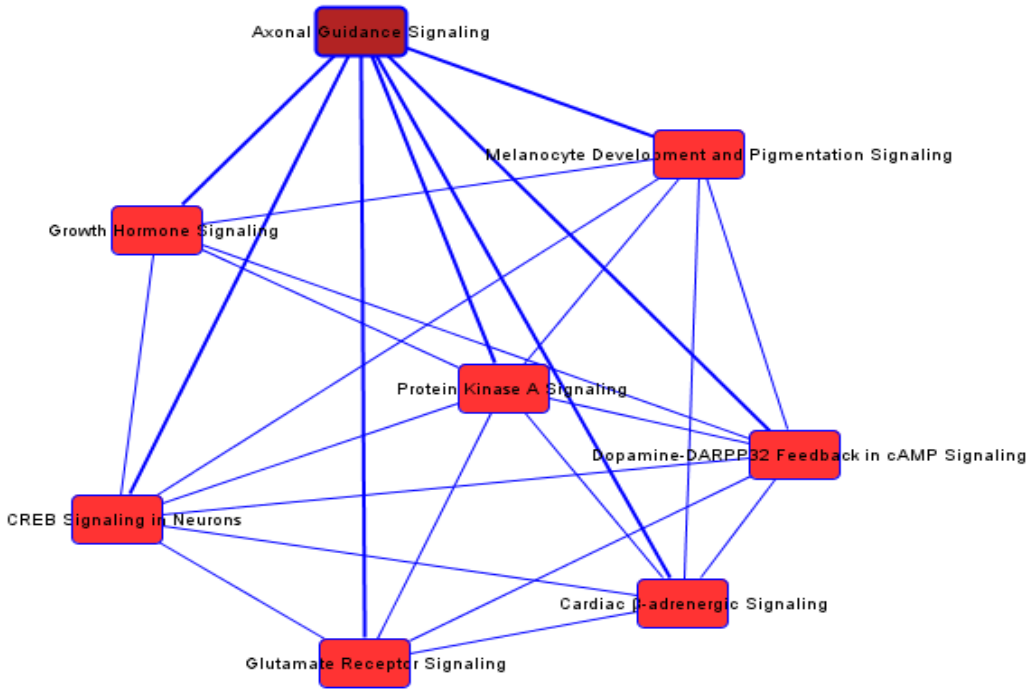


Figure 5: Network Showcasing Pathways Affected by hsa-miR-218

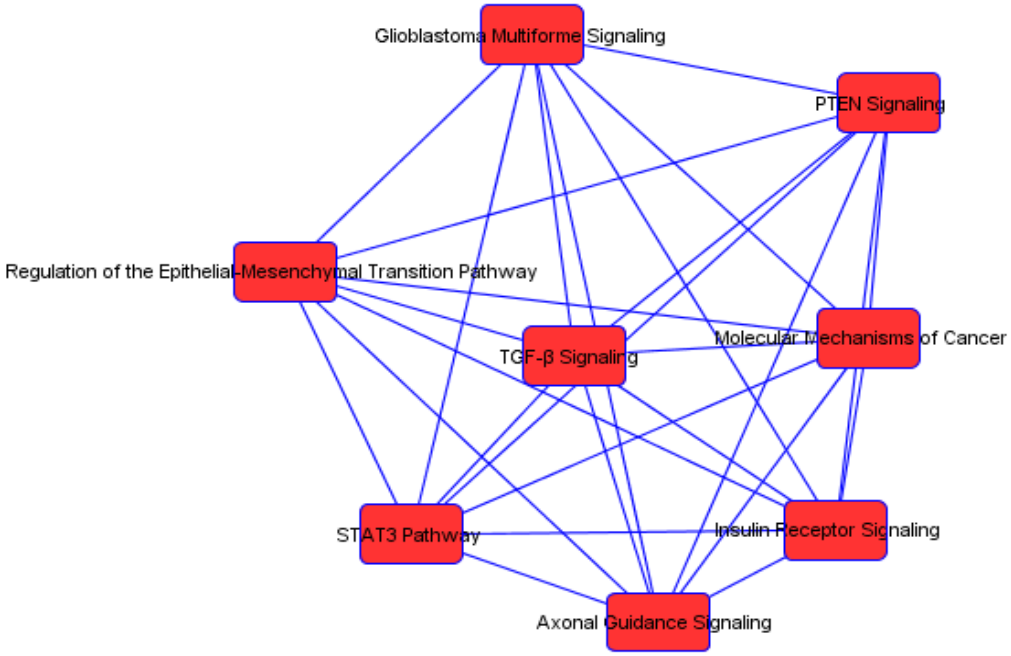


Figure 6: Network Showcasing Pathways Affected by hsa-miR-497

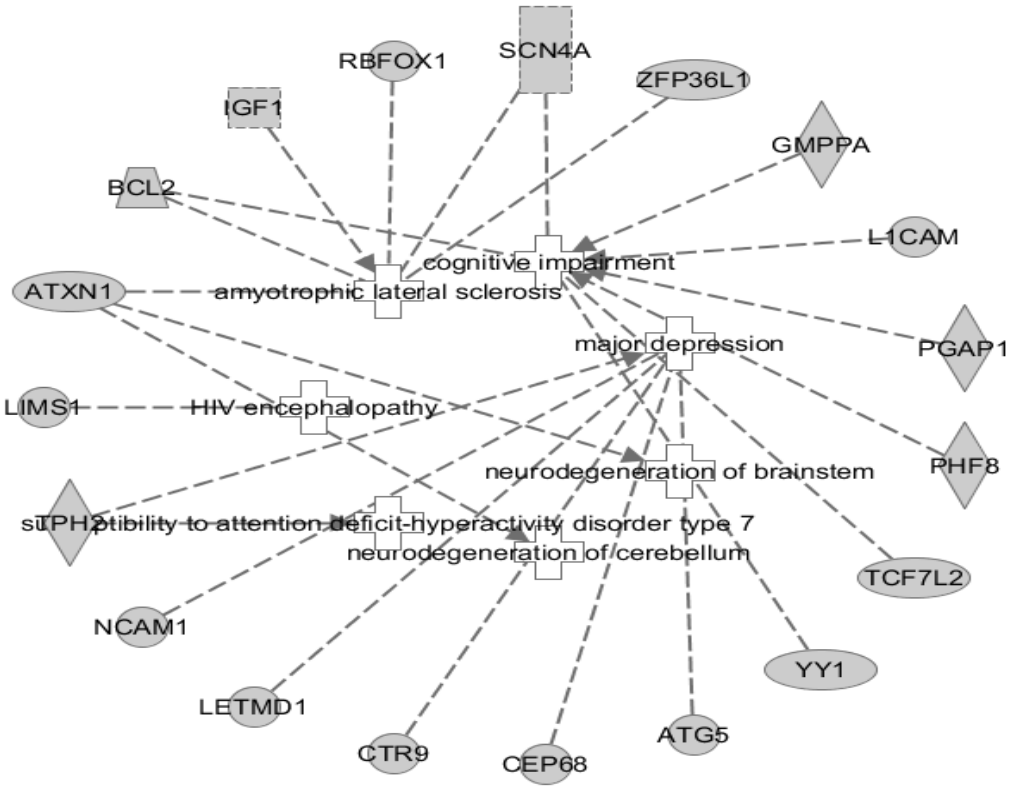


Figure 7: Genes and Their Probable Functions Affected by hsa-miR-629

The above figure represents the most probable genes affected due to miR-629 and the diseases states they regulate. Hsa-miR-629 was commonly regulated in smoking in both uninfected controls and HIV-1 positive/dementia negative.

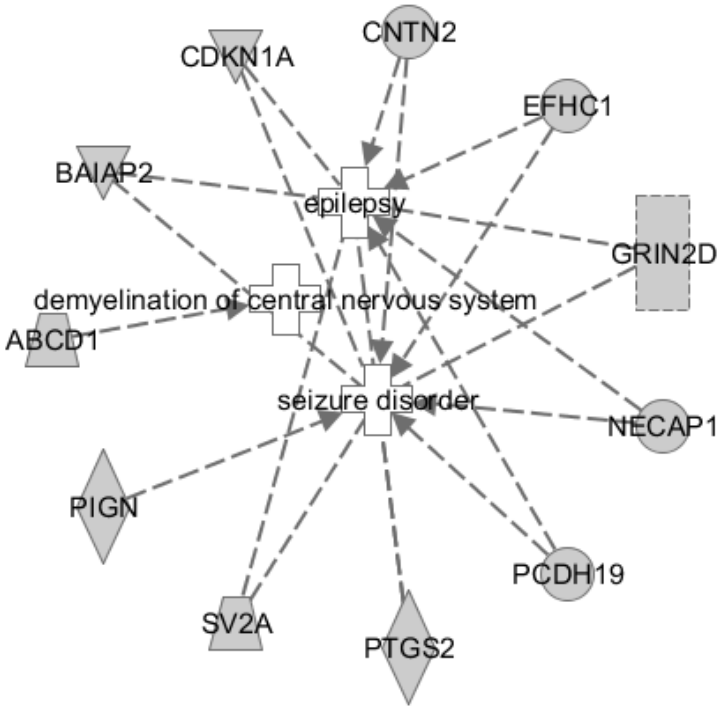


Figure 8: Genes and Their Probable Functions Affected by hsa-miR-558

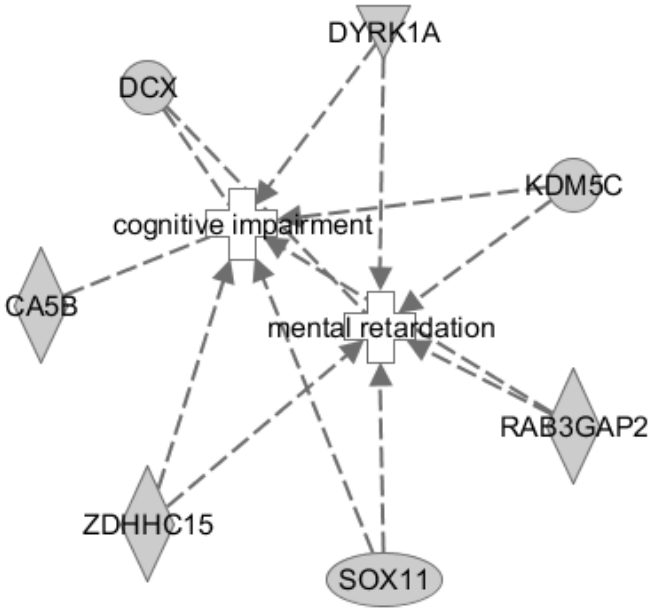


Figure 9: Genes and Their Probable Functions Affected by hsa-miR-645

6.0 DISCUSSION

The first case of HIV-1 reported in the United States was in 1981, and since then 75 million cases globally have been reported. Currently there are 35 million people living with HIV-1 globally. The incidence of HIV-1 has decreased from 3.2 million in 2010 to 2.5 million in 2013, but the prevalence has been increasing steadily, mainly due to availability of advanced medical technologies. Now the aging HIV-1 positive population has bought with it new and complex challenges to the issue of treating an individual with AIDS. We now face another major problem in our quest to stamp out HIV-1, preventing, diagnosing and treating the co-morbidities associated with HIV-1, many of which result in poorer outcomes due to the virus then in those who do not have HIV-1.

The solution is, being able to recognize that an individual is more prone to develop a particular co-morbidity and prevent it by modifying their lifestyles and medication. This approach is far more efficient and effective then treating the ailment.

Several of the miRNA that are found to be deregulated by the behavioral factors, play a role in cognitive processing. Hsa-miR-152 and hsa-miR-148a are both miRNA that were deregulated by smoking in uninfected controls and HIV-1 positive/dementia negative individuals. These two miRNA are significantly involved in neuregulin signaling, and neuregulins are proteins that have been shown to have diverse functions in the development of the nervous system.

6.1 FUTURE DIRECTION

In order to identify the actual deregulating factor of these miRNA, the next step is to find out if the behavioral factor, the virus or a combination of both is the causative factor. A major problem exists in trying to separate these exposures into individual factors and see their individual effect on the miRNA expression. One possible approach is by performing a multivariate analysis, that involves observation and analysis of more than one statistical outcome variable at a time.

6.2 PUBLIC HEATH SIGNIFICANCE

Our study on the effect of behavioral exposures on miRNA deregulation has significant public health relevance. By continuing with this line of study, it is possible to eventually create miRNA biomarkers that would foretell if an individual with certain behavioral exposures would be prone to develop HIV-1 associated neurocognitive disorders. Additionally, these biomarkers could be used not only in prevention and diagnosis of possible HIV associated co-morbidities but also to aid in the determination of the infected individuals' ability to respond to antiviral treatment as well as creating better treatment regimes.

6.3 LIMITATIONS

We faced certain restrictions as we conducted our study. The major limitation being we are using miRNA obtained from PBMC's to observe changes in the brain. We acknowledge this limitation, but as we are not able to obtain brain tissue samples we are forced to use deductive reasoning to

prove our hypothesis. Our next obstacle is that our sample sizes are small, as we did not have accurate information on their cognitive impairment or dementia status on all subjects. Due to the small sample sizes the values calculated do not fully represent the accurate assessment between miRNA regulation in relation to behavioral factors and HAND. Next the variables (behavioral exposures) could not be calculated individually as there was insufficient data and a very small sample size to do an analysis on individuals who only smoked or who only consumed alcohol in each of the established categories (uninfected controls, HIV-1 positive with and without dementia). Another future goal of this study, would be to identify individuals in each category who only have one behavioral exposure, only smoking of tobacco (more than 30 pack years) or only consumption of alcohol (thirteen or more drinks per week). With a larger sample size we would be able to calculate a more accurate measure of the miRNAs that are being regulated either independently by HAND or in conjunction with behavioral factors. Lastly the data assimilated on the HIV-1 positive/dementia negative individuals is subject to change as the MACS evaluation of dementia has changed over the years the data was collected.

APPENDIX

LIST OF REGULATED MiRNA

MiRNA Regulated in Uninfected Controls due to **Smoking**

miRNA	Fold Change	P-value
hsa-let-7a	2.7331	0.0472
hsa-miR-105	-2.9579	0.0438
hsa-miR-106b	7.1113	0.0146
hsa-miR-124#	-22.8650	0.0000
hsa-miR-1269	-27.0344	0.0001
hsa-miR-128	4.9951	0.0414
hsa-miR-130b	3.3704	0.0393
hsa-miR-138-2#	-8.0780	0.0130
hsa-miR-148a	3.4703	0.0328
hsa-miR-152	7.5407	0.0122
hsa-miR-154	7.1934	0.0059
hsa-miR-15b	4.2456	0.0383
hsa-miR-181c	5.9955	0.0056
hsa-miR-181c#	4.2804	0.0407
hsa-miR-182#	34.5823	0.0432
hsa-miR-1826	-33.4262	0.0111
hsa-miR-185	6.0984	0.0325
hsa-miR-187	-7.7124	0.0069
hsa-miR-18a	5.6232	0.0308
hsa-miR-18a#	-2.5769	0.0440
hsa-miR-18b	4.3523	0.0424
hsa-miR-191#	-3.4416	0.0411
hsa-miR-193a-5p	9.9696	0.0027
hsa-miR-194	6.0767	0.0220
hsa-miR-200b#	-11.0050	0.0007

hsa-miR-202#	-5.4450	0.0074
hsa-miR-203	4.3270	0.0349
hsa-miR-20b#	-18.4465	0.0135
hsa-miR-21	3.0545	0.0353
hsa-miR-21#	4.0024	0.0499
hsa-miR-213	5.1263	0.0381
hsa-miR-214	-7.2844	0.0234
hsa-miR-219-1-3p	-7.2195	0.0064
hsa-miR-219-2-3p	-5.7948	0.0424
hsa-miR-22	11.3960	0.0048
hsa-miR-220b	-6.0289	0.0299
hsa-miR-220c	15.1435	0.0200
hsa-miR-23b	3.4094	0.0499
hsa-miR-27a	3.5403	0.0418
hsa-miR-27b	4.0904	0.0406
hsa-miR-28-5p	9.3725	0.0046
hsa-miR-298	-19.7476	0.0001
hsa-miR-299-3p	-161.6296	0.0136
hsa-miR-29b	10.6408	0.0039
hsa-miR-302b#	5.1978	0.0393
hsa-miR-30d#	6.2176	0.0035
hsa-miR-32	6.2167	0.0033
hsa-miR-324-5p	6.0227	0.0299
hsa-miR-325	-29.5116	0.0042
hsa-miR-337-5p	5.9091	0.0077
hsa-miR-346	-8.1868	0.0190
hsa-miR-361-5p	7.6461	0.0031
hsa-miR-362-5p	7.8214	0.0101
hsa-miR-369-5p	8.4129	0.0018
hsa-miR-376c	3.8910	0.0107
hsa-miR-433	6.9599	0.0121
hsa-miR-491-3p	9.0747	0.0103
hsa-miR-494	6.0520	0.0408
hsa-miR-499-3p	7.9450	0.0218
hsa-miR-502-3p	3.4724	0.0454
hsa-miR-508-3p	7.0682	0.0468
hsa-miR-519b-3p	-33.5926	0.0383
hsa-miR-532-3p	5.1133	0.0294
hsa-miR-532-5p	4.3246	0.0276
hsa-miR-551b	-30.8829	0.0002
hsa-miR-556-5p	24.0502	0.0157
hsa-miR-564	4.7380	0.0234
hsa-miR-620	-14.5837	0.0010

hsa-miR-628-3p	-2.1006	0.0285
hsa-miR-629	-2.3568	0.0330
hsa-miR-655	4.4654	0.0090
hsa-miR-874	5.3286	0.0133
hsa-miR-922	-3.9439	0.0061
hsa-miR-98	4.9446	0.0164

MiRNA Regulated in HIV-1 Positive Dementia Negative due to **Smoking**

miRNA	Fold Change	P-value
hsa-let-7g#	9.5690	0.0063
hsa-miR-1245	-42801.7847	0.0119
hsa-miR-1263	38.5143	0.0026
hsa-miR-1283	15.6353	0.0396
hsa-miR-1284	-1093045.0470	0.0015
hsa-miR-146a#	16.5449	0.0019
hsa-miR-148b#	2.9295	0.0183
hsa-miR-152	2.6747	0.0193
hsa-miR-204	15.1371	0.0057
hsa-miR-221#	6.2653	0.0407
hsa-miR-301b	3.5178	0.0357
hsa-miR-302d	7.5929	0.0224
hsa-miR-331-5p	3.6557	0.0488
hsa-miR-379	3.4224	0.0386
hsa-miR-384	13.7745	0.0101
hsa-miR-455-3p	-9.4582	0.0190
hsa-miR-503	7.7328	0.0336
hsa-miR-513-5p	8.1154	0.0204
hsa-miR-517b	9.3679	0.0487
hsa-miR-517c	22.7991	0.0227
hsa-miR-518a-5p	-9.6833	0.0096
hsa-miR-519e#	-77.8612	0.0151
hsa-miR-545#	3.6892	0.0439
hsa-miR-548J	5.2669	0.0322
hsa-miR-562	-12.5317	0.0241
hsa-miR-566	18.8969	0.0389
hsa-miR-607	6.5434	0.0346
hsa-miR-608	5.0932	0.0178
hsa-miR-614	6.8475	0.0295
hsa-miR-617	6.3746	0.0208
hsa-miR-621	29.2438	0.0154
hsa-miR-625	2.2753	0.0408

hsa-miR-629	4.1165	0.0103
hsa-miR-630	3.8683	0.0419
hsa-miR-634	3.6298	0.0439
hsa-miR-658	-411779.5048	0.0329
hsa-miR-672	4.1666	0.0324
hsa-miR-874	19.2739	0.0250
hsa-miR-935	11.0274	0.0185
hsa-miR-937	8.9544	0.0064

MiRNA Regulated in HIV-1 Positive Dementia Positive due to **Smoking**

miRNA	Fold Change	P-value
hsa-miR-1200	363.2536	0.0433
hsa-miR-183#	-15.7520	0.0027
hsa-miR-558	-10.0498	0.0025
hsa-miR-618	-156.1200	0.0111
hsa-miR-649	-7.4997	0.0242

MiRNA Regulated in Uninfected Controls due to **Alcohol**

miRNA	Fold Change	P-value
hsa-let-7e#	-6.2318	0.0191
hsa-miR-10b#	4.3549	0.0250
hsa-miR-1236	-21.3139	0.0194
hsa-miR-124#	-8.8110	0.0445
hsa-miR-125a-3p	-4.4821	0.0319
hsa-miR-1294	7.2144	0.0029
hsa-miR-1304	26.9748	0.0000
hsa-miR-143#	147.9160	0.0026
hsa-miR-147	13.4689	0.0040
hsa-miR-154	-8.2442	0.0298
hsa-miR-200c#	4.3298	0.0413
hsa-miR-213	4.9903	0.0447
hsa-miR-219-1-3p	-9.5618	0.0123
hsa-miR-220	4.9231	0.0201
hsa-miR-23b	4.4617	0.0198
hsa-miR-298	-7.9247	0.0241
hsa-miR-331-3p	12.3765	0.0318
hsa-miR-369-3p	7.5212	0.0062
hsa-miR-455-3p	8.1547	0.0032
hsa-miR-515-5p	5.1602	0.0099
hsa-miR-518a-3p	28.2518	0.0039

hsa-miR-548c-3p	11.6331	0.0003
hsa-miR-551b	-20.0687	0.0316
hsa-miR-556-5p	-33.4100	0.0166
hsa-miR-616	4.4275	0.0484
hsa-miR-665	-11.8406	0.0254
hsa-miR-924	9.0003	0.0106

MiRNA Regulated in HIV-1 Positive Dementia Negative due to **Alcohol**

miRNA	Fold Change	P-value
hsa-miR-1	-31.2739	0.0355
hsa-miR-1206	-77.4577	0.0374
hsa-miR-1245	670457.2010	0.0004
hsa-miR-125a-3p	-8.2404	0.0311
hsa-miR-125b-1#	11.0038	0.0219
hsa-miR-1284	168783.6318	0.0089
hsa-miR-1286	624.4479	0.0125
hsa-miR-1288	1904.8377	0.0118
hsa-miR-1293	25.2395	0.0160
hsa-miR-1304	37.3787	0.0384
hsa-miR-146a#	8.5758	0.0357
hsa-miR-147b	8.4551	0.0296
hsa-miR-181c#	4.4296	0.0314
hsa-miR-186#	9.2406	0.0231
hsa-miR-218-1#	12988.3322	0.0019
hsa-miR-24-1#	8.1806	0.0176
hsa-miR-302a	5.1852	0.0253
hsa-miR-30c-2#	203694.0965	0.0017
hsa-miR-338-3p	8.2353	0.0295
hsa-miR-455-3p	-13.5234	0.0444
hsa-miR-497	21.7973	0.0007
hsa-miR-499-5p	-65.0177	0.0216
hsa-miR-513C	10.4754	0.0064
hsa-miR-519e#	146.8242	0.0057
hsa-miR-556-3p	7.7604	0.0194
hsa-miR-562	24.4164	0.0078
hsa-miR-621	32.6504	0.0009
hsa-miR-640	6.5634	0.0206
hsa-miR-658	9463409.1539	0.0019

MiRNA Regulated in HIV-1 Positive Dementia Positive due to **Alcohol**

miRNA	Fold Change	P-value
hsa-miR-1200	-172.7587	0.0006
hsa-miR-148a#	-77.5513	0.0064
hsa-miR-218-1#	-6.2417	0.0213
hsa-miR-296-3p	-7.8476	0.0384
hsa-miR-410	-4.0707	0.0475
hsa-miR-432#	14.0221	0.0332
hsa-miR-497	-20.1807	0.0009
hsa-miR-518f	-97.1187	0.0221
hsa-miR-645	-609.7084	0.0000
hsa-miR-646	20.1555	0.0277
hsa-miR-663B	17.9712	0.0003
hsa-miR-873	-4.9876	0.0403

MiRNA Regulated in Uninfected Controls due to **Marijuana**

miRNA	Fold Change	P-value
hsa-let-7f	-4.1435	0.0297
hsa-miR-1236	-18.5085	0.0280
hsa-miR-124#	10.2281	0.0025
hsa-miR-1294	11.6364	0.0001
hsa-miR-1304	18.4212	0.0002
hsa-miR-146a	24.6677	0.0365
hsa-miR-146a#	-16.7871	0.0405
hsa-miR-182	3.0618	0.0340
hsa-miR-1826	-36.3647	0.0157
hsa-miR-298	5.8575	0.0361
hsa-miR-30c-1#	58.1894	0.0001
hsa-miR-373	20.7765	0.0110
hsa-miR-424	4.7848	0.0207
hsa-miR-455-3p	6.2242	0.0181
hsa-miR-493	6.4773	0.0170
hsa-miR-508-3p	-9.9381	0.0221
hsa-miR-515-5p	4.5998	0.0187
hsa-miR-518a-3p	24.3918	0.0069
hsa-miR-518d-5p	-6.6338	0.0285
hsa-miR-520D-3P	-101.5054	0.0379
hsa-miR-522	9.1623	0.0374
hsa-miR-548c-3p	8.0224	0.0088
hsa-miR-549	993.0585	0.0023
hsa-miR-551b	0.0488	0.0252
hsa-miR-556-5p	0.0320	0.0264

hsa-miR-638	7.3658	0.0180
hsa-miR-643	10.6075	0.0106
hsa-miR-665	-15.8040	0.0050
hsa-miR-872	-22.6918	0.0290
hsa-miR-892b	43.1518	0.0056
hsa-miR-924	9.3221	0.0074

MiRNA Regulated in HIV-1 Positive Dementia Negative due to **Marijuana**

miRNA	Fold Change	P-value
has-miR-155	2.0672	0.0417
hsa-miR-1183	-6.5058	0.0149
hsa-miR-1225-3P	-4.1301	0.0466
hsa-miR-191	2.3440	0.0205
hsa-miR-218-1#	-338.8942	0.0089
hsa-miR-219-2-3p	10.5538	0.0075
hsa-miR-223	2.4377	0.0477
hsa-miR-302d#	-34.8868	0.0037
hsa-miR-376a	4.3875	0.0380
hsa-miR-411	5.2922	0.0186
hsa-miR-488	-17.9644	0.0139
hsa-miR-490-3p	-26.7345	0.0007
hsa-miR-548L	10.6886	0.0445
hsa-miR-558	22.1775	0.0043
hsa-miR-573	-18.1189	0.0454
hsa-miR-651	9.0292	0.0412
hsa-miR-935	10.6989	0.0070

MiRNA Regulated in HIV-1 Positive Dementia Positive due to **Marijuana**

miRNA	Fold Change	P-value
has-miR-1305	349.1311	0.0034
hsa-miR-101#	-4.5587	0.0221
hsa-miR-1200	-65.4799	0.0082
hsa-miR-1298	15.1064	0.0110
hsa-miR-149#	5.3230	0.0293
hsa-miR-183#	-5.4831	0.0249
hsa-miR-190b	2.6225	0.0427
hsa-miR-215	3.9364	0.0495
hsa-miR-483-3p	7.5973	0.0307
hsa-miR-483-5p	3.4516	0.0351
hsa-miR-558	-6.1437	0.0090

hsa-miR-584	22.9982	0.0263
hsa-miR-616	0.2886	0.0458
hsa-miR-631	-5.5190	0.0071
hsa-miR-645	-193.0356	0.0000
hsa-miR-934	-7.5912	0.0033

BIBLIOGRAPHY

Ances, B. M., & Ellis, R. J. (2007). Dementia and neurocognitive disorders due to HIV-1 infection. *Semin Neurol, 27*(1), 86-92. doi: 10.1055/s-2006-956759

Chen, M. F., Gill, A. J., & Kolson, D. L. (2014). Neuropathogenesis of HIV-associated neurocognitive disorders: roles for immune activation, HIV blipping and viral tropism. *Curr Opin HIV AIDS, 9*(6), 559-564. doi: 10.1097/COH.0000000000000105

Cohen, J. (2000). Searching for the epidemic's origins. *Science, 288*(5474), 2164-2165.

Costiniuk, C. T., & Jenabian, M. A. (2014). Cell-to-cell transfer of HIV infection: Implications for HIV viral persistence. *J Gen Virol*. doi: 10.1099/vir.0.069641-0

Eggers, C., & fur die Deutsche Neuro, A.-A. (2014). [HIV 1-associated neurocognitive disorder : Current epidemiology, pathogenesis, diagnosis and management]. *Nervenarzt, 85*(10), 1280-1290. doi: 10.1007/s00115-014-4082-y

Faria, N. R., Rambaut, A., Suchard, M. A., Baele, G., Bedford, T., Ward, M. J., . . . Lemey, P. (2014). HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. *Science, 346*(6205), 56-61. doi: 10.1126/science.1256739

Fevrier, M., Dorgham, K., & Rebollo, A. (2011). CD4+ T cell depletion in human immunodeficiency virus (HIV) infection: role of apoptosis. *Viruses, 3*(5), 586-612. doi: 10.3390/v3050586

Gottlieb, M. S., Schroff, R., Schanker, H. M., Weisman, J. D., Fan, P. T., Wolf, R. A., & Saxon, A. (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med, 305*(24), 1425-1431. doi: 10.1056/NEJM198112103052401

Hellmuth, J., Milanini, B., & Valcour, V. (2014). Interactions between ageing and NeuroAIDS. *Curr Opin HIV AIDS, 9*(6), 527-532. doi: 10.1097/COH.0000000000000104

Holt, J. L., Kraft-Terry, S. D., & Chang, L. (2012). Neuroimaging studies of the aging HIV-1-infected brain. *J Neurovirol, 18*(4), 291-302. doi: 10.1007/s13365-012-0114-1

Jin, X. F., Wu, N., Wang, L., & Li, J. (2013). Circulating microRNAs: a novel class of potential biomarkers for diagnosing and prognosing central nervous system diseases. *Cell Mol Neurobiol, 33*(5), 601-613. doi: 10.1007/s10571-013-9940-9

Joseph, S. B., Arrildt, K. T., Sturdevant, C. B., & Swanstrom, R. (2014). HIV-1 target cells in the CNS. *J Neurovirol*. doi: 10.1007/s13365-014-0287-x

Kasinski, A. L., & Slack, F. J. (2011). Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer, 11*(12), 849-864. doi: 10.1038/nrc3166

Kedzierska, K., & Crowe, S. M. (2002). The role of monocytes and macrophages in the pathogenesis of HIV-1 infection. *Curr Med Chem, 9*(21), 1893-1903.

Lackritz, E. M. (1998). Prevention of HIV transmission by blood transfusion in the developing world: achievements and continuing challenges. *AIDS, 12 Suppl A*, S81-86.
Lindl, K. A., Marks, D. R., Kolson, D. L., & Jordan-Sciutto, K. L. (2010). HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. *J Neuroimmune Pharmacol, 5*(3), 294-309. doi: 10.1007/s11481-010-9205-z
Maartens, G., Celum, C., & Lewin, S. R. (2014). HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet, 384*(9939), 258-271. doi: 10.1016/S0140-6736(14)60164-1
Martin-Thormeyer, E. M., & Paul, R. H. (2009). Drug abuse and hepatitis C infection as comorbid features of HIV associated neurocognitive disorder: neurocognitive and neuroimaging features. *Neuropsychol Rev, 19*(2), 215-231. doi: 10.1007/s11065-009-9101-6
Mehandru, S., Poles, M. A., Tenner-Racz, K., Horowitz, A., Hurley, A., Hogan, C., . . . Markowitz, M. (2004). Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med, 200*(6), 761-770. doi: 10.1084/jem.20041196
Mothes, W., Sherer, N. M., Jin, J., & Zhong, P. (2010). Virus cell-to-cell transmission. *J Virol, 84*(17), 8360-8368. doi: 10.1128/JVI.00443-10
Nadal, D., Arlettaz, R., & Berger, C. (2014). [Vertical HIV infection is preventable but remains challenging]. *Ther Umsch, 71*(8), 503-508. doi: 10.1024/0040-5930/a000544
O'Driscoll, L. (2006). The emerging world of microRNAs. *Anticancer Res, 26*(6B), 4271-4278.
Peeters, M., Jung, M., & Ayouba, A. (2013). The origin and molecular epidemiology of HIV. *Expert Rev Anti Infect Ther, 11*(9), 885-896. doi: 10.1586/14787210.2013.825443
Post, F. A., Wood, R., & Maartens, G. (1996). CD4 and total lymphocyte counts as predictors of HIV disease progression. *QJM, 89*(7), 505-508.
Ranganathan, K., & Sivasankar, V. (2014). MicroRNAs - Biology and clinical applications. *J Oral Maxillofac Pathol, 18*(2), 229-234. doi: 10.4103/0973-029X.140762
Rico-Rosillo, M. G., Vega-Robledo, G. B., & Oliva-Rico, D. (2014). [The role and importance of the microRNAs in the diagnosis and development of diseases]. *Rev Med Inst Mex Seguro Soc, 52*(3), 302-307.
Rosca, E. C., Rosca, O., Simu, M., & Chirileanu, R. D. (2012). HIV-associated neurocognitive disorders: a historical review. *Neurologist, 18*(2), 64-67. doi: 10.1097/NRL.0b013e318247bc7a
Sharp, P. M., & Hahn, B. H. (2010). The evolution of HIV-1 and the origin of AIDS. *Philos Trans R Soc Lond B Biol Sci, 365*(1552), 2487-2494. doi: 10.1098/rstb.2010.0031
Sharp, P. M., & Hahn, B. H. (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med, 1*(1), a006841. doi: 10.1101/cshperspect.a006841
Tripathi, A., Liese, A. D., Winniford, M. D., Jerrell, J. M., Albrecht, H., Rizvi, A. A., . . . Duffus, W. A. (2014). Impact of Clinical and Therapeutic Factors on Incident Cardiovascular and Cerebrovascular Events in a Population-Based Cohort of HIV-Infected and Non-HIV-Infected Adults. *Clin Cardiol, 37*(9), 517-522. doi: 10.1002/clc.22311
Wannamethee, S. G., Sirivichayakul, S., Phillips, A. N., Ubolyam, S., Ruxrungtham, K., Hanvanich, M., & Phanuphak, P. (1998). Clinical and immunological features of human immunodeficiency virus infection in patients from Bangkok, Thailand. *Int J Epidemiol, 27*(2), 289-295.

Weiland, M., Gao, X. H., Zhou, L., & Mi, Q. S. (2012). Small RNAs have a large impact: circulating microRNAs as biomarkers for human diseases. *RNA Biol, 9*(6), 850-859. doi: 10.4161/rna.20378

Williams, A. E. (2008). Functional aspects of animal microRNAs. *Cell Mol Life Sci, 65*(4), 545-562. doi: 10.1007/s00018-007-7355-9

Yi, R., & Fuchs, E. (2011). MicroRNAs and their roles in mammalian stem cells. *J Cell Sci, 124*(Pt 11), 1775-1783. doi: 10.1242/jcs.069104