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The Influence of Gene Environment Interaction on the Risk of Cognitive Impairment: Reducing Sexual Risk Behaviors and Alcohol Use in HIV-infected Adults

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THE INFLUENCE OF GENE ENVIRONMENT INTERACTION ON THE RISK OF COGNITIVE IMPAIRMENT: REDUCING SEXUAL RISK BEHAVIORS AND ALCOHOL USE IN HIV-INFECTED ADULTS

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To: Dean Michele Ciccazzo
R.Stempel College of Public Health and Social Work

This dissertation, written by Karina Villalba, and entitled The Influence of Gene Environment Interaction on the Risk of Cognitive Impairment: Reducing Sexual Risk Behaviors and Alcohol Use in HIV-Infected Adults, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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William Darrow

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The dissertation of Karina Villalba is approved.

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Dean Lakshmi N. Reddi
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DEDICATION

I dedicate this dissertation to my mother Wilma Villalba, who as early as I can remember instilled in me the desire to learn and taught me the value of education. She has been my rock through all my academic and personal endeavors.

I also dedicate this work to my father, Walter Villalba, and to my son, Gabriel Villalba, who were patient, loving, supportive and encouraged me to keep going throughout this journey. Finally, I dedicate this work to God as the creator and builder of a better world.

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ABSTRACT OF THE DISSERTATION

THE INFLUENCE OF GENE ENVIRONMENT INTERACTION ON THE RISK OF COGNITIVE IMPAIRMENT: REDUCING SEXUAL RISK BEHAVIORS AND ALCOHOL USE IN HIV-INFECTED ADULTS

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Memory deficits and executive dysfunction are highly prevalent among HIV-infected adults. These conditions can affect their quality of life, antiretroviral adherence, and HIV risk behaviors. Several factors have been suggested including the role of genetics in relation to HIV disease progression. This dissertation aimed to determine whether genetic differences in HIV-infected individuals were correlated with impaired memory, cognitive flexibility and executive function and whether cognitive decline moderated alcohol use and sexual transmission risk behaviors among HIV-infected alcohol abusers participating in an NIH-funded clinical trial comparing the efficacy of the adapted Holistic Health Recovery Program (HHRP-A) intervention to a Health Promotion Control (HPC) condition in reducing risk behaviors.

A total of 267 individuals were genotyped for polymorphisms in the dopamine and serotonin gene systems. Results yielded significant associations for *TPH2*, *GALM, DRD2* and *DRD4* genetic variants with impaired executive function, cognitive flexibility and memory. SNPs *TPH2* rs4570625 and *DRD2* rs6277 showed a risk association with

executive function (odds ratio = 2.5, p = .02; 3.6, p = .001). *GALM* rs6741892 was associated with impaired memory (odds ratio = 1.9, p = .006). At the six-month follow-up, HHRP-A participants were less likely to report trading sex for food, drugs and money (20.0%) and unprotected insertive or receptive oral (11.6%) or vaginal and/or anal sex (3.2%) than HPC participants (49.4%, p <.001; 22.5%, p=.05; 15.4%, p=.04). Results also showed that impaired cognitive flexibility and visual memory were associated with increased alcohol use (F = 1, 3.82, $\eta 2 = 0.03$, p = .05; and F = 1, 5.79, $\eta 2 = .05$ p = .02), respectively. These findings provide evidence that dopamine and serotonin polymorphisms influence executive function and memory as well as moderate alcohol use and that HHRP-A was effective in reducing sexual transmission risk behaviors in HIV-infected alcohol abusers.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AUDIT	Alcohol Use Disorders Identification Test
ANN	American Academy of Neurology
ANNK1	Ankyrin Repeat and Kinase Domain 1
ANI	Asymptomatic Neurcognitive Impairment
ADHD	Attention-deficit/hyperactivity Disorder
ACASI	Audio Computer Assisted Self-interview
AVLT	Auditory Verbal Learning Test
CNS	Central Nervous System
CTT	Color Trails Test
CBO	Community-Based Organization
ART	Combination Antiretroviral Therapy
CAPI	Computer Assisted Personal Interview
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
DRD2	Dopamine Receptor D2
DRD4	Dopamine Receptor D4
GALM	Galactose Mutarose Aldose 1-epimerase
HWE	Hardy-Weinberg Equilibrium
HPC	Health Promotion Comparison
HHRP-A	Holistic Health Recovery Program-Adapted

HIV	Human Immunodeficiency Virus
5-HT	5-Hydroxytryptamine
IMB	Information Motivation Behavior Skills
MNI	Mild Neurocognitive Impairment
PFC	Prefrontal Cortex
PCR	Polymerase Chain Reaction
ROCT	Rey-Osterrieth Complex Figure Test
SCT	Short Category Test
SLC6A4	Solute Carrier Family 6 Member 4
SLC6A3	Solute Carrier Family 6 Member 3
SNP	Single Nucleotide Polymorphism
TLFB	Timeline Followback
TPH2	Tryptophan Hydrolase Isoform 2
VNTR	Variable Number Tandem Repeat

CHAPTER I

BACKGROUND

A. Introduction

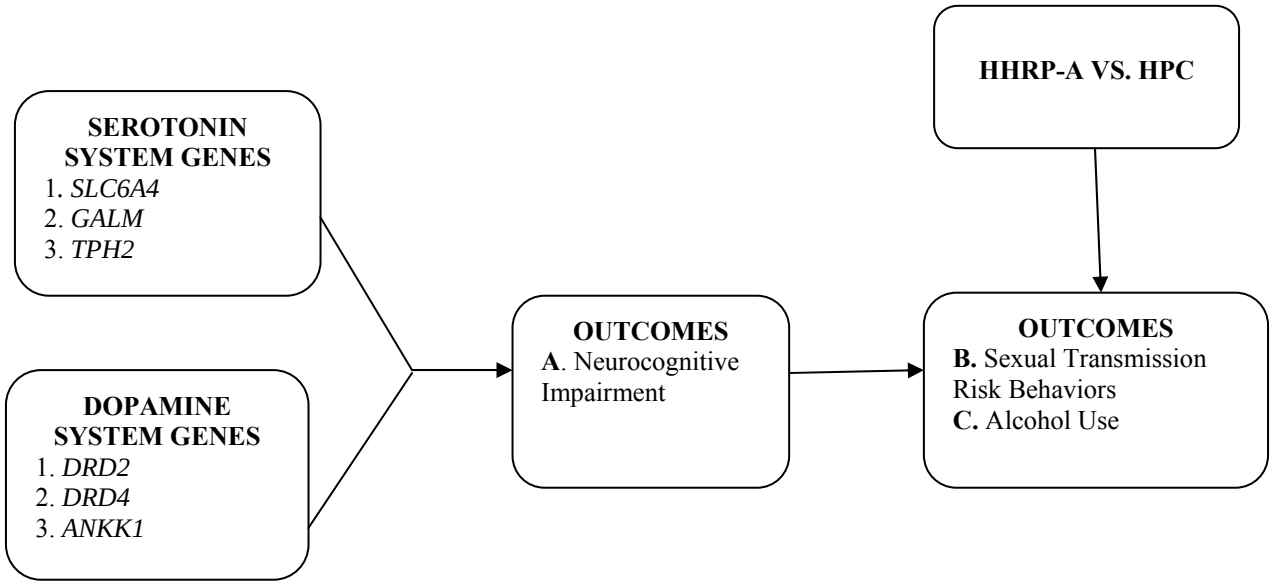
The development of combination antiretroviral therapy (ART) has mitigated the severity of the human immunodeficiency virus (HIV) epidemic. Therapeutic advances have transformed HIV/AIDS from a life-threatening illness to a chronic condition.[1] Despite substantial improvements in life expectancy and a lower incidence of HIV-associated neurocognitive disorders (HAND), neuropsychological and neurocognitive deficits continue to be highly prevalent.[2] Clinical neurocognitive manifestations of HAND in the ART era differ from the typical AIDS dementia complex.[1] In the pre-ART era, a progressive subcortical dementia with motor and cognitive slowing was common. However, today more cortical than subcortical involvement is often reported.[3, 4]

The AIDS Task Force of the American Academy of Neurology (AAN) developed the Frascati criteria which categorized HAND into three conditions.[3] These conditions are based on the number of cognitive domains impaired and the level of interference in everyday life. These conditions are asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD).[5] Although the incidence of HAD has declined, prevalence rates of HAND driven by ANI have increased due to increased longevity of affected patients.[5] ANI is defined as impairment in at least two cognitive domains with one standard deviation below the mean and no impairment in activities of daily living.[5] Neurocognitive deficits continue to be a significant problem in HIV even after the advent of ART.[5] High rates of asymptomatic neurocognitive disorder have been reported in several recent studies. For example,

Simone and colleagues found that 60% of HIV-positive individuals on ART still suffered from some form of neurocognitive impairment.[6] Asymptomatic or minimally symptomatic neurocognitive disorders are more prevalent in individuals in the current ART era than in the pre-ART era.[7] An epidemiological study of 1,555 HIV-infected adults reported that 33% were diagnosed with ANI, 12% with MND and 2% with HAD.[8]

HAND encompasses a range of cognitive impairments, including slowed processing and deficient memory and attention, decreased executive function, and behavioral changes, such as apathy or lethargy.[9] Although this type of impairment is much more subtle than the classic HIV dementia, it still affects daily function, quality of life, antiretroviral adherence, and can increase HIV risk behaviors.[10] The causes of continuing high rates of asymptomatic neurocognitive impairment and, to a lesser degree, mild neurocognitive disorder in the ART era are uncertain. However, the variability in the penetration of antiretroviral drugs across the blood-brain barrier, neurodegeneration caused by toxic inflammatory activation, and lower nadir CD4 T-cell count probably contributed to neurocognitive decline. [5, 7, 11, 12] In addition, aging, co-infection with hepatitis C, drug abuse, and genetic factors may act as moderators of neurocognitive decline.[5] Currently, demographic characteristics, medical comorbidities, and lifestyle behaviors are used to identify individuals who are at risk for HAND.[13] However, additional risk factors such as the role of genetics in relation to HIV-susceptibility and disease progression should also be examined.[10] The accumulated evidence from genetic studies suggests that cognition is heritable, leaving some individuals more vulnerable to develop neurocognitive disorders than others.[14, 15] The contribution of genetic variants affecting the metabolism and activity of dopamine and serotonin system genes are known

to influence these individual differences.[15] Thus, investigating the association between dopamine and serotonin-related genetic polymorphisms and neurocognitive decline is warranted. This research determined whether specific genetic differences in HIV-infected individuals were associated with impaired memory and executive functions, and whether cognitive decline moderated alcohol use and sexual transmission risk behaviors. Behavioral studies have shown convincing evidence of an intricate association between HIV transmission risk behaviors and distal social factors. However, there is not enough information on the effects of biological factors on HIV transmission risk behaviors as presented below.



B. Genes Associated with Neurocognitive Disorders

Cognitive control processes regulating thought and action are multifaceted functions influenced by heritable genetic factors and environmental influences.[15] The consequences of cognitive impairment are seen by the large range of both neurologic and neuropsychiatric disorders that affect the quality of life.[10, 16, 17] Cognitive impairment is

highly heritable, and individual differences in executive function and memory are strongly driven by genetic variations.[18] A study by Friedman and colleagues indicated that individual differences in executive function including inhibiting dominant responses, updating working memory representations, and shifting between task sets, are almost 99% heritable.[19] Additionally, evidence shows that cognition increases linearly from childhood to adulthood by genetic modulation of environmental influences on cognitive functioning.[15] Individuals increasingly select and modify their experiences partly based on their genetic predispositions.[18, 20, 21] Cognitive neuroscience and pharmacology associate dopamine and serotonin as neuromodulators of executive function.[15] Furthermore, studies found associations between serotonin and dopamine polymorphisms with sustained attention, memory, and executive function phenotypes in both clinical and non-clinical populations.[22-25] The most common genes associated with neurocognitive impairment are shown in Table 1.

C. Serotonin Systems

Serotonin pathways arise from the dorsal and ventral raphe nuclei to innervate cortical and subcortical brain regions, including the limbic forebrain, basal ganglia, frontal cortices, thalamus, and the hypothalamus.[26] The neurotransmitter serotonin, 5-hydroxytryptamine (5-HT), is implicated in the pathophysiology of several psychological, behavioral, and psychiatric disorders.[27] Low 5-HT in the central nervous system probably influences impulsivity, aggressive behaviors, increase use of alcohol, and risky health behaviors.[28] The serotonin transport gene *SLC6A4* is one of the most extensively studied genes in the serotonergic pathway.[22, 29] *SLC6A4* encodes the serotonin transporter gene that affects serotonergic neurotransmission by reuptake of synaptic

serotonin, ending neurotransmission.[30] Polymorphisms within *SLC6A4* are known to influence memory regulation, decision making, and response inhibition.[14, 27, 31] In addition, twin studies have shown an additive genetic influence on measures of sustained attention.[32, 33]

The tryptophan hydroxylase isoform 2, *TPH2,* is a promising candidate gene for cognitive functioning because it is the rate-limiting enzyme of 5-HT synthesis.[24] The single nucleotide polymorphism (SNP) rs4570625 may lead to increased risk of depression, attention-deficit hyperactivity disorder (ADHD), autism, and suicide.[34-36] Evidence of *TPH2* gene variations playing a role in cognition comes from studies implicating *TPH2* in the pathophysiology of ADHD and obsessive-compulsive disorder.[36-38] A recent study showed that the SNP rs4570625 on *TPH2* was associated with impaired executive control.[39] Another study, on homozygous TT genotype on SNP rs4570625, showed low anxiety and high extroversion, indicative of low impulse control, which can be correlated to lower prefrontal executive control performance.[24]

D. Dopamine Systems

Dopamine is a neurotransmitter that regulates functional network activities in various regions of the brain. Dopamine neurons are located in the ventral midbrain and the diencephalon.[40] Dopaminergic neurons send projections to the frontal cortex, globus pallidus, striatum, and nucleus accumbens, and are involved in several cognitive functions influencing performance, motor control, reward, and cognition.[41, 42] Dopamine and serotonin systems modulate executive function by co-jointly adjusting neurochemical transmission in the prefrontal cortex (PFC).[15] The PFC plays a central role in top-down control of many higher-order executive tasks. It is involved in learning, memory,

categorization, inhibition control, and cognitive flexibility.[43-45] Activation of D1, D2, D3, and D4 receptors modulate the excitability of receptor cells and PFC neural network activity. [46] The dopamine D2 receptor gene, *DRD2,* is one of the most extensively investigated genes associated with dopamine receptor function, with the *Taq*IA polymorphism being the most frequently studied.[46, 47] The D2 receptor of the *DRD2* gene is associated with PFC mediated behaviors including attentional control, planning, and verbal reasoning.[47] Other areas include visuospatial performance and cognitive flexibility.[48]

The dopamine D4 receptor is widely expressed in the CNS, particularly in the frontal cortex, hippocampus, amygdala and hypothalamus.[33, 49] The *DRD4* gene is located on chromosome 11p15.5 and has a highly variable number of tandem repeats (VNTR) in the coding sequence.[23, 50] The three most common polymorphic variants of the receptor in the human population are D4.4, D4.7 and D4.2. However, they are functionally and pharmacologically nearly indistinguishable from each other.[51, 52] Individuals with D4.7 or more repeats show both reduced binding affinities and receptor densities for dopamine neurotransmission.[53] Several studies have analyzed the association between the D4.7-repeat allele in *DRD4* gene and ADHD.[23, 33] The D4.7 repeat correlates with impulsivity and lower levels of response inhibition.[54] Table 2 shows significant associations found in the literature and relevant to this review.

E. HIV-associated Pathologies in Dopamine and Serotonin Systems

HIV neuropathology is widespread in both the cortical and subcortical brain regions.[13] HIV enters the CNS through infected macrophages/monocytes early in the course of infection and the virus resides primarily in microglia and macrophages.[55]

Microglia and macrophages are the only resident CNS cells capable of increasing HIV infection in the brain.[56] The infection of these cells leads to a cascade of neurotoxic events that damage neurons through the production of secreted viral neurotoxins, neuroinflammation, myelin degradation, and the breakdown of the blood-brain barrier.[57] HIV-related neuropathology is particularly prevalent in areas of the frontal lobes and subcortical structures, especially the putamen and caudate nuclei, the hippocampus, basal ganglia and substantia nigra.[2, 58, 59] Specifically, there is a significant decrease in the levels of homovanillic acid in the cerebrospinal fluid of AIDS patients, results that are probably related to loss of dopamine cell bodies in post-mortem tissues of these patients.[60]

F. HIV-associated Neurocognitive Disorders

Behavior encompasses three functional systems: cognition, emotionality, and executive function.[61] HIV infection can disrupt all three of these functional systems.[5] Cognition is the ability to select, classify, and integrate information. It also includes information storage, retrieval, mental organization, and recognition of information.[61] Executive function is part of a system that acts in a supervisory capacity of the brain through planning, decision-making, and response control for purposeful goal-directed behavior. [62] Memory is a subclass of cognitive function, divided into long-term and short-term (working) memory. Long-term memory is further divided into explicit and implicit memory as shown in Figure 1.[63] Neuropsychological studies have shown an association between HIV-associated neurocognitive impairment and dysfunction of frontostriatal circuits that mediate processing speed, working memory, and executive function.[64-66] For example, these patients suffer from impairment of episodic memory and visual memory,

as well as affective and cognitive-emotional processing.[67] Other studies have documented limited use of higher level encoding strategies, such as semantic clustering and strategic retrieval that may negatively impact medication adherence and cause poor work-related performance.[68, 69] Normal executive function is dependent on the structural and functional integrity of the dorsolateral parietal cortex.[70] Executive dysfunction and poor cognitive flexibility are the most prevalent neurocognitive impairment among HIV-infected adults.[71-73] Risky decision making is also affected by HIV infection that can impact frontostriatal circuits and subcortical regions.[74] For example, HIV-infected adults have the propensity to choose larger immediate rewards over gradually-accumulated smaller rewards that result in overall long-term gains.[74] These observations suggest that HIV-infected adults make more impulsive and risky decisions in their daily lives (e.g., failure to use a condom).[5]

G. HIV-associated Risky Behaviors

Recent studies suggest that, in addition to opiates, cocaine, and methamphetamine, alcohol, cannabinoids, and tobacco may also influence the effects of HIV on the brain.[75] HIV-infected substance abusers are more prone to rapid progressive illness with higher viral loads, decreased immune suppression, and increased cognitive impairments compared to HIV-infected non-substance abusers.[76] In addition, an established trend of high-risk behaviors, often associated with depression is observed in this population.[77] HIV-infected substance abusers are more likely to be non-adherent with antiretroviral medication, a factor that may account for worsening cognitive function, and more rapid disease progression.[78]

There is a high level of comorbidity of alcohol dependence and HIV infection.[79, 80] For example, the rate of heavy drinking among HIV-infected adults is almost twice that of the general public.[81] HIV-infected individuals who abused alcohol showed a nine fold increase in medication noncompliance compared to HIV-infected non-alcohol abusers.[82] Chronic alcohol use is characterized by compulsive and obsessive behaviors which can negatively activate the mesolimbic dopamine reward system and influence the effects of serotonin in the brain.[83, 84] Abnormal dopaminergic transmission is considered one of the pathogenic mechanisms of alcohol dependence.[85] Dopamine is thought to mediate reward mechanisms in mesolimbic neurons and numerous studies have focused on genes related to dopamine function.[41, 46] In contrast, the serotonergic system is believed to modulate alcohol craving and drinking behavior.[86] Alcohol exposure alters several aspects of serotonergic signal transmission in the brain.[87]

The effects of chronic alcohol use on cognitive function are greater for current consumption, higher quantities, longer duration, and older age.[82] A clinical study evaluated the effect of regular daily consumption of six or seven standard alcoholic drinks and found a moderate association with cognitive impairment.[88] Clinical studies show that not all chronic drinkers are at equal risk for brain changes; most suffered mild cognitive impairment which can improve within a year of abstinence.[89, 90] Findings on the effects of alcohol abuse and HIV infection are mixed. Some studies have demonstrated that alcohol abuse and HIV infection have independent effects on cognitive function[91, 92] while others have proposed the possibility of increased vulnerability to cognitive impairment due to an interaction between chronic alcohol use and HIV infection.[79, 93]

Although healthcare practitioners have turned to antiretroviral therapies to avert new infections and to slow down HIV disease progression, alcohol can pose a serious challenge to HIV treatment and prevention.[94] Furthermore, alcohol may interfere with the prevention of HIV transmission by impeding ART adherence and increasing the risk for sexual risk behaviors.[95] Heavy alcohol consumption impairs judgment and cognition which in turn diminishes the perception of risk, leading to unsafe sexual practices and increased risk of HIV transmission.[96] Furthermore, one in three HIV-infected alcohol abusers engaged in unprotected vaginal or anal intercourse with an uninfected partner in the last year.[94] A meta-analysis assessing the association between alcohol and unprotected sex, based on diverse indicators of alcohol consumption (global, situational and event-level), showed significant associations with alcohol use and unsafe sexual practices.[98]

Intervention studies focusing on alcohol use are important due to the wide spectrum of problems associated with sexual transmission risk and antiretroviral adherence among HIV-infected individuals.[99] In general, behavioral interventions are designed to improve HIV treatment adherence and to reduce unsafe sexual practices. Evidence shows that risk reduction interventions are more effective for participants who are not chronic alcohol users.[100, 101] Alcohol use threatens the success of intervention and prevention programs and should be addressed when developing programs for HIV-infected individuals.

H. Significance

Asymptomatic neurocognitive impairment remains a challenge during the ART era.[1] Memory deficits and executive dysfunction are highly prevalent among HIV-

infected adults.[8] These conditions can affect their quality of life, antiretroviral adherence, and HIV risk behaviors.[5] The causes of asymptomatic neurocognitive impairment are still unclear. However, several factors have been suggested including the role of genetics in relation to HIV disease progression.[5] It is suggested that among HIV-infected adults who suffer from impaired cognition, genetically determined polymorphisms in dopamine and serotonin-related genes, may amplify differences in cognitive performance measures compared to healthy subjects. Cognitive functions are influenced by the serotonin and dopamine systems. Thus, genetic differences in the serotonin and dopamine system genes may exacerbate the development of neurocognitive impairment in an individual.[1, 5, 15]

Behavioral studies have shown evidence of an intricate association between HIV transmission risk behaviors and distal social factors. However, there is not enough information on the effects of biological factors on HIV transmission risk behaviors. This dissertation hypothesizes that serotonin and dopamine-related gene polymorphisms compounds the HIV-associated vulnerability to deficits in executive function, cognitive flexibility and memory and these cognitive impairments moderate alcohol use and sexual transmission risk behaviors.

I. Statement of Aims and Hypotheses

Specific Aim 1: To investigate whether serotonin-related polymorphisms (*SLC6A4* 5-HTTLPR, *TPH2* rs4570625 and *GALM* rs6741892) are associated with impaired executive function and memory.

- *Hypothesis 1:* Serotonin-related polymorphisms are associated with impaired executive function and memory in HIV-infected alcohol abusers.

Specific Aim 2: To investigate whether dopamine-related polymorphisms (*DRD4* D4,

ANKK1 rs800461, and *DRD2* rs6277) are associated with impaired cognitive flexibility and executive function.

- *Hypothesis 2:* Dopamine-related polymorphisms are associated with impaired cognitive flexibility and executive function in HIV-infected alcohol abusers.

Specific Aim 3: To evaluate the effectiveness of the adapted Holistic Health Recovery Program (HHRP-A) on reducing alcohol use and HIV sexual transmission risk and to explore whether cognitive impairment moderates alcohol use and sexual transmission risk.

- *Hypothesis 3A:* Participants in the experimental group will show a greater reduction in alcohol use and sexual transmission risk than participants in the comparison group.
- *Hypothesis 3B:* Cognitive impairment moderates alcohol use and sexual transmission risk in HIV-infected alcohol abusers.

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Table 1. Serotonin-and dopamine-related genes associated with cognitive functions

Neurotransmitter	Gene	Genetic variant	Molecular association	Cognitive test	Cognitive association
Dopamine	*DRD4*	VNTR exon 3	7-repeat allele associated with decreased dopamine ability to inhibit camp formation	Go/NoGo task	4-repeat allele showed less accurate response inhibition
Dopamine	*DRD4*	VNTR exon 3	7-repeat allele associated with decreased dopamine ability to inhibit cAMP formation	Stop-signal task	Adults with the 7-repeat allele displayed impaired inhibition
Serotonin	*TPH2*	rs4570625	TPH associated with regulation of serotonin availability	fMRI image test	T allele associated with impaired executive control
Serotonin	*TPH2*	rs4570625	TPH associated with regulation of serotonin availability	Working memory task	G allele associated with fewer errors
Serotonin	*SLC6A4*	5HTTLPR	L allele associated with increased 5-HT uptake compared to S allele	Continuous performance task	L allele associated with higher rates of omission and commission rates
Serotonin	*SLC6A4*	5HTTLPR	L allele associated with increased 5-HT uptake compared to S allele	Working memory task	S/S genotype associated with higher error-rate performance
Serotonin	*SLC6A4*	VNTR intron2	STin2-polymorphisms as transcriptional regulators	Trial making test, Rey auditory verbal learning test	Homozygous STin2.10 associated to cognitive dysfunction in depressed adults

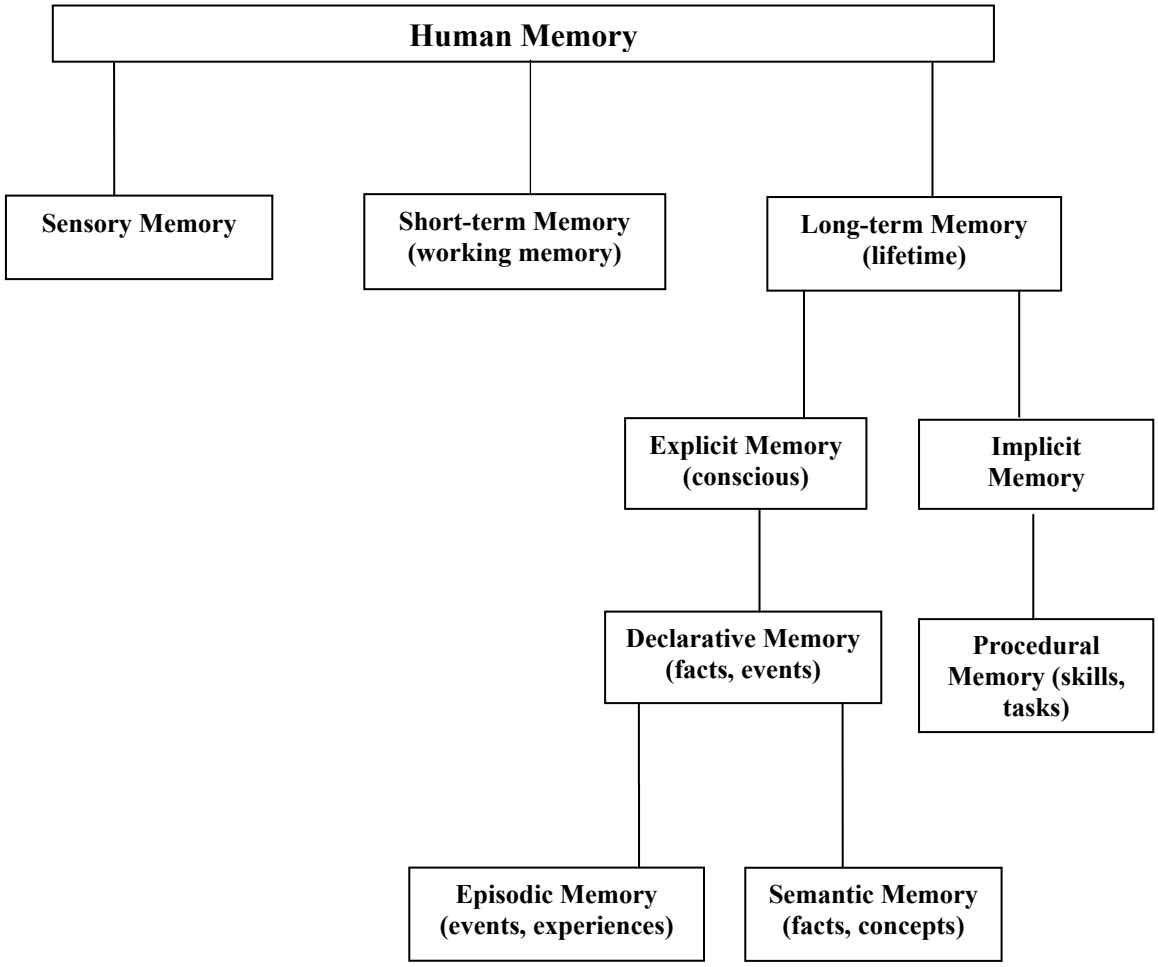
5-HT, serotonin; *SLC6A4*, serotonin transporter; DA, dopamine; *DAT1*, dopamine transporter 1; *DRD2*: dopamine receptor D2; *DRD4*, dopamine receptor D4; fMRI: functional magnetic resonance imaging; SNP, single nucleotide polymorphism; TPH, tryptophan hydroxylase; VNTR, variable number tandem repeat.

Table 2. Characteristics of the most studied SNPs associated with the risk of cognitive decline and dementia

Chromosome nucleotide position	Gene	Genotype	MAF*	SNP-risk allele	PMID**
Chr.19:44892362	*TOMM40*	A/G	G = 0.1336	rs2075650	23207651[102]
Chr. 8:65732478	*MTFR1*	A/G	G = 0.3641	rs10808746	23535033[102]
Chr. 19:44906745	*APOE*	A/G	A = 0.0822	rs769449	24468470[103]
Chr. 2:127137039	*BIN1*	C/T	G = 0.3714	rs744373	19734903[103]
Chr. 6:47484534	*CD2AP*	C/T	C = 0.1905	rs9296559	21460840[103]
Chr. 6:129476939	*LAMA2*	C/T	C = 0.2461	rs2571577	23535033[104]
Chr. 13:85634269	*SLC8A1*	C/T	C = 0.1322	rs9602785	23535033[104]
Chr. 13:85634269	*SLTRK6*	C/T	C = 0.1322	rs9602785	23535033[104]
Chr. 21:45621894	*PCBP3*	G/T	T = 0.1538	rs11701130	23535033[105]
Chr. 7:143412046	*EPHA1*	C/T	C = 0.1878	rs11767557	21460840[105]
Chr. 2:136957689	*PICALM*	A/G	T = 0.3297	rs3851179	19734902[106]
Chr. 2:136957689	*THSD7B*	C/T	C = 0.3880	rs524398	23535033[106]
Chr. 8:27607002	*CLU*	C/T	T = 0.3848	rs11136000	19734902[106]
Chr. 1:207518704	*CR1*	A/G	A = 0.0877	rs6656401	19734903[107]
Chr. 19:51224706	*CD33*	G/T	A = 0.2378	rs3865444	21460840[107]
Chr. 11:6013029	*OR56A1*	C/T	C = 0.2195	rs10769565	23535033[107]
Chr. 5:11043805	*CTNND2*	A/T	T = 0.3030	rs2973488	23535033[108]

*Genome Reference Consortium Human Build 37 patch release 10 (GRCh37.p10) used for nucleotide position (http://www.ncbi.nlm.nih.gov/SNP/)
**PMID PubMed Identification Number

Figure 1. Types of human memory



CHAPTER II. METHODS

A. Data Source

Data collected from the study entitled "Intervening with HIV+ Alcohol Abusers: Influence of Neuro-Behavioral Factors" were used to examine the research aims in this dissertation. The main study was a longitudinal (12 month follow-up) randomized controlled trial designed to reduce HIV sexual transmission risk and substance use, increase the utilization of primary HIV care services and to improve psychosocial health among a sample of HIV-infected alcohol abusers.

The experimental condition was an adaptation of the Holistic Health Recovery Program (HHRP), an evidence-based intervention guided by the Information-Motivation-Behavioral skills (IMB) model.[1] The HHRP program was originally designed for drug abusing populations (See Appendix 1).[2] However, the main study from which this dissertation is derived, used and adapted version (HHRP-A) of the original evidence-based intervention specifically tailored for alcohol abusing populations.

1. Recruitment and Informed Consent

Recruitment was conducted in multicultural, low income, urban areas of Miami-Dade County. These areas are known for their high rates of alcohol and other drugs of abuse, HIV, poverty and lack of health insurance. Thirteen community-based organizations (CBOs) serving the target population agreed to act as recruitment sites. These CBOs were among the largest in Miami-Dade providing outpatient treatment programs for alcohol and other drugs of abuse and mental health services to HIV-positive men and women. Recruiters screened interested potential participants to determine eligibility and proceeded to the informed consent process if all eligibility criteria were

met. The recruiter met each potential participant in a private space and explained the study procedures, the intervention and assessment protocols, the follow-up periods, the confidentiality and compensation. A separate consent form was presented to eligible participants to solicit their participation in the genetic testing and blood draw. They were informed that they could decline to participate in the intervention study or the blood draw and withdraw at any time without adverse consequences.

Since this study collected highly sensitive clinical data, multiple layers of protection for confidentiality were developed: First, a Data Safety Monitoring Board was established for this project. Second, only the participant's number was used on the data collected thus, making it impossible to link a specific participant to questionnaire and genotype data collected. Only the Principal Investigator and project coordinator had access to the password protected and encrypted database linking participants' names and numbers. Finally, a Certificate of Confidentiality was obtained from the National Institute on Alcohol Abuse and Alcoholism.

The inclusion criteria were: between 18 and 60 years old, HIV-positive and willing to present documentation to confirm serostatus, consumed alcohol in the last 3 months with a history of alcohol abuse or dependence within the past 2 years, and at least one episode of unprotected vaginal or anal sex in the past 90 days. Additional criteria included: ability to understand and speak English, ability to understand the informed consent, and ability to provide contact information to facilitate being located for follow-up interviews. Other inclusion criteria included willingness to be randomized to the experimental or control group, not facing immediate incarceration or residence in a restricted environment, and currently not showing overt signs of major psychiatric

disorder. As previously mentioned, blood specimen collection for the study was optional and subject to informed consent. See appendix 2 for further information on the CBOs.

2. Group Randomization

Once participants completed the baseline assessment, they were entered into the study in groups of eight of the same sex. The groups of participants were assigned to receive either the experimental or control condition, using a randomized block design, based on a computer-generated random sequence assignment. Random sequencing was used to control for bias in subject assignments across conditions. To prevent cross-group contamination participants were recruited from different locations. Each cohort was staggered in time to reduce the possibility of interaction and to prevent discussion of their experiences with participants from different cohorts.

3. Treatment Condition: HHRP-A

HHRP is an evidence-based program designed to promote risk reduction behaviors among HIV-positive individuals.[2] While the original intervention (HHRP) included twelve weekly group sessions, the adapted intervention (HHRP-A) included eight, two-hour sessions, delivered twice a week, for four weeks. Some of the topics discussed were: setting and reaching goals, reducing risk behaviors, preventing relapse, adherence to antiretroviral treatment, and coping with stigma and grief. There was an emphasis on relaxation and coping skills training designed to reduce negative mood, and build positive social support networks with peers. Cognitive remediation strategies were incorporated because of the potential for cognitive impairment in this population. Some of these strategies were repetition and review, behavioral games, memory books,

reduction of distraction and fatigue, as well as an ongoing assessment of learned materials with immediate feedback.[3]

4. Treatment Condition: Health Promotion Comparison

The Health Promotion Comparison (HPC) condition focused on educational and didactic methodologies, addressing common health problems such as nutrition, physical fitness, smoking avoidance/cessation and healthy living. HPC did not incorporate behavioral skills training or motivational enhancement techniques. HPC matched HHRP-A in total administration time and format (eight, two-hour sessions). However, the program was condensed and delivered in two days, to reduce the risk of cross-group contamination and the potential for enhancing social support that group sessions repeated over time could engender. A session offering standard care of HIV education was included in the HPC program. It was unethical not to include an HIV education in the comparison group, given the high-risk nature of this population.

B. Materials

1. Selection of Genetic Markers

A group of genetic markers known to correlate with cognitive function was used. Serotonin-and dopamine-related genes were selected using the medical literature which was reviewed for associations with cognitive function.[4-7] The following genes were: 1) solute carrier family 6 (neurotransmitter transporter, serotonin), 2) member 4 (*SLC6A4*), 3) dopamine receptor D2 *(DRD2*), 3) dopamine receptor D4 *(DRD4*), 4) galactose mutarose , aldose 1-epimerase (*GALM)*, 5) solute carrier family 6 (neurotransmitter transporter, dopamine) member 3 *(SLC6A3),* 6) ankyrin repeat and kinase, domain containing 1(*ANKK1)* and 7) tryptophan hydroxylase isoform 2 (*TPH2).* [8-15] The chosen

polymorphisms were further divided into two categories: single nucleotide polymorphisms (SNPs) and variable number tandem repeats (VNTR). A SNP is a single nucleotide change in the DNA sequence code.[16] It is the most common type of stable genetic variation and is bi-allelic.[16] VNTR is a linear arrangement of multiple copies of short repeated DNA sequences that vary in length and are highly polymorphic, making them useful as markers in genetic analysis.[17] Tables 1and 2 include characteristics of the genetic polymorphisms that were analyzed.

2. Genotyping

Genotyping was done by TaqMan® SNP Genotyping Assays (Foster City, CA, USA) using the Bio-Rad CFX96™ System. Genotyping was performed blindly, without knowledge of the clinical status of the participants. Data acquisition and analysis were done on Bio-Rad CFX manager software (2.1). The detection method for allelic discrimination was based on changes in fluorescence. Usually, the software assigns specific dye fluorescence to each of the two alleles amplified, HEX-dye and FAM-dye. If only one dye shows fluorescence then it is indicative of homozygosity. If both dyes are detected, it is indicative of heterozygosity. Life Technologies provided data on which allele was labeled by which dye, typically HEX (allele 1) is the major allele, and FAM (allele 2) is the minor allele. VNTR variants were analyzed using conventional PCR. The primers were from (Integrated DNA Technologies (IDT) Coraville, Iowa, USA). The online IDT SciTools software Oligo Analyzer 3.0 (www.idtdna.com) was used for primer validation. The cycling conditions for the PCR process was optimized for each template and primer pair combination. The amplicons were run on an agarose gel with an

appropriate molecular weight marker for size determination. For protocol details and genetic terminology used in this dissertation see Appendices 3 and 4.

Viral load and $CD4^+$ T cell count came from the documentation collected by the participant's own health care provider within one month from study intake; this information was provided by the participant at baseline. Demographic characteristics were also collected. Timeline Followback (TLFB) assessed sexual transmission risk, alcohol use and other drugs. The Alcohol Use Identification Disorder Test (AUDIT) determined alcohol use severity. Neurocognitive impairment was assessed by the following neurological tests: The Rey-Osterrieth Complex Figure, Short Category, Color Trail A and B, and Auditory Verbal Learning.

3. Neurocognitive Measures

The Rey-Osterrieth Complex Figure Test: It is a widely used measure for visuospatial construction and nonverbal memory.[18] It consists of a complex geometric figure that is copied and then redrawn from memory. Accuracy of correctly copied or recalled elements is measured based on a score from 0 to 36. The figure is divided into 18 components. Each piece is evaluated with respect to its drawing accuracy.[19] This instrument shows high test-retest reliability, with alpha scores for copy accuracy as 0.88 and for recall as 0.87.[20]

Auditory Verbal Learning Test: This test measures verbal memory capacity, retrieval efficiency, and learning.[21] The assessment is based on a five-trial presentation of a 15-noun word list (list A) with a presentation rate of one word per second. A free recall test follows each trial. On completion of trial 5, a single word presentation of a 15-noun word interference list (list B) is presented. The test measures total learning, learning rate,

interference measures, retention measures, and recognition measures with lower scores indicative of greater impairment.[22] This instrument demonstrates high test-retest reliability, with alpha scores ranging from 0.51 to 0.72.[23]

Color Trails 1 Form A and B: The CTT is based on the use of numbered colored circles and universal sign language symbols.[24] Trail A requires the individual to connect colored circles numbered 1-25 as fast as possible using a pencil. Trail B requires the individual to alternate between pink and yellow colored circles numbered 2-25 as fast as possible. The test measures the time to completion, errors, near misses and prompts.[25] Trail A evaluates sustained visual attention while Trail B evaluates cognitive flexibility The test uses the time in seconds the participant needs to complete the test, with higher scores indicating poorer functioning.[24] This instrument demonstrates high test-retest reliability, with alpha scores ranging from 0.85 to 1.00.[24]

The Category Test Short Form-Booklet Format: The SCT assessment consists of five booklets, one for each subtest, with 20 cards per subtest. All of the cards within each subtest are organized according to a single principle. Thus, the test requires the individual to formulate an organizing concept for each subtest. The number of errors on each booklet is added and translated to normalized T-*scores*. Impairment is determined by higher scores indicating poorer functioning.[26] Test-retest coefficients range is from 0.60 to 0.96 depending upon the severity of impairment in the sample.[27]

4. Sexual Risk Behavior and Alcohol Use Measures

Degree of Alcohol Use Severity: AUDIT is used to measure alcohol use patterns. The AUDIT is a 10-item survey that measures alcohol consumption, dependence symptoms and personal and social harm reflective of drinking over the past 30 days.[28]

The AUDIT score measures low-risk alcohol level from 0 to 7; high-risk alcohol level from 8 to 15; harmful alcohol level from 16 to 19; and probably dependency level ≥ 20.[28]

Timeline Followback: The TLFB is a method used to obtain estimates of daily drinking and sexual behavior.[29] It uses a calendar format which helps determine retrospective estimates of the participant's daily drinking and sexual events over the last three months.[29] TLFB was developed originally to assess alcohol use, using memory aids to enhance recall (e.g., key dates serve as anchors for reporting drinking). This method provides a wide range of information including pattern, variability, and the magnitude of drinking.[30] TLFB is also used for sexual behavior. The structure of the TLFB encourages an interactive process whereby memory of one event may facilitate recall of similar or related events.[31] In addition, the TLFB method permits interviewers to obtain enriched contextual information regarding risk behavior. This ability to provide detailed event-level data is especially important for research on the co-occurrence of risky behaviors. Test-retest intraclass correlations from the TLFB showed that all sexual behaviors are reliable, ranging from .86 to .97.[31]

C. Analysis

To standardize cognitive measures, standardized T-*scores* were developed by using multiple linear regression methods analyzing the influence of age, sex, education, and ethnicity on each cognitive test score. These standardized *T-scores* were used to determine cognitive impairment according to the Frascati criteria,[32] presented in Table 3.

Data were evaluated for potential selection bias since 112 participants did not participate in this study. Statistical analyses were performed using Stata v.11 (StataCorp, College Station, TX). Logistic and linear regression were used to explore the associations

between dopamine and serotonin-related genes and cognitive impairment (executive functioning, cognitive flexibility, memory and visual memory). The statistical threshold was set at $P < .05$ and 95% confidence intervals (CIs). Ethnic and gender -specific associations were calculated through stratified analyses. Genotyping counts were tested for Hardy-Weinberg equilibrium (HWE) for each SNP. Associations in the overall sample were assessed by adjusting for sex and ethnicity. By default, the additive genetic model was used but the use of the dominant and recessive models was considered.

Data for alcohol use were transformed using the Box-Cox method.[33] Data for sexual transmission risk were analyzed using nonparametric methods. Initially, the Wilcoxon signed-rank test was used for all the analyses. Transformed data used the t-test and ANOVA methods to measure the outcomes. Change over time on continuous measures was analyzed using two repeated measures analysis of variance (ANOVAs) for treatment condition and time variables. Where change over time in any continuous variable was detected, effect sizes (partial η^2) were reported. Intervention outcomes were assessed by alcohol use and sexual transmission risk. The outcomes were modeled as a function of condition (intervention or control), time (baseline and follow-up), gender, and condition by time interaction. The interaction between neurocognitive measures and alcohol was also measured.

D. References

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Table 1: Variable number tandem repeats analyzed in the study

Polymorphism	Gene Symbol	Gene Name	Chromosome	VNTR Type
5HHTLPR	*SLC6A4*	Solute carrier family 6, member 4	17	44bp insertion/deletion
STin2	*SLC6A4*	Solute carrier family 6, member 4	17	17 bp tandem repeat, 9,10 and 12 copies
DAT	*SLC6A3*	Solute carrier family 6, member 3	5	40 bp, tandem repeat, 3 to 11 copies
D4	*DRD4*	Dopamine receptor D4	11	48 bp tandem repeat, 2 to 10 copies

bp = base pair

Table 2: Single nucleotide polymorphisms analyzed in the study

Ref-SNP ID	Gene Symbol	Gene Name	Chromosome	SNP Type
Rs800461	*ANKK1*	Ankyrin repeat and kinase domain containing 1	11	Amino acid substitution
Rs6277	*DRD2*	Dopamine receptor D2	11	Amino acid substitution
Rs6741892	*GALM*	Galactose mutarotase aldose 1-epimerase	2	Missense
Rs4570625	*TPH2*	Tryptophan hydroxylase isoform 2	12	5' upstream

Table 3. Categories of HIV-associated neurocognitive disorder according to Frascati criteria

	Neurocognitive status*	**Functional status****
Asymptomatic neurocognitive impairment	1 SD below the mean in 2 cognitive domains	No impairment in activities of daily living
Mild neurocognitive impairment or disorder	1 SD below the mean in 2 cognitive domains	Impairment in activities of daily living
HIV-associated dementia	2 SD below the mean in 2 cognitive domains	Notable impairment in activities of daily living

SD=standard deviation.

*Neurocognitive testing should include an assessment of at least five domains, including attention–information processing, language, abstraction-executive, complex perceptual motor skills, memory (including learning and recall), simple motor skills, or sensory, perceptual skills.

**No agreed measures exist for HIV-associated neurocognitive disorder criteria.

CHAPTER III

SEROTONIN-RELATED GENE POLYMORPHISMS AND ASYMPTOMATIC NEUROCOGNITIVE IMPAIRMENT IN HIV-INFECTED ALCOHOL ABUSERS

A. Abstract

Memory deficits and executive dysfunction are highly prevalent among HIV-infected adults. These conditions can affect their quality of life, antiretroviral adherence and HIV risk-taking behaviors. Cognitive impairment is highly heritable and individual differences in executive function and memory are strongly driven by genetic variations. There is strong evidence from cognitive neuroscience associating dopamine as a neuromodulator of executive function. However, the role of the 5-hydroxytryptamine (5-HT) system, in cognition is less clear. The aim of this study was to investigate the potential associations between single nucleotide polymorphisms (SNPs) in the serotonin system genes and cognitive impairments in HIV-infected adults. A total of 267 biologically unrelated individuals were genotyped for polymorphisms *SLC6A4* 5-HTTLPR, *TPH2* rs4570625 and *GALM* rs6741892. To assess neurocognitive functions, the Short Category, the Color Trails, and the Auditory Verbal Learning tests were used. Results yielded statistically significant associations for *TPH2* and *GALM* variants with impaired executive function and memory. The SNP rs4570625 showed a significant association with impaired executive function (odds ratio = 2.5, 95% CI, 1.1-4.9; p = .02). The risk increased in African American males (odds ratio = 4.8, 95% CI, 1.5-14.8; p = .005). SNP rs6741892 was associated with impaired memory (odds ratio = 1.9, 95% CI, 1.2 - 3.1; p = .006), and again the risk increased in African American males (odds ratio = 2.4, 95% CI, 1.2-4.9; p = .02). No significant associations were seen with the 5-

HTTLPR polymorphism. Findings in this study suggest that *TPH2* rs4570625 and *GALM* rs6741892 polymorphisms in the serotonin system influence cognitive control in HIV-infected alcohol abusers.

B. Introduction

The development of ART has drastically improved HIV survival rates in the past few decades.[1] However, HIV-associated neurocognitive disorders remain highly prevalent and continue to represent a significant public health problem.[2] While the incidence of HIV-associated dementia has declined in the ART era, around 50% of HIV-infected individuals will continue to experience some form of neurocognitive decline. With the most prominent decline on deficient memory and attention, decreased executive function, and behavioral changes such as apathy or lethargy.[1, 3] Executive function is an umbrella term that includes sustained attention, response inhibition, working memory, error processing and other complex cognitive abilities.[4] It is a system that acts in a supervisory capacity, is located in the frontal lobes and coordinates complex behavior through planning, decision-making, and response control for purposeful goal-directed behavior.[5, 6] A recent study using a cluster analysis approach showed that executive dysfunction was the most prominent cognitive function in HIV neurocognitive impairment and most likely to affect behavior.[7]

Genetic association studies have revealed statistical correlations between genetic polymorphisms in the dopamine system and measures in executive function.[8-11] Similarly, there is increasing evidence also implicating the 5-HT system but its role in cognition is less clear.[12, 13,14] Behavioral studies have demonstrated that serotonin provides a tonic inhibition to dopamine facilitatory effects related to several behaviors.[15, 16] Similarly,

monoaminergic neurotransmitters, including dopamine and serotonin, may interact within cortical networks to modulate the expression of specific cognitive functions.[17] For example, studies on tryptophan depletion have proposed that 5-HT has a strong neuromodulatory influence over the orbito-frontal-limbic network associated with executive functions.[18-20]

The most widely studied genes of the serotonergic pathway are the tryptophan hydroxylase isoform 2 (*TPH2*) and the serotonin transport gene (*SLC6A4*).[21, 22] *SLC6A4* encodes the serotonin transporter that affects serotonergic neurotransmission by reuptake of synaptic serotonin, ending neurotransmission.[23] Serotonin reuptake variation is linked to a functional polymorphism in the promoter region of the *SLC6A4* gene on chromosome 17q11.1-q12.[23] Polymorphisms within *SLC6A4* have influenced memory regulation, decision making, and response inhibition capabilities.[14, 24, 25] In addition, twin studies have shown an additive genetic influence on measures of sustained attention.[26, 27]

The *TPH2* gene encodes a member of the protein-dependent aromatic acid hydroxylase family.[12] It is the rate-limiting enzyme of 5-HT synthesis in the brain, which transforms tryptophan into 5-hydroxy-tryptophan, the direct precursor of 5-HT.[28, 29] The functional SNP rs4570625 is found within the transcriptional region of *TPH2* on chromosome 12p21.1.[12] Evidence of *TPH2* variations playing a role in cognition comes from studies implicating *TPH2* in the pathophysiology of ADHD and obsessive-compulsive disorder.[30-32] Several studies have shown that the homozygous TT genotype in SNP rs4570625 is associated with poorer executive control compared to GG and GT genotypes.[12, 25] Another study showed a similar association using the NoGo-

anteriorization test, which is an index of prefrontal functioning.[33] These studies showed a compensatory adjustment of deficits in executive control functions.[28, 33]

Another gene of interest is galactose mutarotase (*GALM*). A genome wide association study (GWAS) found a strong association between thalamic and a coding SNP rs6741892 in *GALM* using the tracer [11C]DASB-BPND, used to measure brain serotonin transporter levels. [34] The study demonstrated that SNP rs6741892 accounted for about 50% of the variance in [11C]DASB-BPND binding potential in the thalamus, especially for the TT genotype.[34] *GALM* catalyzes the conversion of beta-D-galactose to alpha D-galactose, which may affect regional neurophysiology, leading to local increases in serotonin release in the brain.[35]

HIV-infected individuals continue to experience neurocognitive deterioration despite virologically successful treatment.[1] Currently, demographic characteristics and medical comorbidities are used to identify individuals who are at risk for HAND.[36] Still the variance in HAND is not completely explained by these factors. Thus, additional characteristics, such as genetic factors associated to HAND susceptibility, should also be investigated.[37] Genetically determined polymorphisms in serotonin-related genes may amplify differences in cognitive performance measures in individuals already with impaired cognition. Thus, it was hypothesized that serotonin-related genetic polymorphism *SLC6A4* 5-HTTLPR, *TPH2* rs4570625 and *GALM* rs6741892 are associated with impaired executive control and memory. We used a number of cognitive measures reported to be valid and reliable measures.[38, 39]

C. Methods

1. Sample

This study used baseline data gathered between 2009 and 2012 as part of a longitudinal randomized controlled trial for HIV-infected adults who abuse alcohol and other drugs. The main study recruited a total of 379 individuals. However, the current study used 267 biologically-unrelated individuals, because112 participants declined to provide blood for genetic testing. Participants were between 18 and 60 years of age, HIV-positive, with a history of drinking alcohol in the last three months, and/or a history of alcohol abuse or dependence in the past two years. An additional inclusion criterion was not showing overt signs of a major psychiatric disorder including psychosis or suicidality. Institutional Review Board (IRB) approval was obtained at Florida International University prior to the start of the study. After full explanation of the study, written informed consent was obtained from all participants.

2. Genotyping

DNA was extracted from whole blood by manual extraction using the QIAamp DNA Mini Kit (Valencia, CA). Genotyping for *TPH2* and *GALM* SNPs was conducted using TaqMan® SNP Genotyping Assays (Foster City, CA) on Bio-Rad CFX96™ real-time PCR instrument (Hercules CA). Polymerase Chain Reaction (PCR) amplifications were performed by using the Probes Supermix, a 2X reaction buffer which contains the necessary components for running PCR. PCR amplifications were performed using the manufacturer's suggestion of 20µl total volume and with the following PCR thermal cycling conditions: enzyme activation at 95^{O}C for two minutes, and 49 cycles of denaturation at 95^{O}C for 5 seconds followed by annealing and extension at 61^{O}C for 5

seconds. For the promoter variant called 5-HTTLPR, Bio-Rad CFX Manager software (version 3.0) was used for data acquisition and genotype assignment. The primer sequences used for the 5-HTTLPR amplification were obtained from a previous study.[35] The sequences were as follow 5'-CCGCTCTGAATGCCAGCACCTAAC-3' (forward primer) and AGAGGGACTGAGCTGGACAACCAC-3' (reverse primer) amplifying a 522-bp for the 16-repeat allele and a 478-bp- for the 14-repeat allele[35]. The 25 µl reaction mixture contained: 1 x PCR amplification buffer (Qiagen, Valencia, CA), 300 µM dNTPs, 0.5 µM of each primer, 0.5 U Taq DNA polymerase (Qiagen) and 50ng of genomic DNA. The temperature cycle consisted of an initial denaturation at 94 ^{O}C for 3 min, followed by 30 cycles of 30 s at 94 ^{O}C, 30 s at 60 ^{O}C and 30 s at 72 ^{O}C, and final extension for 2 min at 72 ^{O}C. PCR products were separated on a 1.5% agarose gel supplemented with gel red and visualized under UV light. All genotypings were performed blindly, without knowing the clinical status or any background data on the samples.

3. Neurocognitive Assessment

All participants were assessed on the same battery of neurocognitive tests and in the same order. Verbal memory and retrieval efficiency were measured with the AVLT, using the version World Health Organization/University of California Los Angeles (WHO/UCLA).[40] Executive function was measured with the SCT.[39] and cognitive flexibility and sustained attention were measured with the CTT Test A & B.[41] Alcohol use and other drugs of abuse were measured using the TLFB method for accurate recall of drug consumption. AUDIT was used to determine patterns of alcohol consumption. AUDIT is a 10-item survey that measures alcohol consumption, dependence symptoms

and personal and social harm reflective of drinking over the past 30 days.[42] The AUDIT scores are: low-risk level (0 to 7), hazardous level (8 to 15), harmful level (16 to 19) and possible dependency ($\geq$ 20).[42] There are no agreed criteria to measure HIV-associated neurocognitive disorders. However, the Frascati criteria have been validated and is widely used to classify HIV impairments.[43] The Fracasti criteria were used to determine cognitive impairment. Therefore, impairment in at least two domains and a cut-off of greater than one standard deviation below the mean was used to determine asymptomatic neurocognitive impairment [44] (Table 2, chapter 2).

Auditory Verbal Learning Test

The AVLT assessment was based on a five-trial presentation of a 15-noun word list (list A) with a presentation rate of one word per second. On completion of trial 5, a single word presentation of a 15-noun word interference list (list B) was presented. The test measured retention, learning and recognition rates with higher scores representing better episodic memory.[45, 46] This instrument demonstrates high test-retest reliability, with alpha scores ranging from 0.51 to 0.72.[47]

Short Category Test

The SCT assessment consisted of five booklets, one for each subtest, with 20 cards per subtest. All of the cards within each subtest were organized according to a single principle. The test required the individual to formulate an organizing concept for each subtest. The number of errors on each booklet was added to determine impairment

with lower scores representing better executive function.[39] Test-retest coefficients range from 0.60 to 0.96, depending upon the severity of impairment in the sample.[48]

Color Trail Test

Color Trail A and B were presented. Trail A required the individual to connect colored circles numbered 1-25 as fast as possible using a pencil. All odd-numbered circles had a pink background while all even-numbered circles had a yellow background.[49] Trail B required the individual to start with a pink colored number one circle and alternate between pink and yellow colored circles numbered 2-25 as fast as possible. The test measured time to completion, errors, near misses and prompts.[49] Trail A evaluated sustained visual attention while Trial B evaluated cognitive flexibility.[41] The test used the raw time in seconds with higher scores indicating poorer functioning.[41] This instrument demonstrates high test-retest reliability, with alpha scores ranging from 0.85 to 1.00.[41]

D. Analysis

Statistical analyses were performed using Stata v.11 (StataCorp, College Station, TX). Data were evaluated for potential selection bias since one hundred and twelve participants did not participate. To standardize cognitive measures for this study, standardized *T-scores* were developed by using multiple linear regression methods analyzing the influence of age, sex, education, and ethnicity on each cognitive test score. Each of the five cognitive domains was included as dependent variables: memory (recognition measures form AVLT), executive function (number of errors on the SCT), sustained attention and cognitive flexibility (time to complete the task for CTT A & B). The continuous predictor was age, and categorical predictors were sex, education and

race/ethnicity. All the predictors in the model were included in each regression, retaining only the variables that significantly contributed to the prediction of cognitive test score. These predictive scores were subtracted from each individual's actual composite score to calculate residual scores. Finally, residual scores were converted to *T-scores* (mean = 50 and SD =10) which were used to determine cognitive impairment.

Logistic regression methods were used to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). All statistical tests were two-tailed, and the threshold for statistical significance was set at $P < .05$. The ORs, with 95% CIs, were used as a measure of effect size. Genotyping counts were tested for Hardy-Weinberg equilibrium (HWE) for each SNP. Ethnic and gender-specific associations were assessed through stratified analyses. Associations in the overall sample were assessed by adjusting for sex and ethnicity/race. By default, the additive genetic model was used, but due to previous associations in the recessive model for *SCL6A4,* the recessive and dominant models were also used.

E. Results

A total of 267 HIV-infected alcohol abusers completed the study. Table 1 provides participant characteristics. The majority of the participants were men 173 (65%). Ethnicity was self-identified: 203 (76%) were African Americans, 21 (8%) Caucasians and 43 (16%) Hispanics. The average age of participants was *mean* = 45.1 (*SD* = 7.1). The majority 190 (69%) completed high school. HIV disease markers, including viral load and CD4 count, were not associated with AIDS diagnosis. About 128 (48%) of the participants had undetectable viral load, and a CD4 count average was 441.4 cells/mm^3 (*SD* = 287). Lifetime alcohol use, on average, was 23.8 years in this sample.

The mean AUDIT score was 16, and 101(38%) scored > 20. The most commonly used drugs besides alcohol were cocaine and marijuana. However, the number of times these drugs were used was negligible compared to alcohol use. Selection bias was not observed since the nested study was comparable to the main study. The Frascati criteria were used to measure neurocognitive impairment. A total of 112 (43%) participants scored below the mean (*T-score*: mean = 50; SD = 10). Asymptomatic neurocognitive impairment, greater than one standard deviation below the mean, was observed in 101 (91%) and mild neurocognitive impairment, greater than two standard deviations below the mean was seen in 11 (9%). Executive function and memory showed the highest impairment in this sample (executive function: *mean* = 58.2 *SD* = 10.9; Memory: *mean* = 40.0 *SD* = 9.1). HIV-associated dementia was not observed.

Genotyping results, including genotype frequencies are presented in Tables 2 and 3. All SNPs were in Hardy-Weinberg equilibrium. Analyses yielded significant associations with executive function and *TPH2* and memory and *GALM* genetic polymorphisms. Whereas, 5-HTTLPR polymorphism did not show an association with cognitive flexibility as previously suggested.[52] The SNP rs4570625 of *TPH2* gene showed an overall association in the dominant model with impaired executive function (odds ratio = 2.5, 95% CI, 1.1-4.9; p =.02). Furthermore, the association showed an increased risk in males (odds ratio = 4.0, 95% CI, 1.6-10.5; *p*= .007), not in females ($P_{\text{interaction}}$ = .08 for sex). Greater risk was observed in African American males (odds ratio 4.8, 95% CI, 1.5-14.8; p = .005). For the SNP rs6741892 of the *GALM* gene, a significant association with impaired memory (odds ratio = 1.9, 95% CI, 1.2-3.1; p =.006) was observed. The risk again was increased in African American males (odds

ratio 2.4, 95% CI, 1.2-4.9; p =.02). Results from this study showed that the associations between serotonin-genes and asymptomatic neurocognitive impairment are male-specific. The interaction between *GALM* and *THP2* polymorphisms with alcohol use was non-significant (p = .65).

F. Discussion

This study provides further evidence for the role of 5-HT in cognition, where functional polymorphisms of two candidate genes in the serotonergic signaling pathway influence executive function and memory. Significant associations were found between *TPH2* SNP rs4570625 and executive dysfunction and *GALM* SNP rs6741892 and impaired memory. Stratification by potential effect modifiers (sex and race) showed an even greater effect in African American males but not in females. For the polymorphism 5-HTTLPR, no statistically significant associations were found with neurocognitive measures. Data suggest that the 5-HTTLPR polymorphism is probably not a risk factor for cognitive impairment and supports previous studies that reported no association between this polymorphism and cognitive abilities or cognitive decline.[50, 51] However, other studies provided evidence of the influence of 5-HTTLPR polymorphism on executive function. For example, a study by Rosier and colleagues evaluated the effect of 5-HTTLPR polymorphism on executive function in ecstasy users and found a significant association with the S allele.[52] Another study, observed impaired executive performance for homozygous SS genotype compared to LL and LS genotypes in patients affected by schizophrenia.[21]

The findings in this study indicate that homozygous TT genotype in SNP rs4570625 showed higher error rates measured by the SCT than TG and GG genotypes

implicating executive dysfunction. These findings parallel and extend those of functional imaging and molecular genetic studies suggesting that polymorphism rs4570625 is a risk marker for executive dysfunction. [12, 28, 30] SNP rs4570625 affects the transcription rate of *TPH2,* which may increase the activity of PFC.[53] The PFC plays a central role in top-down control of many higher-order executive tasks.[54-56] Evidence from a functional image study showed a significant association between SNP rs4570625 and increased activity in several prefrontal and parietal sites during updating of working memory.[28] The authors suggested that the effect of SNP rs4570625 was not specific for attention, impulse control, or working memory, rather it seemed to reflect one common basal cognitive process.[28] Another study showed a similar association using the NoGo-anteriorization test, which is an index of prefrontal functioning.[33] These studies showed a compensatory adjustment of deficits in executive control functions.[33,28] Similarly, results in this study are in line with studies suggesting increased PFC activity due to serotonin dysregulation affecting executive function.[21, 25, 57, 58] Thus, associating executive dysfunction to a wide range of behaviors.

Behavioral studies have demonstrated that executive dysfunction (i.e., poor learning) are central to HIV-neurocognitive impairment and most likely affects behaviors, including adherence to antiretroviral medication and unemployment (or underemployment).[2] Heaton and colleagues found that medically asymptomatic HIV-infected adults with executive dysfunction were twice as likely to be unemployed and perceived greater vocational difficulties than their unimpaired counterparts.[59] Similarly, another study showed that, for recently diagnosed individuals, the key predictors for finding employment were learning and memory.[60]

This study is the first to analyze the functionality of this SNP rs6741892 in relation to 5-HT transporter. Thus, a significant association between rs6741892 and memory measured by AVLT was found in HIV-infected alcohol abusers. The AVLT was used because the repeated presentations of words and their successive testing at various time intervals allowed for the analysis of different learning processes such as acquisition, retention, retrieval, and interference.[45] The thalamus plays a significant role in regulating higher-level brain activity.[61] The dorsomedial nucleus is of particular interest because of its established role in memory and its extensive reciprocal connections with the PFC.[62] Impaired explicit memory in HIV-infected adults is associated with decreased volumes of the thalamus.[63] Thus, results in this study showed a significant association with decreased total learning, as a measure of explicit memory. Explicit memory is further divided into semantic and episodic memory (Figure 1, Chapter 1). Semantic and episodic memory have been associated with neurocognitive deficit in HIV-infected adults.[64] Episodic memory impairment has been associated with limited use of higher level encoding strategies, such as semantic clustering and strategic retrieval.[64] This can lead to issues involving medication non-adherence and problematic work-related issues in HIV-infected adults.[65, 66]

This study has several limitations that should be noted. First, there was relatively low frequency of homozygous TT genotype of the *TPH2* SNP. However, it should be noted that there is relatively low occurrence of TT genotype within the general population. In fact, compared to previous studies, the current study included a rather high proportion of homozygous TT genotype carriers compared to others.[12, 13, 53] Second, the lack of alpha-level corrections due to multiple comparisons. However, multiple

comparisons were necessary due to the exploratory nature of the study, including the analysis of the SNP functionality in the *GALM* gene, as well as the use of all three genetic models.

In general the additive model is used to assess statistical associations of SNPs. While the additive model has sufficient power to detect associations in most situations, there may be occasions where statistical significance is not found, when in fact, there is an association. Consequently, a strength in this study was the use of multiple genetic models to determine associations that may remain undetectable by the exclusive use of the additive model.

G. Conclusion

The current study was the first to explore the relationship between serotonin-related polymorphisms and asymptomatic neurocognitive impairment in a sample of HIV-infected alcohol abusers. The results showed a significant association between *TPH2* rs4570625 and individual differences in executive function and *GALM* rs6741892 with memory. The two associations were male-specific. The present study validates previous results pointing to genetic influences on executive function and memory. Moreover, a significant association between SNP rs6741892 with memory was demonstrated, which may imply SNP rs6741892 as a functional polymorphism in the *GALM* gene affecting 5-TH transport.

H. References

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Table 1. Demographic and clinical characteristics of main and current study participants

Baseline characteristics	Main study n = 112	Current study N = 267	P values
Age mean (DS)	44.1 (7.7)	45.1(7.1)	.66
Sex, No (%)			.72
Male	67 (60)	173 (65)	
Female	45 (40)	94 (34)	
Education No (%)			.24
8th grade or less	13 (12)	19 (7)	
High school diploma	73 (65)	190 (69)	
Some college	26 (23)	57 (24)	
Race/Ethnicity No (%)			.26
Caucasian	17 (15)	21 (8)	
African-American	80 (72)	203 (76)	
Hispanic	15 (13)	43 (16)	
Alcohol use mean (DS)			
Number of standard drinks (past 90 days)	100 (50.1)	190 (100.1)	.10
Lifetime	22 (10.5)	23.8 (10.9)	.24
AUDIT score	14 (7.5)	16 (8.0)	.09
Other drugs, mean (DS)			
Number of times cocaine use (past 90 days)	23.5 (16.8)	33.5 (19.8)	.25
Number of times marijuana use (past 90 days)	19.3 (12.5)	25.6 (20.9)	.63
HIV Characteristics mean (DS)			
CD4 count	412.9 (318.4)	441.4 (286.9)	.73
Viral load No (%)			.16
Undetectable	45 (40)	128 (48)	
50-10,000	39 (35)	80 (30)	
10,001 - 30,000	8 (7)	29 (11)	
30,000 or more	20 (18)	29 (11)	
Taking ART	76 (68)	216 (81)	.84
Cognitive measures, mean (DS)			
Executive skills *T-scores*	50.1 (9.0)	48.2 (10.9)	.93
Memory skills (learning) *T-scores*	45.9 (10.1)	48.2 (9.1)	.18
Memory skills (recall) *T-scores*	48.1 (9.8)	40.0 (10.5)	.11
Cognitive flexibility *T-scores*	40.4 (10.4)	45.7 (10.8)	.09
Sustained attention *T-scores*	53.9 (9.3)	56.0 (9.8)	.10

Table 2. Genotype frequencies and cognitive scores for AVLT and SCT tests

Genes	**SNP**	**Genotype**	**N (%)**	**Cognitive *T-scores* Mean (SD)**
TPH2	rs4570625	AA	93 (36%)	63.2 (10.1)
		AB	120 (46%)	59.5 (13.3)
		BB	47 (18%)	57.6 (15.1)
GALM	rs6741892	AA	90 (35%)	50.1 (10.2)
		AB	114 (43%)	47.1 (9.0)
		BB	60 (22%)	47.4 (9.8)

Table 3. TPH2 and GALM associations with cognitive impairment stratified by sex and race/ethnicity (ORs and 95% CIs)

Chr[a]	Position	Gene	Variant	Allele[b]	Domain	Alleles	Sex[d]	Race[e]	Sex-Race[f]
12	12:71938143	*TPH2*	rs4570625	G >T	Executive function	2.5 (1.1 to 4.9)	4.0 (1.6 to 10.5)	3.3 (1.4 to 7.6)	4.8 (1.5 to 14.8)
						P = 0.02	*P* = 0.01	*P* = 0.007	*P* = 0.005
2	2:38689828	*GALM*	rs6741892	A >T	Memory	1.9 (1.2 to 3.1)	2.3 (1.2 to 4.2)	1.9 (1.1 to 3.6)	2.4 (1.2 to 4.9)
						P = 0.006	*P* = 0.009	*P* = 0.02	*P* = 0.02

[a]Chromosomal locations are based on the Genome Reference Consortium. [b]Alleles are based on Genome Reference Consortium. [c]ORs adjusted for alcohol use, CD4 cell count, adherence to antiretroviral medication, sex and self-reported race/ethnicity. [d]$OR_{stratafied}$ by sex [e]$OR_{stratified}$ by race. [f]$OR_{strafied}$ by sex and race.
Chr., chromosome; OR, odds ratio; CI confidence interval.

CHAPTER IV

DRD2 AND *DRD4* GENES RELATED TO COGNITIVE DEFICITS IN HIV-INFECTED ADULTS WHO ABUSE ALCOHOL

A. Abstract

HIV-infected individuals continue to experience neurocognitive deterioration despite virologically successful treatments. There is evidence suggesting that neurocognitive impairment is heritable and individual differences in executive function and memory are strongly driven by genetic variations. The contribution of genetic variants affecting the metabolism and activity of dopamine may influence these individual differences. The present study investigated the influence of two candidate dopaminergic gene polymorphisms (*DRD4* and *DRD2*) on executive function and memory. They were measured by the Short Category (SCT), Color Trail (CTT) and Rey-Osterrieth Complex Figure Tests (ROCT). Participants were an ethnically diverse sample of 267 HIV-infected adults that were genotyped for polymorphisms in the *DRD4* 48bp-variable number tandem repeat (VNTR), *DRD2* rs6277 and *ANKK1* rs1800497. Results showed significant associations with the SNP rs6277 and impaired executive function (odds ratio = 3.6, 95% CI, 2.4-8.7; p = .001) and cognitive flexibility (odds ratio = 1.7, 95% CI, 1.3-2.6; p = .01). The results were further stratified by race and sex and significant results were seen in males (odds ratio = 3.5, 95% CI, 1.5-5.5; p = .008) and in African Americans (odds ratio = 3.1, 95% CI, 2.3-3.5; p = .01). In addition, *DRD4* VNTR 7-allele was significantly associated with executive dysfunction. Specifically, the 7-absent group performed worse than the 7-present group, in total errors committed in the SCT. Results support the notion that dopaminergic variations influence differences in

cognition and contribute to an understanding of the relation between genes and cognitive impairments in HIV-infection.

B. Introduction

Human immunodeficiency virus (HIV) is a global epidemic that affects approximately 36 million people worldwide.[1] In addition to its deleterious effects on the cell-mediated immune system, HIV can also damage cells in the central nervous system and lead to HIV-associated neurocognitive disorders (HAND).[2] The manifestations of HAND have significantly changed in response to the introduction to antiretroviral therapy (ART). For example, the incidence of HIV-associated dementia has declined. However, the prevalence for asymptomatic and mild neurocognitive impairment have increased with increased longevity.[3] HAND encompasses a range of cognitive impairment that includes deficient memory and attention, decreased executive function, and behavioral changes, such as apathy or lethargy.[4]

Dopamine is a neurotransmitter that regulates functional network activities in various regions of the brain.[5] Dopamine neurons are located in the ventral midbrain and are involved in several cognitive functions that influence performance, motor control, reward, and cognition.[5-7] Studies investigating the relationship between HIV infection and brain pathology have revealed that the HIV virus can cause damage to dopamine-rich areas including the basal ganglia, substantia nigra, and the frontal cortex.[8-10] For example, a neuroimaging study measured basal ganglia volume using magnetic resonance imaging reported selective basal ganglia atrophy in adults with HIV-associated neurocognitive disorder compared to their HIV-negative counterparts.[11] Another study using the same method showed that atrophy of the caudate nucleus was significantly associated with

cognitive impairment in asymptomatic HIV-infected adults compared to the seronegative control group.[12]

Dopamine receptors, D1, D2, D3, and D4 modulate the excitability of receptor cells and prefrontal neural network activity.[13] The SNP rs1800497 (also known as *TaqIA*) of the D2 receptor gene *DRD2* is one of the most extensively investigated genes in reference to neuropsychiatric disorders.[13, 14] This *DRD2*-associated polymorphism is located within the coding region of a neighboring gene, *ANKK1*, and is associated with a reduced number of dopamine binding sites in the brain.[15] The SNP rs1800497 is located more than ten kilobase-pairs downstream from the coding region of the *DRD2* gene in chromosome 11q23 and is, therefore, unlikely to alter *DRD2* directly.[16] Proximity of the two genes may reflect functional relationship and may be associated with dopaminergic phenotypes by being in linkage disequilibrium.[17, 18] Polymorphism *DRD2* SNP rs6277 has been reported to affect D2 receptor density in the striatum.[19] Several studies have shown that SNP rs6277 is associated with prefrontal cortex mediated behaviors including attentional control, planning and verbal reasoning.[14] A study on cognitive flexibility showed that SNP rs6277 was a strong predictor for learning from negative reward prediction errors by avoiding those responses linked to negative outcomes.[17, 20]

The dopamine D4 receptor is widely expressed in the CNS, particularly in the frontal cortex, hippocampus, amygdala and hypothalamus.[5, 21] The dopamine D4 receptor *DRD4* gene is located on chromosome 11p15.5 and has a highly variable number of tandem repeats in the coding sequence.[22] The polymorphism is a 48bp VNTR sequence in exon 3, encoding the third intracellular loop of D4 receptor.[23] The most common polymorphic variants of the receptor are D4.7, and D4.4.[24, 25] Individuals with D4.7

repeat show both reduced binding affinities and receptor densities for dopamine neurotransmission.[26] The D4.7 repeat is correlated with impulsivity and lower levels of response inhibition.[27] Several studies have analyzed the association between the D4.7-repeat allele in *DRD4* gene and attention-deficit hyperactivity disorder (ADHD).[21, 28] The present study explores potential associations with *DRD2* rs6277, *ANNK1* rs1800497 and *DRD4* 48bp VNTR polymorphism and cognitive functions in HIV-infected alcohol abusers.

C. Methods

1. Participants

This study utilized a cross-sectional design, using baseline data gathered between 2009 and 2012 as part of a longitudinal randomized controlled trial for reducing risk behaviors among HIV-infected alcohol abusers. Recruitment was made in densely populated, multicultural, low income, urban areas of Miami-Dade County, Florida. Participants were between 18 and 60 years of age, HIV-positive and willing to present documentation to confirm serostatus, consumed alcohol within the last 3 months, with a history of alcohol abuse or dependence within the past 2 years, and, at the time of recruitment, were not showing overt signs of major psychiatric disorders. Additionally, availability to provide blood specimen was required. All participants provided signed informed consent as approved by the Institutional Review Board (IRB) at Florida International University.

Participants were evaluated for alcohol use by the Timeline Followback (TLFB) and the Alcohol Use Disorders Identification Test (AUDIT) test. All participants were assessed using the same battery of neurocognitive tests and in the same order. Nonverbal

memory was measured with the RCFT. Cognitive flexibility and sustained visual attention was measured with the CTT test A & B, and executive function was measured with the SCT.

2. *Genotyping*

DNA was extracted from whole blood by manual extraction using the QIAamp DNA Mini Kit (Valencia, CA). SNPs rs6277 and rs1800497 were genotyped using the TaqMan® SNP Genotyping Assays (Foster City, CA). Allelic discrimination analysis was performed on the Bio-Rad CFX96™ real-time PCR machine (Hercules CA).

For VNTR D4, Bio-Rad CFX Manager software (version 3.0) was used for data acquisition and genotype assignment. The primer sequences used for the D4 amplification were obtained from a previous study.[29] The sequence was as follows: 5'CTGCTGCTCTACTGGGC 3' sense and 5'GTGCACCACGAAGGAAGG 3' antisense The 25 µl reaction mixture contained: 1 x PCR amplification buffer (Qiagen, Valencia, CA), 300 µM dNTPs, 0.5 µM of each primer, 0.5 U Taq DNA polymerase (Qiagen) and 50ng of genomic DNA. The temperature cycle consisted of an initial denaturation at 94 ^{O}C for 5 min, followed by 30 cycles of annealing for 40 s at 54 ^{O}C, extension for 40 s at 72 ^{O}C, denaturing for 40s at 94^{O}C, and then the final extension for 6 min at 72 ^{O}C. The amplification products were separated on a 3% agarose gel electrophoresis according to the number of repeats. The size of the amplified fragments was from 500 bp to 750 bp (2-7 copies of the 48pb repeat). These genetic markers were chosen based on prior evidence of the SNPs conferring risk to neurocognitive deficits or a theoretical association with executive function.

3. Neurocognitive measures

The neurocognitive test battery included standardized measures of multiple domains of cognitive function selected for their sensitivity to HIV-associated neurocognitive impairment. The neurocognitive tests were assessed in the following domains:

1. *Visual Memory*-Rey-Osterrieth Complex Figure Test evaluated visuospatial construction and nonverbal memory.[30] It consists of a complex geometric figure that is copied and then redrawn from memory.[31] Copy and accuracy of correctly copied or recalled elements was measured based on a score from 0 to 36. The figure was divided into 18 components. Each piece was evaluated with respect to its drawing accuracy with higher scores indicating better accuracy. High test-retest reliability scores 0.88 for copy and 0.87 for recall [31]
2. *Sustained attention*- Color Trail Test part A evaluated sustained attention.[32] Trail A required the individual to connect colored circles numbered 1-25 as fast as possible using a pencil. All odd-numbered circles had a pink background, while all even-numbered circles had a yellow background.[33] The test measured time in seconds to complete with higher scores indicating poor performance. High test-retest reliability, scores ranging from 0.85 to1.00.[32]
3. *Cognitive flexibility*- Color Trail Test part B evaluated cognitive flexibility. Participants were presented with numbered colored circles that required to start with a pink colored number one circle and alternate between pink and yellow colored circles as fast as possible.[32] The test

measured time in seconds to complete with higher scores indicating poor performance. High test-retest reliability scores ranging from 0.85 to 1.00.[32]

4. *Executive function*-The Short Category Test evaluated executive function. It consisted of five booklets with 20 cards per subtest and required the individual to formulate an organizing concept for each subtest.[34] The number of errors on each booklet was added and the total number of errors determined impairment with lower scores representing better executive function.[35] Test-retest coefficients range from 0.60 to 0.96 depending upon the severity of impairment in the sample.[34]

The neurocognitive tests were completed at baseline. Trained personnel administered the tests in the same order and according to standardized procedures.

4. Alcohol Use

The TLFB method assessed alcohol use and other drugs of abuse. This method obtains estimates of substance use by using a calendar format and providing retrospective estimates of the participant's substance use over the last three months.[36] The AUDIT is a screening tool that is sensitive to early detection of high-risk drinking behaviors as shown in Table 1.[37]

D. Analysis

Since 112 (29%) individuals chose not to participate in this study, data were evaluated for potential selection bias. Statistical analyses were performed using Stata v.11 (StataCorp, College Station, TX). Logistic regression and linear regression analyses

were used to explore the association between dopamine-related genes and cognitive impairment (executive functioning, cognitive flexibility and visual memory). The statistical threshold was set at $P < 0.05$ and 95% confidence intervals (CIs). Ethnic and gender -specific associations were calculated through stratified analyses. Genotyping counts were tested for HWE for each SNP. For the *DRD4* polymorphism, the Pearson's X^2 and t-test were used to compare group differences. For *DRD4* 48 bp VNTR, alleles were grouped in short (S; < 7 repeat) and long (L; ≥ 7 repeat) as described in previous studies.[38, 39] For statistical analysis, participants were placed in one of two genotype groups 7-allele present (homozygous for the short allele) or 7-allele absent (heterozygous or homozygous for the long allele).

To standardize cognitive measures for this study, standardized T-*scores* were developed by using multiple linear regression methods analyzing the influence of age, sex, education, and ethnicity on each cognitive test score. Each of the cognitive domains was included as dependent variables. The continuous predictor was age, and the categorical predictors were sex, education and race/ethnicity. For each regression, all the predictors were included in the model, retaining only the variables that significantly contributed to the prediction of cognitive test score. The β weights of each of these predictors in the final model, as well as the standard error of each regression model, were used to calculate predicted scores on each test. These predictive scores were subtracted from each individual actual composite score to calculate residual scores. Residual scores were then converted to *T-scores* (mean = 50; SD = 10). T-*scores* were used to determine cognitive impairment according to the Frascati criteria.[40] For the cognitive domains,

scores were developed as follows: executive function (SCT), visual memory (RCFT) sustained attention (CTT A) and cognitive flexibility (CTT B).

E. Results

Of the 379 participants recruited for the main study, 70% (N = 267) provided blood samples. The participants were 94 (34%) females and 173 (65%) males. The average age in the sample was: (males: *mean* = 45.1 *SD* = 7.1; females: mean = 45.3 *SD* = 65.9). The majority of participants self-identified as African-American 203 (76%), followed by Hispanic 43(16%) and Caucasian 21 (8%). A total of 190 (69%) had completed high school. At baseline, participants provided recent (within one month from intake) lab tests of CD4 count and viral load. Lab reports showed viral load as undetectable for 128 (48%) of the sample and an average CD4 count of 440 cells/mm^3 (SD = 287). The overwhelming majority of participants, 219 (81%) reported currently taking antiretroviral medication. Selection bias was not observed when participants' characteristics in the main study were compared to those in the present study. Results demonstrated that the participants were similar in age, education, sex, ethnicity and HIV clinical characteristics as shown in Table 2.

1. Alcohol and other drugs of abuse

The TLFB determined alcohol and other drugs use. Questions included a total number of standard drinks consumed in the last 90 days, the total number of heavy drinking days (< 5standard drinks) in the last 90 days, and lifetime alcohol use. A standard drink is defined as 12 oz of beer, 5 oz of wine, 1.5 oz of liquor all of which contain approximately 13.6 g of absolute alcohol.[41] Results showed a mean AUDIT score of 16, which is categorized as a harmful drinking level. In addition, a total of 101 (38%)

of the participants scored > 20 which is indicative of possible alcohol dependence. Lifetime alcohol use averaged 23.8 years for this sample. Additional detailed information on other substance use was also assessed. The main drugs used, besides alcohol, were cocaine and marijuana, with an average use in the last 90 days of 33 and 25 times, respectively.

The Frascati criteria were used to measure asymptomatic neurocognitive impairment, (1 standard deviation below the mean in at least 2 cognitive domains). Results for the neurocognitive measures were below average (*T-score*: mean = 50 SD = 10) with the exception of sustained attention (mean = 56.0, SD = 9.8). The cognitive domains with the lowest average scores were cognitive flexibility (mean = 45.7; SD = 10.8) and executive function, with the lowest scores representing better cognition (*mean* = 58.2; SD = 10.9).

2. DRD2 polymorphism and cognitive flexibility

Results of the analyses are presented in Tables 3 and 4. All SNPs were in Hardy-Weinberg equilibrium. The SNP rs6277 of *DRD2* gene showed an overall association with impaired cognitive flexibility (odds ratio = 1.7, 95% CI, 1.3-2.6; p = .01) and with executive function (odds ratio = 3.6, 95% CI, 2.4-8.7; p = .001). The association between SNP rs1800497 and cognitive flexibility was non-significant. Results were stratified by sex and race for cognitive flexibility and executive function. Testing show an increased risk for executive function impairment in African Americans (odds ratio = 3.1, 95% CI, 2.3-3.5; p = .001), and an even greater risk for males (odds ratio = 3.5, 95% CI, 1.5-5.5; p = .008). There was, a significant gender interaction for cognitive flexibility ($p_{interaction}$ = 0.013 for sex), but not for executive function ($p_{interaction}$ = .35 for sex). Interaction with

alcohol was not significant (p = .32) and no significant gene-gene interactions for *DRD4* and *DRD2* were found (results not shown).

3. DRD4 48bp VNTR polymorphism and executive function

The allele frequencies for *DRD4* 48bp VNTR were similar to those observed in African populations in other studies.[42, 43] In this study, the most frequently detected alleles of the 48bp VNTR of the D4 receptor were for *DRD4*-allele 4 (353/484, 72.9%), and *DRD4*-allele 7 (66/484, 13.7%). To a lesser degree *DRD4*-allele 2 (38/484, 7.8%), *DRD4*-allele 3 (7/484, 1.5%), *DRD4*-allele 5 (11/484, 2.3%), and *DRD4*-allele 6 (9/484, 1.8%) were also present. The nine and ten repeat alleles were not detected in this study population. The genotype distribution of the 242 participants is shown in Table 5. One hundred and eighty-six participants were grouped into the 7-absent allele group (< 7 repeats), and 56 were grouped into the 7-present allele group (≥7 repeats). When comparing allele groups, the 7-allele present and 7-allele absent groups did not differ in sex, race/ethnicity, alcohol use, or CD4 count. The 7-absent allele group mean score was associated with a higher rate of error in SCT measuring executive function than the 7-present group (mean: 0.17, 95% CI 1.17-1.29; p = 0.008). In addition, a multiple linear regression with executive function as the dependent variable and age, sex, alcohol use, genotype group and race/ethnicity as the independent variables showed that *DRD4* 7-absent allele and age had a significant effect on executive function. Whereas, sex, alcohol use and race/ethnicity did not show a significant effect (data not shown).

F. Discussion

This study provided evidence that suggests genetically determined differences in *DRD2* gene polymorphism (rs6277) and in the *DRD4* gene (48 VNTR) are associated

with impaired executive function and cognitive flexibility. However, no associations were found with SNP rs1800497. It is well-recognized that genes are likely to affect more than one cognitive function, and variations in cognitive functions are likely to be influenced by more than one gene.[44] Similarly, this study showed that the *DRD2*, SNP rs6277 is associated with impairment in two cognitive domains: executive function and cognitive flexibility. Conversely, executive function is influenced by *DRD2* and *DRD4* genetic polymorphisms. Although recent publications stress the need to consider gene-gene interactions, our results showed no such interactions.[44]

The present study found a significant association between *DRD2* genetic polymorphisms and executive dysfunction and impaired cognitive flexibility. The study showed that SNP rs6277 CC homozygous genotype was less efficient in inhibitory control as it took them more time to complete the task than TT and CT genotypes. Results of this study are consistent with those that reported an association between CC genotype and poorer working memory performance and executive functioning.[45, 46,32]

However, results are mixed in the literature regarding the precise influence of the SNP rs6277 *DRD2* on distinct cognitive processes and the effect's direction respectively.[47] For example, one study showed that high circulate *DRD2* binding potential in the TT genotype was associated with a higher rate of error in the Wisconsin Card Sorting Test, measuring executive function.[48] Conversely, Colzato and colleagues reported lower inhibitory control in SNP rs6277 CC homozygous genotype, which was associated with lower levels of *DRD2* striatal dopamine availability.[49] This incongruence may be explained by a recent analysis on the regional specificity of SNP rs6277 in the *DRD2* gene.[50] The *DRD2* genotypes lead to differences in binding affinities and

availabilities in striatal versus extrastriatal regions.[50] In all extrastriatal regions the CC genotype is associated with the highest *DRD2* binding potential, whereas the CC genotype is associated with the lowest binding potential in the striatum.[51] Thus, the SNP rs6277 in the *DRD2* gene changes the receptor's affinity and regulates *DRD2* availability, but its effect differs depending on the brain region under investigation.[50, 51] Neuroimaging studies suggest that the functional SNP rs6277 is associated with *DRD2* density changes across the cortex and thalamus.[50] For example, a meta-analysis showed a significant association between the SNP rs6277, C allele and schizophrenia.[52] Furthermore, studies have proposed that higher cortical *DRD2* density is a risk factor for executive dysfunction as shown for schizophrenia.[19, 53, 54] Thus, our results suggest that higher cortical *DRD2* density may also be considered a risk factor for HIV-associated neurocognitive impairment.

The *DRD4* 48bp VNTR polymorphism has been previously linked to ADHD phenotypes.[28, 55-58] In particular, the specific allele (7-repeat) of the 48pb VNTR polymorphism in the coding region of *DRD4* may be a risk factor for the development of ADHD.[28] ADHD is known to alter prefrontal cognitive functions that are often related to dopaminergic dysfunction. [59] Thus, following previous studies on ADHD, this study sought to assess whether cognitive functions (cognitive flexibility and executive function) were associated with the *DRD4* 48bp VNTR polymorphism in HIV-infected adults. Results in this study showed that the 7- absent allele group was significantly associated with executive dysfunction. The effect of the *DRD4* VNTR on executive function reported herein is comparable with a familial study that reported a significant association between the 7-absent allele group and lower scores in working memory and executive

function.[55] Similarly, several studies on *DRD4* VNTR showed that *DRD4* 7-absent allele group was associated with worse cognitive functioning than the *DRD4* 7-present allele group.[57, 60] However, the results of this study conflicts with the findings of other similar studies. One study found poorer inhibitory performance in the 7-present allele group versus the 7-absent allele group.[56] Another, found that 7-present allele group performed better that the 7-absent allele group on verbal memory, but for visuo-constructive ability (WAIS-III Block Design) and set shifting (WCST) the 7-absent allele group performed better than the 7-present allele group.[61]

This poses important questions with respect to the relationship between genetic risk and neurocognitive performance. There are several potential explanations for these conflicting results. First, higher and lower than average levels of synaptic dopamine may lead to neurocognitive impairment.[62] This is a particularly interesting since the 7-present allele is associated with reduced receptor functioning.[61] Second, the combinations of certain risk genotypes rather than one single risk genotype may lead to the presence of cognitive dysfunction.[63] Third, Boontra and colleagues suggested that the investigation of haplotypes rather than genotypes may yield stronger associations.[61] These relationships have not been fully tested and required further research, especially since cognitive endophenotypes are important for HIV-associated neurocognitive impairments.

Due to the exploratory nature of the study, multiple statistical comparisons were made. Because of the low power of the study to detect smaller effect sizes, some important associations may not have emerged as statistical significant. These results should be viewed with caution and should be replicated before definitive conclusion can be drawn. Alternatively, these results can serve as an initial point for future research in

cognitive phenotypes for HAND in adults. Molecular genetics, as applied in the present study, offers further analytic insight of the analysis besides behavioral assessment and neuroimaging, and may present a reasonable instrument for the dissociation of different executive control processes. Since most of the polymorphisms have a relative small effect on cognition, to detect an effect, a larger sample is optimal. In addition to the genes analyzed in this study, other genes related to cognitive function should be included in the future.

In summary, the present study provides evidence that genetically determined differences in the SNP rs6277 *DRD2* gene and *DRD4* 48bp VNTR may be a risk factors for executive function and cognitive flexibility deficits. Furthermore, rs6277 showed an association with impairment in two cognitive domains (executive function and cognitive flexibility) while executive function seems to be influenced by *DRD2* and *DRD4* genetic polymorphisms. Finally, *DRD4* 48bp VNTR (7-allele absent group) was associated with executive dysfunction, which is in line with the recent proposal that either higher or lower levels of synaptic dopamine may lead to neurocognitive impairment.

G. References

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Table 1. Alcohol Use Disorders Identification Test (AUDIT) scores

	Significance of Scores
0 -7	Low risk of alcohol-related harm
8-10	High risk of experiencing alcohol-related harm
11-19	Already experiencing significant alcohol-related harm
20+	Possible alcohol dependent advisable further testing to determine dependence.

Table 2. Demographic and clinical characteristics of main study and current study participants

Demographic Characteristics	Main study n = 112	Current study N = 267	P values
Age, mean (DS)	44.1 (7.7)	45.1(7.1)	.66
Sex, No (%)			.72
Male	67 (60)	173 (65)	
Female	45 (40)	94 (34)	
Education No (%)			.24
8th grade or less	13 (12)	19 (7)	
High school diploma	73 (65)	190 (69)	
Some college	26 (23)	57 (24)	
Race/Ethnicity No (%)			.26
Caucasian	17 (15)	21 (8)	
African-American	80 (72)	203 (76)	
Hispanic	15 (13)	43 (16)	
Alcohol use, mean (DS)			
Number of standard drinks (past 90 days)	100 (50.1)	190 (100.1)	.10
Lifetime	22 (10.5)	23.8 (10.9)	.24
AUDIT score	14 (7.5)	16 (8.0)	.09
Other drugs, mean (DS)			
Number of times cocaine use (past 90 days)	23.5 (16.8)	33.5 (19.8)	.25
Number of times marijuana use (past 90 days)	19.3 (12.5)	25.6 (20.9)	.63
HIV Characteristics, mean (DS)			
CD4 count	412.9 (318.4)	441.4 (286.9)	.73
Viral load No (%)			.16
undetectable	45 (40)	128 (48)	
50-10,000	39 (35)	80 (30)	
10,001 - 30,000	8 (7)	29 (11)	
30,000 or more	20 (18)	29 (11)	
Taking ART	76 (68)	216 (81)	.84
Cognitive measures, mean (DS)			
Executive skills *T-scores*	50.1 (9.0)	48.2 (10.9)	.93
Memory skills (learning) *T-scores*	45.9 (10.1)	48.2 (9.1)	.18
Memory skills (recall) *T-scores*	48.1 (9.8)	40.0 (10.5)	.11
Cognitive flexibility *T-scores*	40.4 (10.4)	45.7 (10.8)	.09
Sustained attention *T-scores*	53.9 (9.3)	56.0 (9.8)	.10
Visual Memory *T-scores*	47.9 (11.9)	43.1 (13.8)	.09

Table 3. *DRD2* and *ANKK1* associations with cognitive domains

Chr.	Position[a]	Gene	Variant	Alleles[b]	Domain	OR (CI%)
11	11:113412737	DRD2	rs6277	C/T	Cognitive flexibility	1.7 (1.3 to 2.6) p = .01
11	11:113412737	DRD2	rs6277	C/T	Executive function	3.6 (2.4 to 8.7) p = .001
11	11:113400106	ANKK1	rs1800497	C/T	Cognitive flexibility	1.1 (0.7 to 1.8) p = .71

Table 4. *DRD2* associations with cognitive flexibility and executive function by gender, race/ethnicity groups and alcohol use (ORs and 95% CIs)

	Females	Males	Hispanics	African American	Alcohol Use
DRD2 rs6277 (executive function)	1.3	3.5 (1.5 to 5.5) p = .008	2.6	3.1 (2.3 to 3.5) p = .01	2.6
	$p_{interaction}$ = .35		$p_{interaction}$ = .05		
DRD2 rs6277 (cognitive flexibility)	0.9	1.8 (1.2 to 2.9) p =.01	1.9	1.5	1.6 (1.4 to 2.4) p =.03
	$p_{interaction}$ = .013		$p_{interaction}$ =.72		$p_{interaction}$ = .32

Table 5. D4 Receptor 48bp repeat genotype group classification

D4 receptor 48bp repeat genotype	N	%	Genotype group
2/2	8	3.3	1
2/3	2	0.8	1
2/4	20	8.4	1
3/4	2	0.8	1
3/6	3	1.0	1
4/4	140	57.8	1
4/5	5	2.0	1
4/6	6	2.6	1
4/7	40	16.7	2
5/7	6	2.4	2
7/7	10	4.2	2

Group 1: 7-absent group: < 7-fold repeat of the 48bp repeat of D4 receptor
Group 2: 7-present group: ≥ 7-fold repeat of the 48bp repeat of D4 receptor

CHAPTER V

A RANDOMIZED CONTROL TRIAL OF A MANUAL-GUIDED RISK REDUCTION INTERVENTION FOR HIV-INFECTED ALCOHOL ABUSING INDIVIDUALS

A. Abstract

Improved HIV treatments and decreased mortality from AIDS-related illness have been paralleled by increased prevalence of HIV infection. HIV infection transmission risk is compounded by alcohol abuse and related risky sexual behaviors. Interventions that target alcohol use are central to improving the health of HIV-infected alcohol abusers. This study randomized 284 HIV-infected alcohol abusers to either the adapted Holistic Health Recovery Program (HHRP-A) or the Health Promotion Comparison program (HPC). Overall HIV sexual transmission risk behaviors decreased in both the experimental HHRP-A and HPC control groups. Cognitive impairment was measured as a moderator for alcohol use. Results showed that impaired cognitive flexibility and visual memory were associated with increased alcohol use. ($F = 1, 3.82, \eta 2 = 0.03, p = .05$;, and $F = 1, 5.79, \eta 2 = .05$ $p = .02$) respectively. At the six-month follow-up, HHRP-A participants were less likely to report trading sex for food, drugs and money (20.0%) and unprotected insertive or receptive oral (11.6%) or vaginal and/or anal sex (3.2%) than HPC participants (49.4%, $p < .001$; 22.5%, $p = .05$; 15.4%, $p = .04$). The number of times that sex was traded for food, money and drugs decreased in men in the HHRP-A experimental group (food: $Z = 2.2$ $p = .03$; money: Z 3.4 $p = .001$; drugs: $Z = 3.1$ $p = .002$). Similarly, men and women exhibited decreases in the number of episodes of sex following alcohol consumption (men: $Z = 2.0$ $p = .04$; women: $Z = 2.3$ $p = .02$). This

study suggests that HHRP-A is an effective intervention program for the reduction of sexual transmission risk behaviors in HIV-infected alcohol abusers.

B. Introduction

The reduction of HIV transmission risk behaviors among HIV-infected individuals remains a major health priority. Depression, substance use, and stressful, traumatic experiences have been identified as predictors of sexual transmission risk.[1, 2] The impact of heavy alcohol use in HIV-infected individuals has not been extensively examined.[3, 4] Approximately 5% of the total US population abuses alcohol. However, the prevalence of alcohol abuse in HIV-infected individuals is between 30 to 60%.[5, 6] HIV-infected alcohol abusers are more prone to rapidly progressing illness with higher viral loads, increased immune suppression, and cognitive impairments compared to HIV-infected alcohol non-abusers.[3] Behavioral factors associated with alcohol abuse can also increase HIV disease progression, interfering with antiretroviral therapy adherence.[4] Alcohol consumption impairs judgment and cognition which diminishes risk perception, therefore increasing HIV risk transmission.[7] Over 70% of people infected with HIV remain sexually active after diagnosis and one-third engages in unprotected sexual behavior.[8]

An area of recent interest is on the interaction between alcohol and HIV on cognition. Regular daily consumption of six or seven standard alcoholic drinks may be associated with moderate cognitive impairment.[9] On the other hand, individuals affected by both alcohol abuse and HIV infection may suffer a compounded deficit in cognitive performance which may affect adherence to antiretroviral medication and treatment outcomes.[10] Clinical research shows that not all chronic drinkers are equally at risk for

brain changes; most suffered mild cognitive impairment that improved within a year of abstinence.[11, 12]

A study reported high rates of knowledge on how to prevent HIV transmission among infected persons, but uncertainty about how to make these changes was a factor for not changing their behavior.[13] Efforts to reduce risk of HIV transmission and alcohol abuse have been guided by several models of health behavior change.[14] One theoretical approach that has effectively predicted HIV-related health behaviors in diverse samples is the Information-Motivation-Behavioral Skills (IMB) model.[15, 16] The model states that HIV prevention information and motivation work through preventive behavioral skills to influence risk reduction behaviors.[17] The Holistic Health Recovery Program (HHRP) is an evidence-based intervention guided by the IMB model for HIV-infected drug users.[18] The HHRP-A is an adapted version of the HHRP program designed for alcohol abusers based on the IMB model. The HHRP-A is a manual-guided intervention designed to reduce sexual transmission risk and alcohol use in HIV-infected alcohol abusers.[19] In addition, the HHRP-A incorporated cognitive remediation strategies by using materials and techniques that minimize the effects of cognitive difficulties. This study evaluated the effectiveness of the IMB-based, manual-guided intervention using HHRP-A to reduce sexual transmission risk and alcohol use in HIV-infected alcohol abusers.

C. Methods

1. Sample

A total of 284 HIV-infected adults were recruited from CBOs in Miami, Florida, between January 2009 and November 2012. The slow recruitment process was planned to help reduce treatment group cross-contamination and group assignment bias. The

inclusion criteria for the participants were age between 18 and 60 years, HIV-positive and willing to present documentation to confirm serostatus, alcohol consumption within the last three months and/or a history of alcohol abuse or dependency within the past two years, and at least one episode of unprotected vaginal or anal sex in the past 90 days. Additional criteria included: ability to understand and speak English, understand and give informed consent, provide contact information for follow-up interviews, willingness to be randomized to the experimental or control group, not facing immediate incarceration or residence in a restricted environment, and no evidence of major psychiatric disorders. The Institutional Review Board of Florida International University approved the research protocol, and all participants provided signed informed consent prior to participating in the study.

2. Neurocognitive Assessment

The following tests measured neurocognitive deficits at baseline. 1) The *Short CategoryTest*- measured executive function by abstract concept formation and learning,[20] 2) The *Rey-Osterrieth Complex Figure Test*-measured visual memory,[21] 3) The *Auditory Verbal Learning Test*-measured retention rates, learning rates and recognition,[22, 23] 4) The *Color Trail Test part A*-measured sustained attention,[24] and 5) The *Color Trail Test part B*-measured cognitive flexibility.[24] The Frascati criteria were used to measure impairment.[25]

3. Study Design

This study was a prospective randomized clinical trial where participants were assigned to the experimental or control groups. Participants were entered into the study in cohorts of eight of the same gender. Cohorts were assigned to receive either the

experimental or control condition according to a computer-generated random sequence. The random sequencing controlled for bias in subject assignments across conditions. The experimental group used the HHRP-A intervention program designed to promote risk reduction behaviors among HIV-infected alcohol abusers.[26] The HHRP-A intervention manual was highly structured and involved both didactic presentations of material as well as experiential exercises. Participants attended eight sessions lasting two hours, twice a week, for four weeks. The intervention addressed harm reduction skills training, relapse prevention and reduction in HIV sexual transmission risk behaviors. Each session was co-facilitated by two counselors using a nonjudgmental, motivational enhancing therapeutic style. Cognitive remediation strategies were incorporated because of the potential for cognitive impairment in this population. Some of these strategies included emphasis on structure and consistency, repetition and review, behavioral games and memory books, as well as ongoing assessment of new learning and retention, with immediate provision of feedback.

The Health Promotion Comparison (HPC) group focused on educational and didactic methodologies, addressing common health problems such as nutrition, physical fitness, and healthy living. The HPC did not incorporate behavioral skills training or motivational enhancement techniques. The HPC matched HHRP-A in total administration time and format (eight, two-hour sessions). However, the program was condensed and delivered in two days to reduce the risk of cross-group contamination and the potential for enhancing social support that group sessions repeated over time could engender. A standard care HIV education component was included in the HPC because it

was considered ethically irresponsible not to include HIV education in a comparison condition, given the high-risk nature of this population.

Assessments were conducted at baseline and six-month follow-up. The timeline follow-back (TLFB) method was used to measure alcohol use and sexual transmission risk behaviors. TLFB uses a calendar format that provides retrospective estimates of the participant's substance use and sexual conduct over the last 90 days.[27] Alcohol abuse was defined by the amount consumed (e.g., at risk, heavy) or by the consequence of its use (e.g., abuse, dependence).[4] Alcohol abuse is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as > 4 drinks on an occasion or >14 drinks in a week for men, and > 3 and > 7 for women, respectively.[28] In addition, computer assisted personal interview, and audio computer assisted self-interview were used for baseline data. Data were collected at baseline and six-month follow-up for all measures. Participants were offered incentives for participation as follows: $50 gift card to local stores upon completing the baseline data collection; $25 gift card upon completing post-intervention assessments; $15 cash or gift card for attendance at each group session; and $5 cash for transportation.

4. Outcome Measures

At the baseline and six-month follow-up assessments, participants reported on alcohol use and sexual transmission risk behaviors. The number of vaginal, anal and oral sex events without a barrier and times participant traded sex for food, money, or drugs, and number of sex events directly following alcohol consumption were used to measure sexual transmission risk. The number of drinking days, heavy drinking days (≥ 5 standard drinks), and the number of standard drinks were used to measure alcohol use.

Report of any or no incident of trading sex for food, drugs and money, sex directly following alcohol consumption, unprotected insertive or receptive oral, anal or vaginal sex were compared as categorical variables at six-month follow-up by group assignment (HHRP-A versus HPC). Hazard ratios (HRs) were calculated as measures of relative risk; 95% confidence intervals and p-values from Chi-square or Fisher's exact 2-tailed tests were used for significance testing.

Analyses were conducted with SPSS version 20 (IBM, 2011). Continuous measures were quantities consumed or frequency of alcohol use and sexual transmission risk events. Data for alcohol use were transformed using the Box-Cox method,[29] and sexual transmission risk used nonparametric methods. Initially, the Wilcoxon signed-rank test was used for all the analyses. Transformed data were analyzed using the t-test and ANOVA methods to measure the outcomes. Change over time on continuous measures was analyzed using two repeated measures by analyses of variance (ANOVAs) for treatment condition and time variables. Where change over time in any continuous variable was detected, effect sizes (partial η^2) were reported. The intervention outcomes were assessed by alcohol use measured by total number of standard drinks in the last 90 days and the total number of heavy drinking days ($\geq$ 5 standard drinks) in the last 90 days. Sexual transmission risk was measured by number of insertive and receptive anal or vaginal sex events without a condom, number of episodes receiving or giving oral sex without a barrier, number of times the participant traded sex for food, drugs and money, and number of sex events directly following alcohol consumption. The outcomes were modeled as a function of condition (intervention or control), time (baseline and follow-up), gender, and condition by time. The interaction between neurocognitive measures and

alcohol was also measured in both conditions. Cognitive impairment was measured by normalized *T*-scores in this sample. Normalized *T*-scores were developed by using multiple linear regression methods analyzing the influence of age, sex, education, and ethnicity on each cognitive domain. The normalized *T-scores* (mean = 50 and SD =10) were used to determine cognitive impairment.

D. Results

A total of 284 participants were randomized to either the control group 140 (49.3%) or the experimental group 144 (50.7%). There was no significant difference between the groups in age, or gender (Table 1). The predominant race for both groups was African American (control group: 71%; the experimental group: 72%). They did not differ significantly by ART use and immunologic or virologic status. Demographic, behavioral and clinical characteristics for the control and experimental groups are presented in Table 1. According to Frascati criteria a total of 109 (41%) of the participants in the sample scored below the mean, in the domains of executive function, cognitive flexibility and memory (*T-score*: mean = 50; SD =10). Asymptomatic neurocognitive impairment, greater than one standard deviation below the mean was observed in 98 (90%) of these individuals, and mild neurocognitive impairment, greater than two standard deviations below the mean was observed in 11 (10%). (Frascati definition Table 3, Chapter 2). The proportion with neurocognitive impairment at baseline did not differ significantly between the two groups.

1. Alcohol use

Analyses of the intervention impact on the outcomes for alcohol consumption revealed an overall significant decrease in alcohol consumption by time ($F = 3, 162, \eta 2 =$

0.54, *P* = <.01) for the experimental and the control groups, but there were no significant differences between experimental and control groups in alcohol use reduction. When cognitive impairment was measured as a moderator for alcohol use. Results showed that impaired cognitive flexibility and visual memory were associated with increased alcohol use. (F = 1, 3.82, η2 = 0.03, p = .05; and F = 1, 5.79, η2 = .05 p = .02), respectively.

2. Sexual transmission risk

Proportions of study participants who reported HIV risk behaviors at six-month follow-up differed by intervention group assignment; in all cases, participants in the experimental condition had better risk reduction outcomes. At baseline trading sex for food, drugs or money and number of sex events directly following alcohol consumption were the most frequent HIV risk behaviors reported. At the six-month follow-up, HHRP-A participants were less likely to report trading sex for food, drugs and money (20.0%) and unprotected insertive or receptive oral (11.6%) or vaginal and/or anal sex (3.2%) than HPC participants (49.4%, p <.001; 22.5%, p=.05; 15.4%, p=.04) shown in Table 2. Additionally, a significant decrease for trading sex for food, money and drugs (food: Z = 2.2 p = .03; money: Z 3.4 p = .001; and drugs: Z = 3.1 p = .002), as well as decreased number for vaginal sex events without a barrier (Z = 1.9 p = .05) was observed over time in study participation, which was statistically significant only in men. Both men and women had similar statistically significant decreases in number of reported sex events directly following alcohol consumption (men: Z = 2.0 p = .04; women: Z = 2.3 p = .02). No significant changes for number of anal or oral sex events without a barrier were reported. The control group showed a small decrease in trading sex for food, money or

drugs, sex following alcohol consumption. However, this decrease was not significant as shown in Table 3.

3. Attrition analysis

Of the 278 participants who completed the baseline assessment, 185 (66%) also completed the six-month follow-up assessment. Of the 94 (34%) participants who did not complete the follow-up assessment, three died, one went to jail, and 90 were excluded from the analyses due to incomplete data and/or were lost to follow-up. Some of the reasons for loss to follow-up included seeking health care at another clinic and not returning to complete the assessment for unknown reasons. No difference ($p > .35$) in attrition was seen by study condition, demographic factors, or baseline sexual transmission risk behaviors (vaginal, anal and oral sex without a barrier, trading sex for food, money, or drugs, and number of sexual events after alcohol use) or for alcohol use (total number of standard drinks in the last 90 days and total number of heavy drinking days in the last 90 days).

E. Discussion

The study evaluated the efficacy of the HHRP-A intervention program and showed a significantly lower level of HIV transmission risk behaviors at the six-month follow-up assessment in the experimental compared to the control group. Proportions reporting sexual transmission risk behaviors including trading sex for food, money, or drugs and unprotected sex were 49% to 80% lower six months post-intervention among HHRP-A participants versus comparison group members. Additionally, alcohol use decreased for number of heavy drinking days (≥ 5 standard drinks) and total number of standard drinks in both the intervention and control groups.

Alcohol abuse has been associated with increased involvement in sexual risk behaviors, including sex under the influence, trading sex for drugs or money, and vaginal and/or anal sex events.[30] Very few HIV intervention programs address alcohol abuse as part of their intervention; our results are encouraging in that respect.[31-34] In addition, a significant interaction effect was found between alcohol use and cognitive impairment in the domains of cognitive flexibility and visual memory which were associated with increased alcohol use. These results are similar to clinical studies showing that alcohol abuse is associated with impairments in specific neuropsychological functions.[12] However, some studies have suggested an increase in vulnerability to cognitive impairment due to the interaction between chronic alcohol use and HIV infection.[35, 36] For example, one study examined the effect of current heavy alcohol use and HIV infection on cognitive performance and found a synergistic effect for the heaviest current drinkers (> 6 drinks per occasion) and HIV infection on motor and visuomotor speed.[37] Individuals affected by both alcohol dependency and HIV infection may suffer compounded deficit in cognitive performance which may affect ART adherence and treatment outcomes.[10] The results in this study showed a similar trend of lower functioning in cognitive areas affected by both alcohol and HIV infection.

Alcohol use may not only interfere with prevention strategies, but may threaten the success of emerging biomedical approaches to HIV prevention.[4, 38, 39] Behavioral interventions such as HHRP-A specifically tailored for HIV-infected alcohol abusers are necessary for this population. To our knowledge this is the first HIV intervention study that demonstrated specific reductions in sexual transmission risk behaviors in HIV-infected alcohol abusers. The current study also provided important information about

which sexual transmission risk behaviors are most common among HIV-positive alcohol abusers. These behaviors include trading sex for money and drugs, unprotected sex, and sex following alcohol use. These preliminary results showed that HHRP-A is an effective intervention for sexual transmission risk-reduction behaviors. However, the highest decrease in sexual risk behaviors were observed mostly in men. These results may be related to the women's economic dependency on men, as well as to the men's cooperation in decreasing sexual risk behaviors. Thus, it is important to incorporate risk reduction behavior strategies that are specifically geared for men and women respectively.

There were a few limitations in the ability to draw conclusions in this study. First, for ethical reasons, the participants who were active in alcohol or drug abuse treatment programs could not be excluded. Thus, we were not able to determine the extent to which HHRP-A components alone had an impact on alcohol use at the six-month follow-up. This is an important issue that should be addressed in the future. Second, although significant differences were found for sexual transmission risk behaviors the high loss to follow up (34%) limits the interpretability of the findings. Last, alcohol use and sexual transmission risk were assessed by self-reports alone allowing for either overestimation or underestimation of the number of sexual events or quantity of alcohol consumed.

Some of the strengths in this study were the ethnic diversity of the sample and the representation of patients with neurocognitive impairment. Second, the HHRP-A program covered a range of topics relevant to HIV-infected alcohol abusers and provided cognitive remediation strategies to facilitate learning and retention. Moreover, the treatment condition was manual-guided, and procedures for assuring the integrity of the

treatment conditions were used. Finally, this population was recruited from Miami-Dade County, Florida, the metropolitan area with the highest HIV prevalence in the nation: our results may be relevant for other highly-impacted urban areas.[40]

In conclusion, findings from this study, although preliminary, suggest that enhancing substance abuse treatment with comprehensive interventions that address the special needs of HIV-infected alcohol abusers can provide important benefits. Implementation of interventions, such as HHRP-A that target individuals already infected with HIV appear to be essential to control the spread of HIV. These interventions will need to meet the special needs of substance-using HIV-infected population and should be incorporated into addiction treatment.

F. References

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Table 1. Baseline Demographic, behavioral and clinical characteristics of the control and experimental groups

Characteristics	**Control n = 140 (%)**	**Experimental n = 144 (%)**	P value
Age in years, mean (SD)*	44.9 (7.5)	45.4 (6.8)	.70
Male	77 (55)	89 (62)	.67
Ethnic background			.43
Caucasian	13 (9)	20 (14)	
African American	99 (71)	104 (72)	
Hispanic	19 (14)	23 (16)	
Education			.44
Some college	29 (21)	37 (26)	
High school diploma	53 (38)	48 (33)	
Some school no diploma	58 (41)	59 (41)	
HIV characteristics Mean (SD*)			
CD4+ T-lymphocyte count	469 (303.8)	466 (328.9)	.42
Viral load undetectable	60 (43)	76 (53)	.56
Taking antiretroviral therapy (ART)	100 (71)	114 (79)	.24
Alcohol Use and Sex Behaviors Mena (SD*)			
Lifetime alcohol use years	23.5 (11.2)	23.2 (10.8)	.88
Currently in an alcohol treatment facility	84 (60)	97 (67)	.15
In the last 12 months in an alcohol treatment facility	50 (40)	47 (33)	.09

Table 1. Continued…….

Characteristics	Control group n = 140	Experimental group n = 144	P value
Traded sex for food, drugs or money in last 90 days	59/130 (45.3)	54/149 (36.2)	.12
Had any sexual event directly following alcohol consumption in last 90 days	69/117 (59.0)	106/143 (74.1)	**.009**
Had insertive or receptive oral sex without a barrier in last 90 days	37/132 (28.0)	48/144 (33.3)	.34
Had vaginal and/or anal sex without a barrier in last 90 days	25/132 (18.9)	29/144 (20.1)	.80
Cognitive measures, Mean T scores (SD*)			
Executive function T-scores	50.1 (10.4)	44.7 (10.1)	**.001**
Cognitive Flexibility T-scores	46.5 (10.5)	43.2 (10.9)	.36
Sustained attention T-scores	45.5 (10.8)	41.1 (10.2)	.29
Visual memory T-scores	50.5 (10.3)	51.1 (10.3)	.44
Memory T-scores	39.6 (9.1)	39.8 (10.5)	.46
Proportion with Neurocognitive Impairment	49/133 (35%)	60/134 (41%)	.19

*SD standard deviation

Table 2. HIV risk behaviors at six-month follow-up by assignment

All HIV Risk Behaviors	Group	Number (%) Reporting Behavior	Hazard Ratio (95%CI)*	P value
Traded sex for food, drugs and money, last 90 days	Experimental	19/95 (20.0)	0.41 (0.27 to 0.64)	**<.001**
	Control	42/85 (49.4)		
Sex events directly following alcohol consumption, last 90 days	Experimental	15/106 (14.1)	0.89 (0.43 to 1.8)	.75
	Control	11/ 69 (15.9)		
Insertive or receptive oral sex without a barrier, last 90 days	Experimental	11/95 (11.6)	0.51 (0.26 to 1.0)	.05
	Control	18/80 (22.5)		
Vaginal and anal sex without a barrier in last 90 days	Experimental	3/95 (3.2)	0.2 (0.06 to 0.70)	**.04**
	Control	12/78 (15.4)		

*95%CI 95% Confidence Interval

Table 3 HIV risk behaviors at six-month follow-up by assignment and gender

Measure	Intervention	Women		Men	
*Traded sex for shelter or food last 90 days	Experimental	Z = 1.2 p = .24		**Z = 2.2 p = .03**	***r* = 0.31**
	Control	Z = 0.7 p = .45			
*Traded sex for money last 90 days	Experimental	Z = 1.2 p = .21		**Z = 3.4 p = .001**	***r* = 0.35**
	Control			Z = 0.6 p =.56	
Traded sex for drugs last 90 days	Experimental	Z = 0.9 p = .36		**Z = 3.1 p = .002**	***r* = 0.25**
	Control	Z = 0.5 p = .62		Z = 0.9 p = .34	
Sex events directly following alcohol consumption last 90 days	Experimental	**Z = 2.3 p = .02**	***r*= 0.30**	**Z = 2.0 p = .04**	***r* = 0.34**
	Control	Z = 0.9 p = .32		Z = 1.3 p = .20	
Vaginal sex without a barrier last 90 days	Experimental	Z = 0.9 p = .32		**Z = 1.9 p = .05**	***r* = 0.29**
	Control	Z = 1.5 p = .07		Z = 0.5 p = .61	
Sex events without a barrier last 90 days	Experimental	Z = 1.5 p = .13		**Z = 2.7 p = .008**	***r* = 0.37**
	Control	Z = 0.7 p = .49		**Z = 2.2 p = .02**	***r* = 0.27**

The effect size was calculated by dividing the Z by the square root of N ($r = Z/\sqrt{N}$) using the Wilcoxon Signed-rank Test.
*Measures for trade sex for food or money in the control group were not calculated because of the low number of participants.

CHAPTER VI

CONCLUSION

A. Summary of Conclusions

HIV-infected individuals continue to experience neurocognitive deterioration despite viral suppression due to successful ART treatment.[1] Currently, demographic characteristics, and medical comorbidities are used to identify individuals who are at risk for HAND.[2, 3] However, additional risk factors such as the role of genetics in relation to HIV-neurocognitive susceptibility should be investigated.[4] Memory deficits, and executive dysfunction are highly prevalent among HIV-infected adults. These conditions can affect their quality of life, antiretroviral adherence, and HIV risk behaviors.[5, 6] Cognitive functions are influenced by the serotonin and dopamine systems. Thus, genetic differences in the serotonin and dopamine system genes may exacerbate the development of neurocognitive impairment in an individual.[1, 5, 7, 8] The study's overarching hypothesis was to determine whether specific genetic differences in HIV-infected alcohol abusers were correlated with executive dysfunction, impaired cognitive flexibility and memory, and whether these cognitive deficits moderated alcohol use and sexual transmission risk behaviors.

The third chapter investigated the potential associations between single nucleotide polymorphisms in the serotonin system genes and cognitive impairment in HIV-infected adults. A total of 267 biologically unrelated individuals were genotyped for polymorphisms SLC6A4 5-HTTLPR, TPH2 rs4570625 and GALM rs6741892. The SCT, CTT A&B and AVLT tests were used to assess cognitive functions. Results showed a

significant association for *TPH2* and *GALM* variants with executive function and memory. This study provided further evidence for the role of serotonin in cognition, where *TPH2* and *GALM* gene polymorphisms affect 5-HT signaling pathway influencing executive function and memory. These findings parallel and extend those of functional imaging studies and molecular genetics suggesting that the *TPH2* genetic variant rs4570625 is a risk marker for cognitive impairment. [9-11] Moreover, a significant association was found between *GALM* polymorphism and memory, which may imply SNP rs6741892 as a functional polymorphism in the *GALM* gene affecting 5-TH transport.[12]

The fourth chapter investigated the influence of two candidate gene polymorphisms (*DRD4* and *DRD2*) in the dopamine system. Executive function and cognitive flexibility domains were measured by the SCT, CTT A&B and ROCT tests. Participants were genotyped for polymorphisms in the *DRD4* 48bp-variable number tandem repeat (VNTR), *DRD2* rs6277 and *ANKK1* rs1800497. This study found significant associations with *DRD2* and *DRD4* genes and impaired cognition. SNP rs6277 of the *DRD2* gene showed a significant association in two cognitive domains (executive function and cognitive flexibility). *DRD4* 48bp VNTR (7-allele absent group) was significantly associated with executive dysfunction, which is in line with a recent proposal that either higher or lower levels of synaptic dopamine may lead to neurocognitive impairment.[13] In summary, these studies suggest a compounded effect of genetic influence and HIV infection on cognition in HIV-infected individuals.

The fifth chapter randomized 284 HIV-infected alcohol abusers to either the adapted Holistic Health Recovery Program (HHRP-A) or the Health Promotion Comparison program (HPC). The HHRP-A is based on the IMB model; it is a manual-guided intervention designed to reduce sexual transmission risk and alcohol use in HIV-infected alcohol abusers.[14] The intervention phase was evaluated at the six-month follow-up for sexual transmission risk and alcohol use. Results showed a significant decrease for trading sex for food, money and drugs in men but not in women. Additionally, men and women exhibited decreased number of sex events following alcohol use. Finally, cognitive impairment was measured as a moderator for alcohol use. Results showed that impaired cognitive flexibility and visual memory were associated with increased alcohol use. This study suggests that HHRP-A is an effective intervention for the reduction of sexual transmission risk behaviors in HIV-infected alcohol abusers.

Public health genomics is an area of public health that focuses on the effective translation of genomics research into population health benefits. Genomics plays a role in most chronic diseases and these chronic diseases are partly the result of how genes interact with environmental and behavioral risk factors.[16-19] Thus better understanding of the genetic interaction with the environment can help not only clinical practitioners identify, develop and evaluate screening tools, but this can also help develop interventions that can improve health and prevent disease.

The evidence for variability in genetic susceptibility to cognitive impairment as previously demonstrated has laid the groundwork for an approach to intervention development and implementation informed by genomics which may pave the way to a more personalized approach to prevention depending on the patient genotype.[16] Similarly,

the outcomes from this dissertation may provide further understanding of how genetic predispositions influence the neuromodulatory serotonin and dopamine systems relevant to cognitive impairment and risk behaviors in HIV-infected alcohol abusers. More personalized treatments including clinical markers, such as cognitive and genetics tests, may not only assist in determining who is more at risk but, may be able to assist in determining the type intervention strategies that can be used when a prevention program is developed and implemented. Interventions that target alcohol use is central to improve the health of HIV-infected alcohol abusers.[15] The HHRP-A intervention program is an effective method to reduce sexual transmission risk among HIV-infected alcohol abusers. Enhancing substance abuse treatment with this kind of comprehensive intervention that addresses the special needs of HIV-infected alcohol abusers is needed and should be further evaluated.

This dissertation had two main limitations, first, the lack of alpha-level corrections due to the multiple comparisons that were made, however, these comparisons were necessary because of the exploratory nature of the studies in this dissertation. Thus, results should be view with caution and must be replicated. Second, it was not possible to evaluate the efficacy of the HHRP-A intervention for reducing alcohol use due to the participants were also active in alcohol or drug abuse treatment programs. Thus, future studies should include individuals not currently enrolled in a substance abuse treatment program. Similarly, in the future genetic studies on cognition should include, in addition to the polymorphisms analyzed in this dissertation, other genes related to cognitive function since most of the polymorphisms have relative small effects on cognition. In summary, the results in this dissertation can serve as an initial point for future research in

cognitive phenotypes for HAND in adults. These findings provide evidence that dopamine and serotonin polymorphisms influence executive function and memory as well as moderate alcohol use and that HHRP-A was effective in reducing sexual transmission risk behaviors in HIV-infected alcohol abusers.

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APPENDICES

Appendix 1. HHRP Fact sheet

HOLISTIC HEALTH RECOVERY PROGRAM

A Group-level Intervention for HIV-positive and HIV-negative Injection Drug Users
FACT SHEET

Program Overview
The Holistic Health Recovery Program (HHRP) is a 12-session, manual-guided, group level program to promote health and improve quality of life.

The primary goals of HHRP are health promotion and improved quality of life. More specific goals are abstinence from illicit drug use or from sexual risk behaviors; reduced drug use; reduced risk for HIV transmission; and improved medical, psychological, and social functioning. HHRP is based on the Information-Motivation-Behavioral Skills (IMB) model of HIV prevention behavioral change. According to this model, there are three steps to changing behavior: Providing HIV prevention information, motivation to engage in HIV prevention and opportunities to practice behavior skills for HIV prevention.

Core Elements
The core elements of HHRP are:
- Teaches skills to reduce harm of injection drug use and unprotected sexual activities.
- Teaches negotiation skills to reduce unsafe sexual behaviors with sexual partners and teaches skills to heal social relationships.
- Teaches decision making and problem solving skills using cognitive remediation strategies.
- Teaches goal setting skills including developing action plans to achieve goals.
- Teaches skills to manage stress, including relaxation exercises and understanding what aspects of the stressful situation can, and cannot, be controlled.
- Teaches skills to improve health, health care participation, and adherence to medical treatments.
- Teaches skills to increase clients' access to their own self-defined spiritual beliefs, in order to increase motivation to engage in harm reduction behaviors.
- Teaches skills to increase awareness of how different senses of self can affect self-efficacy and hopelessness.

Target Population
HHRP targets HIV-positive and HIV-negative injection drug users.

Program Materials
- Program manuals for both HIV-positive and HIV-negative injection drug users are available, which include all the materials required to implement the intervention.

Research Results
Implementation of HHRP produced the following results:
- Decrease in addiction severity.
- Decrease in risk behavior.
- Significant improvement in behavioral skills, motivation, and quality of life.

For More Information on the Holistic Health Recovery Program
Currently, CDC does not offer trainings for Holistic Health Recovery Program (HHRP). However, the intervention implementation materials are available for download from Yale University School of Medicine, Department of Psychiatry - http://info.med.yale.edu/psych/3s/training.html

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Appendix 2. Community-based organizations assessed by the main study

Name	Website
Borinquen Health Care Center	http://www.borinquenhealth.org/
Florida Department of Health: Broward County Department Sexually transmitted Disease Program	http://browardchd.org/default.aspx
Center for Haitian Coalition	http://www.hccinc.org/
South Florida Provider Coalition Spectrum Programs	http://www.drugrehabcomparison.com/facility/florida/miami/spectrum-programs-inc-south-florida-provider-coalition/
The Salvation Army	https://donate.salvationarmyusa.org
Care Resource	http://www.careresource.org/
Empower U, Inc.	http://www.empower-u-miami.org/
The Village	http://www.villagesouth.com/
South Florida AIDS Network	http://www.jacksonhealth.org/services-sfan.asp
The Center for Positive Connections Support & Resource Center	http://www.aidsnet.org/newmain/providers/centerpositive
South Beach AIDS Project Inc.	http://www.miami.com/south-beach-aids-project-inc
Miami Beach Community Health Center, Inc.	http://www.miamibeachhealth.org/
Helen B. Bentley Family Health Center, Inc.	http://www.helenbbentleyfamilyhealthcenter.com/

Appendix 3. Additional Tables for Methods Section

Table 4. Real-time PCR Protocol

Reaction Setup	Per Reaction (µl)
SsoFast™ Probes Supermix 2X	5.00
TaqMan® SNP Genotyping Assay 20X	0.50
DNase-free water	1.50
Template DNA	3.0

Table 5. Cycling Conditions on CFX96 PCR System

Cycling Step	Temperature	Time	No. of Cycles
Denature	95 ^{o} C	2 min	1
Annealing	95 ^{o} C	5 sec	49 X
Extension	72^{o} C	5 sec	

Appendix 3. Continuation.....

Table 6. PCR Protocol

Reaction Setup	Per Reaction (µl)
PCR buffer 10X	2.5
dNTP mix	1.0
Primer (forward)	0.5
Primer (reverse)	0.5
Taq DNA polymerase	0.5
DNase-free water	17.0
Template (DNA)	3.0

Table 7. Cycling Conditions on MyCycler PCR System

Cycling Step	Temperature	Time	No. of Cycles
Denature	94^{o} C	30 sec	1
Annealing	60 ^{o} C	30 sec	30 X
Extension	72^{o} C	2 min	
Final Extension	72 ^{o} C	2 min	

Appendix 4. Genetic terminology used in this dissertation

Term	Definition
Allele	A known variation (version) of a particular gene
Base pair (bp)	When quantified refers to the physical length of a sequence of nucleotides
Carrier	A healthy person who is a heterozygote for a recessive trait.
Denaturation	Reversible disruption of hydrogen bonds between nucleotides converting a double-stranded DNA molecule to single-stranded molecules. Heating or strong alkali treatment result in denaturation of DNA.
DNA (deoxyribonucleic acid)	The large double-stranded molecule carrying the genetic code. It consists of four bases (adenine, guanine, cytosine and thymine), phosphate and ribose.
Chromosome	Structure in a cell nucleus that carries the genes.
Gene	Physical and functional unit of heredity that carries information from one generation to the next, which is the entire DNA sequence necessary for the synthesis of a functional polypeptide or RNA molecule. In addition to the coding regions (exons), a gene may have non-coding intervening sequences (introns) and transcription-control regions
Genome-wide association study (GWAS)	Simultaneous investigation of up to five million genetic variants covering the whole genome in complex genetic diseases.
Genotype	The diploid genetic formula at one or more loci.
Genotype-environment (GXE) interaction	This term refers both to the modification of genetic risk factors by environmental risk and protective factors and to the role of specific genetic risk factors in determining individual differences in vulnerability to environmental risk factors

Appendix 4. Continuation.....

Hardy-Weinberg equilibrium (HWE)	In an infinitely large population, gene and genotype frequencies remain stable as long as there is no selection, mutation, or migration. For a bi-allelic locus where the gene frequencies are p and q: p2+2pq+q2 = 1.
Linkage Disequilibrium	The tendency for two 'alleles' to be present on the same chromosome (positive LD), or not to segregate together (negative LD). As a result, specific alleles at two different loci are found together more or less than expected by chance. The same situation may exist for more than two alleles. Its magnitude is expressed as the delta (D) value and corresponds to the difference between the expected and the observed haplotype frequency.
MAF	Minor allele frequency (MAF) refers to the frequency at which the least common allele occurs in a given population.
PCR	Polymerase chain reaction. A technique that allows amplification of specific DNA segments in a very short time.
rs	Reference SNP ID
SNP	is a single nucleotide change in the DNA sequence code It is the most common type of stable genetic variation and is bi-allelic
VNTR	is a linear arrangement of multiple copies of short repeated DNA sequences that vary in length and are highly polymorphic, making them useful as markers in genetic analysis

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