Genetic and Neural Factors in Alcohol-Related Cognition

HM20013938

Approved by IRB 8/24/2018

Table 1. Overview and schedule of proposed training activities and their alignment with training goals (TG).

Domain	Activity Description	Proposed Award Year				
		Y1	Y2	Y3	Y4	Y5
Mentored Training	Meet with Dr. Dick to select/discuss directed readings, develop of theoretical models, and track progress (4 hours/month Y1-Y5; TG #1)	X	X	X	X	X
	Meet with Dr. Goldman to discuss directed readings from literatures on clinical alcohol misuse, externalizing behaviors, and their genetic basis (1 hour/month Y1-Y5; TG #1).	X	X	X	X	X
	Meet with Dr. Aliev to select/discuss directed readings and develop/review genetic and genomic statistical analyses (4 hours/month Y1-Y5; TG #2)	X	X	X	X	X
	Meet with Dr. Moeller to develop and discuss the design, progress, and results of the neuroimaging study (1 hour/month Y3-Y5; TG #1-2)			X	X	X
	[*Meet with Dr. Parrott to develop the design and implementation of alcohol administration (1 hour/month Y2-Y3; TG #1-2)*]		[*X*]	[*X*]		
	Lab rotation with Dr. Goldman at NIAAA's Laboratory of Neurogenetics to learn advanced molecular genetics and alcohol misuse investigation techniques (5 days in Y1; TG #1-2)	X				
	Lab rotation with Dr. Parrott at the University of Georgia to learn advanced alcohol administration and aggression laboratory techniques (3 days in Y2; TG #1-2)		X			
	[VCU *Lab visit by Dr. Parrott to evaluate the implementation of alcohol administration protocol (3 days in Y3; TG #1-2)*]			[*X*]		
Conferences	Present results at annual meetings of Behavior Genetics Association annual meeting and Research Society on Alcoholism (Y2-Y5)		X	X	X	X
Courses	HGEN 502: Advanced Human Genetics - 3 hours/week in Y1. A comprehensive study of specific areas in human genetics.	X				
	HGEN 603: Mathematical and Statistical Genetics - 3 hours/week in Y1. Provides an introduction to the rudiments of theoretical and applied mathematical population genetics.	X				
	HGEN 617: Genetic Analysis of Complex Traits - 3 hours/week in Y2. Introduces the theory/practice of analysis of complex human traits.		X			
	HGEN 619: Quantitative Genetics - 3 hours/week in Y2. The effects of genes and environment on complex human traits.		X			
	[*IPAS 600: The Biological Basis of Addiction - 3 hours/week in Y3.*]			[*X*]		
	[*RHAB 644: Alcohol and Human Behavior - 3 hours/week in Y3.*]			[*X*]		
Grant Writing	Prepare (Y4-Y5) [*and submit (Y5)*] an R01 application.				X	X
Responsible Conduct of Research	Biennial human subjects research re-certification through the Collaborative Institutional Training Initiative (CITI) (Y1, Y3, Y5)	X		X		X
	VCU- and department-wide ethics workshops and discussions with mentors and student scholars (Y1-Y5)	X	X	X	X	X
	CCTR 520: Fundamentals of Research Regulation - 2 hours/week in Y1. The regulations that govern translational and clinical research.	X				
	OVPR 601: Scientific Integrity - 1 hour/week in Y1. A survey of contemporary issues relating to responsible conduct in research.	X				
	OVPR 602: Responsible Scientific Conduct - 1 hour/week in Y2. A survey of issues relating to responsible conduct in research.		X			
	OVPR 603: Responsible Conduct of Research - 1 hour/week in Y2. This course will develop and refine skills needed to solve problems involving relevant topic areas of responsible scientific conduct.		X			
Workshops	Attend University of Washington Summer Institute in Statistical Genetics (3 weeks in Y1)	X				

SPECIFIC AIMS

Aggressive behavior in the context of alcohol misuse is a substantial public health concern. Genetic predispositions have been identified for both aggression and alcohol misuse. However, the biological factors that predispose individuals towards alcohol-influenced aggression remain poorly understood. Specifically, the extent to which alcohol-influenced aggression is genetically-influenced is unknown. Further, it remains to be seen whether alcohol-influenced aggression is uniquely heritable, or is simply the by-product of genetic risk factors for both alcohol misuse and aggression. If alcohol-influenced aggression is found to be substantially genetically-influenced, the specific genetic and neural pathways through which these effects are transmitted must then be investigated. The goal of the research activities in this K01 proposal is to fill these gaps in our knowledge.

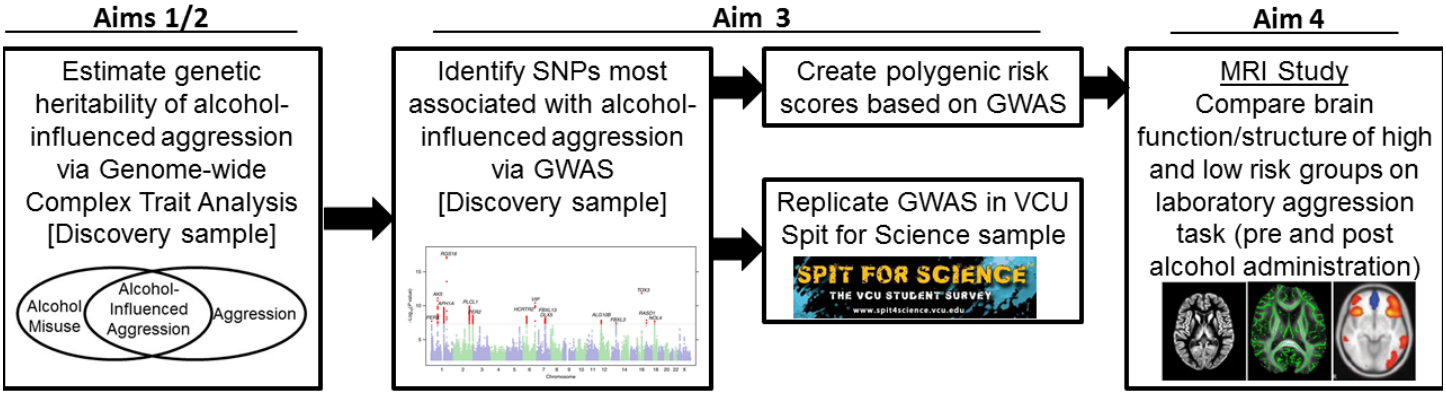
The overall objective of the research activities in this K01 award proposal is to examine the genetic factors and neural mechanisms that promote alcohol-influenced aggression. The proposed research activities will address the following research aims and test their associated hypotheses:

Aim 1: Examine the extent to which genetic factors influence the extent to which individuals act aggressively in the context of alcohol misuse. *Hypothesis 1:* Genome-wide complex trait analysis (GCTA) will reveal that alcohol-influenced aggression exhibits substantial genetic heritability.

Aim 2: Examine whether genetic factors for alcohol-influenced aggression exist above-and-beyond genetic risk factors for general aggressiveness and alcohol misuse. *Hypothesis 2:* After statistically controlling for genome-wide associations with non-alcohol-influenced aggression and alcohol misuse, significant genetic heritability will still be observed for alcohol-influenced aggression via GCTA.

Aim 3: Identify the specific genetic loci and polymorphisms that are most strongly linked to alcohol-influenced aggression and create polygenic risk scores from these findings. *Hypothesis 3:* Genome-wide association study (GWAS) techniques will identify specific gene polymorphisms associated with alcohol-influenced aggression.

Aim 4: Identify functional and structural brain mechanisms that transmit the genetic effects on alcohol-influenced aggression. *Hypothesis 4:* Polygenic risk scores for alcohol-influenced aggression will be associated with greater laboratory aggression, through changes in brain structure and function.



This proposal capitalizes on the resources available through [*NIH-funded publicly-available datasets from the database of Genotypes and Phenotypes (dbGaP)*] and VCU's Spit for Science (S4S) project, on which my primary mentor, Dr. Danielle Dick is a Principal Investigator. Drs. Goldman and Aliev (Co-Mentors) also play substantial roles in many of these projects. I would begin by analyzing existing data from [*the dbGaP discovery sample*], then seeking to replicate these findings with ongoing data collection for Spit for Science at my current institution, VCU. Finally, I would conduct a new MRI brain imaging study, using S4S participants (recruited based on their underlying genetic risk profiles) analyses in order to leverage my existing skillset in neuroimaging and laboratory aggression measurement and further accomplish the proposed research aims.

Accomplishing these aims would advance my long-term goal to become an independent alcohol investigator who integrates across multiple disciplines to study the factors that influence alcohol-influenced aggression. These activities would set the stage for R01-level applications to investigate the environmental factors that influence these effects, and biologically-informed interventions that might ameliorate them.

RESEARCH STRATEGY

A. Significance

A1. Aggressive behavior is a substantial public health concern in the United States, with approximately 1.2 million violent crimes occurring in 2015[1]. Such violent crimes (e.g., aggravated assault, murder, rape) resulted in over 1.5 million non-fatal injuries and over 17,000 deaths in 2015[2]. Previous estimates of these aggressive acts' financial toll was approximately $177 billion[3]. Violent crime is occurring with greater frequency in recent years, rendering the investigation and treatment of aggression an urgent issue[1]. Alcohol is a significant factor in at least 40% of criminally violent acts[4]. These frequency rates hold across juvenile and adult populations[5] and soar to approximately 66% in the context of intimate partner violence[5]. One in three college undergraduates experienced alcohol-influenced aggression and sexual assault rates on college campuses are drastically magnified by alcohol misuse[5,70]. These statistics lead to the inevitable conclusion that alcohol-influenced aggression is a significant threat to public health and necessitates further research into its underlying bases.

A2. Alcohol is not merely correlated with greater aggression, but numerous laboratory experiments have shown that alcohol consumption *causes* increased aggression[6-8]. Further, there exists a dose-response curve, in which greater doses of alcohol cause greater corresponding levels of aggressive behavior[9]. Alcohol's ability to increase aggression is likely due to its ability to reduce inhibitory psychological processes and increase negative emotions such as anger and frustration[10].

A3. Despite alcohol's profound effect on aggression, there is substantial variability among individuals in the extent of the alcohol-aggression link[11]. Indeed, not all alcohol misuse results in aggressive acts, yet the reasons for the underlying variability in this phenomenon remain incompletely understood. Given that variability in both aggression and alcohol misuse is substantially influenced by genetic components[12-13], it is likely that such heritable factors substantially contribute to individual differences in alcohol-influenced aggression.

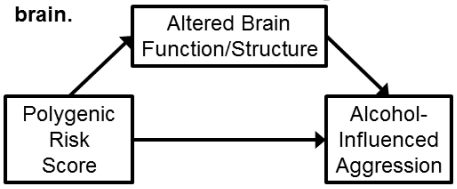
A4. To date, there has been little-to-no investigation of the genetic basis of alcohol-influenced aggression. A substantial body of research has developed around the genetic basis of alcohol misuse and aggression, treating these outcomes separately. This has produced a critical gap in the understanding of aggression, alcohol consumption, and their interactive effects.

A5. Behavioral genetics should factor in the contextual nature of human behaviors. Attempts to identify the genetic basis of alcohol misuse and aggressive phenotypes are aided by modeling the complexity inherent in them[14-15]. For instance, one study found that a genetic risk score for aggression's effect on intimate partner violence was moderated by a laboratory alcohol adminstration[16]. Ignoring these contextual, environmental factors may underlie some of the irreproducibility of genetic associations with aggressive phenotypes. This project will model aggressive phenotypes alongside the contextual element of alcohol misuse, and use polygenic risk scores to predict such alcohol-induced aggression.

A6. Another beneficial development for genetic approaches to alcohol-influenced behaviors is the incorporation of neuroscientific approaches to test mechanistic hypotheses. Genetic effects on complex behaviors are likely mediated by changes in brain structure and function[17] (see Figure 1 for conceptual model).

The added granularity provided by these neural mechanisms is critical for understanding how genetic influences impact outcome, and potentially for developing effective treatments for alcohol misuse. The proposed approach, which brings together polygenic, contextual, and neural mechanistic effects, is in line with the NIAAA Strategic Plan's goal to identify genomic and non-genomic mechanisms of alcohol effects.

Figure 1. Conceptual model through which genetic influences on alcohol-influenced aggression are mediated by functional and structural changes to the brain.



B. Innovation

The proposed research details a novel approach to understanding the biological basis of alcohol-influenced aggression. This project will advance the field in several ways. First, the genetic basis of alcohol misuse and aggression have been frequently investigated in separation, but rarely together. The proposed research will serve as the first systematic investigation of the genetic basis of alcohol-influenced aggression. Second, the proposed research will use existing data from the NIAAA-funded Collaborative Studies on Genetics of Alcoholism (COGA) project[18] [*and four other publicly-available genomic datasets*] to develop novel polygenic risk scores for alcohol-influenced aggression. These risk scores would be a resource to the field and could be used by other researchers to examine this under-investigated behavioral phenotype. The proposed research will use these polygenic risk scores to identify at-risk individuals from another NIAAA-funded longitudinal project, Spit for Science[19]. I will collect new data from these individuals using neuroimaging techniques and laboratory alcohol administration and aggression tasks. Building off of these ongoing projects is an efficient use of resources

given the immense expense of longitudinal genotyping projects. Further, employing a sample of alcohol-dependent probands and a normative undergraduate sample will enhance the generalizability of the findings obtained in this research.

In addition to these innovative aspects, the research and training activities associated with this proposal will provide novel training for in genetic, genomic, and statistical methods, and conceptual knowledge of alcohol misuse phenotypes. I will combine this new skillset with my existing expertise in neuroimaging and aggression to begin a career of investigating alcohol misuse and its ability to magnify violence.

C. Approach

The proposed research seeks to identify whether alcohol-influenced aggression has a substantially heritable genetic component (Aim I), that this heritability exists above-and-beyond genetic risk factors for general aggressiveness and alcohol misuse (Aim II), that specific genetic polymorphisms will be associated with alcohol-influenced aggression and these can be translated into polygenic risk scores (Aim III), and that these risk scores will exert their effects on alcohol-influenced aggression through specific alterations in brain function and structure (Aim IV). I will pursue these aims using [*a discovery sample comprised of five NIH-funded genomic datasets that are publicly available through the database of Genotypes and Phenotypes (dbGaP) and a replication sample from*] the NIAAA-funded VCU Spit for Science Student Survey.

Discovery Sample from dbGaP

The GWAS discovery sample (N = 31,123) will be drawn from five ongoing and completed, multisite studies on the genetic basis of substance dependence and related behavior, available via dbGaP (Table 1).

Table 1. List of dbGaP projects used in discovery sample (N = 31,123).

Project	*Funding Source*	*Sample Size*	*Participants*	*dbGaP ID*
Yale-Penn Alcohol Dependence GWAS (ADGWAS)[73]	*NIAAA/NIDA*	*2,909*	*Alcohol-dependent participants + controls*	*phs000425.v1.p1*
Collaborative Studies on Genetics of Alcoholism (COGA)[20]	*NIAAA*	*17,783*	*Alcohol-dependent probands + genetic relatives, + controls*	*phs000092.v1.p1*
Collaborative Genetic Study of Nicotine Dependence (COGEND)[74]	*NCI*	*2,900*	*Nicotine-dependent probands + genetic siblings, + controls*	*phs000092.v1.p1*
Family Study of Cocaine Dependence (FSCD)[75]	*NIDA*	*1,044*	*Cocaine-dependent participants + controls*	*phs000092.v1.p1*
Australian GWAS of Heroin Dependence (GWASHD)[76]	*NIDA*	*6,487*	*Heroin-dependent participants + controls*	*phs000277.v1.p1*

Participants. Across the discovery sample, participants exhibit substantial demographic variance (Table 2). The COGA sample, which is roughly generalizable to the other samples, exhibited substantial socioeconomic diversity (54.6% completed high school, median income: $35,000, income range: $1,000-150,000).

Table 2. Demographics of dbGaP projects used in discovery sample.

Project	*Sex*	*Age Range*	*Age M(SD)*	*Ethnicity/Race*
ADGWAS	*46.9% F, 53.1% M*	*18-79*	*37.4 (11.8)*	*35.1% ED, 64.9% EM*
COGA	*53.7% F, 46.3% M*	*18-97*	*39.83 (14.6)*	*65.8% ED, 26.2% EM*
COGEND	*61.8% F, 38.2% M*	*25-47*	*36.5 (5.5)*	*55.4% ED, 44.6% EM*
FSCD	*50.3% F, 49.7% M*	*18-60*	*37.0 (8.8)*	*48.3% ED, 51.7% EM*
GWASHD	*47.0% F, 53.0% M*	*18-58*	*35.6 (9.2)*	*100% ED*

ED=European-Descent, EM=Ethnic-Minority

Genotyping and imputation. Participants provided blood samples to biologic repositories, on which whole-genome genotyping was performed. Several genome-wide Illumina arrays were used, including the Human OmniExpress 12.VI, the Omni2.5 BeadChip, and the Human610-Quad BeadChip.] Genotypic data were checked for minor allele frequency (SNPs excluded with frequencies < 3%), pedigree structure/Mendelian inconsistencies, with samples imputed to 1000 Genomes[21] using SHAPEIT[22] and then IMPUTE2[23].

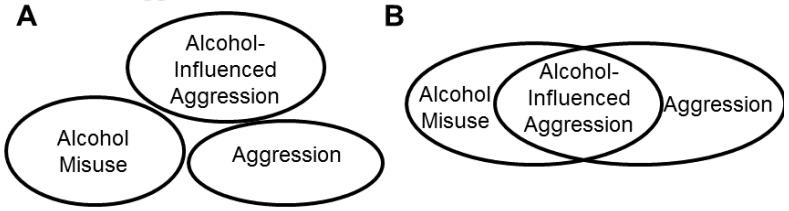
Phenotype measures. Participants completed the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA)[24] [*or variants of this instrument (e.g., Semi Structured Assessment for Drug Dependence and Alcoholism) that included our specific questions-of-interest*]. The SSAGA includes reports of whether participants have previously performed physically-aggressive acts, and whether the acts were performed while participants had consumed alcohol or not (e.g., “have you ever physically injured anyone on purpose?”). Greater alcohol-influenced aggression is indicated by a greater number of *yes (with alcohol)* responses to such questions and non-alcohol-influenced aggression is indicated by a greater number of *yes (no alcohol)* responses. The SSAGA also assesses the extent to which participants have previously engaged in specific aspects of alcohol misuse (e.g., binge drinking)[25].

Analytic Plan

Genome-wide complex trait analyses (GCTA). The initial analysis of the [*discovery*] dataset will seek to decompose phenotypic variance into the proportions that can be explained by genetic (h^2) and

environmental/measurement-error factors[26]. GCTA will quantify the heritability of aggressive, alcohol-misusing, and alcohol-aggressive phenotypes. GCTA will then be applied to determine the extent whether alcohol-influenced aggression has distinct heritability from general aggression and alcohol misuse (Figure 2A) or shares substantial genetic overlap with these two related phenotypes (Figure 2B).

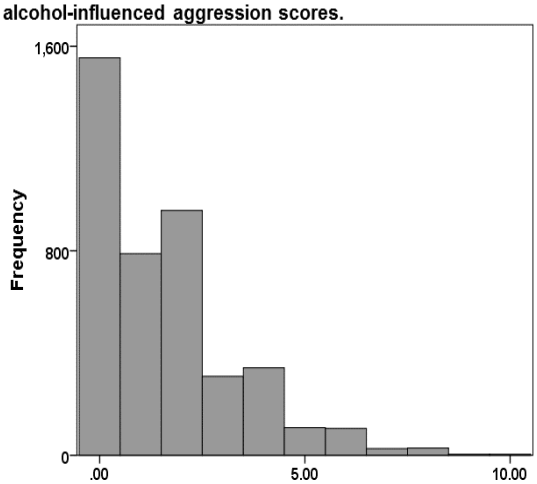
Figure 2. Competing conceptual models of the heritability of alcohol-influenced aggression.



These analyses will be conducted with the GCTA software toolkit v1.26[27], using a restricted maximum likelihood method to compute a bivariate genetic relationship matrix by correlating the genetic similarity between each participant. Then, these genetic similarity estimates are modeled as random effects predictors of each phenotype: alcohol-influenced aggression, non-alcohol-influenced aggression, and alcohol misuse. Fixed-effects covariates will include age, ethnicity/race, sex, and socioeconomic status. To prevent heritability inflation, any two participants who share a second-cousin degree of relatedness or more will be excluded from subsequent analyses. A heritability (h^2) estimate will be calculated for alcohol-influenced aggression, *controlling* for the heritability of non-alcohol-influenced aggression and alcohol misuse phenotypes. A substantial heritability estimate ($h^2 > 0.1$, $p < .05$) for alcohol-influenced aggression will serve as support for the unique genetic basis for this phenotype.

Genome-wide association study (GWAS). After the GCTA, GWAS will be used to identify single nucleotide polymorphisms (SNPs) and clusters of SNPs that are significantly associated with alcohol-influenced aggression[28-29]. Due to the skewness of the alcohol-influenced aggression scores (see Figure 3), quasi-Poisson regression analyses will be used to accommodate such a distribution using the Bioconductor v2.1 package[30] and SNPTEST v2.5[31]. Specifically, alcohol-influenced aggression scores will be regressed onto principal components of the available SNPs using an additive model, excluding non-autosomal SNPs, with demographics factors as covariates. Due to racial stratification, separate GWAS will be performed for participants of African, American, East Asian, European, South Asian, and Mixed Race descent[71]. Participants will be empirically assigned to these ancestral super-populations using the 1KGP population base and the Mahalanobis' distance between each population[71]. I will meta-analyze these separate sets of GWAS findings METAL v3.0[32], which will produce meta-analytic '% variance explained' effect size estimates and *p* values for each SNP. [*In addition to this meta-analytic approach, I will test for gene-by-race/ethnicity moderation using a kernel-machine approach to estimate whether each SNP's association with alcohol-influenced aggression varies across the discovery sample's diverse racial/ethnic groups, using the Robust Joint Tests package*[77].] After correction for genetic inflation and weighting based on sample size, I will calculate polygenic risk scores for alcohol-influenced aggression [based on *SNPs meeting increasingly stringent significance thresholds: .5, .4, .3, .2, .1, .05, .01, .001, .0001. The threshold that maximizes the variance explained (R^2) in alcohol-related aggression will be used for risk score calculation*[33].] Using an additive approach, I will sum across the number of alleles that are significantly linked to the phenotype, weighted by their respective effect size estimates from the GWAS. For example, if the A allele (versus the corresponding G allele) of a given SNP is significantly associated with greater alcohol-influenced aggression, then each copy of the A allele will be initially coded as +1 (GG = 0, GA = 1, AA = 2). These scores will then be multiplied by the GWAS effect size estimate for that given SNP. This polygenic risk scores will capture aggregate genetic risk for alcohol-influenced aggression in a manner that represents the current state of the field[34].

Figure 3. Frequencies of a subset of COGA participants' alcohol-influenced aggression scores.



Feasibility

Preliminary phenotypic analyses on the alcohol-influenced aggression scores from the COGA dataset, assessed among a subset of 9,273 participants, revealed a substantially-variable, albeit positively-skewed, distribution of alcohol-influenced aggression (Figure 3). Scores were distributed across the possible 0-10 range of the score, median = 1.00, *M* = 1.57, *SD* = 1.73. These initial estimates of phenotypic frequencies suggest that there will be sufficient proportions of participants to adequately power the analyses in this proposal.

Power analysis. Power calculations for the GCTA heritability analysis[35], which assumed an underlying

heritability of h^2 = 0.2, a type I error rate of 0.05, and a variance of SNP-derived genetic relationships of .00002, suggested that 80% power for the proposed GCTA can be obtained with 4,430 participants. The [*discovery*] sample far exceeds this sample size at over [*31,000*] participants. GWAS power analyses, implemented via the GWAS.PC v1.0 package for R statistical software[35] were also performed to determine the minimum effect size (in units of % variance explained) that [*the discovery sample could detect with 80% power. Calculations were based on the planned detection of multiple, additive SNP markers, an alpha level of .05, and a minor allele frequency of 0.05, revealing that the proposed GWAS is able to detect effect sizes of 0.09% variance-explained and larger. However, this is likely an optimistic assessment as this power analysis did not account for the moderating effects of ethnicity/race and the genetic relatedness of participants (yet recent research has indicated that GWAS power is not significantly affected by underlying genetic relationships between participants[72]).*]

Potential Problems and Alternative Strategies

The proposed GWAS on the [*discovery*] sample may not yield any statistically-significant SNPs that are associated with alcohol-influenced aggression. If this is the case, polygenic risk scores will be obtained from the largest GWAS projects for both aggression (the EAGLE consortium[37]) and alcohol misuse (Psychiatric Genomics Consortium[38]). An alcohol-influenced aggression risk score will be computed by multiplying the aggression and alcohol misuse polygenic risk scores with one another, to statistically simulate the interactive effect of these genetic dispositions. There is a current push in genomics research for sample sizes that are unattainable by any individual investigator (e.g., N > 50,000)[15]. The proposed research outlined in the [*discovery and replication*] datasets are admittedly underpowered to detect a complex phenotype such as alcohol-influenced aggression. However, the proposed research activities will provide invaluable training that will be employed in well-powered datasets throughout my career as an alcohol investigator.

Sample II: Spit for Science - The VCU Student Survey

The proposed research will attempt to replicate the findings from the [*discovery*] sample in a new sample of undergraduate students enrolled in VCU's Spit for Science (S4S) project[19] (Phase I). Replicating the polygenic factors obtained in a discovery dataset, in an independent sample, is a critical step in building cumulative scientific knowledge[40]. Among a subset of participants in the Spit for Science sample, brain imaging will be used to identify the neural mechanisms of polygenic risk for alcohol-influenced aggression (Phase II).

Phase I - Replication in S4S Sample

Participants. At the outset of each academic year, incoming first-year VCU undergraduates are invited to participate in the S4S project via an online survey, and they have the option to provide a saliva sample that will be genotyped. Compensation is $10. From 2011-2014, S4S has enrolled six consecutive cohorts (68% average enrollment from incoming freshman, average cohort N = ~2,500[19]). Of those individuals who completed the initial online component, 97% provided a saliva sample for genotyping. Participants in the sample reflect general demographics among VCU students, which are relatively diverse (40% male, 60% female; 51% White, 19% African American, 15% Asian, 6% Hispanic/Latino, and 9% other/multiracial/unknown). Increasing freshman class sizes have magnified the S4S cohort sizes in recent years to approximately 3,300 participants per cohort.

Genotyping and imputation. Genotyping is performed at Rutgers University Cell and DNA Repository (RUCDR) using the Affymetrix BioBank array. The 653,000 gene variants included in this array contain 296,000 common GWAS framework variants for imputation as well as 357,000 functional variants. Quality control uses AffyPipe analysis pipeline[39] to exclude Off Target Variants, SNPs missing > 5% of genotypes, samples missing > 2% of genotypes, and SNPs missing > 2% of genotypes after sample filtering. Samples will be imputed to 1000 Genomes[21] using SHAPEIT[22] and then IMPUTE2[23].

Phenotype measures. The same SSAGA[24] questions from the [*discovery samples*] about alcohol-influenced aggression will be added to the existing online survey administered to all new S4S participants at the onset of their participation. The S4S online survey already includes demographic assessments. These surveys will be administered in the fall of 2018, 2019, and 2020.

Analytic Plan

[*S4S data will undergo the same GWAS and risk score procedures as employed in the discovery dataset.*]

Feasibility

This first phase of Aim II builds upon the already successful and ongoing S4S project, by simply adding a short online survey to the existing S4S battery of questionnaires. However, undergraduates at VCU differ from the [*diverse members of the discovery sample*] in ways that may suppress the amount of alcohol-influenced aggression among the S4S sample (e.g., lower levels of alcohol misuse and dependence). A pilot sample of VCU undergraduates were asked about their aggressive behavior and whether it occurred in the context of

alcohol misuse or not. Of the 166 female and male participants surveyed, 53.6% indicated that they had participated in a physical fight in the past 5 years (30.1% had been in more than 1 fight). Of those who had previously fought, 38.2% participated in this fight under the influence of alcohol. These initial estimates suggest that 1 out of 5 VCU undergraduates (i.e., 20.48%) have perpetrated a substantial act of physical aggression under the context of alcohol misuse. This prevalence rate indicates that the much larger pool of potential S4S participants is likely to exhibit alcohol-influenced aggression at a sufficient rate to test associations with polygenic risk scores.

Power analysis. The expected S4S sample size of 10,000 students will provide 80% power to detect a genome-wide effect of 0.35% variance explained in alcohol-influenced aggression, or larger. [*The S4S GWAS is likely to be under-powered, yet performing this replication GWAS will serve as a valuable training in how to collect genomic data and use it to replicate previous GWAS results.]*

Potential Problems and Alternative Strategies. The S4S sample GWAS may not replicate the profile of SNPs found in the [*discovery*] sample, as the S4S sample is smaller and is comprised of undergraduate students. However, replication is a crucial step in genomic science and this is as important training experience.

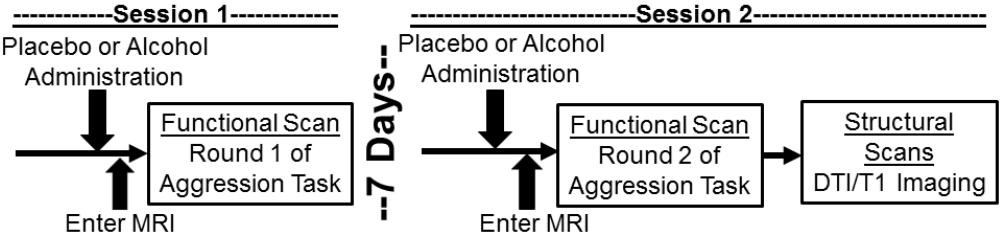
Phase II - Investigating Neural Mechanisms in an S4S Sub-Sample

Participants. From the 2018-2020 cohorts of the S4S sample, I will recruit [*202*] individuals with low (*N* = [*101*]) and high (*N* = [*101*]) polygenic risk scores for alcohol-influenced aggression for a brain imaging experiment. Participants [*will be equal numbers of males and females and*] are expected to reflect the ethnic/racial makeup of the larger S4S sample which is relatively diverse (40% male, 60% female; 51% White, 19% African American, 15% Asian, 6% Hispanic/Latino, and 9% other/multiracial/unknown). Ages will range from 21-35 to ensure that only adults complete the study, that they can legally consume alcohol, and that aging effects do not influence our MRI data.

Recruitment and Screening. I will recruit VCU students who enrolled in the 2018-2020 cohorts of the S4S study, using their existing contact information in the S4S participant database. Participants will be recruited based on their polygenic risk scores that were obtained from the [*discovery*] GWAS. The S4S GWAS will not be used to establish the polygenic risk scores used to recruit these participants as they draw from the same pool of individuals. Establishing polygenic effects on behavior requires replication in an independent sample, as the use of dependent samples artificially inflates association statistics[41]. Recruitment based on polygenic risk scores will proceed by contacting individuals from the lowest end of the distribution of polygenic risk scores (low risk group) and the highest end of the distribution of risk scores (high risk group), working from both ends of the distribution towards the median. Individuals who are contacted based on their risk scores will be screened with an online survey various conditions that would make the alcohol administration and MRI scanning procedures unsafe or invalid (see Protection of Human Subjects for a detailed list) and will be contacted to schedule an appointment if they pass these criteria[42,43].

MRI [*Sessions*]. *[The MRI study will take the form of a two-session experiment, in which alcohol or placebo beverages are administered to each participant in a counterbalanced order. In Session 1,*] participants will arrive at VCU's Collaborative Advanced Research Imaging (CARI) facility where they will repeat the screening procedures and then will practice the computer tasks that they will perform in the scanner. Then, participants will be told that they will receive [*either]* a placebo [*or*] an alcoholic beverage, but that they will not be informed of the nature of each beverage until the end of the study (see Figure 4 for the MRI scan timeline). [*By random assignment,*] participants will then consume [*either a placebo beverage of orange juice with 4ml ethyl alcohol added to the rim or an alcoholic beverage consisting of a 1:5 ratio of 95% ethyl alcohol and orange juice[43]. The alcohol dose will be proportional to participants' body weight and sex, due to sex differences in body fat composition and alcohol metabolism (females: 0.9 g/kg ethyl alcohol; males: 1.0 g/kg ethyl alcohol)[43,44]. This alcohol dose typically increases participants' breath-alcohol-concentration to .08%, the legal driving limit in Virginia[43,44].*] After consuming the beverage, participants will then be placed in the MRI scanner and complete a validated laboratory aggression paradigm that allows individuals to "hurt" a fictitious opponent with an aversive noise blast, while undergoing echo-planar functional MRI (EPI). In this task, participants compete against an opponent to press a button faster, and the loser of the competition then hears an aversive noise blast. Aggression is operationalized as the

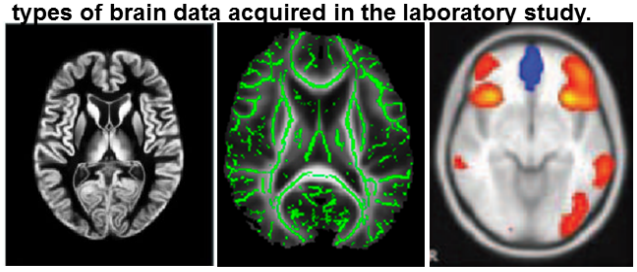
Figure 4. Timeline of both MRI sessions.



volume of the noise blast (65-105 decibels) that participants select for a fictional opponent who repeatedly provokes them by selecting extremely loud noise blasts. After completing these scans, [*participants will leave and return to the CARI center 7 days later for Session 2. Participants will consume either a placebo (if they consumed an alcoholic beverage in Session 1) or an alcoholic beverage (if they consumed an alcoholic beverage in Session 1), and then will be returned to the scanner to complete the aggression paradigm a second time against a new provocative opponent.]* Participants will be told that both of their opponents are same-sex VCU undergraduates, when in fact, they are computer programs.

After completing the aggression task, participants will undergo diffusion tensor imaging (DTI), which quantifies the integrity of white matter pathways[45], and a high-resolution T1 scan, which quantifies gray matter density[46] (see Figure 5 for a depiction of each brain imaging modality). Participants will be removed from the MRI scanner and allowed to relax in a nearby testing room until their BAC is between .02-.04%. [*At the end of Session 2, an experimenter will briefly interview each participant to assess their suspicion of our deception during the study, debriefed as to the true purposes of the study, provided with counseling resources in case of distress, and then paid $100 for their participation. Participants who guess that they had no actual partners or that the goal of the study was actually to measure aggression will be excluded from all data analyses*]

Figure 5. Prototypic examples (top-down-view) of the types of brain data acquired in the laboratory study.

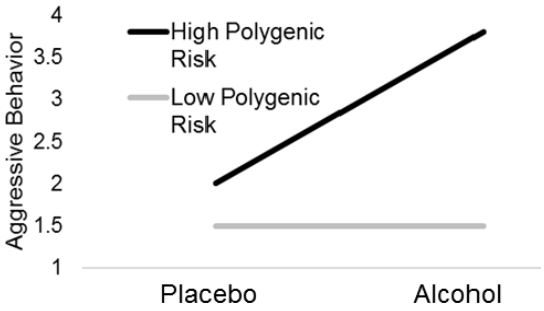


T1 Gray Matter | **DTI White Matter** | **EPI Functional**

Analytic Plan

A [*mixed*]-effects general linear model (GLM) will test the test the prediction that the high polygenic risk group will select louder noise blasts after alcohol administration, and that this effect would be absent from those with low polygenic risk (Figure 6). All MRI analyses will be conducted with FSL v.5.0)[47-54]. Using FSL, GLMs will compare the two polygenic risk groups' estimates of white matter integrity (i.e., fractional anisotropy values)[51,52], gray matter density[53], and functional blood-oxygen-level-dependent (BOLD) signal[47-49] across [*two specific brain networks: the dopaminergic reward circuit, the prefrontal control circuit (Figure 7). Aggression is predicted by exaggerated activity in the brain's reward network and by blunted activity in the lateral prefrontal cortex's regulatory regions[42], and alcohol operates in a similar manner on both of these networks[78], rendering them likely mediators of polygenic risk.*]

Figure 6. Anticipated results comparing polygenic risk groups on their aggressive behavior, after alcohol or placebo.



BOLD estimates will be obtained from the aggression trials of the task. Multiple comparisons corrections will be conducted using nonparametric threshold-free cluster enhancement[54]. Mediation models will then be fit in which the effect of polygenic risk score group on aggression (after the alcohol administration) will be mediated by functional and structural brain estimates from brain regions that were significantly different between the two polygenic risk groups (Figure 8). The PROCESS macro for SPSS will be used to conduct nonparametric bootstrapping (using 5,000 bootstrapped resamples) to test the significance of each neural mechanism's indirect effect[55].

Figure 8. Mediation models that will be fit to the S4S sample, with brain data articulated as mediators.

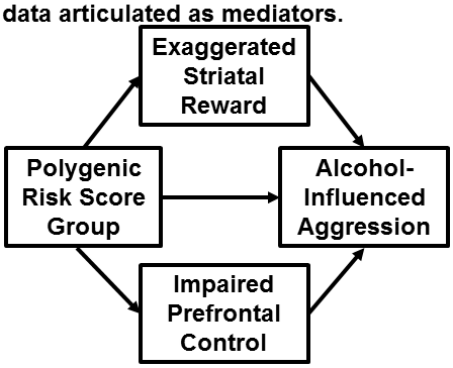
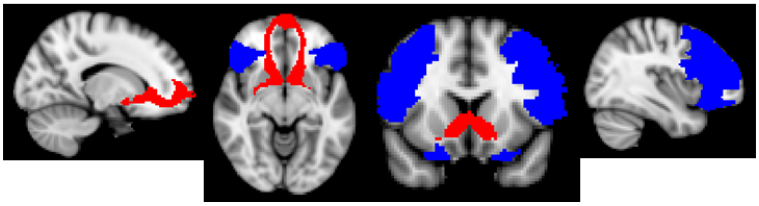


Figure 7. Brain regions expected to be modulated by polygenic risk for alcohol-related aggression. Red = reward circuit, blue = prefrontal control circuit.



Feasibility

I have performed numerous functional and structural MRI experiments using these laboratory aggression procedures and statistical techniques[56-65]. [*My lab has also completed a complex MRI study using this aggression paradigm, which recruited 112 participants over 1 year, suggesting that*

the large MRI sample size in this proposal can be readily obtained at VCU.] Prior research using this laboratory aggression paradigm has shown that aggression on this task is reliably increased by alcohol administration[66] and can be safely and effectively administered in the MRI scanner[80]. Therefore, this task is well-adapted for the MRI environment, and for alcohol administration.

Power analysis. FMRIPOWER v1.0[67] [*and powerMediation[79]*] packages were used to analyze pilot fMRI data from previous research on differences between groups (that are at-risk for aggression) in aggression-related brain activity [42]. Using the ventral striatum (a core node of the brain's reward circuit[68]) as the region-of-interest, [*mediation modeling will have at least 80% power to detect a small indirect effect (0.09%) between risk groups with 198 participants, 99 per group[69]. Based on previous work with this aggression paradigm, 4 of these participants will likely be suspicious of our deception. As such, a sample size of 202 participants was selected (101 per group).*]

Potential Problems and Alternative Strategies

Participants in the low and high polygenic risk groups will likely differ on other variables (e.g., physical health) that might serve as problematic confounds. To address this, participants from each group will be closely-matched on age, sex, race, and socioeconomic status. The groups will be equated in physical and mental health as the screening procedures exclude any individuals with diagnosed physical or mental pathologies. There are also risks posed by the MRI environment, alcohol administration, and aggression task procedures. I have extensive MRI experience, participants undergo extensive screening procedures, and participants are under constant supervision by CARI center staff who are certified in safe MRI and alcohol administration procedures. CARI center staff are prepared to effectively handle any potential medical emergencies. If a brain abnormality is observed, a licensed radiologist is available through the CARI facility for consultation. The success of this study hinges on effective participant recruitment. Multiple offshoot studies using S4S data have been successful (e.g., an F31-funded fMRI study in Dr. Dick's lab that recruits S4S participants for based on polygenic risk scores for externalizing behaviors). Further, the recruitment pool is large enough to provide many potential participants who meet this study's inclusionary criteria.

Benchmarks for Success

Table 3 summarizes the timeline of the major tasks for both Aim I and Aim II projects. These milestones provide benchmarks for success. [*All manuscript submissions will target flagship journals of behavioral genetics and alcohol misuse (e.g., Addiction Biology, Nature Genetics).*]

Table 3. Research activities by award year (Y).

Activity	Y1	Y2	Y3	Y4	Y5
[*Discovery*] data preparation	X				
[*Discovery*] data analysis	X	X			
[*Discovery manuscript submission*]		[*X*]			
S4S genotyping data collection	X	X			
S4S genotyping data analysis			X		
[S4S genotyping manuscript submission]			[*X*]		
S4S neuroimaging data collection			[*X*]	X	
S4S neuroimaging data analysis					X
[S4S neuroimaging manuscript submission]					*[X]*
R01 application preparation				X	X
[*R01 application submission*]					[*X*]

Summary and Future Directions

The proposed research integrates across disciplines, methodological and statistical approaches to investigate a costly and complex phenomenon: alcohol-influenced aggression. The studies will estimate the extent to which alcohol-influenced aggression is genetically heritable, and whether this heritability is distinct from general forms of aggressiveness and alcohol misuse. These studies will also identify specific genome-wide polymorphisms that are linked to alcohol-influenced aggression and will seek to replicate these findings in an independent sample. Further, I will build upon these discoveries by combining neuroimaging with a laboratory alcohol administration to identify the neural pathways through which these genetic risk factors alter brain structure and functional responses to aggression after alcohol administration. These research and training activities are a logical extension from my current skillset and will enable him to pursue a career as an independent alcohol misuse researcher. I will use the skillset gained from the proposed projects to apply for an R01 to conduct a larger investigation of the environmental factors (e.g., childhood trauma) that interact with the genetic risk factors for alcohol-influenced aggression. The ultimate goal of such investigation is to construct and validate effective interventions that reduce violent behavior in the context of alcohol misuse. The novel training and research experiences that I will gain under this K01 award will provide the foundation for these future career steps.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Across the proposed award timeline, I will receive substantial training in the responsible and ethical conduct of research on human participants. Virginia Commonwealth University's (VCU) Office of Research and Innovation offers regular education and training activities in responsible conduct of research. In year 1, I will enroll in two VCU courses: CCTR 520: Fundamentals of Research Regulation (2 lecture hours, 2 credits) and OVPR 601: Scientific Integrity (1 lecture hour, 1 credit). In year 2, I will enroll in two VCU courses: OVPR 602: Responsible Scientific Conduct (1 lecture hour, 1 credit) and OVPR 603: Responsible Conduct of Research (1 lecture hour, 1 credit). In addition, regular workshops are offered by various VCU departments, centers, and institutes in areas such as the informed consent process, adverse and reportable events in research, vulnerable populations, bio-safety, data management, and conflicts of interest. I will attend these regular workshops and will also gain informal research ethics training in interactions with my primary and co-mentors. Further, my weekly laboratory meetings with doctoral students and undergraduate research assistants will regularly involve discussions of the responsible conduct of research. As a VCU researcher, I have completed the Basic, Social/Behavioral, and Biomedical courses in responsible conduct of research from the Collaborative Institution Training Initiative (CITI). I will keep these certifications up-to-date via biennial online courses.