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Drinking Motives Underlying Internalizing and Externalizing Pathways to Alcohol Misuse in College Students

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Drinking Motives Underlying Internalizing and Externalizing Pathways to Alcohol Misuse in College Students

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

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Table of Contents

List of Tables v

List of Figures viii

List of Abbreviations xi

Abstract xiv

Chapter 1. A Global Introduction to the Construct of Alcohol Misuse 1

I. Alcohol misuse and college students 4

II. Etiology of alcohol misuse 6

III. Heterogeneity in alcohol misuse 9

IV. Intermediate mechanisms in internalizing and externalizing pathways 12

V. Drinking motives as intermediate phenotypes 14

VI. Aims of this dissertation project 18

Chapter 2. Sample and Methods: The Spit for Science Study 21

I. Study Design 21

II. Participants 23

III. Measures 23

IV. Genotyping 29

Chapter 3. Internalizing And Externalizing Typologies Of Alcohol Misuse In College Students 32

I. Specific Aim 32

II. Methods 34

III. Results 36

IV. Summary and Discussion 49
Chapter 4. Epidemiology and Development of Drinking Motives Across College 53
I. Specific Aim 53
II. Methods 56
III. Results 59
IV. Summary and Discussion 75
Chapter 5. Genetic Etiology of Drinking Motives 79
I. Specific Aim 79
II. Methods 82
III. Results 86
IV. Summary and Discussion 111
Chapter 6. The Internalizing Pathway and Alcohol Misuse 114
I. Specific Aim 114
II. Methods 118
III. Results 121
IV. Summary and Discussion 132
Chapter 7. Discussion and Conclusions 135
I. Summary of Findings 135
II. Implications 138
III. Limitations 143
IV. Conclusions and Future Directions 144
List of References 146
Author's Vita 164

List of Tables

Table 2.1 Timeline of assessment for four cohorts of college students enrolled in the Spit for Science Study 23

Table 2.2 Descriptive statistics for alcohol misuse measures 26

Table 2.3 Descriptive statistics for drinking motive scores 27

Table 2.4 Descriptive statistics for internalizing measures 28

Table 2.5 Descriptive statistics for drinking motive scores 29

Table 2.6 Descriptive statistics for psychosocial/environmental measures 30

Table 3.1. Correlations between alcohol misuse measures 37

Table 3.2. Correlations between internalizing measures 38

Table 3.3. Correlations between externalizing measures 38

Table 3.4 Factor loadings for an exploratory factor analysis of externalizing measures.... 38

Table 3.5. Model fit indices for the latent profile analysis 39

Table 3.6. Latent class comparisons on drinking motives and other outcome measures in the latent profile analysis 43

Table 3.7. Latent class comparisons on demographic characteristics in the latent profile models 43

Table 3.8. Model fit indices for the growth mixture model analysis 45

Table 3.9. Latent class comparisons on drinking motives and other outcome measures in the parallel process growth mixture models 48

Table 3.10. Latent class comparisons on demographic characteristics in the parallel process growth mixture models 48

Table 4.1. Cross-time correlations for drinking motives across five waves of assessment 59

Table 4.2. Within-time correlations between motive subscale scores 60

Table 4.3. Multiple linear regression results of demographic and environmental factors predicting mean drinking motives 63

Table 4.4. Correlations between drinking motives and internalizing, externalizing, and alcohol use measures 64

Table 4.5. Estimates of cross-lagged path coefficients for drinking motives and internalizing, externalizing, and alcohol misuse outcomes predicting each other across three intervals 65

Table 4.6. Within-wave correlations between drinking motives and internalizing, externalizing, and alcohol misuse outcomes in the cross-lagged models 75

Table 5.1. Heritability estimates (h^2) for drinking motives from genome-wide complex trait (GCTA) analysis in five ancestry subgroups and meta-analysis 87

Table 5.2. Genomic annotation for loci with three or more SNPs reaching a suggestive level of association (p < 5e-05) with drinking motives 97

Table 5.3. Top results of gene-based enrichment meta-analysis tests for association with drinking motives 109

Table 5.4. Top results of pathway-based enrichment meta-analysis tests for association with drinking motives 110

Table 6.1. Endorsement of lifetime screening criteria for five primary anxiety disorders in a sample of college students 121

Table 6.2. Loci of top association peaks from a meta-analysis of genome-wide association scans for anxiety disorder phenotypes in college students from five genetic ancestry populations 125

Table 6.3. Genomic inflation values for genome-wide association analyses and meta-analyses for two anxiety-related phenotypes 127

Table 6.4. Top results from a meta-analysis of gene-based association results for an anxiety disorder case-control status (CC) or factor score (FS) 131

Table 6.5. Logistic and linear regression results for prediction of anxiety-related traits using polygenic risk scores based on genome-wide association results from the ANGST consortium meta-analysis..... 132

List of Figures

Figure 1.1. Conceptual dimensions of motivations in Cooper's (1994) Drinking Motives Questionnaire defined by valence (positive-negative) and source (internal-external).... 16

Figure 3.1. Endorsement patterns for standardized internalizing, externalizing, and alcohol misuse measures in the three class latent profile model solution 41

Figure 3.2. Distribution of drinking motive scores between the latent classes 44

Figure 3.3. Parallel growth trajectories in internalizing, externalizing, and alcohol misuse measures across four years of college in the three latent class model solution 47

Figure 4.1. Example illustration of the cross-lagged model 58

Figure 4.2. Mean values of drinking motive scores across five waves of assessment ..61

Figure 4.3. Cross-lagged model of social drinking motives and binge drinking frequency. 66

Figure 4.4. Cross-lagged model of enhancement drinking motives and binge drinking frequency 67

Figure 4.5. Cross-lagged model of coping drinking motives and binge drinking frequency 68

Figure 4.6. Cross-lagged model of conformity drinking motives and binge drinking frequency 69

Figure 4.7. Cross-lagged model of social drinking motives and alcohol use disorder symptoms (AUDsx) 70

Figure 4.8. Cross-lagged model of enhancement drinking motives and alcohol use disorder symptoms (AUDsx) 71

Figure 4.9. Cross-lagged model of coping drinking motives and alcohol use disorder symptoms (AUDsx) 72

Figure 4.10. Cross-lagged model of conformity drinking motives and alcohol use disorder symptoms (AUDsx) 73

Figure 5.1. Manhattan plot of genome-wide association meta-analysis results for social drinking motives 89

Figure 5.2. Manhattan plot of genome-wide association meta-analysis results for enhancement drinking motives 90

Figure 5.3. Manhattan plot of genome-wide association meta-analysis results for coping drinking motives 91

Figure 5.4. Manhattan plot of genome-wide association meta-analysis results for conformity drinking motives 92

Figure 5.5. QQ plot of genome-wide association within-ancestry and meta-analysis results for social motives 93

Figure 5.6. QQ plot of genome-wide association within-ancestry and meta-analysis results for enhancement motives 94

Figure 5.7. QQ plot of genome-wide association within-ancestry and meta-analysis results for coping motives 95

Figure 5.8. QQ plot of genome-wide association within-ancestry and meta-analysis results for conformity motives 96

Figure 5.9. Manhattan plot of genome-wide association analysis results for coping drinking motives in Europeans 100

Figure 5.10. Manhattan plot of genome-wide association analysis results for enhancement drinking motives in Europeans 101

Figure 5.11. Regional association plot of –log(*p*) values in the *FBLN2* gene region for enhancement motives in the cross-ancestry GWAS meta-analysis 102

Figure 5.12. Regional association plot of –log(*p*) values in the *PECR* gene region for enhancement motives in the European ancestry GWAS 103

Figure 5.13. Regional association plot of –log(*p*) values in the chromosome 5 29.2-29.8Mb region for coping motives in the cross-ancestry meta-analysis 104

Figure 5.14. Regional association plot of –log(*p*) values in the *GRIN3A* gene region for coping motives in the European ancestry GWAS 105

Figure 5.15. Regional association plot of –log(*p*) values in the *LOC390617* region for coping motives in the European ancestry GWAS 106

Figure 5.16. Regional association plot of –log(*p*) values in the chromosome *SIRT1* gene region for conformity motives in the cross-ancestry meta-analysis 107

Figure 6.1. Genome-wide association meta-analysis results for anxiety disorder case-control (CC) status in a sample of college students from five genetic ancestry populations 123

Figure 6.2. Genome-wide association meta-analysis results for an anxiety disorder factor score (FS) in a sample of college students from five genetic ancestry populations 124

Figure 6.3. Genome-wide association results for anxiety disorder case-control (CC) status in the European subset (*n* = 1919) of a sample of college students 128

Figure 6.4. Regional plot of –log(*p*) values of association with case-control status in Europeans in a genome-wide significant locus on chromosome 4 129

List of Abbreviations

ADs ..... Anxiety disorders

AFR ..... African ancestry group

AGO ..... Agoraphobia

AIC ..... Akaike's information criteria

AMR ..... American ancestry group

ASB ..... Antisocial behavior

AUD ..... Alcohol use disorder

AUDsx ..... Alcohol use disorder symptoms

BAS ..... Behavioral activation system

BFI ..... Big Five Inventory

BIC ..... Bayesian information criteria

BIS ..... Behavioral inhibition system

BP ..... Base pair position

CC ..... Anxiety disorder case-control status

CFA ..... Confirmatory factor analysis

CHR ..... Chromosome

DMQ ..... Drinking Motives Questionnaire

DNA ..... Deoxyribonucleic acid

EAS ..... East Asian ancestry group

EFA ..... Exploratory factor analysis

FDR ..... False discovery rate

FS ..... Anxiety disorder factor score

eQTL ..... expression quantitative trait locus

EUR ..... European ancestry group

GAD ..... Generalized anxiety disorder

GCTA ..... Genome-wide complex trait analysis

GWAS ..... Genome-wide association study

h^2 ..... Heritability estimate

HWE ..... Hardy-Weinberg equilibrium

IBS ..... Identical-by-state

Indel ..... Small insertion/deletion

INFO ..... Imputation information score

LD ..... Linkage disequilibrium

LMR ..... Lo-Mendell-Rubin goodness of fit test

MAC ..... Minor allele count

MAF ..... Minor allele frequency

MDD ..... Major depressive disorder

NIAAA ..... National Institute on Alcohol Abuse and Alcoholism

PAN ..... Panic disorder

PC ..... Ancestry principal component

PHO ..... Specific phobia

PRS ..... Polygenic risk score

QC ..... Quality control

RDoC ..... Research Domain Criteria

REML ..... Restricted maximum likelihood estimation

S4S ..... Spit for Science study

SAD ..... Social anxiety disorder

SAS ..... South Asian ancestry group

ssBIC ..... Sample size-adjusted Bayesian information criteria

SCL-90 ..... Hopkins Symptom Checklist – 90

SNP ..... Single nucleotide polymorphism

SSAGA ..... Semi-structured assessment for the genetics of alcoholism

UPPS .. Urgency Premeditation Planning Sensation Seeking Impulsivity Behavior Scale

UTR .. Untranslated region upstream/downstream of the protein-coding region of a gene

Y1F ..... Year 1 fall semester survey wave

Y1S ..... Year 1 spring semester survey wave

Y2S ..... Year 2 spring semester survey wave

Y3S ..... Year 3 spring semester survey wave

Y4S ..... Year 4 spring semester survey wave

Abstract

DRINKING MOTIVES UNDERLYING INTERNALIZING AND EXTERNALIZING TRAJECTORIES OF ALCOHOL MISUSE

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Alcohol misuse, including heavy episodic use and negative consequences, is a major public health concern and a particular problem among college students. The etiology of alcohol misuse is not well resolved, with multiple and often contradictory factors implicated in its development. Genetic factors influence alcohol misuse but few specific genes have been identified. A potential reason for these challenges is that alcohol misuse is phenotypically and genetically heterogeneous; that is, there are multiple causal pathways underlying its development. Previous typologies have suggested that distinct internalizing and externalizing pathways are involved, with corresponding differences in profiles of personality, temperament, and comorbid psychopathology. Drinking motives, specifically drinking for positive reinforcement versus negative reinforcement motives, map intuitively onto such pathways and may provide a mechanism explaining their development. The aim of this project was to utilize drinking

motives as intermediate phenotypic measures to investigate genetic and environmental factors contributing to the hypothesized diverging internalizing and externalizing pathways to alcohol misuse in a prospective, longitudinal sample of college students. Mixture modeling approaches identified distinct internalizing and externalizing subgroups with both quantitative and qualitative differences in traits/symptoms. The externalizing subgroup had a broader risk profile and elevated levels of both types of drinking motives, while the internalizing subgroup had specifically elevated levels of internalizing symptoms and negative reinforcement motives. Longitudinal analyses indicated stability of drinking motives throughout college and differential associations between positive/negative reinforcement motives and internalizing, externalizing, and alcohol misuse measures. Cross-lagged structural equation models pointed to a causal direction of effect of positive reinforcement motives on alcohol misuse. Finally, a series of genetic association analyses identified some promising genes and genetic variants underlying drinking motives and internalizing psychopathology, though their genetic etiologies remain largely inconclusive. The results of this project tie together several parallel lines of research on alcohol misuse and in the broader psychiatric genetics field. Findings support the existence of distinct, though not wholly separate, internalizing and externalizing subgroups, and suggest that the intermediate mechanisms of drinking motives are a valuable tool through which to understand these heterogeneous pathways to alcohol misuse.

Chapter 1. A Global Introduction to the Construct of Alcohol Misuse

In 2016, the Office of the Surgeon General of the United States released a comprehensive report detailing the epidemic of addiction facing the nation (Office of the Surgeon General, 2016). This report was the first of its kind to address the health consequences of alcohol and illicit drug use and, like its historical predecessor on cigarette smoking, focused a spotlight on alcohol and substance addiction as one of the country's top public health priorities. Substance use and misuse are leading contributors to the global public health burden, and alcohol misuse is responsible for the lion's share of this burden due to its widespread prevalence. It is estimated that alcohol misuse costs the U.S. almost $250 billion each year (Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015) and accounts for costs over 1% of the GDP in nations throughout the world (Rehm et al., 2009). These costs are both direct and indirect, from health consequences of drinking to ancillary increases in crime, legal costs, and productivity losses. In addition, alcohol misuse accounts for 3.8% of global mortality and 4.6% of the global burden of disease and injury (Rehm et al., 2009).

Although its public health impact is quantifiable, the definition of what is considered alcohol misuse is not so unequivocal. The terms "alcoholism" and "addiction" have long been in colloquial use to describe repetitive drunkenness or continued drinking in the face of negative consequences (Nathan, Conrad, & Skinstad, 2016), yet there is no medical test for addiction and no threshold of a certain number of

drinks or frequency of drinking above which one becomes an alcoholic. The criteria has historically been subjective and heavily influenced by notions of religion and morality (Nathan et al., 2016). As the field of psychiatry became more systematic about developing a reliable and valid nosology throughout the 1900s, alcohol misuse came to be defined first as a personality disorder, then as the behavioral disorder of Alcoholism, and then as two separate disorders: Alcohol Abuse (AA) and Alcohol Dependence (AD), emphasizing the distinctive aspects of harmful use and compulsive use, respectively (Sellman, Foulds, Adamson, Todd, & Deering, 2014). Most recently, these two disorders have been reunited into the single diagnostic construct of Alcohol Use Disorder (AUD; with mild, moderate, and severe categories) in the current version of the *Diagnostic and Statistical Manual for Mental Disorders* (DSM-5, American Psychiatric Association, 2013). This classification reflects new evidence that harmful use and compulsive use differ in severity, not in kind (Borges et al., 2010; Goldstein et al., 2015; Lago et al., 2017). However, an AUD diagnosis comes at a mid-point or an end-point of an ongoing, escalating trajectory of heavy alcohol use; it is not possible to go to bed abstinent and awake an alcoholic. Therefore the construct of alcohol misuse is best conceptualized with consideration of both the harmfulness of individual occasions of alcohol consumption and the longitudinal patterns that determine whether consumption results in negative consequences.

Alcohol use can be considered *misuse* if it causes harm in the domains of health, social, or occupational functioning. Acute consumption of large quantities of alcohol, or *binge drinking*, can cause immediate physical harm including overdose (alcohol poisoning) and organ damage, as well as increased risk for physical and sexual assault,

accidents, and injuries (Kuntsche, Kuntsche, Thrul, & Gmel, 2017). Binge drinking is defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as four or more drinks in one sitting for women and five or more for men. Binge drinking as a regular pattern of consumption, on five or more days per month, is considered by the NIAAA as *heavy alcohol use*. AUD, on the other hand, captures patterns of heavy/risky use that persist despite chronic interference with job performance or social obligations as well as the physiological processes that occur with long-term use such as tolerance and withdrawal. Like most psychiatric disorders, the criterion of clinically significant impairment is an important consideration for making a diagnosis. However, numerous negative consequences of alcohol use (to one's self and to others) can occur in the absence of an AUD diagnosis (Office of the Surgeon General, 2016); this domain can thus be more broadly considered as *alcohol problems*.

Frequency/quantity of consumption, binge drinking behaviors, and alcohol problems are highly correlated outcomes that demonstrate a substantial shared etiology (Dick, Meyers, Rose, Kaprio, & Kendler, 2011; Whitfield et al., 2004). Item response theory models have shown that AUD criteria form a unidimensional continuum but only tap into the upper end of the underlying distribution of alcohol problems, while alcohol consumption measures discriminate the lower end of the same spectrum (Borges et al., 2010). However, there are some important distinctions between these domains. Binge drinking and heavy consumption have peak prevalence in young adulthood, while AUD prevalence remains relatively stable throughout younger and older adulthood (Kuntsche et al., 2017; Substance Abuse and Mental Health Services Administration, 2014). There is also evidence that commonly comorbid disorders such as depression and anxiety

show uniquely elevated rates of alcohol problems but not consumption heaviness (e.g. Schry & White, 2013). Broadly speaking, all aspects of alcohol use and misuse are correlated, but subtle qualitative distinctions between the dimensions are important to consider. For the purposes of this manuscript, alcohol misuse is considered in terms of the two domains of heavy consumption (primarily binge drinking) and alcohol problems (primarily AUD symptoms).

I. Alcohol misuse and college students

With a definition of alcohol misuse in hand, its prevalence, consequences, and correlates may be identified. In recent years, alcohol misuse in college students has been a particular focus of study, with the NIAAA launching a major initiative on underage and college drinking research in 2004. There is good reason for this: college attendance rates have been rapidly rising over the past 50 years (Snyder, de Brey, & Dillow, 2016), and alcohol use, particularly heavy/binge drinking, appears to be concentrated in this population. Most students have initiated alcohol use before the end of college, and over 60% report drinking in the past month (Johnston, O'Malley, Bachman, Schulenberg, & Miech, 2014). Nearly two-thirds of regular drinkers in college report recent binge drinking (Substance Abuse and Mental Health Services Administration, 2014), and 40% report experiencing one or more AUD symptoms (Knight et al., 2002) with 20% meeting diagnostic criteria for AUD (Blanco et al., 2008). Negative consequences including assault and academic and legal problems are prevalent in college students (White & Hingson, 2014), and they are at particularly heightened risk for drunk driving and overdose (Hingson, Zha, & Weitzman, 2009).

Notably, there is a higher prevalence of alcohol use and AUDs in college students than their age-matched, non-college attending peers, despite a lower prevalence of illicit drug use and despite the fact that college-bound high school students have lower levels of alcohol use than their peers (Blanco et al., 2008; Johnston et al., 2014).

This evidence suggests that college attendance or the college environment itself propagates alcohol misuse. This could be in part due to the coincidence of the typical college time period with a peak age for heavy alcohol use (Substance Abuse and Mental Health Services Administration, 2014) and onset for AUDs (Kessler et al., 2005a). The comparisons with non-college peers, however, indicate that this is not the sole factor. The beginning of college marks the onset of a massive transition in an individual's roles and responsibilities, where students often leave homes and families and begin to be independently accountable for their own lives. At the same time, they are typically exposed to many new peers, social influences, opportunities for personal growth and self-identity development, and a freedom previously unparalleled in adolescence. Numerous aspects of the college environment provide opportunities for such freedoms to manifest into alcohol use/misuse: the low supervision in general and tolerance for alcohol-related violations specifically, campus traditions and social groups like Greek organizations that encourage heavy alcohol use, the mixture of students below and above the legal drinking age in one social group which facilitates access for underage drinkers, and the high density of available alcohol on and around campuses (Dowdall & Wechsler, 2002; Ham & Hope, 2003; Merrill & Carey, 2016).

The prevalence and consequences of alcohol misuse in college students alone make it a valuable public health outcome to study. However, perhaps more importantly,

college intersects with the beginning or early stages of a lifelong drinking trajectory for most individuals (Borsari, Murphy, & Barnett, 2007). Alcohol misuse is not an isolated phenomenon, but a complex process that unfolds across development in heterogeneous ways (Savage et al., in press; Tarter & Vanyukov, 1994; Windle et al., 2008). Understanding the origins of alcohol misuse during this early period and the reasons for its prevalence in the context of the college environment can lead to important insights for prevention, intervention, and treatment throughout the lifespan.

II. Etiology of alcohol misuse

AUDs and alcohol misuse fit into the broader diagnostic category of addictive disorders, which share the common feature of the repeated use of a drug (or behavior) that activates the brain's reward system, coupled with a loss of control over such use (American Psychiatric Association, 2013). Among mental disorders, substance addictions are unique in being contingent on an environmental exposure (i.e. alcohol), without which an individual cannot develop an addiction. This reinforces the developmental nature of alcohol and addictive disorders; their onset cannot occur if the drug is not accessible, and they cannot progress without its continued availability. Current theories conceptualize addictive disorders as multi-stage diseases involving three major processes: 1) binge/intoxication, 2) withdrawal/negative affect, and 3) preoccupation/craving (Koob et al., 2004; Koob & Le Moal, 2008). These stages involve distinct neurobiological systems (Koob & Volkow, 2016) and may reflect a shift in the balance from reward-seeking (positive reinforcement) processes to pain/withdrawal-avoidance (negative reinforcement) processes that occurs in the development of

addictions. However, alcohol is a drug with both stimulant and sedative effects (Erblich, Earleywine, Erblich, & Bovbjerg, 2003; Kreusch, Vilenne, & Quertemont, 2013), which has been shown to both induce positive feelings and to bring about relief from negative feelings (Bacon & Ham, 2010), with effects differing greatly between individuals. It is important then to consider these positive and negative reinforcement processes and their part in the developmental stages of addiction.

This multi-process pattern of addiction is reflected in the epidemiological and clinical literature, in which a diverse set of intrapersonal and psychosocial factors has been shown to correlate with alcohol misuse. The epidemiological literature has been remarkably consistent in demonstrating that most common psychiatric and substance use disorders fall into two underlying categories: the "internalizing" and the "externalizing" domains (Kendler, Prescott, Myers, & Neale, 2003; Krueger, 1999). Current theory suggests that disorders of the externalizing spectrum share the core features of disinhibition, impulsivity, and sensation-seeking (Dick et al., 2010; Krueger et al., 2002), while the internalizing spectrum is marked by negative affect, risk aversion, and punishment sensitivity (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Brown & Barlow, 2009; Carvalho et al., 2014). AUD is considered part of the externalizing spectrum, along with other drug use disorders and antisocial behavior (conduct disorder in children), yet it is also strongly associated with disorders and traits on the internalizing spectrum such as mood and anxiety disorders (Hasin, Stinson, Ogburn, & Grant, 2007; Kessler, Chiu, Demler, & Walters, 2005b). Robust risk factors for alcohol misuse include seemingly contradictory personality traits ranging from impulsivity to behavioral inhibition and extraversion to shyness (Lynam & Miller, 2004; Page, 1989;

Wardell, O'Connor, Read, & Colder, 2011; Whelan et al., 2014). AUD also occurs at elevated rates alongside virtually all other psychiatric and substance use disorders, with the highest correlations for externalizing disorders (0.40 – 0.67) and lower but still substantial correlations (0.22 – 0. 33) for internalizing disorders (Kessler et al., 2005b; Krueger & Markon, 2006). Such patterns indicate that conceptualizations of alcohol misuse as a single unitary construct are untenable and highlight the importance of considering multiple possible pathways in its development.

A number of reasons could explain these observed patterns. Comorbidity between traits/disorders can arise a) when there is a shared etiology between them (i.e. the same underlying cause leading to multiple distinct outcomes), b) when one trait directly causes the other, c) when two traits are actually alternate forms of expression of the same underlying entity, or d) when the comorbid existence of two traits is actually itself a separate entity that merely shares signs/symptoms with both (Krueger & Markon, 2006). It is not necessary that the mechanism of comorbidity be the same between alcohol misuse and its many correlated traits and disorders. Identifying these mechanisms, though, can lead to a better understanding of alcohol misuse.

Although alcohol misuse has been the focus of decades of intensive research, much remains unknown about its etiology. It is well established that not all individuals are at equal risk for developing alcohol misuse, and that biological predispositions make some people more vulnerable than others. As has been extensively reviewed (Agrawal et al., 2012; Dick, Prescott, & McGue, 2009; Hart & Kranzler, 2015; Palmer et al., 2012), the heritability of alcohol use and misuse is around 50%, but there has thus far been little success in identifying the actual genes involved. Heritability is defined as the

proportion of inter-individual variance in a *phenotype* (trait, behavior, or other observable outcome) in a population that can be attributed to genetic factors. It is estimated mathematically by comparing the phenotypic similarity between individuals with varying degrees of known genetic similarity such as twins, siblings, cousins, and unrelated individuals (Neale & Cardon, 1992). More recent methods have been developed to estimate heritability directly from similarities in the DNA code of unrelated individuals (Yang, Lee, Goddard, & Visscher, 2011). However, these methods have several limitations and can only provide information about the genetic effects in aggregate and not which genes and biological processes drive individual differences in risk. Only a few genes have demonstrated robust, replicable associations with alcohol misuse (Dick et al., 2015), and either individually or cumulatively these account for less than 3% of the variance in alcohol misuse (Hart & Kranzler, 2015) – a far cry from the estimated heritability. Although gene identification efforts have been challenging for the whole psychiatric genetics field (Sullivan, Daly, & O'Donovan, 2012), alcohol misuse has remained particularly intractable in the face of growing successes with other similarly complex phenotypes (CONVERGE Consortium, 2015; Otowa et al., 2016; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

III. Heterogeneity in alcohol misuse

A potential explanation for the slow progress in conclusively understanding the etiology of alcohol misuse at both the phenotypic and genetic levels is that the construct of alcohol misuse may not be a single, uniform entity with a single underlying causal pathway, but rather a heterogeneous mix of different pathways that result in a similar

observable outcome. Such heterogeneity would mean that there are many distinct sets of risk and protective factors contributing to alcohol misuse that differ vastly between individuals, whereby two people could experience a similar set of symptoms but due to entirely different causes. When such individuals are categorized together in a study of association of a certain risk factor with alcohol misuse, the magnitude of statistical association of this true risk factor for individual A will be diluted by the presence of individual B – for whom this factor has no effect on risk – in the same outcome category. When not accounted for in the definition of the outcome, etiological heterogeneity has the effect of changing the profile of risk/resilience factors from a few predictors that have robust effects in smaller subgroups to a large number of factors that each have quite modest statistical associations when their effects are spread out over the whole population.

This maps well onto what is seen for psychosocial predictors of alcohol misuse, for which a multitude of temperaments, personality profiles, and dimensions of psychopathology are associated with an increase – albeit modest – in risk. The same cannot yet be said conclusively of its genetic etiology, but the fact that very few associations have been identified despite substantial research efforts suggests a similar profile of many associated genes with small effects, so small in fact that studies of thousands of individuals remain underpowered to detect them. It is notable that one of the few genes that has been consistently replicated, the *ALDH2* gene which is involved in alcohol metabolism, has a very large effect on reducing alcohol misuse in the East Asian populations where a particular variant is common (Hart & Kranzler, 2015). However, this variant's effect size is much smaller in European populations where it is

less common (Macgregor et al., 2009), illustrating the attenuating effect that can occur when combining samples with multiple causal pathways.

The concept of (phenotypic) heterogeneity in alcohol misuse is not new. A long history of theory and research has put forward several typologies for classifying individuals with alcohol use disorders into distinct subgroups based on their clinical characteristics, course of symptoms or recovery, comorbidities, and/or associated risk factors (Leggio, Kenna, Fenton, Bonenfant, & Swift, 2009). Most prominent and lasting among these are the typologies proposed by Cloninger et al. (1988) and Babor et al. (1992), which both proposed two types of alcoholism differing in severity, age of onset, family history of alcoholism, and correlated psychopathologies. Though there were some distinctions, these typologies largely agreed on the existence of an early-onset, severe, familial subgroup with high rates of polysubstance use and other psychopathology and antisocial traits, and a second subgroup with later onset, fewer problems/consequences, and anxious personality traits. More recent approaches using mixture modeling to statistically parse out patterns in the data has supported the existence of a more severely affected and more highly heritable “behavioral disinhibition” class and a second “affect regulation” class with high levels of neuroticism and depression (Sintov et al., 2010). Similarly, others have found a severe/externalizing class versus an internalizing class of individuals with alcohol use disorders (Hildebrandt, Epstein, Sysko, & Bux, 2017). Multiple longitudinal studies have also lent support to the idea that childhood internalizing and externalizing traits form two distinct developmental pathways leading to alcohol misuse outcomes later in life (Conrod, Stewart, Comeau, & Maclean, 2006; Mezquita, Ibáñez, Moya, Villa, & Ortet, 2014; Zucker, 2008).

Some evidence also suggests that the mechanisms of comorbidity with alcohol misuse differ for the internalizing and externalizing domain. Multivariate twin studies, using the same mathematical principles as described above, are able to disaggregate the genetic and environmental influences on the covariance between multiple traits in addition to the variance of each individual trait. In one such study, Kendler et al. (2003) found that genetic influences on AUD where shared with externalizing disorders, while environmental influences on AUD were shared with internalizing disorders. Edwards, Larsson, Lichtenstein, and Kendler (2011a) similarly identified a correlated environmental, but not genetic, liability between anxiety/depression symptoms and intoxication frequency in early adolescence, though this decreased at older ages. Molecular genetic studies have begun to find similar patterns of genetic correlations between traits (Cho et al., 2017). These findings lend further support to the typology of a strongly genetically influenced externalizing pathway versus a more environmentally influenced internalizing pathway to alcohol misuse.

IV. Intermediate mechanisms in internalizing and externalizing pathways

If such distinct pathways exist, what is the means by which they lead from individual differences in predispositions to the eventual development of problematic alcohol use? There is evidence to suggest that the shared genetic influences on alcohol misuse and other externalizing disorders are due to a common mechanism of impulsivity and behavioral undercontrol manifested in each of these outcomes (Dick et al., 2010; Krueger et al., 2002). The orientation towards immediately rewarding stimuli, despite potential negative consequences, is thought to drive polysubstance use and

engagement in other gratifying behaviors that should otherwise be deterred by their negative health, legal, and interpersonal consequences. Internalizing disorders, on the other hand, tend to predict subsequent incidence of alcohol problems (Birrell, Newton, Teesson, Tonks, & Slade, 2015; Boschloo et al., 2013; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003) and are theorized to have a causal role in their development because of the use of alcohol to self-medicate and reduce symptoms of anxiety and depression (Bacon & Ham, 2010; Vorspan, Mehtelli, Dupuy, Bloch, & Lépine, 2015; Weiss, Griffin, & Mirin, 1992). Some environmental factors such as trauma exposure predict both internalizing disorders and alcohol misuse, with effects on alcohol misuse potentially mediated through internalizing traits (Schwandt, Heilig, Hommer, George, & Ramchandani, 2013). However, it should be noted that alcohol misuse is also linked to subsequent onset of internalizing symptoms, particularly depression (Fergusson, Boden, & Horwood, 2009; Swendsen et al., 1998), so the causal direction of association is not incontrovertible.

Evolutionary theory provides further context for understanding the mechanisms of the internalizing and externalizing pathways. Recent changes in thinking towards the classification of psychopathology have led the National Institute of Mental Health to propose a new diagnostic system, the Research Domain Criteria (RDoC), in an effort to better map mental disorders onto their underlying neurobiological systems (Cuthbert & Insel, 2013; Insel et al., 2010). Two of these systems, “negative valence” and “positive valence”, represent the fundamental evolutionary drives to avoid negative or painful stimuli and approach positive or rewarding stimuli, respectively. These aversive and appetitive motivations make up core aspects of personality and temperament

(Neuroticism/Negative Affect and Extraversion/Positive Affect) that are found in virtually every system that has been proposed to classify human personality (Elliot & Thrash, 2010). They are carried out by distinct neurobiological systems, the Behavioral Inhibition System and the Behavioral Activation System (Gray, 1982). These systems are differentially linked to anxiety/negative affect and impulsivity/positive affect, respectively – mapping intuitively onto the internalizing and externalizing domains of psychopathology. Studies have also demonstrated a differential autonomic nervous system (cortisol) response to stress, with an intrinsically under-aroused stress response system present in children with externalizing disorders and a heightened stress response in those with internalizing disorders (Bae et al., 2015; Hartman, Hermanns, de Jong, & Ormel, 2013).

Collectively, the evidence indicates distinct mechanisms driving internalizing and externalizing pathways to alcohol misuse. For those with an internalizing predisposition, alcohol may be a means to obtain relief from negative affect (particularly in response to stress) and achieve a neutral or positive affect state. For those with an externalizing predisposition, alcohol misuse may stem from the impulsive pursuit of the rewarding effects of ethanol and insensitivity to negative consequences that may result. These hypotheses, however, remain to be validated, and the intermediate mechanisms linking biological and psychological predispostitions to distal alcohol use outcomes are yet to be well understood.

V. Drinking motives as intermediate phenotypes

A potential tool to validate and investigate these heterogeneous pathways is the use of intermediate phenotypes, or "endophenotypes", which sit in the mediational pathway between a biological (or psychological) predisposition and the eventual manifestation of a trait or disorder (Gottesman & Gould, 2003; Hines, Ray, Hutchison, & Tabakoff, 2005). Drinking motives, the reasons why people consume alcohol and what they hope to achieve by drinking, present a clear mechanism by which divergent internalizing and externalizing pathways may lead to alcohol misuse and elucidate the intermediate mechanisms by which such pathways may unfold.

The most prominent model of drinking motives, developed by Cooper (1994), proposes four distinct types of drinking motives that fall under two dimensions: valence (negative versus positive reinforcement) and source (internal versus external) – see Figure 1. Negative reinforcement motives reflect drinking to obtain relief from negative emotions or escape unpleasant states while positive reinforcement motives capture drinking that occurs to achieve positive mood states or enjoy the pleasurable aspects of alcohol. Internal motives are driven by one's own desires or feelings, while external motives are driven by social or environmental influences. Drinking motives have repeatedly demonstrated robust, proximal associations with measures of alcohol consumption and alcohol problems (Carpenter & Hasin, 1998; Kuntsche, Knibbe, Gmel, & Engels, 2005). Most research has focused on coping and enhancement motives, which predict frequency/quantity of alcohol use, binge drinking, and alcohol use disorders, though coping motives show somewhat stronger associations with alcohol use disorders/alcohol problems and enhancement motives with heavy alcohol use. There has been far less research on conformity and social drinking motives, but these

have more often shown associations with normative rather than pathological drinking and have weaker effects (Kuntsche et al., 2005).

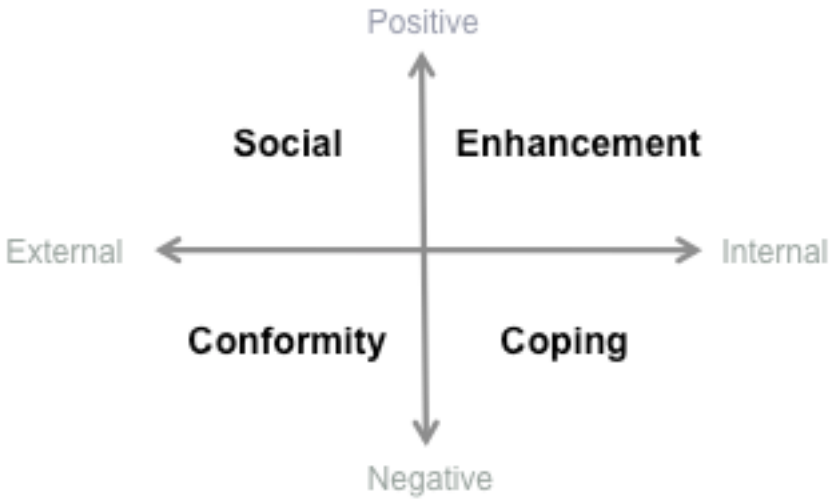

Figure 1.1. Conceptual dimensions of Cooper's Drinking Motives Questionnaire defined by valence (positive-negative) and source (internal-external).

The connections between negative/positive reinforcement motives and internalizing/externalizing pathways to alcohol misuse are intuitive, but there also exists some empirical support for drawing these parallels. Internalizing traits/disorders such as depression, anxiety, and neuroticism have all been linked to higher levels of coping motives (Comeau, Stewart, & Loba, 2001; Mezquita, Stewart, & Ruipérez, 2010; Theakston, Stewart, Dawson, Knowlden-Loewen, & Lehman, 2004; Windle & Windle, 2012), while impulsivity, sensation-seeking, disinhibited behavior, and attentional biases towards reward cues are associated with enhancement motives (Adams, Kaiser, Lynam, Charnigo, & Milich, 2012; Colder & O'Connor, 2002; Comeau et al., 2001). Mediational models have shown direct evidence for pathways from neuroticism to alcohol misuse via coping motives and from sensation seeking to alcohol misuse via

enhancement motives (Adams et al., 2012; Littlefield et al., 2011; Mezquita et al., 2010). There is also some evidence that drinking motives are heritable, with modest to moderate estimates ranging from 11-40% (Agrawal et al., 2008; Kristjansson et al., 2011; Mackie, Conrod, Rijsdijk, & Eley, 2011; Prescott, Cross, Kuhn, Horn, & Kendler, 2004), fulfilling a requisite criterion for an endophenotype linking genes to behavior. A few studies even suggest that drinking motives may mediate the pathways between genetic predispositions and AUD outcomes (Littlefield et al., 2011; Prescott et al., 2004; Young-Wolff, Kendler, Sintov, & Prescott, 2009), a compelling link for their intermediate role and mechanism.

VI. Aims of this dissertation project

Despite the wealth of evidence linking drinking motives to alcohol misuse, there has been virtually no research on the etiology of drinking motives themselves. A few twin studies have estimated the latent genetic risk for drinking motives but to date no molecular genetic studies have been conducted. If drinking motives are to be useful intermediate phenotypes to understand heterogeneous alcohol misuse outcomes, it is important to understand their genetic and environmental etiology and to learn what factors shape their development during college, a critical time period for the formation of alcohol use behaviors. Establishing the utility of drinking motives as an endophenotype would also bring further credibility to the internalizing/externalizing typology. Understanding the etiology of drinking motives could provide insight into the etiology of alcohol misuse and identify actionable mechanisms to incorporate into tailored prevention, intervention, and treatment efforts.

The primary goal of this dissertation project is thus to utilize drinking motives as intermediate phenotypic measures to investigate genetic and environmental factors contributing to diverging pathways to alcohol misuse, which are hypothesized to correspond to internalizing and externalizing subtypes. Data is derived from the Spit for Science study (Dick et al., 2014), a longitudinal, prospective study of college students involving both genotyping and self-report surveys. This study design allows for investigation of biological, psychological, and social aspects of drinking motives and their relationship to alcohol misuse across the span of the college years. Multiple analytic methods are employed to evaluate the utility of drinking motives as intermediate indicators of alcohol misuse pathways and to investigate their etiology. Given the suggestive evidence that the internalizing pathway may have a direct causal role on the development of alcohol use, additional attention is also directed towards further investigation of this pathway.

In Chapter 2, a description of the sample and measures collected in the Spit for Science study is provided. Methods that are common to all of the analyses in this project are described here and may be used as a reference for the sample and measures used for all analyses detailed in subsequent chapters.

Chapter 3 presents an exploration of patterns of internalizing and externalizing symptoms and alcohol misuse in this sample using mixture models to identify whether the proposed internalizing/externalizing typology of alcohol misuse is valid. Latent classes identified in these models are compared on their endorsement of each type of drinking motive to determine whether drinking motives can reliably index different types of alcohol misuse pathways.

In Chapter 4, results from longitudinal analyses are presented to demonstrate the developmental stability and change in drinking motives across the college years and how these patterns relate to changes in alcohol misuse and internalizing and externalizing psychopathology. To provide further insight into the factors shaping students' drinking motives, linear models of various environmental risk and protective factors predicting drinking motives are also tested.

Chapter 5 presents a detailed investigation into the genetic etiology of each of the four drinking motives. This chapter uses multiple methods to identify genetic variants, genes, biological pathways, and aggregate genomic influences underlying individual differences in drinking motives, and to interpret them in the context of their functional implications. Genetic overlap in the etiology of drinking motive subtypes is also investigated as a means to assess whether the drinking motive dimensions have common or distinct biological causes.

In Chapter 6, the focus is shifted towards the internalizing pathway, employing methods to identify genes underlying internalizing psychopathology. These analyses are directly relevant to understanding the etiology of internalizing symptoms; however, the secondary goal is to identify genes that can be used in future work to test causal models of the relationship between internalizing psychopathology and alcohol misuse.

This set of studies provides insight into the genetic, environmental, and developmental etiology of the understudied constructs of drinking motives, and evaluates their suitability to index divergent pathways to alcohol misuse in college students. The results provide further validation of the existence of distinct internalizing and externalizing pathways to alcohol misuse, illustrate the mechanisms of these

pathways, and point to important targets for future efforts to reduce the public health burden of alcohol misuse.

Chapter 2. Sample and Methods: The Spit for Science Study

I. Study Design

Data for this project comes from an ongoing parent study, "Spit for Science: The VCU Student Survey" (NIAAA-R37 AA011408), also referred to as "S4S" (Dick et al., 2014). The S4S study is a research initiative conducted at Virginia Commonwealth University with the aim of building a comprehensive understanding of the genetic and environmental influences on mental health and substance use in college students, and how these unfold and interact across the college years. It is a university-wide effort involving students, faculty, staff, and administrators across departments, with the goal of engaging the student population and incorporating results into university programs to improve student wellbeing. College is a particularly important time to conduct this type of research, not only because it is the time when many mental health and substance problems begin to manifest, but also because it provides an environment with many opportunities and resources to intervene upon this risk (Dick & Hancock, 2015).

S4S is a longitudinal, prospective study involving a self-report survey, repeated annually, and an optional DNA component. Data collection began in the fall of 2011 and the study enrolled four cohorts of incoming students between 2011 and 2015. Participants were recruited via letters sent to their homes in the summer prior to beginning their first semester of college (with information about the study for both students and parents) and in email announcements to their university email account. At

the beginning of the school year in their freshman fall semester, students were emailed a confidential invitation link to enroll in the study and participate in an online survey.

All first-time freshmen aged 18 years and older were eligible to participate, and students who were below the age of 18 were sent an invitation to enroll if they aged in later in their freshman year. Freshmen who did not complete the survey in the fall were given a second opportunity to enroll in the spring semester in concert with a follow-up survey that was administered to participants who enrolled in the fall. Participants who completed the online survey received $10 and a t-shirt in compensation and were then able to provide a saliva sample for DNA collection under the supervision of a research assistant, for which they received an additional $10 compensation. Each subsequent spring that participants were enrolled at the university, they were invited to participate in a follow-up online survey with similar content to the initial assessment, and received $10 for each survey completion (see Table 2.1 for a timeline of data collection).

The self-report surveys assessed a wide range of behaviors and characteristics, including alcohol use outcomes, drinking motives, symptoms of internalizing and externalizing psychopathology, personality, and environmental exposures (e.g. family and peer influences, trauma exposure). Data was collected and managed by the secure REDCap system (Harris et al., 2009) hosted at Virginia Commonwealth University. All participants provided informed consent for both the survey and the DNA collection component. The S4S study and this dissertation project were approved by the Institutional Review Board of Virginia Commonwealth University (Approval #HM20007408).

Table 2.1 Timeline of assessment for four cohorts of college students enrolled in the Spit for Science Study.

Cohort	Fall 2011	Spring 2011	Fall 2012	Spring 2012	Fall 2013	Spring 2013	Fall 2014	Spring 2014	Fall 2015	Spring 2015	Fall 2016	Spring 2016
2011	Fresh.	Fresh.		Soph.		Jr.		Sr.		--		--
2012			Fresh.	Fresh.		Soph.		Jr.		Sr.		--
2013					Fresh.	Fresh.		Soph.		Jr.		Sr.
2014							Fresh.	Fresh.		Soph.		Jr.

II. Participants

Four cohorts of incoming freshmen students have been enrolled in the study thus far (*N* = 9,889), with *n* = 2,310 to 2,707 in each cohort. Participation rates have been consistently high across cohorts, with 63-68% of the eligible incoming students enrolling in the study each year. Of those who enrolled in the fall of freshman year (Y1F), 75% completed the freshman spring follow-up survey (Y1S), and the retention rates for the subsequent surveys were 59% (sophomore spring; Y2S), 52% (junior spring; Y3S), and 48% (senior spring; Y4S) of students from the initial study sample who were still enrolled at the university. These rates are quite high in comparison to comparable surveys in other university populations (Dick et al., 2014).

The demographic characteristics of the sample are consistent with those of the overall VCU student population. The sample is 61.5% female, with self-reported race/ethnicity of 0.5% American Indian/Native Alaskan, 16.3% Asian, 18.9% African American, 49.4% Caucasian, 6.0% Hispanic/Latino, 6.2% multiracial, 0.7% Native Hawaiian/Pacific Islander, and 1.9% unknown/unreported. Nearly all participants, 91% of the total sample (*n* = 9,036), also provided a DNA sample, and genotyping has been completed for three of the first four cohorts.

III. Measures

All measures were collected via a confidential online survey. Participants were emailed an individual link to this survey and could complete it at a time and location of their choosing, within approximately two months from the initiation of each data collection wave. Participants were required to select a response to each item, although

all items had the option “I choose not to answer”. The survey was broad in scope and covered a range of psychological and behavioral outcomes and risk/resilience factors, drawing largely from psychometrically validated scales that have previously been established. To reduce participant burden and facilitate the desired breadth of content area, many scales were administered in an abbreviated version. Some items were only assessed at particular waves/years due to developmental relevance or a timely topical interest. The first survey in the freshman year was considered a baseline metric and thus assessed some temporally stable traits (i.e. personality) and lifetime measures of psychopathology and environmental exposures up to the beginning of college only once, while later assessments focused on the change in such outcomes in the intervening time since the previous survey. The specific sets of measures utilized in this project are described in more detail below.

Alcohol Misuse. In each survey, participants are asked if they have initiated alcohol use (consumed one full drink of alcohol, using the NIAAA definition of a standard drink), and if so, they are asked about a number of different alcohol use behaviors, including frequency of binge drinking (number of days drinking >4 drinks for women and >5 drinks for men) and typical consumption frequency (number of days drinking per month) and quantity (number of drinks per drinking occasion). Binge drinking as a specific question was added partway through the study; for the earlier waves, typical quantity/frequency measures are used to infer typical frequency of drinking at binge levels (number of days per month). Participants who have initiated alcohol use are also asked about symptoms of *DSM-5* Alcohol Use Disorder (AUDsx) using the validated Semi-Structured Assessment for the Genetics of Alcoholism

(SSAGA; Bucholz et al., 1994). Descriptive statistics for alcohol misuse measures are shown in Table 2.2.

Table 2.2 Descriptive statistics for alcohol misuse measures.

Measure	Time	N	Min	Max	Mean	SD
AUDsx	Y1F	6462	0	11	2.32	2.48
AUDsx	Y1S	4194	0	11	2.45	2.51
AUDsx	Y2S	3617	0	11	2.59	2.56
AUDsx	Y3S	2485	0	11	2.56	2.52
AUDsx	Y4S	1452	0	11	2.88	2.68
Binge frequency	Y1F	4270	0	16	1.52	3.36
Binge frequency	Y1S	6005	0	16	1.91	3.55
Binge frequency	Y2S	4049	0	16	2.33	3.45
Binge frequency	Y3S	2630	0	16	2.47	3.61
Binge frequency	Y4S	1513	0	16	2.54	3.67

Note: AUDsx = DSM-5 alcohol use disorder symptoms

Drinking Motives. In each survey, participants who had initiated drinking completed an abbreviated version of the Drinking Motives Questionnaire – Revised (Cooper, 1994). This scale proposes a theoretical model of drinking motives based on two dimensions: source (internal versus external) and valence (positive and negative), in which source reflects whether the motive is individually or socially driven, and valence reflects whether the motive is for negative or positive reinforcement (relief from negative emotions/affective states or obtainment of positive ones). Thus there are four subscales whose items are summed to create scores: Drinking to Cope (internal, negative, e.g. “because it helps me when I feel depressed or nervous”), Drinking to Enhance (internal, positive, e.g. “because it gives me a pleasant feeling”), Drinking to Conform (external, negative, e.g. “to get in with a group I like”) and Drinking to Socialize (external, positive, e.g. “because it makes social gatherings more fun”). Responses are on a Likert-like scale from *1 = Strongly Agree* to *4= Strongly Disagree* (reverse coded). Four items per

each subscale were assessed in the Y1F, Y1S, and Y2S surveys, and the one best-performing item (based on factor loadings) from each subscale was included in the Y3S and Y4S surveys due to space limitations. However, descriptive statistics (see Table 2.3) and correlations across waves showed that the single-item scores performed similarly to the multi-item scale scores.

Table 2.3 Descriptive statistics for drinking motive scores.

Measure	Time	N	Min	Max	Mean	SD
Conformity	Y1F	5852	1	4	1.44	0.73
Conformity	Y1S	4866	1	4	1.42	0.72
Conformity	Y2S	4027	1	4	1.43	0.74
Conformity	Y3S	1726	1	4	1.52	0.80
Conformity	Y4S	1489	1	4	1.53	0.82
Coping	Y1F	5832	1	4	1.84	0.96
Coping	Y1S	4838	1	4	1.86	0.96
Coping	Y2S	4029	1	4	2.04	0.95
Coping	Y3S	1721	1	4	1.94	0.99
Coping	Y4S	1482	1	4	1.97	1.00
Enhancement	Y1F	5849	1	4	2.91	0.87
Enhancement	Y1S	4865	1	4	2.95	0.84
Enhancement	Y2S	4024	1	4	2.86	0.84
Enhancement	Y3S	1733	1	4	2.97	0.85
Enhancement	Y4S	1492	1	4	2.98	0.88
Social	Y1F	5869	1	4	2.94	0.84
Social	Y1S	4894	1	4	3.00	0.82
Social	Y2S	4042	1	4	3.04	0.83
Social	Y3S	1738	1	4	3.07	0.82
Social	Y4S	1495	1	4	3.07	0.79

Internalizing Psychopathology. An abbreviated version of the Hopkins Symptom Checklist-90 (Derogatis & Cleary, 1977) was included in each survey. This instrument has 4 items measuring current levels of anxiety symptoms (e.g. “spells of terror or panic”, “worrying too much about things”) and 4 items measuring current

depression symptoms (e.g. “feeling no interest in things”, “feeling hopeless about the future”). The questions ask participants to rate how much discomfort each symptom has caused them in the past 30 days, from *1 = Not at all* to *5 = Extremely*, and item responses are summed for each subscale. The personality trait of neuroticism, a core component of the internalizing spectrum (Hettema, Neale, Myers, Prescott, & Kendler, 2006), was assessed in the initial survey using a subset of 3 items from the Big Five Inventory (BFI; John & Srivastava, 1999). Table 2.4 presents descriptive statistics for these internalizing measures.

Table 2.4 Descriptive statistics for internalizing measures.

Measure	Time	N	Min	Max	Mean	SD
SCL-90 depression sum score	Y1F	7788	4	20	8.74	3.70
SCL-90 depression sum score	Y1S	7387	4	20	9.76	3.92
SCL-90 depression sum score	Y2S	4685	4	20	9.49	4.04
SCL-90 depression sum score	Y3S	2870	4	20	9.36	4.01
SCL-90 depression sum score	Y4S	1597	4	20	9.35	4.05
SCL-90 anxiety sum score	Y1F	7793	4	20	6.79	3.13
SCL-90 anxiety sum score	Y1S	7388	4	20	6.90	3.26
SCL-90 anxiety sum score	Y2S	4686	4	20	6.57	3.08
SCL-90 anxiety sum score	Y3S	2871	4	20	6.66	3.18
SCL-90 anxiety sum score	Y4S	1599	4	20	6.64	3.24
BFI neuroticism score	Y1F	9804	3	15	8.42	2.9

Externalizing Psychopathology. In each survey, behaviors in the externalizing domain were assessed via antisocial behavior (ASB) items from the SSAGA (e.g. destruction of property, theft, carrying a weapon) and lifetime and past-year use of illicit drugs such as cannabis, cocaine, sedatives, stimulants, and opioids. Due to limited information in the questions and low endorsement rates for individual illicit drugs at each wave, a count of the number of different classes of illicit drugs (cannabis, sedatives,

stimulants, cocaine, opiates) taken over the lifetime was calculated for each individual to index propensity towards polysubstance use. The surveys also included assessments of impulsivity-related dimensions with the Conscientiousness subscale of the BFI and the UPPS-P scale (Lynam, Smith, Whiteside, & Cydera, 2006). Descriptive statistics for these measures are shown in Table 2.5.

Table 2.5 Descriptive statistics for drinking motive scores.

	Time	N	Min	Max	Mean	SD
ASB	Y1F	9739	0	18	2.15	2.27
ASB	Y1S	7422	0	9	0.49	1.16
ASB	Y2S	3650	0	9	0.45	1.05
ASB	Y3S	2882	0	8	0.43	1.04
ASB	Y4S	1602	0	7	0.38	0.98
BFI conscientiousness score	Y1F	9808	3	15	13.19	1.88
UPPS negative urgency	Y1F	9230	1	4	2.18	0.74
UPPS lack of premeditation	Y1F	9267	1	4	1.78	0.59
UPPS lack of perseverance	Y1F	9262	1	4	1.68	0.55
UPPS sensation seeking	Y1F	9260	1	4	2.91	0.67
UPPS positive urgency	Y1F	9232	1	4	2.00	0.72
Polysubstance count	Max	9889	0	5	0.93	1.26

Note: ASB = Antisocial behavior

Psychosocial/Environmental Risk and Protective Factors. The S4S surveys assess numerous environmental and psychosocial constructs that have demonstrated associations with alcohol use and psychopathology. Among these are parenting behaviors, specifically the Involvement and Autonomy Granting subscales of Steinberg's Parenting Style scale (Steinberg, Lamborn, Dornbusch, & Darling, 1992), peer deviance (the proportion of one's friends who engage in deviant behaviors such as getting drunk and cutting school, as described by Kendler, Jacobson, Myers, and Eaves [2008]), other dimensions of personality in the BFI, and exposure to traumatic events

such as an assault or natural disaster, measured by the Life Events Checklist (Gray, Litz, Hsu, & Lombardo, 2004). These measures are described in Table 2.6.

Table 2.6. Descriptive statistics for psychosocial/environmental measures.

	Time	N	Min	Max	Mean	SD
Parental involvement	Y1F	7368	3	12	8.17	2.18
Parental autonomy granting	Y1F	7398	3	12	9.61	2.08
Peer deviance	Y1F	9725	0	24	8.49	5.22
Peer deviance	Y1S	7433	0	24	9.06	5.15
Peer deviance	Y2S	4714	0	24	8.70	4.92
Peer deviance	Y3S	2888	0	24	8.31	4.68
Peer deviance	Y4S	1593	0	24	8.28	4.61
Traumatic events	Lifetime	9811	0	5	1.89	1.31
Traumatic events	Pre-College	9721	0	5	1.53	1.22
Traumatic events	During College	8044	0	5	1.02	1.15

IV. Genotyping

Information about genotyping for this sample has been described in detail elsewhere (Webb et al., 2017). Briefly, DNA was extracted from saliva samples collected via Oragene kits and isolated according to manufacturers' protocol (see also Dick et al., 2014). Samples were genotyped on the Axiom BioBank Array, Catalog Version 2 (Affymetrix Inc., Santa Clara, CA). The array is designed to assay ~653,000 single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels) including ~296,000 common variants that serve as a backbone for imputation and genome-wide analyses, and ~357,000 variants with predicted functional consequences, including non-synonymous, loss of function, known disease-causing, splice altering, expression quantitative trait (eQTL), and pharmacogenetics-related loci. The imputation panel is designed to capture additional genetic diversity present in African ancestral

populations. Basic quality control procedures were first applied to remove poor quality SNPs (missingness >5%, Hardy-Weinberg equilibrium (HWE) p values < 10e-6) and individual samples (genotyping rate < 98%, heterozygosity outliers, phenotypic/genotypic sex discordance, excess relatedness). Following this, samples were imputed to the 1000 Genomes phase 3 all-ancestries reference panel (The 1000 Genomes Project Consortium, 2015) using SHAPEIT2 for phasing (Delaneau, Marchini, & Zagury, 2012) and IMPUTE2 for imputation (Howie, Donnelly, & Marchini, 2009).

The ethnic diversity in this sample required careful quality control and analytic procedures to avoid inducing spurious results due to population stratification. Genetic ancestry principal components (PCs) were derived from the 1000 Genomes (phase 3) full reference population and projected onto the S4S samples to identify genetically homogenous ancestral groups for analysis, as described by Webb et al. (2017). After this procedure, individuals from five continental ancestral super-populations were available for analysis: Africa (AFR), America (AMR), East Asia (EAS), Europe (EUR), and South Asia (SAS). Within-group ancestry PCs were then calculated within each of these super-populations in order to capture fine-grained differences in allele frequencies that could contribute to residual population stratification. All principal component analyses were conducted using the software package EIGENSTRAT (Patterson, Price, & Reich, 2006). Within groups, additional quality control steps were taken to remove reference population outliers and those with excess relatedness (pi-hat > 0.1). Ancestry-specific filtering on HWE and allele frequency was also used to remove poor quality and uninformative SNPs, as described in more detail as relevant to the specific analyses presented in the following chapters.

Chapter 3. Internalizing And Externalizing Typologies of Alcohol Misuse in College Students

I. Specific Aim

The purpose of the analyses described in this chapter is to empirically validate the existence in this sample of the theorized internalizing and externalizing subtypes of alcohol misuse described in the introduction, and to test whether drinking motives can serve as indicators of these classes. The typologies of alcoholics proposed by Cloninger et al. (1988) and Babor et al. (1992) represent heuristics for grouping individuals into categories based on observable symptoms, clinical characteristics, and associated correlates. These are theoretical models for which the classification criteria are largely driven by expert opinion and/or clinical observations; however, typologies can also be empirically determined by the use of statistical models to identify patterns in the data. These models, known as mixture models (also commonly called latent class or latent profile models), are person-centered approaches that seek to reduce the heterogeneous patterns of responses in a multivariate set of indicators to more homogenous subgroups of individuals whose similarity in item endorsement is presumed to be driven by their membership in unobserved, or *latent*, classes (McCutcheon, 1987). Latent class membership can then parsimoniously describe the variance between individuals in patterns of endorsement of the indicators.

This mixture modeling approach is ideal for identifying subtypes of individuals who might otherwise be grouped into a single outcome category (e.g. AUD diagnosis) despite differences in underlying etiology. In a previous study, Sintov et al. (2010) identified three latent classes of individuals with AUD based on psychiatric comorbidities and personality traits. They found a Mild class with low comorbidity, a Moderate class with high comorbidity of depression and elevated neuroticism scores, and a Severe class with high comorbidity across all disorders as well as the greatest impairment in functioning. It was also found that the Severe class had stronger familial influences than the other classes, suggesting that genetic factors may be more relevant for this type of alcohol misuse.

In addition to modeling the latent underlying structure of responses to a cross-sectional set of indicators, it is also possible to apply mixture modeling approaches to longitudinal data to identify distinct classes of trajectories in stability and change in a trait across time. This method, known as growth mixture modeling (Bauer & Curran, 2003), estimates the intercept, slope, and higher order factors underlying the rate of change in a trait and identifies heterogeneous combinations of these growth parameters that best describe the trajectories in subgroups within the data. A multivariate extension of this model is the parallel process growth mixture model, which applies the same principals to detect latent classes of growth trajectories in multiple traits assessed simultaneously. Such models can be used to identify whether different groups of individuals have distinct developmental trajectories of, e.g., alcohol misuse in parallel with their development of other forms of psychopathology.

The specific aim of the analyses presented in this chapter was thus to apply the mixture modeling approaches of latent profile analysis and parallel process growth mixture modeling to the internalizing, externalizing, and alcohol misuse measures assessed in S4S to see whether subtypes of alcohol misuse differing on internalizing and externalizing characteristics emerged in this college student sample. To assess whether drinking motives could reliably serve as indicators of the resulting subtypes found in the models, we then compared these classes on their endorsement of each type of drinking motive.

II. Methods

The sample for the analyses in this chapter includes the full S4S cohort described in Chapter 2. All participants in the S4S study were assessed on one or more variables included in the analyses, with n = 12 dropped for missingness across all variables (leaving an analytic sample n = 9,877).

Measures included in these analyses are the internalizing items (anxiety/depression symptoms; neuroticism), externalizing items (anti-social behavior; illicit drug use; impulsivity; conscientiousness), and alcohol misuse items (binge frequency; AUD symptom count) described in Chapter 2. First we conducted a series of exploratory factor analytic models to identify the structure of the measures within each domain. We then incorporated these measures into mixture models in two ways. In a latent profile analysis, we included mean values of all traits with repeated measures in order to capture the trait-like values of these measures that best fit with the idea of latent classes representing a stable, characteristic typology. In a complementary growth

mixture model approach, we used the repeated measures from each domain (mean anxiety/depression symptoms, antisocial behavior symptoms, binge frequency, AUD symptoms) to model sets of parallel trajectories of change in these outcomes from freshman to senior year.

Both mixture models were run in Mplus version 7 (Muthén & Muthén, 2011) using one- through six-class solutions and maximum likelihood estimation with robust standard errors to account for missingness and non-normality of the variables. The best fitting model was chosen based on comparison of the Akaike's Information Criteria (AIC; Akaike, 1987), Bayesian Information Criteria (BIC; Schwartz, 1978), and sample size-adjusted BIC (ssBIC; Sclove, 1987). Each of these indices tests the goodness of fit of the model to the data while accounting for the number of estimated parameters (parsimony). As mixture models are probabilistic by nature and assign each individual to a latent class with some level of uncertainty, we compared model entropy and average posterior probabilities of class membership to see how well the latent class assignments matched individuals to a latent class. We also employed the Lo-Mendell-Rubin test (LMR; Lo, Mendell, & Rubin, 2001), which assesses the hypothesis that a *k*-1 class model fits the data better than a *k* class model. A non-significant *p* value (>.05) suggests that the model with one fewer class fits the data better than the model tested.

After latent classes were identified, we tested their relationships with drinking motives and other outcomes of interest. To account for the probabilistic nature of the latent class assignment, multiple imputation was used. Data from each participant was copied *n* times for an *n*-class solution, then one copy was assigned to each class and weighted by the conditional posterior probability of membership in that class (c.f.

Bucholz, Hesselbrock, Heath, Kramer, & Schuckit, 2000). This approach allows for the treatment of latent classes as a categorical variable for comparisons without losing information by treating the probabilistic class assignment as absolute values. Associations between latent class membership and mean drinking motives, demographic characteristics, and other relevant outcomes were then analyzed in R version 3.4 (R Core Team, 2017) using weighted least squares regression for continuous outcomes with the *stats* package and weighted chi-square tests with the *weights* package for categorical outcomes. Sex and mean age across available assessments were included as covariates for the regression analyses.

III. Results

Latent structure of measures. The first set of analyses focuses on exploring the nature of the measures from the three domains of interest (internalizing, externalizing, and alcohol misuse) to verify whether the empirical structure of these measures fit with the theorized structure and to determine which indicators to use in the mixture models. For the alcohol use/misuse and internalizing domains, high correlations were found between all items (Tables 3.1-3.2) and a single latent factor was able to account for 44-47% of the variance across multiple measures/time points. Examination of the scree plots indicated that a one-factor solution was the best fit for the underlying structure of each domain, although the patterns of correlation for each pair of measures (except binge drinking frequency with consumption) suggested that each measure captured some unique information. For the externalizing measures, however, the pattern was less clear. Table 3.3 shows the correlations for these items across time.

Exploratory factor analysis indicated that a correlated four-factor solution best fit the data, explaining 38% of the variance (Table 3.4). The factors could be roughly categorized as: 1) ASB; 2) Behavioral Undercontrol; 3) Urgency; and 4) Reward Sensitivity. Although the best choice of indicators to use was ambiguous from the EFA, previous research has indicated that the externalizing domain has two qualitatively distinct dimensions: aggression and impulsiveness/sensation seeking (Ingole, Ghosh, Malhotra, & Basu, 2015). Further, the negative/positive urgency facets of impulsivity measured by the UPPS have been shown to map onto personality traits of neuroticism and negative emotionality more so than externalizing personality traits (Whiteside & Lynam, 2001). Therefore, the measures of ASB, conscientiousness, sensation-seeking, and polysubstance use were retained for use in the mixture models given their representation of the empirical factors, evidence from previous research of the relevance of these factors to the externalizing domain (Dick et al., 2010; Krueger et al., 2002; Whiteside & Lynam, 2001), and availability of those measures in the largest subset of the sample.

Table 3.1. Correlations between alcohol misuse measures.

	Wave				
Measures	Y1F	Y1S	Y2S	Y3S	Y4S
AUDsx & binge frequency	0.41	0.41	0.47	0.48	0.48
AUDsx & consumption	0.43	0.43	0.43	0.46	0.49
Binge frequency & consumption	0.87	0.85	0.87	0.85	0.85

Note: All p's < 5e-100. AUDsx = alcohol use disorder symptoms, consumption = grams of ethanol per month,

Table 3.2. Correlations between internalizing measures.

	Wave				
Measures	Y1F	Y1S	Y2S	Y3S	Y4S
Depression & anxiety	0.70	0.68	0.68	0.68	0.71
Depression & neuroticism	0.51				
Anxiety & neuroticism	0.44				

Note: All p's < 5e-100.

Table 3.3. Correlations between externalizing measures.

	1	2	3	4	5	6	6	8
1. ASB - Y1F	--							
2. BFI Conscientious	-0.17	--						
3. BFI Extraversion	0.06^^	0.11	--					
4. UPPS Lack of Perseverance	0.16	-0.49	-0.09	--				
5. UPPS Lack of Premeditation	0.21	-0.36	0.11	0.43	--			
6. UPPS Negative Urgency	0.22	-0.20	0.01*	0.16	0.32	--		
7. UPPS Positive Urgency	0.19	-0.21	0.09	0.15	0.34	0.55	--	
8. UPPS Sensation Seeking	0.16	0.02^	0.25	-0.08^	0.10	0.09	0.25	--
9. Polysubstance Use	0.35	-0.12	0.08^^	0.12	0.16	0.11	0.08	0.14

*Note: All p's < 5e-100 except where noted. *p > .05, ^p < .05, ^^p < 5e-10*

Table 3.4. Factor loadings for exploratory factor analysis of externalizing items.

Item	F1	F2	F3	F4
ASB Y1F	**0.57**	0.00	0.03	0.07
ASB Y1S	**0.66**	0.03	-0.03	0.02
ASB Y2S	**0.71**	-0.02	-0.03	0.00
ASB Y3S	**0.67**	-0.03	0.06	-0.05
ASB Y4S	0.14	0.18	0.02	**0.40**
Conscientiousness	-0.03	**-0.62**	-0.08	0.05
Extraversion	-0.1	-0.17	0.02	**0.42**
Lack of Perseverance	-0.01	**0.78**	-0.04	-0.02
Lack of Premeditation	-0.03	**0.48**	0.20	0.21
Negative Urgency	0.03	0.10	**0.58**	0.02
Positive Urgency	0.00	-0.01	**0.90**	-0.01
Sensation Seeking	0.04	-0.19	0.18	**0.39**
Polysubstance Use	0.13	0.15	-0.11	**0.42**

Note: Bolded values represent the strongest loading factor for each item.

Latent profile analysis. With these measures included (and averaged across waves, in the case of repeated measures), latent profile models were fit to the data. Model fit comparisons for the 1- through 6-class model are displayed in Table 3.5. The AIC/BIC/sBIC did not indisputably identify a best-fitting class; however, as Bucholz et al. (2000) and Nylund, Asparouhov, and Muthén (2007) note, these criteria are not always ideal for model selection as they tend to suggest splitting the dataset into infinitely smaller classes, especially when the sample size is large. The three-class solution was thus chosen as the best fit, given the evidence from the LMR test that it fit better than a four-class solution as well as the higher entropy of the model.

Table 3.5. Model fit indices for the latent profile analysis.

Classes	-2LL	AIC	BIC	sBIC	Entropy	LMR
1	-116822.385	233680	233810	233753		
2	-111923.251	223902	224104	224015	0.857	0
3	**-108281.774**	**216639**	**216913**	**216792**	**0.887**	**0**
4	-106981.52	214059	214404	214252	0.883	0.523
5	-105824.041	211764	212181	211997	0.854	0.175
6	-104630.19	209396	209885	209669	0.860	0

Note: Bolded text indicates the chosen latent class solution. -2LL = -2 loglikelihood; AIC =Akaike's Information Criteria; BIC = Bayesian Information Criteria; sBIC = sample-size adjusted BIC; LMR = Lo-Mendell-Rubin test

Endorsement rates on the measures used to conduct the analysis are shown for each class in the three-class model in Figure 3.1 (standardized z-scores to facilitate direct comparison of different scales). The first class, including 70% of the sample, was characterized as "Low Risk" and demonstrated low levels of all internalizing, externalizing, and alcohol misuse measures. The second class, "Internalizing", comprised 19% of the sample and had elevated (+1 SD) rates of anxiety and depression symptoms and neuroticism, low sensation-seeking, low conscientiousness,

and moderately elevated (+0.3 SD) levels of anti-social behavior, polysubstance use, and AUD symptoms, but low levels of binge drinking. The third class, “Externalizing” (10%), conversely had low levels of internalizing symptoms, scores on conscientiousness comparable to the Internalizing class, high levels of all other externalizing measures, and particularly high levels of AUD symptoms (+1 SD) and binge drinking (+2.5 SDs).

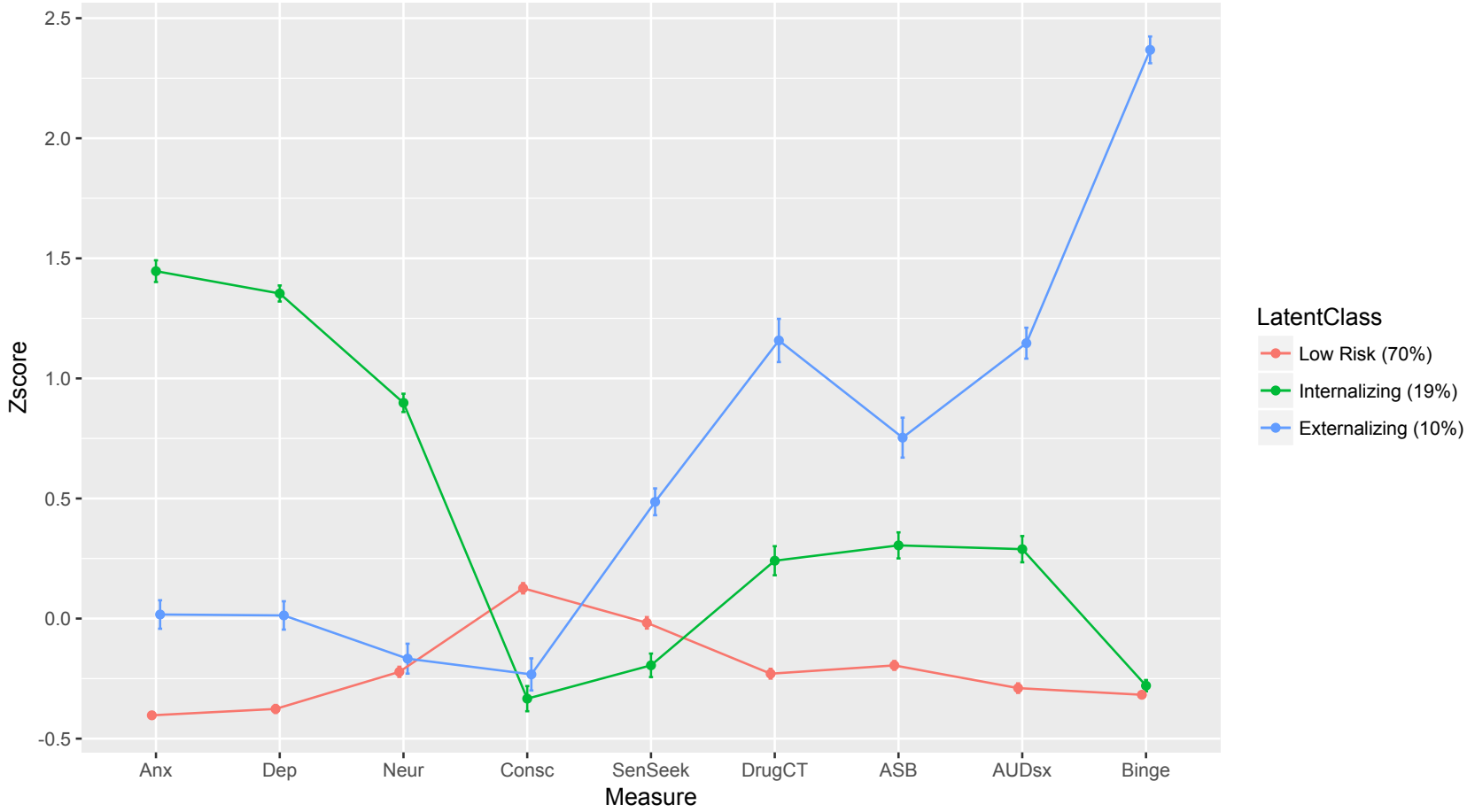

Figure 3.1. Endorsement patterns for standardized internalizing, externalizing, and alcohol misuse measures in the three-class latent profile model solution.

Note: Anx = anxiety, Dep = depression, Neur = neuroticism, Consc = Conscientiousness, SenSeek = sensation seeking, DrugCT = polysubstance count, ASB = antisocial behavior, AUDsx = alcohol use disorder symptoms, Binge = binge drinking frequency

Class comparisons on drinking motives and other relevant outcomes are shown in Table 3.6. The Internalizing and Externalizing classes had higher levels of all four drinking motives than did the Low Risk class (p < 3e-10). The Externalizing class also had higher Enhancement and Social motives than the Internalizing class. The Internalizing class had slightly higher Conformity and Coping motives than the Externalizing class, although these differences were not statistically significant. A comparison of the distribution of drinking motives in each class can be found in Figure 3.2. The latent classes also differed on nearly every personality trait and outcome measured, as seen in Table 3.6. In comparison to the Low Risk class, the Internalizing and Externalizing classes both had elevated levels of each impulsivity dimension and lower levels of agreeableness, conscientiousness, and resilience. The Externalizing and Internalizing classes also differed on some of these traits, with the Externalizing class having greater lack of premeditation and resilience and the Internalizing class having higher negative urgency. The only dimension for which the Internalizing and Externalizing classes varied in their direction of difference from the Low Risk class was that of extraversion, for which the Internalizing class had significantly lower levels and the Externalizing class had significantly higher levels. The latent classes also differed on demographic characteristics (Table 3.7), with a higher proportion of females and White students in the internalizing class and a higher proportion of males and White students in the externalizing class, relative to the Low Risk class.

Table 3.6. Latent class comparisons on drinking motives and other outcome measures in the latent profile analysis.

	Internalizing vs. Low Risk		Externalizing vs. Low Risk		Internalizing vs. Externalizing	
Measure	*B*	*p*	*B*	*p*	*B*	*p*
Conformity Motive	0.19	3.5E-39	0.13	3.0E-13	-0.06	0.0068
Coping Motive	0.57	2.2E-205	0.54	8.3E-118	-0.04	0.1846
Enhancement Motive	0.14	1.2E-15	0.54	9.2E-140	0.41	9.7E-60
Social Motive	0.10	2.9E-10	0.49	1.8E-118	0.38	1.9E-55
Agreeableness	-0.81	8.6E-78	-0.60	8.8E-27	0.21	0.0013
Extraversion	-1.02	4.0E-68	0.94	1.6E-35	1.96	9.3E-110
Openness	0.13	0.0016	0.12	0.0257	-0.01	0.8601
Lack of Perseverance	0.20	1.7E-66	0.15	3.5E-24	-0.05	0.0067
Lack of Premeditation	0.18	3.6E-49	0.27	2.3E-64	0.09	9.5E-07
Negative Urgency	0.57	2.5e-318	0.42	3.2E-104	-0.15	2.3E-12
Positive Urgency	0.32	4.8E-108	0.36	1.4E-81	0.04	0.0647
Resilience	-0.86	6.6E-136	-0.15	5.0E-04	0.71	1.9E-43

Note: Text colors correspond to the latent classes shown in Figure 3.1, with the color of the class having the higher mean value displayed for each outcome (black text indicates no significant differences after multiple testing correction for 14 tests, adjusted p = .0036).

Table 3.7. Latent class comparisons on demographic characteristics in the latent profile models.

Measure		N (%) Low Risk	N (%) Internalizing	N (%) Externalizing	χ^2	p
Sex	Female	4223 (61)	1391 (74)	422 (41)	300.86	< 2e-16
	Male	2678 (39)	491 (26)	602 (59)		
Ethnicity	American Indian	32 (0)	13 (1)	6 (1)	304.90	< 2e-16
	Black	1239 (18)	281 (15)	91 (9)		
	Asian	1536 (22)	242 (13)	94 (9)		
	Hispanic	421 (6)	113 (6)	59 (6)		
	Multi	428 (6)	131 (7)	56 (5)		
	Hawaiian/ Pacific Islander	47 (1)	12 (1)	8 (1)		
	Other	25 (0)	10 (1)	4 (0)		
	White	3102 (45)	1076 (57)	701 (69)		

Note: Counts in each cell are raw values; percentages are weighted.

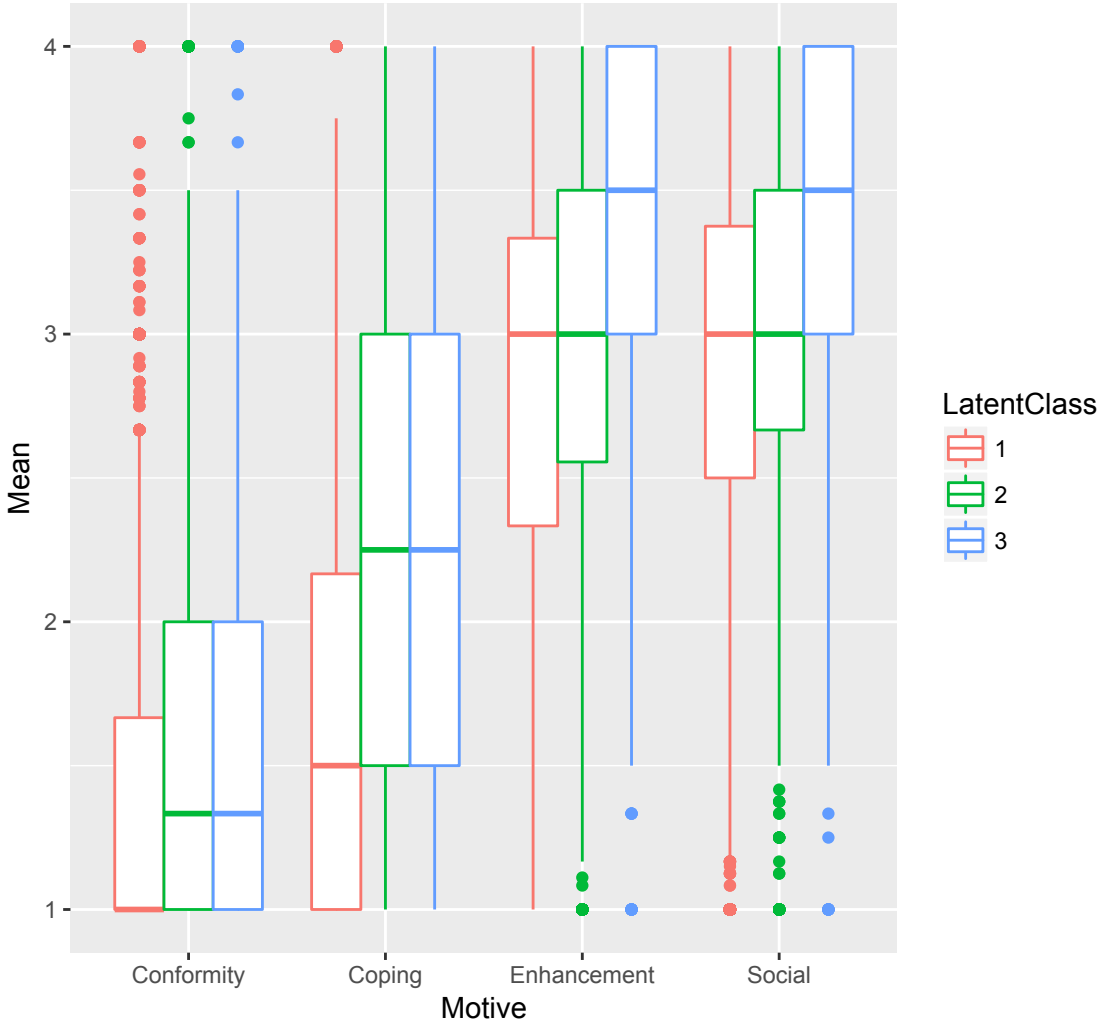

Figure 3.2. Distribution of drinking motive scores between the latent classes.

Growth mixture model. A limitation of the latent class model in the previous section is that it focuses on a single or averaged time point of traits that may be developmentally dynamic. We thus conducted parallel process growth mixture modeling of four traits with repeated measures across the four years of the S4S study: 1) internalizing symptoms, a mean of the SCL-90 anxiety and depression scores which

were highly correlated at each wave; 2) anti-social behaviors; 3) binge drinking frequency; and 4) AUD symptoms. As shown in Table 3.8, the choice of best fitting model was not immediately clear based on fit indices, and estimation problems began plaguing models with higher number of classes despite increasing the number of random starts to obtain a replicated log-likelihood. The three-class solution was chosen based on the LMR test and model entropy.

Table 3.8. Model fit indices for the growth mixture model analysis.

Classes	-2LL	AIC	BIC	sBIC	Entropy	LMR
1	-182767	365755	366546	366197		
2	-179196	358638	359523	359133	0.923	0
3	**-177465**	**355202**	**356180**	**355748**	**0.869**	**0.0001**
4	-176582	353463	354535	354062	0.846	0.09
5	-175431	351186	352352	351837	0.859	1*
6*	-174688	349726	350986	350429	0.874	1*

*Note: Bolded text indicates the chosen latent class solution. *Model did not converge or non-positive definite matrix*

Patterns of the four processes across time for each class are shown in Figure 3.3. Except for binge drinking, levels of all outcomes remained relatively stable across time with flat slopes. Class 1 (8% of the sample) was characterized by higher levels of AUDsx and binge drinking at all waves and with a decreasing trajectory of binge drinking after Y1F. Class 2 (9%) had the highest levels of ASB peaking at Y2S, intermediate levels of alcohol misuse, and modestly higher levels than the other classes of internalizing symptoms. However, the temporal stability of each of these outcomes and the mostly parallel slopes between classes suggests that this model may be capturing only quantitative differences in severity rather than meaningful qualitative differences between individuals, which is the aim of mixture models.

Class comparisons on drinking motives (Table 3.9) indicated that both Class 1 and 2 had higher levels than Class 3 of all motives, and that Class 1 had higher levels than Class 2 of these same motives except conformity. Class 1 was particularly elevated on Enhancement/Social motives. Class 1 also had higher levels (relative to both Class 2 and 3) on virtually all other indicators, although Class 3 had higher levels of agreeableness. There was a higher proportion of males and Whites in both Classes 1 and 2 relative to Class 3 (Table 3.10).

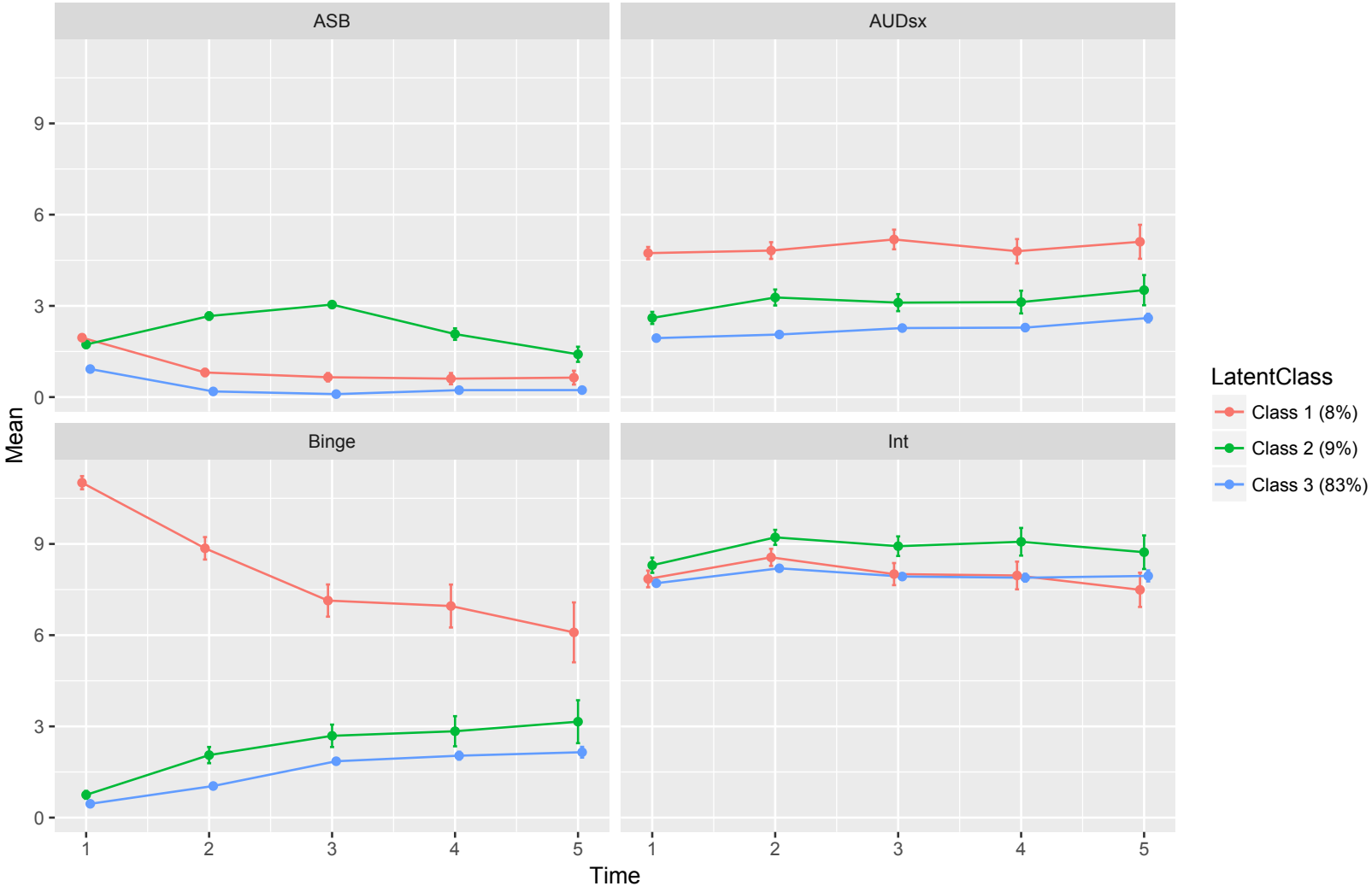

Figure 3.3. Parallel growth trajectories in internalizing, externalizing, and alcohol misuse measures across four years of college in the three latent class model solution.

Table 3.9. Latent class comparisons on drinking motives and other outcome measures in the parallel process growth mixture models.

	Class 1 vs. Class 3		Class 2 vs. Class 3		Class 1 vs. Class 2	
Measure	*B*	*p*	*B*	*p*	*B*	*p*
Conformity Motive	0.08	9.3E-06	0.05	0.0028	0.03	0.2336
Coping Motive	0.37	1.2E-48	0.22	7.3E-20	0.15	0.0000
Enhancement Motive	0.51	6.8E-115	0.09	1.5E-05	0.42	9.0E-47
Social Motive	0.45	1.8E-90	0.07	0.0010	0.38	2.9E-39
Agreeableness	-0.63	1.6E-27	-0.71	6.9E-38	0.08	0.2744
Extraversion	1.22	1.8E-54	-0.01	0.9085	1.23	2.9E-33
Openness	0.03	0.6300	0.07	0.1942	-0.04	0.5604
Lack of Perseverance	0.10	3.9E-10	0.08	4.7E-08	0.02	0.4126
Lack of Premeditation	0.23	9.5E-47	0.12	6.3E-16	0.11	2.6E-07
Negative Urgency	0.31	1.5E-51	0.13	7.6E-11	0.18	5.6E-12
Positive Urgency	0.29	1.1E-48	0.10	2.3E-07	0.19	<5E-100
Resilience	0.05	0.3404	-0.07	0.1275	0.11	0.0659

Note: Text colors correspond to the latent classes shown in Figure 3.3, with the color of the class having the higher mean value displayed for each outcome (black text indicates no significant differences after multiple testing correction for 14 tests, adjusted p = .0036).

Table 3.10. Latent class comparisons on demographic characteristics in the parallel process growth mixture models.

Measure		N (%)	N (%)	N (%)	χ^2	p
		Class 1	Class 2	Class 3		
Sex	Female	352 (45)	381 (44)	5299 (65)	300.86	< 2e-16
	Male	437 (55)	481 (56)	2842 (35)		
Ethnicity	AI	5 (0.6)	7 (0.8)	39 (0.4)	304.90	< 2e-16
	Black	68 (9)	117 (14)	1421 (18)		
	Asian	80 (10)	112 (13)	1679 (21)		
	Hispanic	45)6)	54 (6)	493 (6)		
	Multi	45 (6)	56 (7)	514 (6)		
	Hawaiian/PI	9 (0.4)	5 (0.6)	53 (0.7)		
	Other	3 (0.4)	8 (0.9)	28 (0.3)		
	White	528 (67)	497 (58)	3848 (48)		

Note: Counts in each cell are raw values; percentages are weighted

IV. Summary and Discussion

This set of analyses reflected a broad investigation of internalizing, externalizing, and alcohol-related psychopathology in college students and the relation of these domains to drinking motives. We found that the structure of internalizing and externalizing symptoms/traits previously identified in the literature was largely upheld in this sample, although the externalizing domain had somewhat lower consistency between measures. This may not be as homogenous a set of traits/behaviors as the internalizing domain, as some others have also found (Ingole et al., 2015). Using mixture models, we found that divergent internalizing and externalizing subtypes emerged that had distinctive patterns of alcohol misuse and differed substantially on drinking motives and a variety of traits and behaviors.

Our findings lend additional support to the theoretical and empirical literature proposing the existence of internalizing and externalizing subtypes of alcohol misuse (Babor et al., 1992; Cloninger et al., 1988; Sintov et al., 2010). These results are particularly consistent with the typology found by Sintov and colleagues (2010), in which the more severely affected class among a sample of alcohol-dependent participants had higher levels of externalizing traits and a broad array of comorbid conditions, whereas the moderately affected class had higher rates of internalizing traits and comorbid internalizing disorders specifically. Their severe/externalizing class also demonstrated higher heritability. In the present analyses, the Externalizing class exhibited much higher rates of multiple domains of alcohol misuse and reported elevated levels of all four types of drinking motives, while the Internalizing class had specifically elevated rates of AUD symptoms but not binge drinking and negative reinforcement but not

positive reinforcement motives. This class also differed from both the Externalizing and Low Risk classes by having a lower level of positive affect (extraversion).

Using parallel process growth mixture models, we also found that the internalizing, externalizing, and alcohol misuse domains are relatively consistent across the college years, or at least that there do not appear to be qualitatively distinct classes of trajectories that separate subgroups of individuals. Results from these models showed almost exclusively quantitative differences between groups, with those highest in a trait at one point remaining highest across all waves. However, there was some evidence of a separation of internalizing/externalizing domains here as well, as the class with the highest levels of binge drinking/AUDsx (Class 1) also had higher levels of impulsivity-related traits and extraversion/sensation-seeking and marginally lower levels of neuroticism than the class with the highest levels of internalizing symptoms (Class 2). As in the latent profile model, alcohol misuse appears to relate most strongly to measures from the externalizing domain, though there is a distinct, weaker path of association from the internalizing domain – which is more clearly separated by using a typology like in the latent profile analysis. However, an important caveat here is that the anti-social behavior measures did not perform as expected in this sample, having weak correlations with other externalizing domain traits and in fact being highest in the growth model in the class with highest internalizing symptoms. It is unclear why such unexpected results occurred, but these may be a function of the small number of ASB items in the sample and low rates of endorsement. The college student sample is likely to have lower levels of illegal/anti-social behaviors than the general population and thus the structure of the externalizing domain, particularly with regard to these behaviors,

may not be representative of the structure that has been seen in other (adult) populations. Alternatively, ASB may not be as clearly a discriminator of externalizing behavior as previously believed, at least in the context of other psychopathology.

The results of this study also provide important insights regarding drinking motives. Drinking motives do appear to be useful measures to index distinct pathways of alcohol misuse: a broadband externalizing pathway with heightened risk of all forms of all drinking motives and all types of alcohol misuse, and a specific internalizing pathway to AUD, rather than binge/heavy use, with a specifically elevated level of negative reinforcement motives. These findings are consistent with a number of other studies that have found negative reinforcement (coping) motives to be associated with AUD/alcohol problems while positive reinforcement (enhancement) motives are more strongly associated with heavy use and frequency/quantity, and less consistently AUD (Kuntsche et al., 2005). Further, these results also map on to research identifying a specific association between mood/anxiety disorders and AUD but not frequency/quantity measures (Savage et al., 2016; Schry & White, 2013) and a broadband association between conduct disorder, ASPD, illicit drug use, and impulsivity dimensions and a range of alcohol use and misuse behaviors (Comeau et al., 2001; Stautz & Cooper, 2013). The fact that such patterns of endorsement of drinking motives map onto different subtypes of alcohol misuse provides a compelling link between these two fields of research.

A few important limitations should be noted to contextualize the interpretation of these results. First, the resulting class structure of mixture models is highly dependent on the choice of indicators included in the model. Therefore we may have identified very

different latent classes had other items been used to index internalizing/externalizing behavior or alcohol misuse. Comparison between the latent profile model and the parallel process growth mixture model suggests that including repeated measures of these items would not substantially improve the classification; however, other measures not included in the S4S study might better index the true underlying nature of these domains. Related, many of the measures were constructed from reduced scales of previously validated instruments and were assessed via self-report, so issues of validity and accuracy in the measures are not trivial. However, the consistency of these results with previous classification studies and their coherence within the theoretical model lends credence to their validity. We will return to a further discussion of these results and their implications in Chapter 7.

Our findings validate the existence of the hypothesized internalizing and externalizing pathways to alcohol misuse and provide some initial evidence that drinking motives both index and can be used to better understand these two correlated but distinct pathways. In the following chapter, we begin to explore the nature of how drinking motives fit into these pathways and how they develop across time.

Chapter 4. Epidemiology and Development of Drinking Motives Across College

I. Specific Aim

Despite being robust proximal predictors of numerous alcohol use/misuse behaviors (Kuntsche et al., 2005), the epidemiology of drinking motives themselves has been rarely studied. As we have established in the previous chapter that drinking motives appear to be intermediate factors in the pathway between (genetic) predispositions and alcohol misuse outcomes, it is a key next step to resolve this gap in the existing scientific knowledge.

As potential endophenotypes or intermediate outcomes of interest, knowledge of some of the basic epidemiological aspects of drinking motives is needed: how reliable are they, how they change (or don't change) across time, and what factors are associated with the development of different types of motives – and might thus be used to modify them or predict individual risk. Surprisingly, although drinking motives have been studied often in relationship to alcohol use/misuse outcomes (Kuntsche et al., 2005) and even in several mediational models linking them to basic personality/temperament domains (Adams et al., 2012; Littlefield et al., 2011; Mezquita et al., 2010), we know little about their correlates or developmental course. In particular, some risk/protective factors like parenting behaviors, peer deviance, and trauma/stress exposure have been robustly associated with alcohol misuse (Stone, Becker, Huber, & Catalano, 2012) but their relationships with drinking motives – and how these

relationships might differ between drinking motive types – has not yet been examined. Knowledge about these basic aspects of drinking motives could help researchers to think about how they might be better utilized to understand the (genetic) etiology of alcohol misuse.

In addition, for motives to serve as useful endophenotypes of alcohol misuse in future research and application we must first establish the mechanism by which their association with alcohol misuse occurs. Critically, the issues of whether their association is due to a causal relationship or a shared underlying liability must be resolved because that will determine the level at which intervention/treatment efforts must be directed in order to be most effective. For example, if one's drinking motives have a direct causal effect on alcohol misuse behaviors, efforts to reduce an individual's alcohol misuse might best be carried out through cognitive therapy to modify motivations for drinking into a healthier framework. Alternatively, if a confounding shared liability drives the association, efforts aimed at changing motivations would not address the underlying cause that leads one to engage in harmful alcohol use. It is similarly unknown whether the relationships between drinking motives and internalizing and externalizing traits/symptoms stem from a common predisposition, a causal effect of internalizing/externalizing psychopathology driving motivations to drink, or a causal effect of alcohol consumption leading to changes in psychopathology due to acute/chronic exposure or withdrawal. Again, lack of clarity in the causal directions of relationships between all of these outcomes can lead to ineffective or inefficient application of prevention and treatment efforts.

There are few ways of getting around this issue in most psychological research due to the inability to ethically conduct randomized control trials of such outcomes. However, some quasi-experimental designs can work around this challenge using longitudinal, prospectively collected data, as is available in S4S. One such design is the cross-lagged structural equation model, a robust test for spuriousness that can give insight into whether a correlation between two variables is likely due to confounding versus a directional association (Kenny, 1975; Kenny & Harackiewicz, 1979). The logic of the design is that the time-lagged associations between two (or more) variables at multiple waves can point to which variable is the stronger, and therefore more likely causal, predictor of the other variable. If the contemporaneous correlation between two variables is a function of confounding, each variable at time *n* should be an equally good predictor of the other at time *n*+1 because their association goes through the same path of the unobserved confounding factor. However, in a causal association, the causal factor should be a more reliable predictor of later values of the other factor and thus the cross-lagged correlations between measures at *n* and *n*+1 time points should be unequal.

Although coping and enhancement drinking motives are established predictors of later alcohol use outcomes, there is also some evidence for reciprocal causality, whereby alcohol use/misuse affects future drinking motives. Two cross-lagged studies of drinking motives have been previously conducted in Dutch samples, with conflicting results. One with young adolescents (age 13-16) found no evidence for reciprocity across a one-year period (Schelleman-Offermans, Kuntsche, & Knibbe, 2011), while one with adults (mean age 53) found that drinking frequency had a stronger influence

on motives than vice-versa across a three-month period (Crutzen, Kuntsche, & Schelleman-Offermans, 2013). Recently, a third study of young adult Swedish males undergoing military conscription found the opposite directional effect of enhancement motives leading to higher alcohol consumption and problems (Labhart, Kuntsche, Wicki, & Gmel, 2016). These limited results indicate that the relationship between motives and alcohol misuse is dynamic and likely shifting during the period of young adulthood when patterns of drinking behaviors (especially problem behaviors) are being cemented. There is a corresponding need for an investigation of how these associations unfold across time and an incorporation of how internalizing and externalizing psychopathology may drive the development of such relationships.

The aim of the set of analyses presented in this chapter is thus to conduct basic descriptive analyses about drinking motives and their environmental risk and protective factors, and to investigate the etiology of their relationships with alcohol misuse and internalizing/externalizing psychopathology using cross-lagged models to tease apart the question of causality versus shared etiology.

II. Methods

These analyses involve the full S4S sample described in Chapter 2, using repeated measures of drinking motives, alcohol misuse, internalizing (mean anxiety/depression) and externalizing (ASB) symptoms assessed at each wave. We also examined mean drinking motives in the context of demographic measures (age, sex, ethnicity) and environmental variables (parenting, peer deviance, trauma exposure) assessed by the same self-report survey.

Data analysis involved three parts. First, we examined the correlations between drinking motives across waves to measure their temporal stability. Second, we conducted linear regression analyses to examine how demographic and environmental characteristics predicted mean levels of each type of drinking motive. Third, we investigated the cross-sectional relationships between drinking motives, alcohol misuse, and internalizing and externalizing psychopathology using correlations and their longitudinal relationships using the cross-lagged panel design. A total of eight cross-lagged models were run: four with each of the drinking motives examined separately, times two sets of separate models for binge drinking frequency and AUD symptoms as the alcohol misuse outcome. An example of the structure of the model is visualized in Figure 4.1. These analyses were conducted using structural equation modeling to specify the relationships between variables, and were carried out using the OpenMx package (Boker et al., 2011) in R using full information maximum likelihood estimation. We used data from Y1F to Y3S, given that only two cohorts have completed the Y4S wave and so the sample size for this wave is small for the complexity of model fitting.

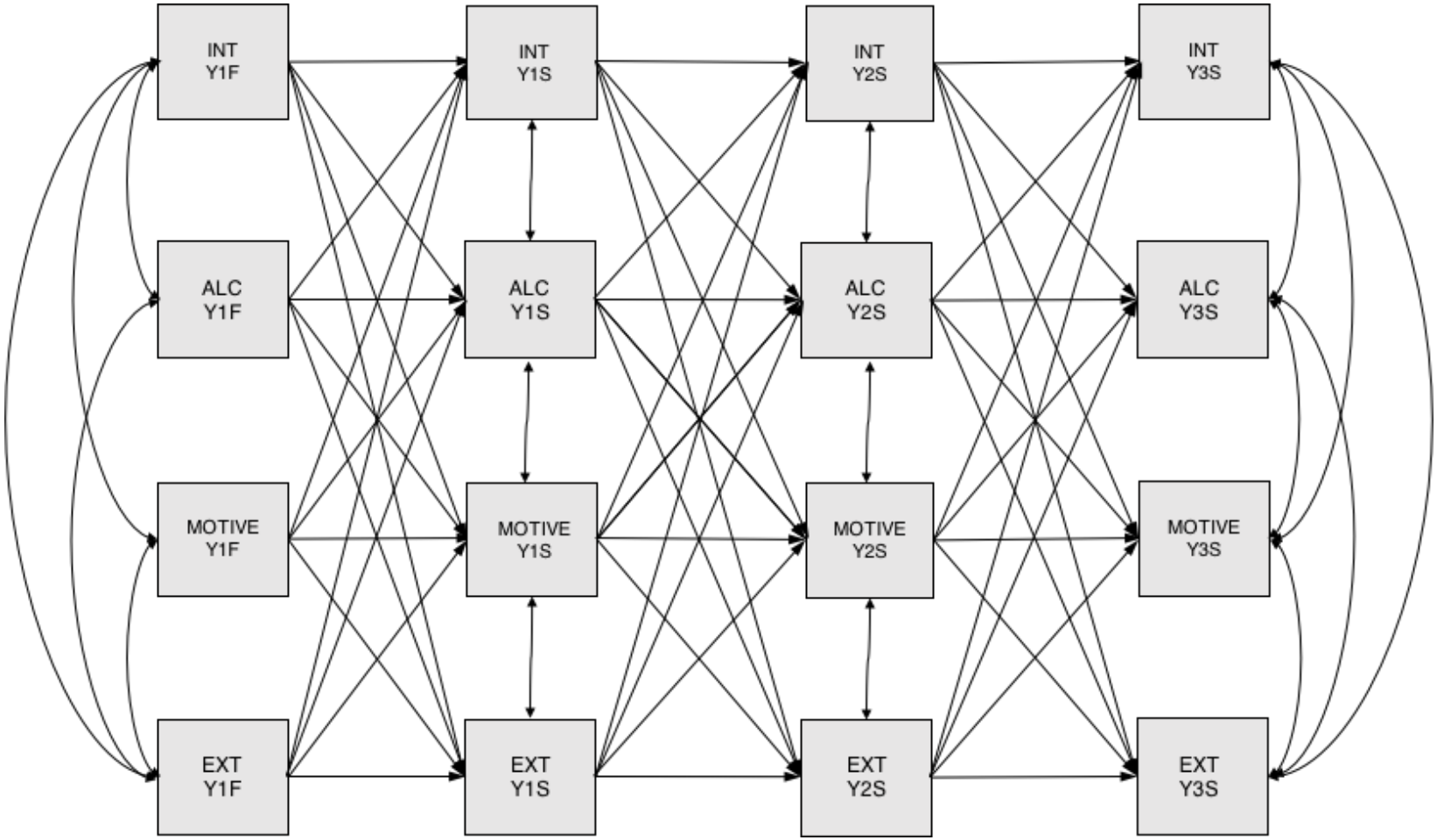

Figure 4.1. Example illustration of the cross-lagged model.

III. Results

Correlations between each of the motives across time are shown in Table 4.1. Although there was some evidence of a decay in the strength of correlation with time (e.g. year 1 with year 4), each pair of temporally adjacent time points was moderately correlated with a high statistical significance (p < 5e-100). Conformity motives showed the weakest correlations across time but also had the lowest levels of endorsement as described in Chapter 2. Mean drinking motive scores across college are shown in Figure 4.2 and demonstrate that endorsement of all four drinking motives increased slightly across time but remained relatively stable. Within-time correlations between the four subtypes of motives are presented in Table 4.2. The patterns were similar at each wave, and showed strong correlations within the positive valence dimension (social – enhancement), modest correlations between positive valence motives and conformity, and modest/moderate correlations between coping and the three other motives.

Table 4.1. Cross-time correlations for drinking motives across five waves of assessment.

Wave	Social	Enhancement	Coping	Conformity
Y1F-Y1S	0.467	0.519	0.460	0.432
Y1F-Y2S	0.408	0.389	0.356	0.350
Y1F-Y3S	0.335	0.334	0.324	0.295
Y1F-Y4S	0.313	0.347	0.255^^	0.279
Y1S-Y2S	0.517	0.476	0.403	0.370
Y1S-Y3S	0.409	0.407	0.417	0.302
Y1S-Y4S	0.383	0.416	0.377	0.260^^
Y2S-Y3S	0.450	0.430	0.428	0.460
Y2S-Y4S	0.481	0.445	0.402	0.207^^
Y3S-Y4S	0.550	0.558	0.557	0.431

Note: All p's < 5e-100 except where noted. ^^p < 5e-10

Table 4.2. Within-time correlations between motive subscale scores.

Time	Motive			
		Social	Enhancement	Coping
Y1F	Enhancement	0.630		
	Coping	0.270	0.300	
	Conformity	0.110	0.040^	0.260
Y1S	Enhancement	0.600		
	Coping	0.290	0.330	
	Conformity	0.130	0.050^	0.280
Y2S	Enhancement	0.560		
	Coping	0.340	0.320	
	Conformity	0.140	0.130^^	0.230
Y3S	Enhancement	0.500		
	Coping	0.290	0.340	
	Conformity	0.190^^	0.120^^	0.260
	Enhancement	0.480		
Y4S	Coping	0.320	0.340	
	Conformity	0.230	0.130^^	0.290

Note: All p's < 5e-100 except where noted. ^p < .001, ^^p < 5e-10

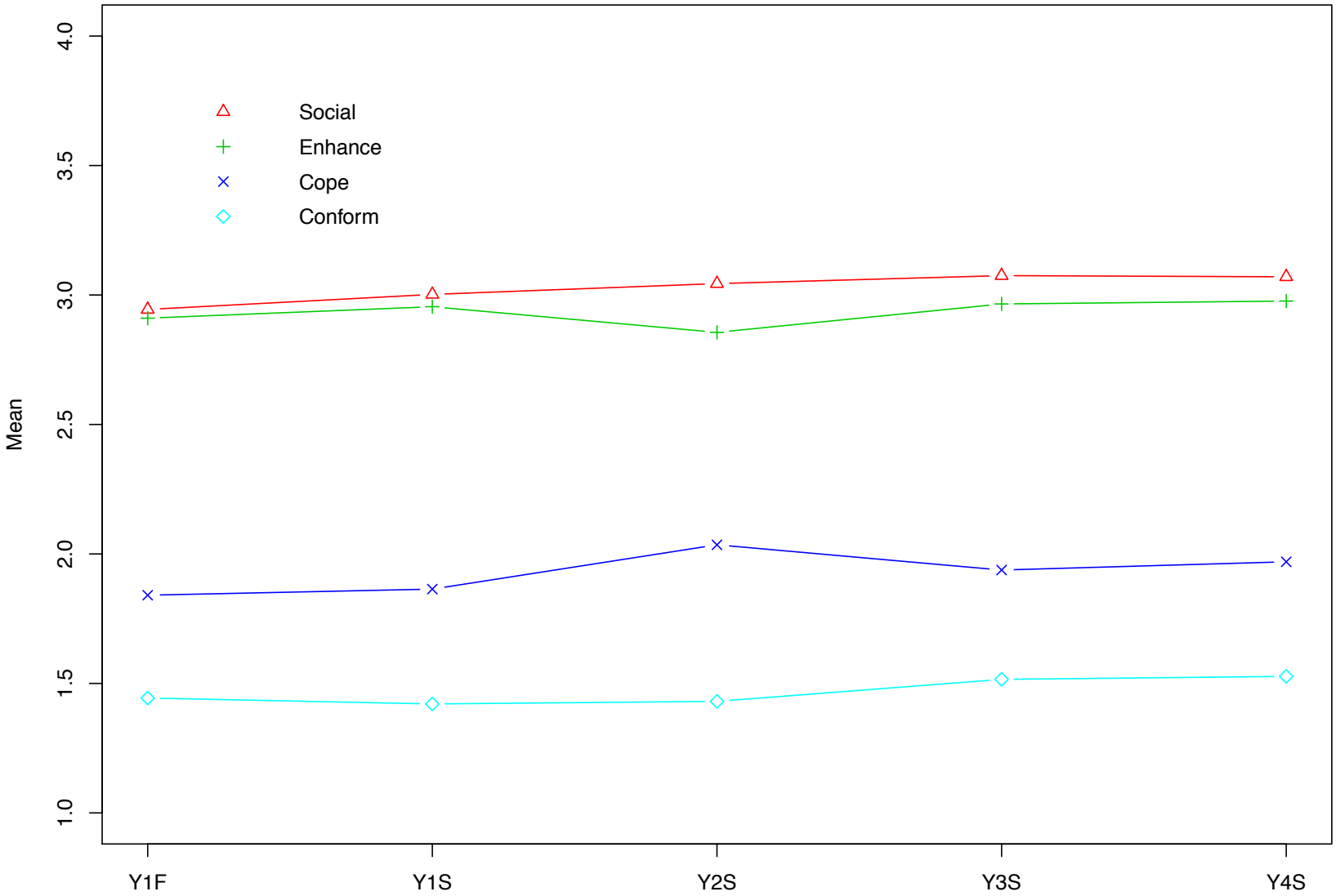

Figure 4.2. Mean values of drinking motive scores across five waves of assessment.

Results from the linear models of environmental predictors of drinking motives are displayed in Table 4.3. Males had higher levels of conformity motives than females, and older age was also modestly associated with higher levels of this type of motive. Students from ethnic minorities generally had lower or not significantly different levels of all four drinking motives as compared to White students; however, Asian ethnicity was associated with higher levels of externally driven motives (conformity and, marginally, social). Lifetime trauma exposure was uniquely associated with higher levels of coping motives, in fact even showing the reverse direction of effect for social motives, with higher level of trauma exposure predicting lower mean social motives. Parental autonomy granting was associated with lower levels of all drinking motives, while parental involvement predicted higher levels of positive reinforcement (social and enhancement) motives. Peer deviance was associated with higher levels of all motives except conformity.

Table 4.3. Linear regression results of demographic and environmental factors predicting drinking motives.

	Social		Enhancement		Coping		Conformity	
Predictor	Estimate	P	Estimate	P	Estimate	P	Estimate	P
Sex (male)	0.04	0.05	0.01	0.69	-0.01	0.71	**0.13**	**7E-14**
Age	0.02	0.04	0.01	0.39	0.01	0.27	**0.02**	**6E-03**
Ethnicity								
American Indian	-0.03	0.86	0.04	0.81	0.05	0.75	-0.19	0.14
Asian	0.08	7E-03	**-0.15**	**4E-07**	0.04	0.20	**0.21**	**2E-16**
Black	-0.02	0.44	**-0.10**	**2E-04**	**-0.10**	**5E-04**	**-0.09**	**1E-05**
Hispanic	0.01	0.72	-0.03	0.43	**-0.13**	**4E-03**	-0.06	0.10
Multiracial	-0.01	0.71	0.03	0.52	0.00	0.92	-0.05	0.17
Hawaiian/ Pacific Islander	0.04	0.74	-0.15	0.19	-0.02	0.91	-0.07	0.50
Unknown	-0.08	0.30	-0.11	0.18	-0.07	0.44	-0.02	0.77
Trauma count (during college)	0.02	0.06	0.02	0.08	0.03	0.03	**0.04**	**1E-05**
Trauma count (lifetime)	**-0.03**	**4E-04**	-0.01	0.51	**0.03**	**4E-03**	-0.02	0.04
Parental involvement	**0.02**	**1E-04**	**0.03**	**7E-08**	-0.01	0.05	0.00	0.23
Parental autonomy granting	**-0.02**	**1E-06**	**-0.02**	**1E-05**	**-0.04**	**4E-15**	**-0.03**	**2E-14**
Mean peer deviance	**0.07**	**2E-16**	**0.07**	**2E-16**	**0.04**	**2E-16**	0.00	0.20

Note: Reference category for the Ethnicity measure is White. Bolded values are significant after multiple testing correction for 8 predictor variables, adjusted p = .0062.

Contemporaneous correlations between drinking motives and internalizing, externalizing, and alcohol misuse measures are presented in Table 4.4 (Y1F correlations presented for items with repeated measures, which showed similar associations at each wave). Negative reinforcement (particularly coping) motives were most strongly correlated with internalizing measures of anxiety, depression, and neuroticism, while positive reinforcement motives were most strongly associated with sensation seeking and polysubstance use. Antisocial behavior and conscientiousness were similarly correlated with both dimensions of drinking motives. Coping motives had the strongest correlation with AUD symptoms (r = 0.30), while social/enhancement motives were most strongly correlated with alcohol consumption measures of binge frequency and grams of ethanol consumed per month (r = 0.25-0.31).

Table 4.4. Correlations between drinking motives and internalizing, externalizing, and alcohol use measures.

Measure	Social	Enhancement	Coping	Conformity
SCL90 - Anxiety	-0.01	0.01	0.25**	0.12**
SCL90 - Depression	0.03	0.03	0.29**	0.08**
BFI - Neuroticism	0.00	0.01	0.18**	0.04*
Antisocial behavior	0.19**	0.19**	0.21**	0.10**
BFI - Conscientiousness	-0.08**	-0.05*	-0.11**	-0.13**
UPPS - Sensation Seeking	0.17**	0.19**	0.07*	0.04*
Polysubstance use count	0.23**	0.24**	0.13**	-0.07
AUD symptoms	0.18**	0.22**	0.30**	0.16**
Binge frequency	0.25**	0.27**	0.14**	0.05
Grams ethanol/month	0.28**	0.31**	0.15**	0.02

*Note: *p < .01, **p < 5e-10*

Results from the cross-lagged models are presented in Tables 4.5 – 4.6 and Figures 4.3 – 4.10. Eight models in total were tested, in which the internalizing and

externalizing phenotypes remained the same but the alcohol misuse outcomes (binge drinking or AUD symptoms) and the drinking motives varied between models. The estimates of most interest for comparison are the reciprocal cross-lagged paths within a set (e.g. alcohol misuse at Y1F predicting drinking motives at Y1S versus drinking motives at Y1F predicting alcohol misuse at Y1S), although the autoregressive paths (the same outcome predicting itself at a later wave) also indicate stability/change in the phenotype across time. Because there are more than two waves of data, there are multiple intervals between waves and thus multiple sets of cross-lagged paths (see Figure 4.1). These can be compared to see how the relationships between outcomes change throughout different years of college.

The tests of equal cross-lagged paths provide a global test of whether each set of cross-lagged paths is equivalent. As shown in Table 4.5, the cross-lagged paths with internalizing and externalizing traits could largely be constrained to equality without a significant decrease in model fit, indicating that this cross-prediction could not be differentiated from confounding. Standardized coefficients for these paths were small (0.00 – 0.07). The one exception to this trend was for enhancement motives, where the larger coefficients in the direction from motives to internalizing symptoms (0.04-0.07 vs. 0.00 – 0.02) suggested a direct and potentially causal association.

Table 4.5. Goodness-of-fit chi-square tests for longitudinal models in which pairs of cross-lagged path coefficients between drinking motives and outcomes of interest are constrained to equality.

Outcome	Social	Enhancement	Coping	Conformity
	p	*p*	*p*	*p*
Binge	**7E-12**	**2E-11**	0.222	**0.008**
AUD	**0.021**	**8E-06**	0.104	0.077
Internalizing	0.192	**2E-04**	0.086	0.933
Externalizing	0.858	1.000	0.137	0.378

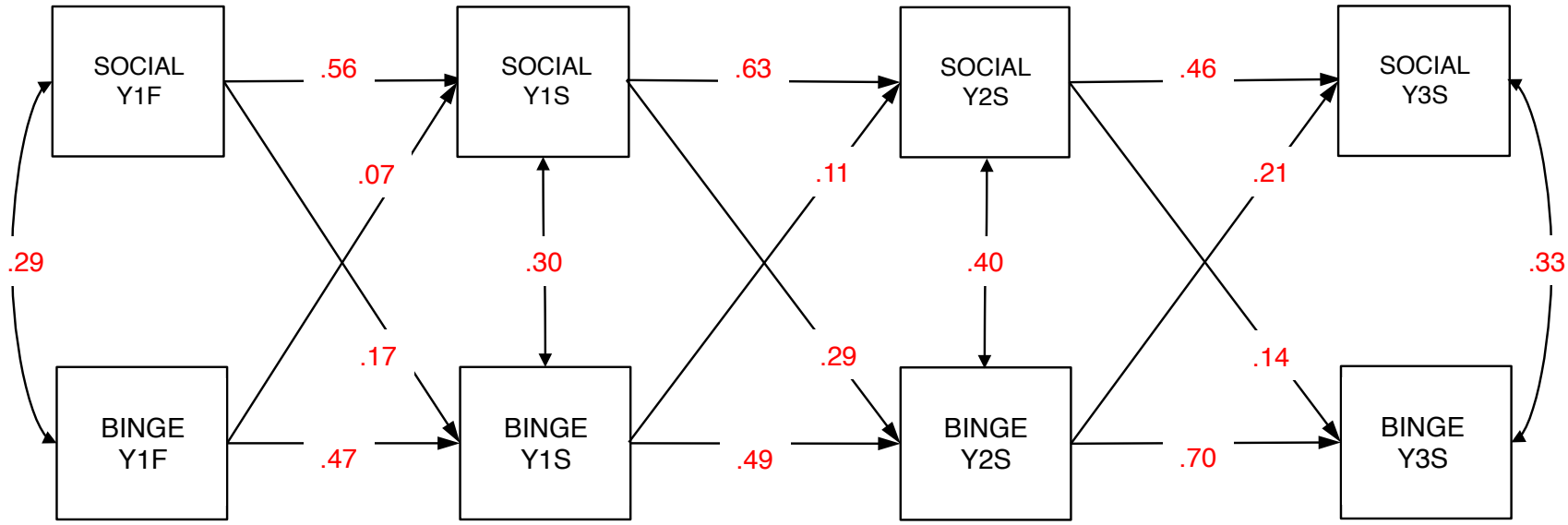

Figure 4.3. Cross-lagged model of social drinking motives and binge drinking frequency.

Note: Coefficients in red are significant, p < .05.

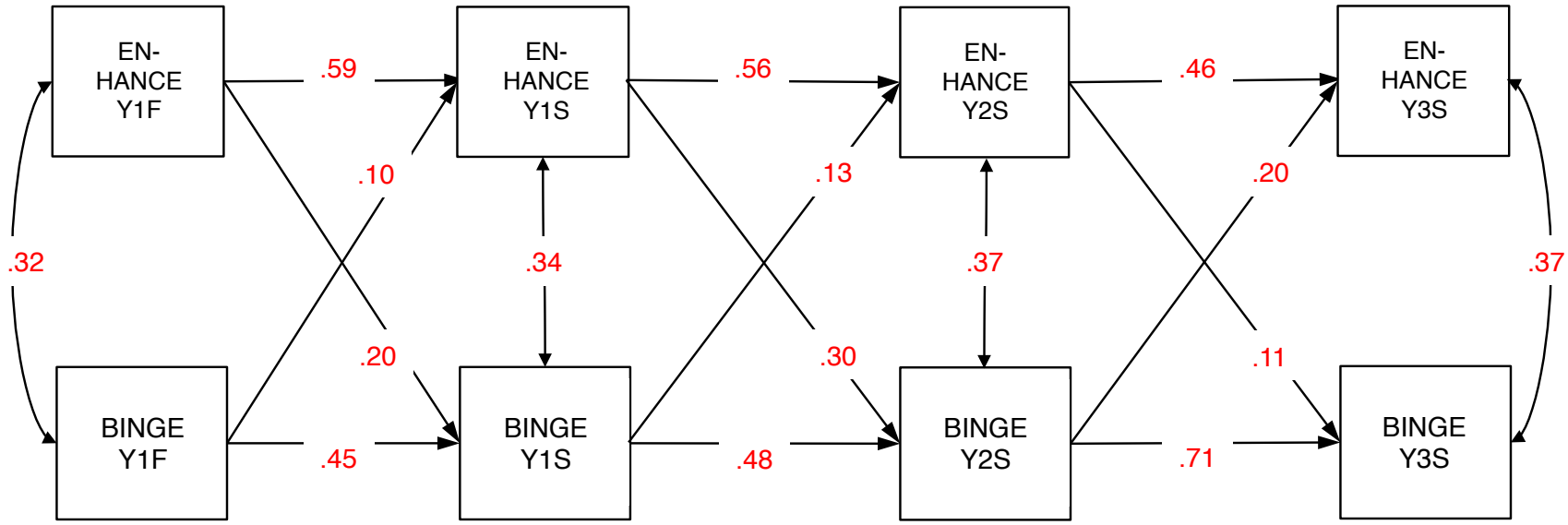

Figure 4.4. Cross-lagged model of enhancement drinking motives and binge drinking frequency.

Note: Coefficients in red are significant, p < .05.

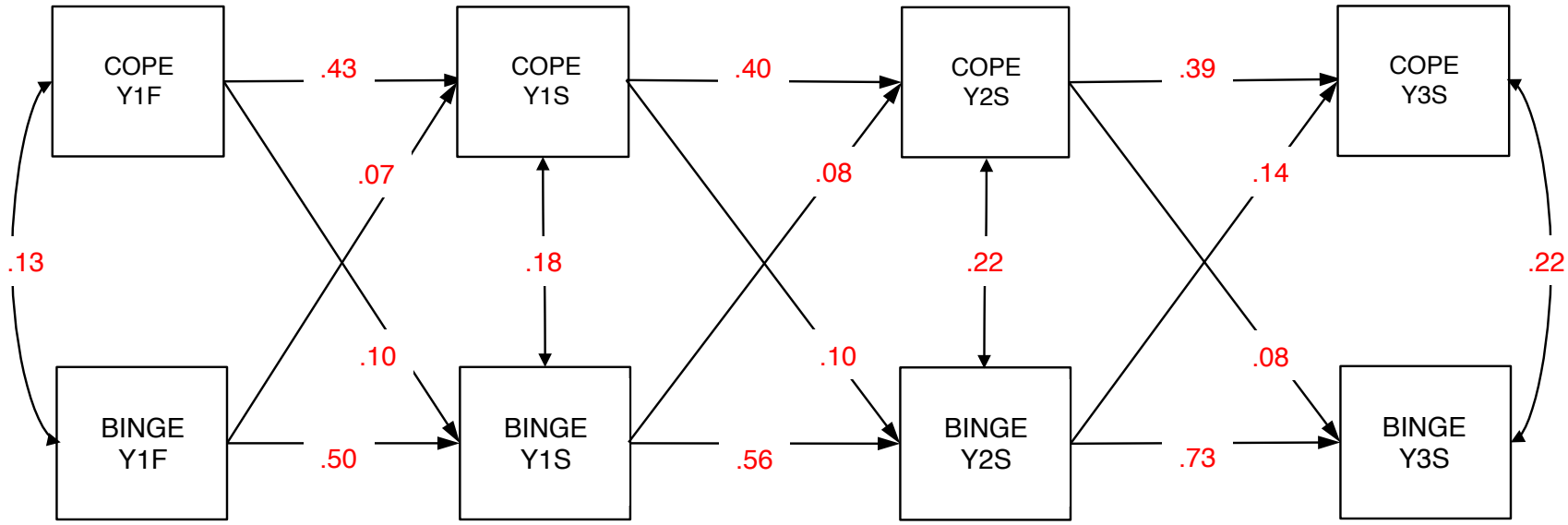

Figure 4.5. Cross-lagged model of coping drinking motives and binge drinking frequency.

Note: Coefficients in red are significant, p < .05.

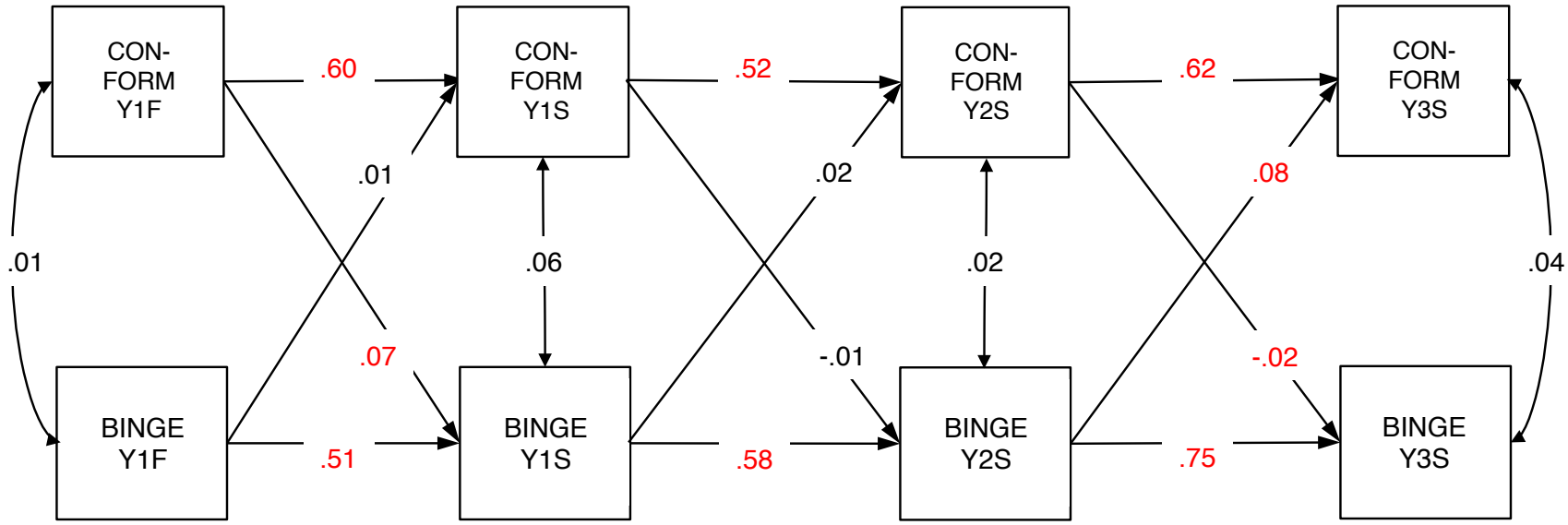

Figure 4.6. Cross-lagged model of conformity drinking motives and binge drinking frequency.

Note: Coefficients in red are significant, p < .05.

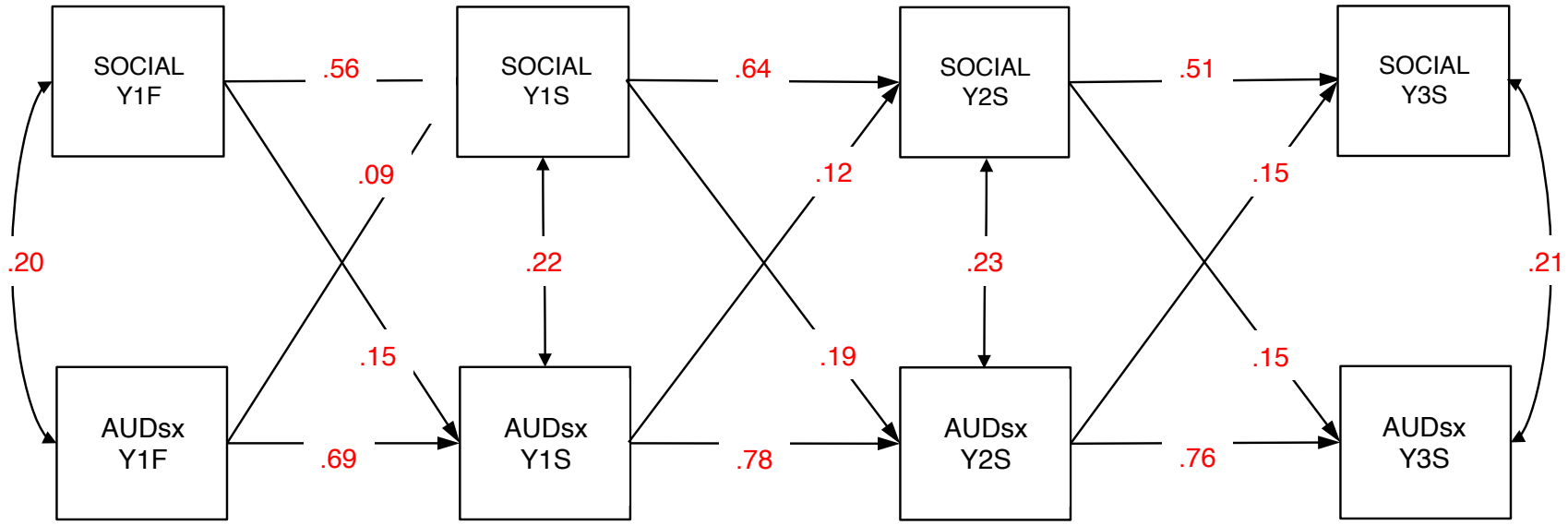

Figure 4.7. Cross-lagged model of social drinking motives and alcohol use disorder symptoms (AUDsx).

Note: Coefficients in red are significant, p < .05.

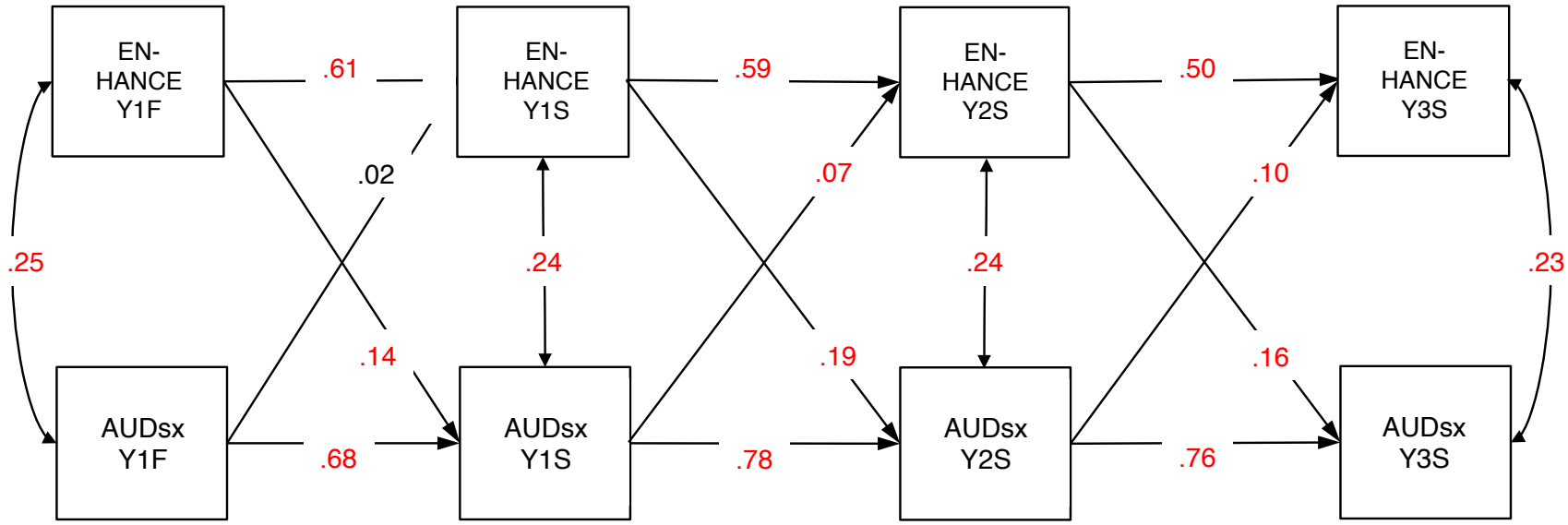

Figure 4.8. Cross-lagged model of enhancement drinking motives and alcohol use disorder symptoms (AUDsx).

Note: Coefficients in red are significant, p < .05.

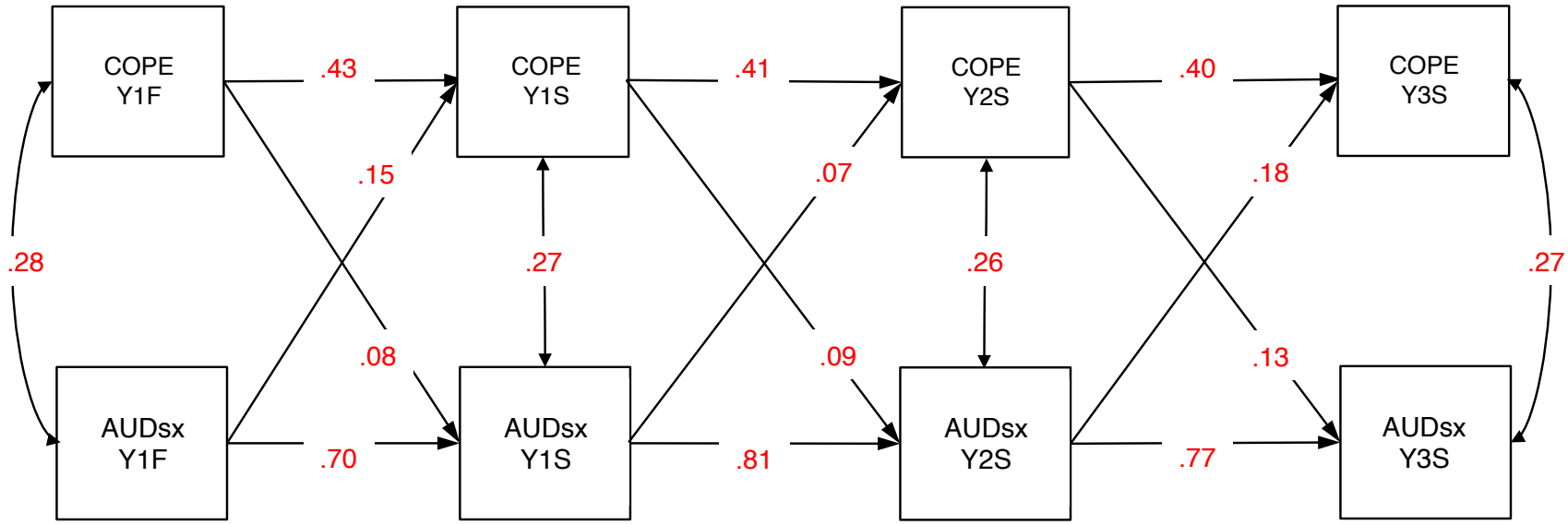

Figure 4.9. Cross-lagged model of coping drinking motives and alcohol use disorder symptoms (AUDsx).

Note: Coefficients in red are significant, p < .05.

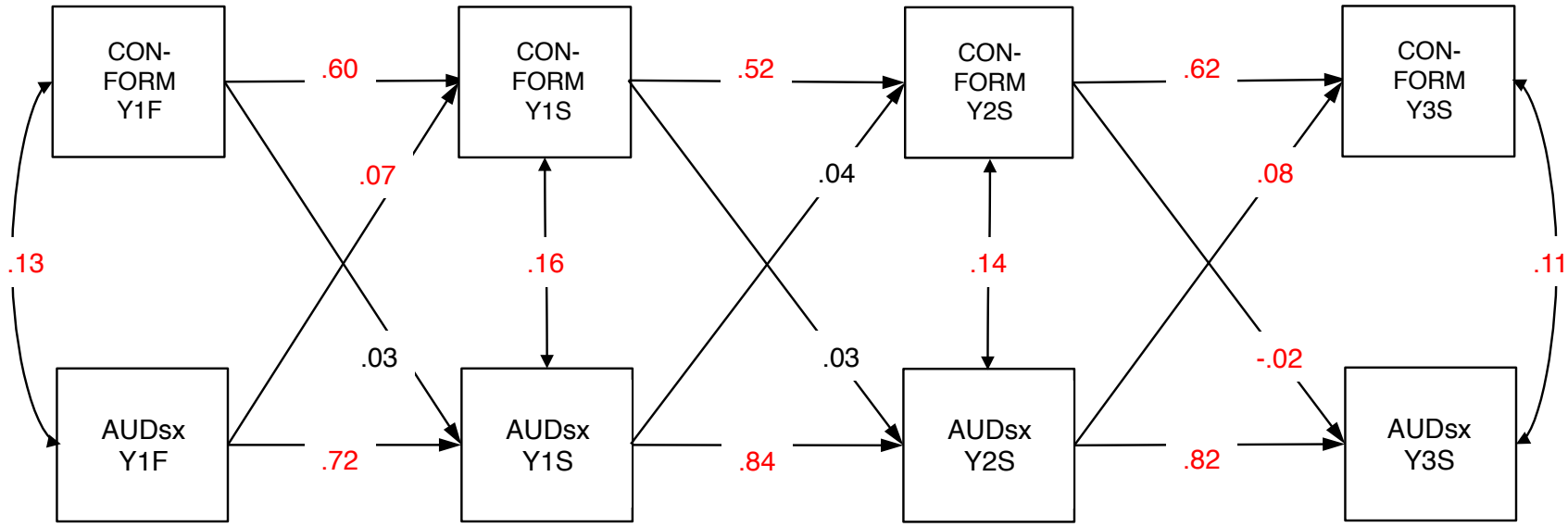

Figure 4.10. Cross-lagged model of conformity drinking motives and alcohol use disorder symptoms (AUDsx).

Note: Coefficients in red are significant, p < .05.

We focus therefore on estimates of the drinking motives cross-lagged paths with the alcohol misuse outcomes, which are presented in Figures 4.3 – 4.10. All estimates are standardized and can be directly compared. Tests of equality in the cross-lagged paths (Table 4.5) indicated that the relationship between drinking motives and alcohol misuse was not simply a function of confounding, as there was a significant decrease in fit when constraining the paths to equality for social motives and binge/AUD, enhancement motives and binge/AUD, and conformity motives and binge frequency. The direction of these relationships were such that drinking motives were stronger predictors of later alcohol misuse than vice versa, although this association appeared to attenuate or reverse in the last wave (year 3). For coping motives, the cross-lagged paths could be constrained to equality without a significant decrease in model fit.

The cross-lagged paths only represent the strength of the directional association of one outcome predicting another at a later wave, but there are also co-temporal correlations between outcomes at each wave that encompass the factors (including confounding) driving a cross-sectional association between them. These within-wave correlations are presented in Table 4.6. The estimates indicate modest correlations between all four drinking motives and internalizing/externalizing symptoms not due to a direct causal pathway. As for alcohol misuse, there were also modest co-temporal correlations with drinking motives that were strongest for the positive reinforcement motives with binge drinking and for the negative reinforcement motives with AUD symptoms.

Table 4.6. Within-wave correlations between drinking motives and internalizing, externalizing, and alcohol misuse outcomes in the cross-lagged models.

		Motive			
Wave	Outcome	Social	Enhancement	Coping	Conformity
Y1F	AUD	0.198	0.247	0.276	0.130
	Binge	0.287	0.322	0.131	0.013
	Internalizing	0.032	0.042	0.297	0.128
	Externalizing	0.198	0.201	0.208	0.085
Y1S	AUD	0.224	0.236	0.271	0.158
	Binge	0.299	0.336	0.183	0.056
	Internalizing	0.048	0.080	0.297	0.126
	Externalizing	0.036	0.061	0.111	0.068
Y2S	AUD	0.231	0.243	0.264	0.139
	Binge	0.398	0.373	0.224	0.016
	Internalizing	0.086	0.101	0.277	0.098
	Externalizing	0.027	0.052	0.107	0.051
Y3S	AUD	0.210	0.231	0.270	0.105
	Binge	0.332	0.366	0.223	0.038
	Internalizing	0.091	0.094	0.280	0.110
	Externalizing	0.037	0.078	0.090	0.006

Note: All correlations are significant, $p < .05$.

IV. Summary and Discussion

In this set of analyses, we have uncovered several insights about the nature of drinking motives in college students. First, drinking motives are relatively stable throughout college. Second, some robust environmental predictors of alcohol misuse like parental autonomy granting and peer deviance are also similarly associated with multiple different types of drinking motives, although others such as trauma exposure have more specific effects. Third, while the relationship between drinking motives and internalizing/externalizing phenotypes is largely non-directional, we have found

evidence that there is a direct and potentially causal link between drinking motives and later alcohol misuse outcomes, particularly for positive reinforcement motives.

These findings have promising implications for the use of drinking motives as endophenotypes or intermediate indices of internalizing and externalizing pathways to alcohol misuse. Their stability across time is important for establishing that these are trait-like outcomes linked to enduring temperamental dimensions (e.g. personality), and thus are viable for aiding gene identification efforts (Gottesman & Gould, 2003). However, their moderate correlations across time also point to their mutability and potential to be changed via environmental interventions. This is particularly important given evidence from the cross-lagged models that drinking motives may be directly linked to later increases/decreases in alcohol misuse outcomes, and thus may be a useful target for intervention/treatment efforts to modify alcohol behaviors, perhaps through cognitive therapies to restructure one's motives for using alcohol.

The difference in the nature of the relationship structure between positive and negative reinforcement (specifically coping) motives with alcohol misuse also points to distinct mechanisms at play that differ between internalizing and externalizing pathways. A causal pathway from genes/temperament to alcohol misuse via positive reinforcement motives suggests that the underlying factors influencing the externalizing pathway to alcohol misuse are mediated by positive reinforcement drinking motives, perhaps originating from impulsivity/reward-seeking neurobiology. An association between negative reinforcement motives and alcohol misuse in an internalizing pathway that is due to confounding factors, or a shared etiology, suggests that both outcomes are driven by the same underlying factors but not through a mediational path, perhaps due

to something such as trauma exposure or a temperamental predisposition increasing both simultaneously.

Although speculative at this stage, if such distinct mechanisms exist they would call for very different efforts for prevention/treatment, as well as different strategies for identifying the genetic and environmental risk factors for these subtypes. However, it is important to remember that even if there are two distinct mechanisms at play, this does not mean that there is no overlap between these pathways at the level of the individual. There were modest to moderate correlations between all four types of drinking motives in this sample, indicative of a general factor underlying higher motivation to drink (for any reason) rather than complete separation between individuals who drink for different reasons. Others have also found that those who drink for coping and enhancement drinking motives do not form two discrete groups (Littlefield, Vergés, Rosinski, Steinley, & Sher, 2013). Therefore while the cross-lagged models indicated a difference in mechanisms linking drinking motives to alcohol misuse, it is possible that multiple processes co-occur within any one individual, leading to overlapping increases in drinking motives in both the positive and negative reinforcement domains.

These results also provide a developmental link between the two previous cross-lagged studies investigating the direction of association between drinking motives and alcohol use behaviors in an older and younger sample, respectively. The study of young adolescents (Schelleman-Offermans et al., 2011) found no directional prediction, while the study of adults (Crutzen et al., 2013) found that it was alcohol use driving changes in one's drinking motives rather than vice versa. Our findings that drinking motives, particularly positive reinforcement motives, are the driver of alcohol misuse in early

college suggest that this is a dynamic process which might emerge in young adulthood and change across the lifespan. These results are also consistent with what was found in a cross-lagged model in a sample of young adult males (Labhart et al., 2016), in which a directional association was evident at that age linking enhancement motives to higher alcohol consumption and problems, Such a shift would be in line with the Koob model (Koob & Volkow, 2016) of early motivations for reward seeking (the “binge” phase) that change after continued alcohol use. Suggestions that the direction of association begins to attenuate or even reverse in our sample (particularly for binge drinking behavior) hints at a developmental shift that merits continued long-term follow-up in research.

Chapter 5. Genetic Etiology of Drinking Motives

I. Specific Aim

As has been established in the previous chapters, drinking motives appear to have an important link to the development of alcohol misuse and may be promising intermediate phenotypes to aid in the understanding of its etiology. However, in order for drinking motives to have some utility as an endophenotypes for gene identification, they must themselves be heritable and lie in the genetic pathway(s) to alcohol misuse. Endophenotypes, conceptually, are less genetically complex subunits in a (biological) pathway that in constellation make up a more complex disorder or behavior like alcohol misuse (Gottesman & Gould, 2003). If the challenge in gene identification for these more complex phenotypes comes from their genetic heterogeneity – i.e. resulting from a mixture of numerous possible genetically influenced pathways – then focusing on a homogenous intermediate mechanism in each pathway should serve to increase power to detect associated genetic variants. However, the selection of the correct intermediate mechanism is critical. An endophenotype must therefore be genetically influenced, and its genetic influences must filter through to an outcome phenotype if it is to be useful in gene identification for that outcome.

Less stringent criteria have been proposed for “intermediate phenotypes” or “biomarkers” as cousins of the endophenotype construct (Kendler & Neale, 2010). These are considered to be phenotypes that segregate with a disease/trait, as

endophenotypes do, but do not necessarily lie in the causal pathway from genes to the outcome of interest. They may instead be a correlated by-product of the same causal pathway, or may be a result of the disease/trait itself. Nevertheless, they can have the same potential benefits as endophenotypes in improving statistical power if they are also less heterogeneous entities, and can point to biological processes important in the development of the disease/trait of interest. Drinking motives might meet the classic criteria for endophenotypes for alcohol misuse or might be less directly linked intermediate phenotypes; they may even be both depending on which type of drinking motive is considered, as the results from the previous chapter suggested. Regardless, the robust and consistent evidence of their proximal association with alcohol misuse suggests that they will be useful investigative tools.

There has been little research on the etiology of drinking motives, and even less on their genetic etiology. A few twin studies have been conducted to estimate the heritability of drinking motives (Agrawal et al., 2008; Kristjansson et al., 2011; Mackie et al., 2011; Prescott et al., 2004; Young-Wolff et al., 2009), although some of these are limited in generalizability due to the focus on specific samples (e.g. females or smokers only). These studies have examined the DMQ and related Alcohol Use Inventory (Wanberg & Horn, 1983) subscales and estimated that their heritability ranges from 11% to 40%. There is also some evidence from twin studies that drinking motives mediate the latent genetic overlap between depression and AUD via coping motives (Young-Wolff et al., 2009), and between personality traits (neuroticism & impulsivity) and AUD via coping and enhancement motives, respectively (Littlefield et al., 2011; Prescott et al., 2004), mirroring the mediation pathways found at the phenotypic level

(Adams et al., 2012; Mezquita et al., 2010). However, to date, no molecular genetic research has been conducted on drinking motives.

Psychiatric and behavioral genetics research has developed exponentially over the past two decades, with statistical analysis of latent genetic influences using twin and family methods quickly being supplemented (or in some cases supplanted) by molecular genetic studies of families using linkage analysis, then candidate gene association studies, and most recently hypothesis-free methods of genome-wide association (GWAS) and sequencing studies. In addition, our understanding of the functional impact of molecular genetic variation has rapidly evolved with new experimental studies and projects like ENCODE (ENCODE Project Consortium, 2012) and ROADMAP (Roadmap Epigenomics et al., 2015) that have begun to document the numerous ways in which changes in the DNA code can impact downstream biological processes. Gene identification efforts for psychiatric and behavioral outcomes have now begun to achieve some measure of success (e.g. Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Unfortunately, such successes have generally not been obtained until hundreds of thousands of participants have been collected for a study, as it is now recognized that the effect sizes for genetic variants impacting these outcomes is smaller than previously expected (O'Donovan, 2015; Sullivan et al., 2012).

As endophenotypes should be less complex and lie closer in the biological pathways to the actual genes, they should require smaller samples to achieve statistical power equivalent to that of a study of, for example, a psychiatric disorder. They should also, in theory, be able to provide insight into the biological process(es) underlying the genetic association for the complex disorder/trait that they index. Our aim for the

analyses in this chapter is thus to conduct a series of molecular genetic analyses on the drinking motives outcomes collected in S4S in order to begin to understand their genetic etiology and provide insight into the genetic etiology of alcohol misuse. We estimate the genome-wide heritability of these motives, investigate the specific variants, genes, and pathways underlying their heritability, and attempt to uncover the extent to which genetic influences are shared between or specific to each type of drinking motive.

II. Methods

The subsample used for this set of analyses includes the first three cohorts of the S4S study who have thus far been genotyped and have passed the basic quality control procedures described in Chapter 2 (n = 6,325). The sample was further restricted to include only unrelated individuals for unbiased genetic analysis and to exclude those with missing phenotype information for drinking motives variables – i.e. those who never initiated alcohol use during the time the measures were collected or those who chose not to answer the relevant survey questions (analytic n = 4,855).

Because analyses in the previous chapters have shown substantial stability of drinking motives between assessments, the analyses here used mean scores across waves for the four drinking motives subscales as the outcome phenotype. We also utilize relevant covariates to control for possible confounding effects on genetic associations with the phenotypes, which included sex, age (mean across waves where drinking motives were measured), and within-ancestry principal components.

Data analysis for this aim involved three major components. First, we estimated the heritability of drinking motives that could be attributable to measured genetic

variants through the use of genome-wide complex trait analysis (GCTA; Yang et al., 2011). This method uses restricted maximum likelihood (REML) estimation to regress the phenotypic similarity between individuals on their genetic similarity based on their proportion of shared alleles identical-by-state (IBS) at individual loci (single nucleotide polymorphisms; SNPs) across the genome. Because there will be few alleles shared in the sample when the minor allele frequency (MAF) of a particular SNP is low, we filtered SNPs for a MAF > .01 before calculating the genomic relatedness matrix between individuals in each ancestry superpopulation in the sample. GCTA was then run separately in each ancestry group using the first 10 within-ancestry PCs, age, and sex as covariates, and the resulting heritability estimates were meta-analyzed with a fixed-effects, inverse variance-weighted scheme.

In the second phase of data analysis, we conducted a GWAS of the imputed genetic variants. SNPs were filtered for imputation quality (INFO score > 0.5) and ancestry specific MAF corresponding to a minor allele count (MAC) > 100. Previous work has established that a MAC > 40 allows for reliable statistical estimation (Bigdeli, Neale, & Neale, 2014); we use a more conservative threshold because the somewhat skewed distribution of the phenotypes may lead to biased parameter estimates due to small numbers of observations at the extremes of the distribution. Within-ancestry PCs to include as covariates were decided based on their association with the phenotype in a stepwise linear regression to avoid overfitting of the model, as the expected effect size of an individual SNP is very small and may be lost with overfitting (c.f. Webb et al., 2017), Analyses were conducted with an additive, frequentist model for the association test using the software SNPTEST (Marchini, Howie, Myers, McVean, & Donnelly, 2007),

again running separately in each ancestry group and meta-analyzing the association test for each SNP. This software allows for the treatment of imputed variants as dosages rather than absolute allele counts to reflect the probabilistic nature of imputation. METAL (Abecasis et al., 2012) was used for meta-analysis of the ancestry-specific results after filtering, and we present only the results for SNPs that were available in a sample size of at least 1000 individuals, meaning that the SNP had to pass quality control filters in either the AFR, EUR, or a combination of two or more ancestry sub-groups to be certain that spurious results from small samples were not given undue consideration. Multiple testing correction was performed by using a Bonferroni-corrected threshold for genome-wide significance of 5x10-8 and calculating false discovery rates (FDR) using the *qvalue* package for R/Bioconductor (Storey, Bass, Dabney, & Robinson, 2015).

After conducting GWAS at the individual variant level, we applied gene-based and pathway-based association testing to identify whether the SNP association signal was enriched at these aggregate levels. Although any individual locus in the genome is unlikely to have a robust effect on a complex outcome, a number of small individual DNA variants in different loci may lead to a similar biological effects due to similar changes in a protein or biochemical pathway, and such effects in aggregate may have a larger association with the phenotype that is easier to detect than any single SNP. Enrichment testing therefore examines whether the association signal (typically indexed by the association test statistic or p value) in a group of SNPs annotated to a particular gene or set of genes is larger than that of a set of SNPs drawn at random from the genome. We conducted these tests using MAGMA (de Leeuw, Mooij, Heskes, &

Posthuma, 2015), a software that uses principal components analysis to extract the association signal from a set of SNPs while accounting for the LD structure between them that could otherwise inflate or deflate the true signal. We annotated SNPs to their genomic locations based on the human reference genome GRCh37 build and used the publically available genomes from the 1000 Genomes project as reference panels for the LD structure of the five ancestral continental superpopulations (The 1000 Genomes Project Consortium, 2015). After annotation of the SNPs to genes, genes were also grouped into pathways based on the curated canonical pathways dataset, which includes the Kyoto Encyclopedia of Genes and Genomes [KEGG], REACTOME, and BIOCARTA pathways, and the Gene Ontology [GO] gene sets, all obtained from the Molecular Signatures Database (Liberzon et al., 2015). These pathways represent groups of genes whose products are involved in known metabolic and regulatory biochemical processes. Gene- and pathway-based analyses were all conducted on the ancestry-specific SNPTEST results and meta-analyzed, using an inverse variance-weighted Stouffer's Z test to combine the enrichment Z statistic across subsamples.

The third phase of data analysis involved an investigation of the genetic overlap between the four drinking motives. This was conducted using two methods: a bivariate extension of GCTA (Lee, Wray, Goddard, & Visscher, 2011) and LD score regression (Bulik-Sullivan et al., 2015). Bivariate GCTA uses raw genotypic data to estimate the covariance between two phenotypes as a function of the genetic relatedness between individuals measured on both phenotypes, while LD score regression uses GWAS summary statistics to estimate genetic correlation between traits from the inflation of

SNPs' association signal for each trait generated from their correlation via linkage disequilibrium with true causal SNPs in neighboring regions.

III. Results

Heritability estimates from GCTA are displayed in Table 5.1. There was wide variation in estimates between ancestry subgroups, but the meta-analysis estimates ranged from 14% (coping) to 22% (enhancement). However, none of the meta-analytic estimates were significantly differentiable from zero in this sample due to their large standard errors.

Table 5.1. Heritability estimates (h^2) for drinking motives from genome-wide complex trait (GCTA) analysis in five ancestry subgroups and meta-analysis.

Motive	Ancestry	h^2	SE	p	N	95% CI
Social	AFR	0.08	0.32	0.40	1046	
	AMR	0.00	0.56	0.50	474	
	EAS	0.25	0.80	0.38	455	
	EUR	0.18	0.15	0.12	2533	
	SAS	1.00	1.12	0.07	312	
	Meta	***0.16***	***0.13***			***-0.09 - 0.42***
Enhancement	AFR	0.00	0.31	0.50	1044	
	AMR	0.00	0.55	0.50	473	
	EAS	0.03	0.81	0.49	455	
	EUR	0.31	0.16	0.02	2537	
	SAS	0.00	1.19	0.50	312	
	Meta	***0.22***	***0.13***			***-0.04 - 0.48***
Coping	AFR	0.67	0.34	0.02	1047	
	AMR	0.22	0.55	0.35	473	
	EAS	1.00	0.75	0.06	453	
	EUR	0.00	0.15	0.50	2533	
	SAS	0.00	1.10	0.50	308	
	Meta	***0.14***	***0.13***			***-0.11 - 0.39***
Conformity	AFR	0.06	0.33	0.43	1044	
	AMR	0.39	0.55	0.25	471	
	EAS	1.00	0.80	0.09	454	
	EUR	0.13	0.14	0.17	2536	
	SAS	0.41	1.11	0.36	312	
	Meta	***0.16***	***0.13***			***-0.09 - 0.40***

Note: AFR = African ancestry group; AMR = American; EAS = East Asian; EUR = European; SAS = South Asian; CI = confidence interval

Manhattan plots for the SNP-based association results are shown in Figures 5.1-5.4, with their corresponding QQ plots in Figures 5.5-5.8. The Manhattan plots show the cross-ancestry meta-analysis results for SNPs available (post-quality control) in at least 1000 individuals. The QQ plots show little evidence of inflation that could indicate bias or population stratification; in fact the median chi-square statistic was in most cases underinflated (less than 1). There was no evidence for any loci reaching the genome-

wide significance threshold (5e-08), and little evidence of even suggestive association peaks (5e-05) for social motives; but for the other three motive types, at least one genome-wide significant locus was identified in addition to several suggestive peaks.

The genome-wide significant locus for enhancement motives included 2 SNPs found only in the European ancestry group, located on chromosome 3 in the *FBLN2* (fibulin 2) gene, which codes for an extracellular matrix protein involved in organ development and differentiation. For coping motives, one genome-wide significant SNP was found atop a peak in an intergenic region on chromosome 5 with no nearby genes. For conformity motives, one genome-wide significant SNP in a peak of 40 suggestive SNPs was found on chromosome 12 in the *SIRT4* (sirtuin 4) gene. More information on these loci is provided in Table 5.2. Because true association signals should be found in peaks, rather than lone SNPs, reflecting the LD structure of the population, we provide further information on suggestive loci only when three or more SNPs in the same position (+/- 10kb) pass the suggestive significance threshold.

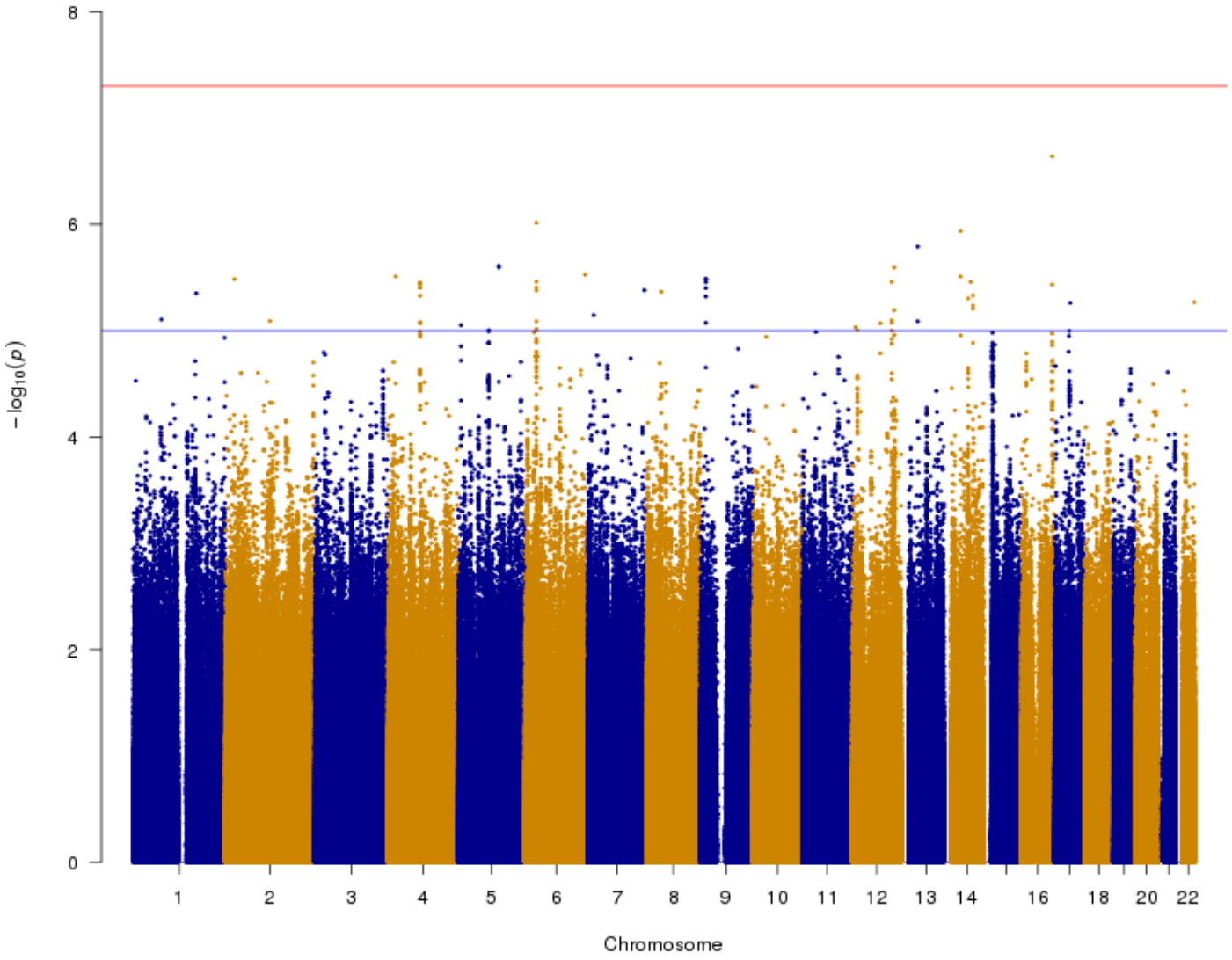

Figure 5.1. Manhattan plot of genome-wide association meta-analysis results for social drinking motives.

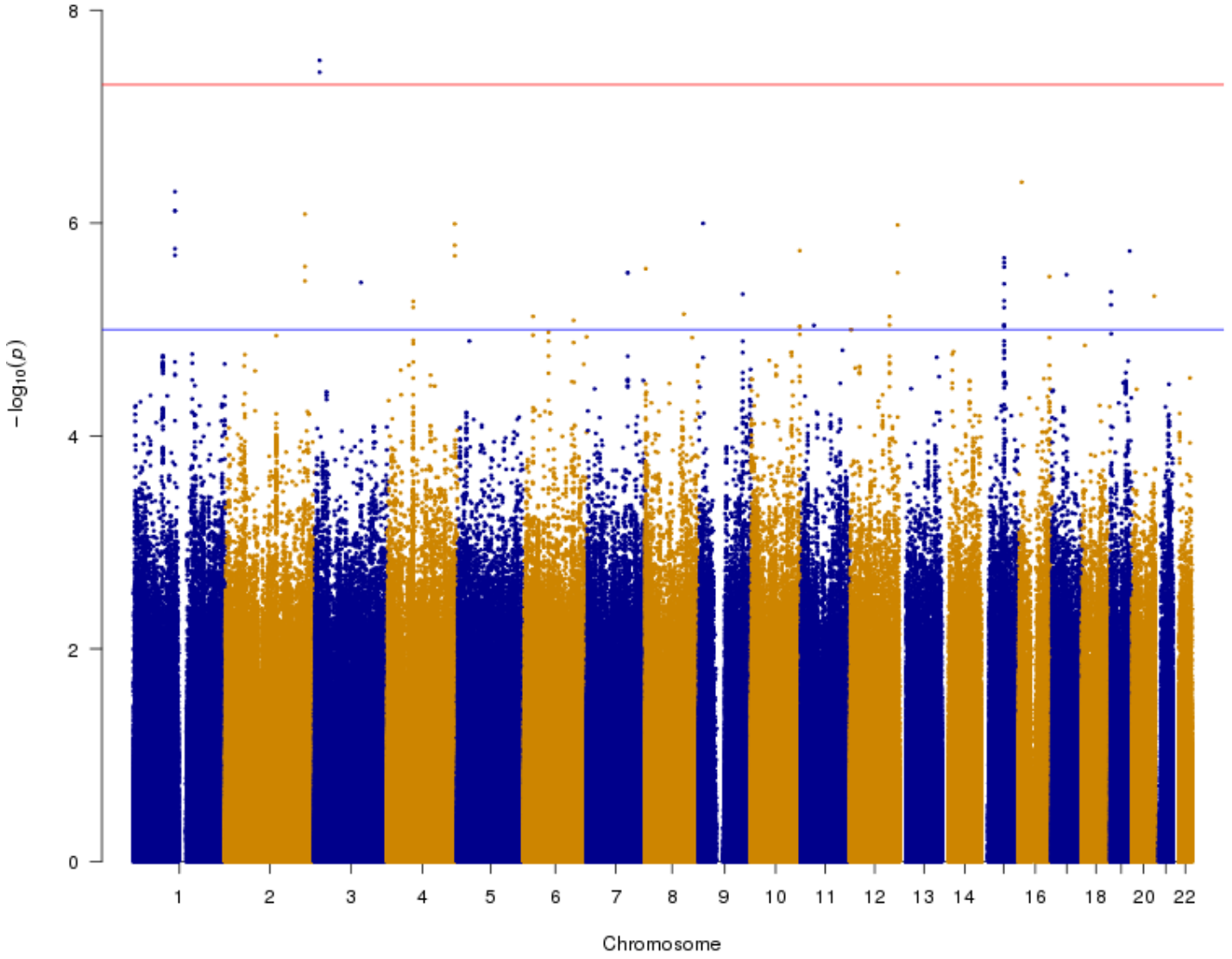

Figure 5.2. Manhattan plot of genome-wide association meta-analysis results for enhancement drinking motives.

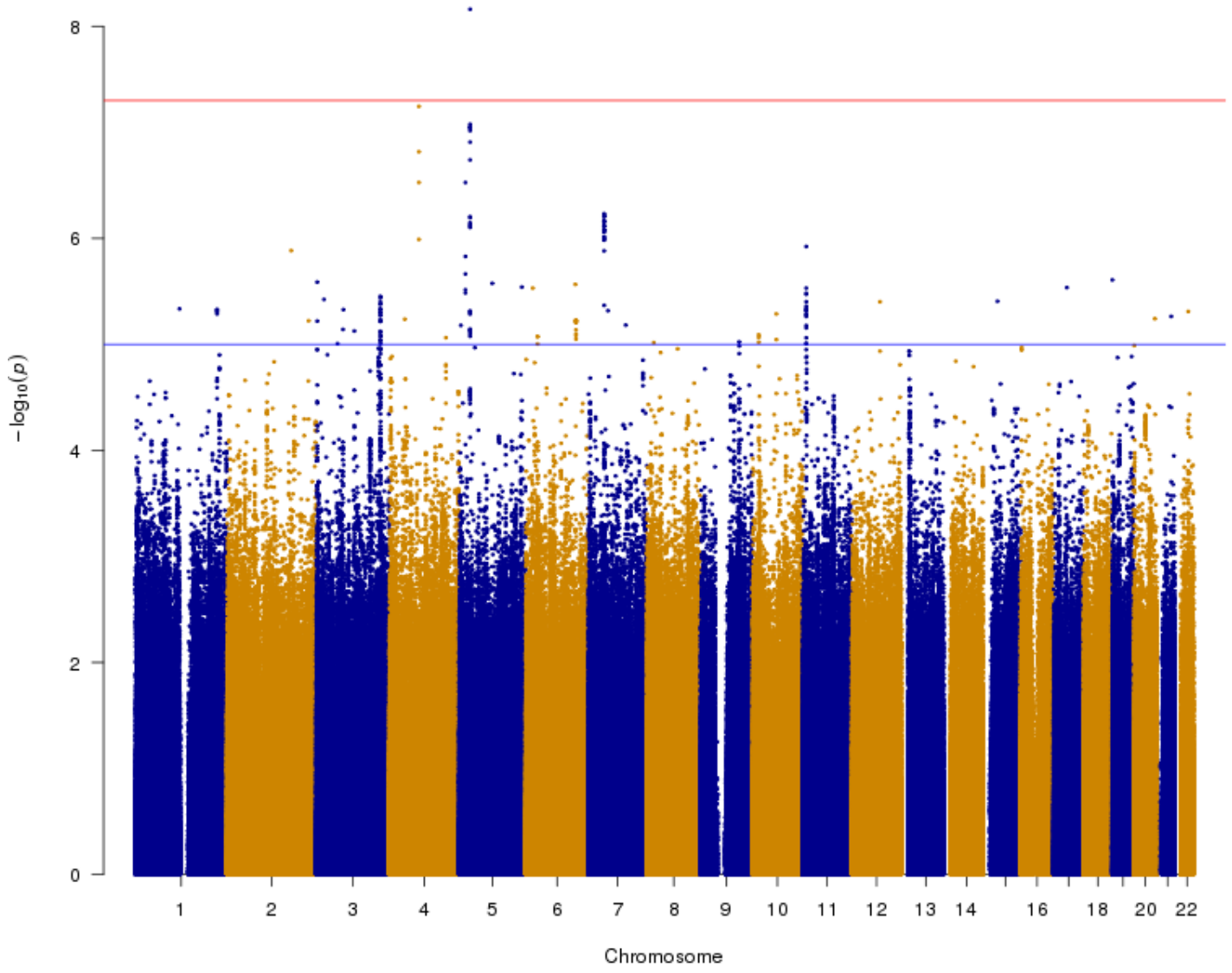

Figure 5.3. Manhattan plot of genome-wide association meta-analysis results for coping drinking motives.

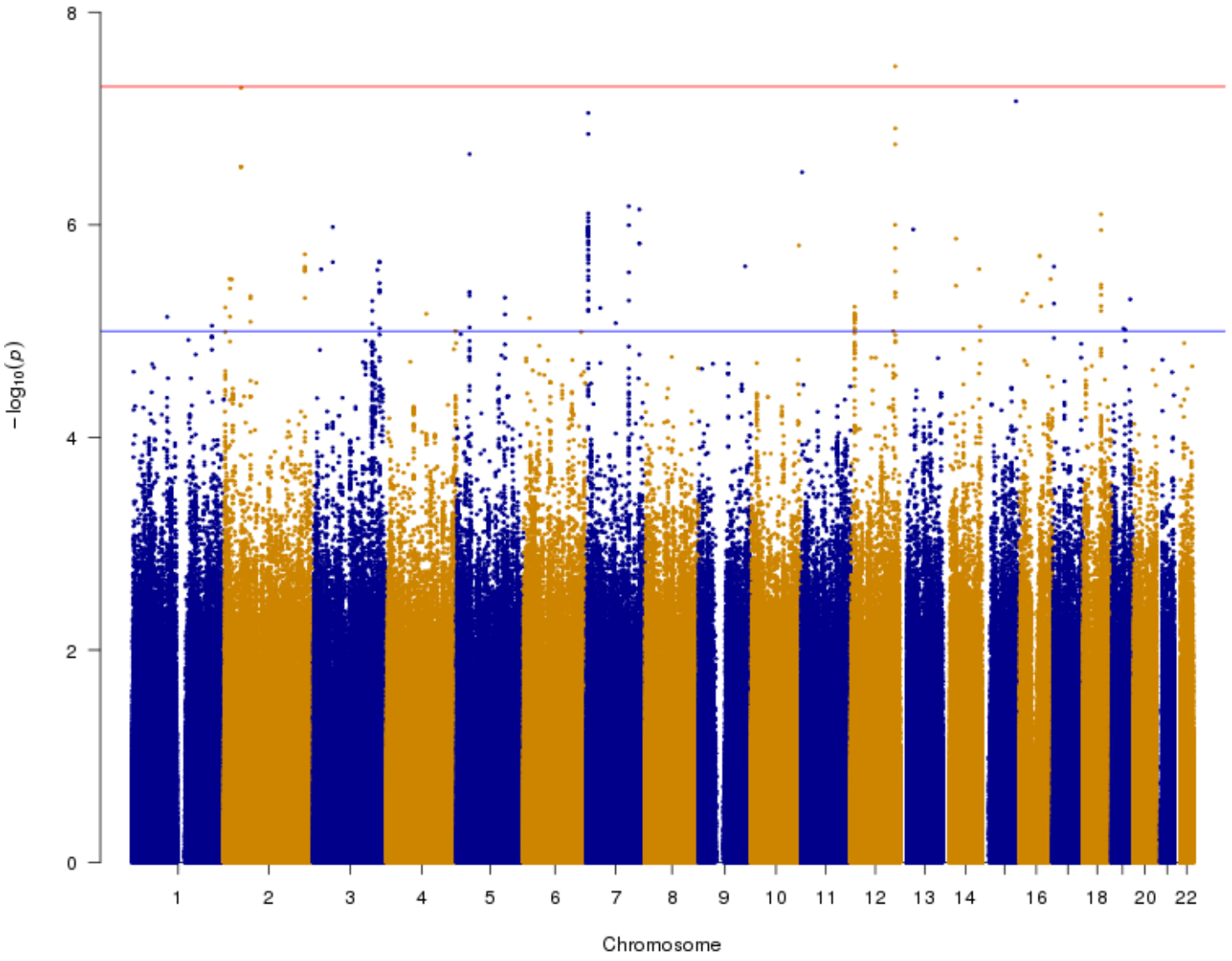

Figure 5.4. Manhattan plot of genome-wide association meta-analysis results for conformity drinking motives.

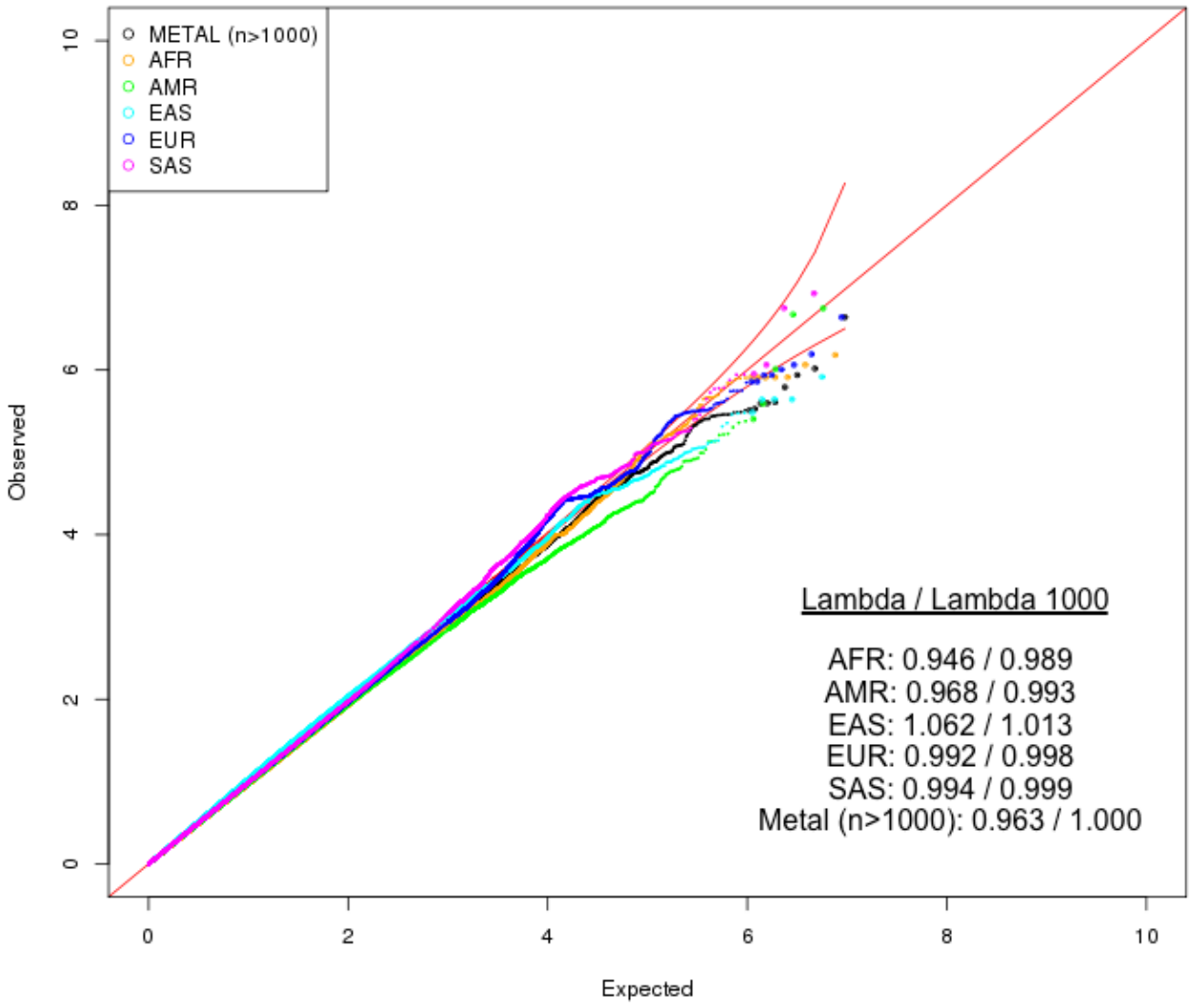

Figure 5.5. QQ plot of genome-wide association within-ancestry and meta-analysis results for social motives.

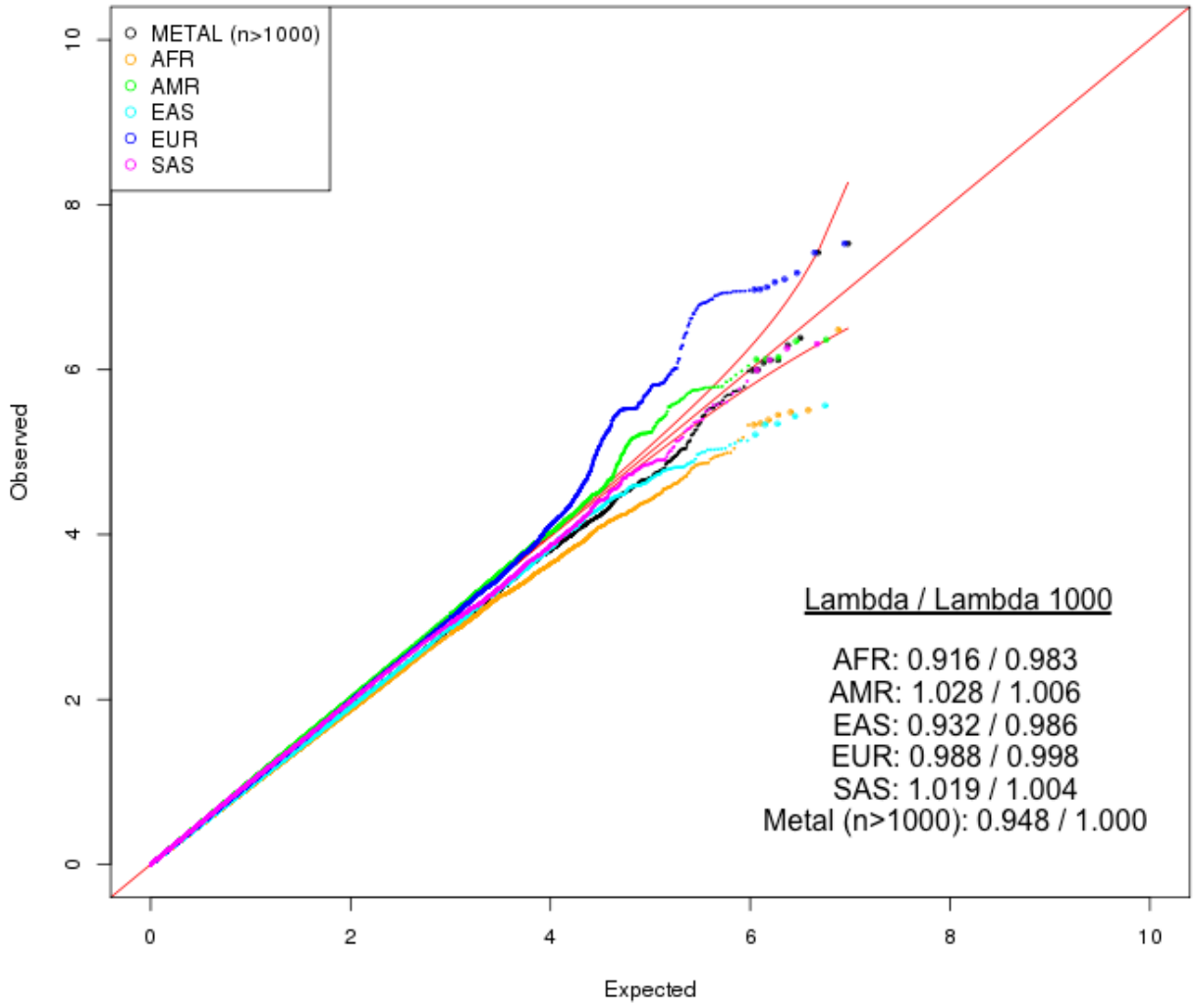

Figure 5.6. QQ plot of genome-wide association within-ancestry and meta-analysis results for enhancement motives.

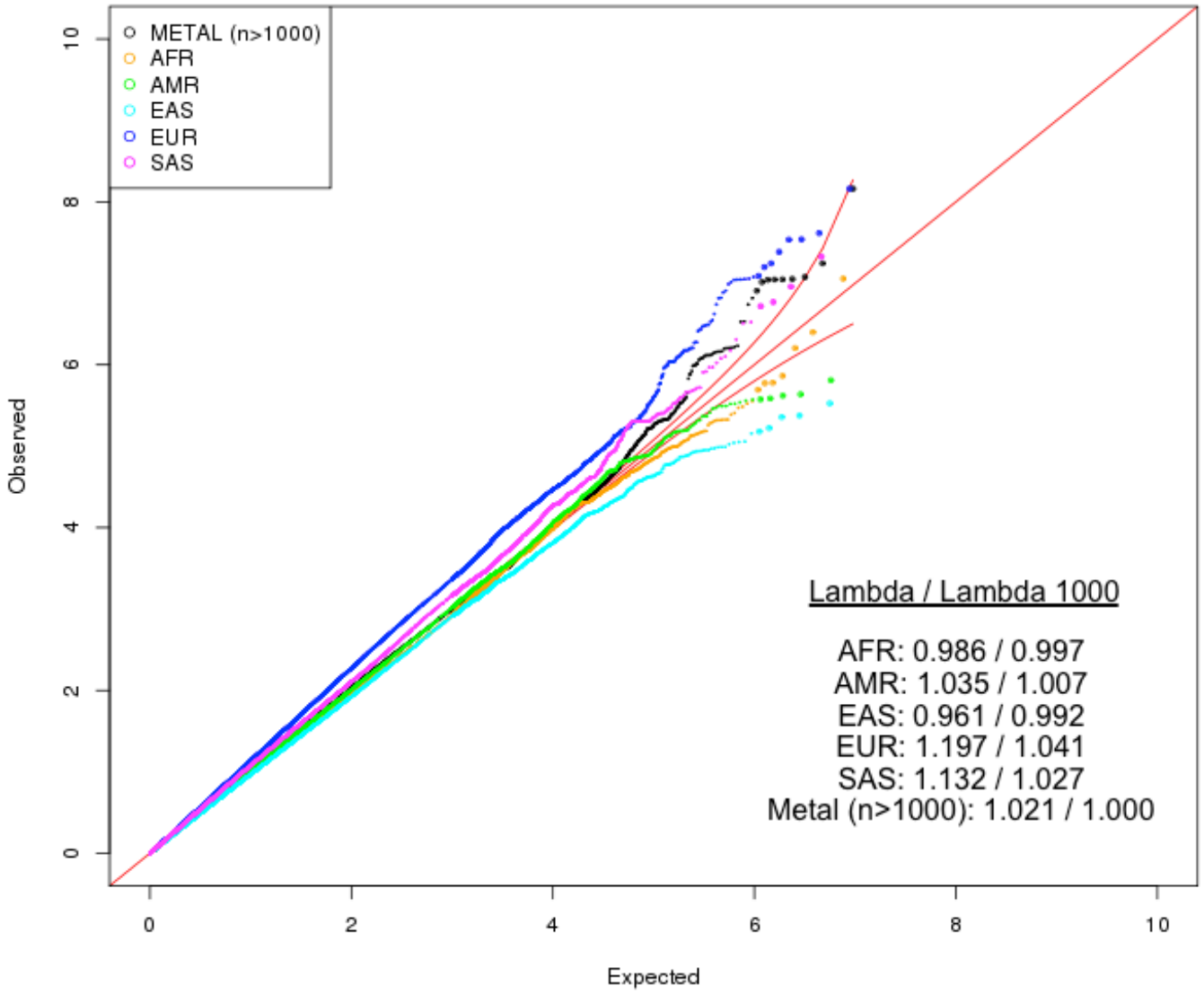

Figure 5.7. QQ plot of genome-wide association within-ancestry and meta-analysis results for coping motives.

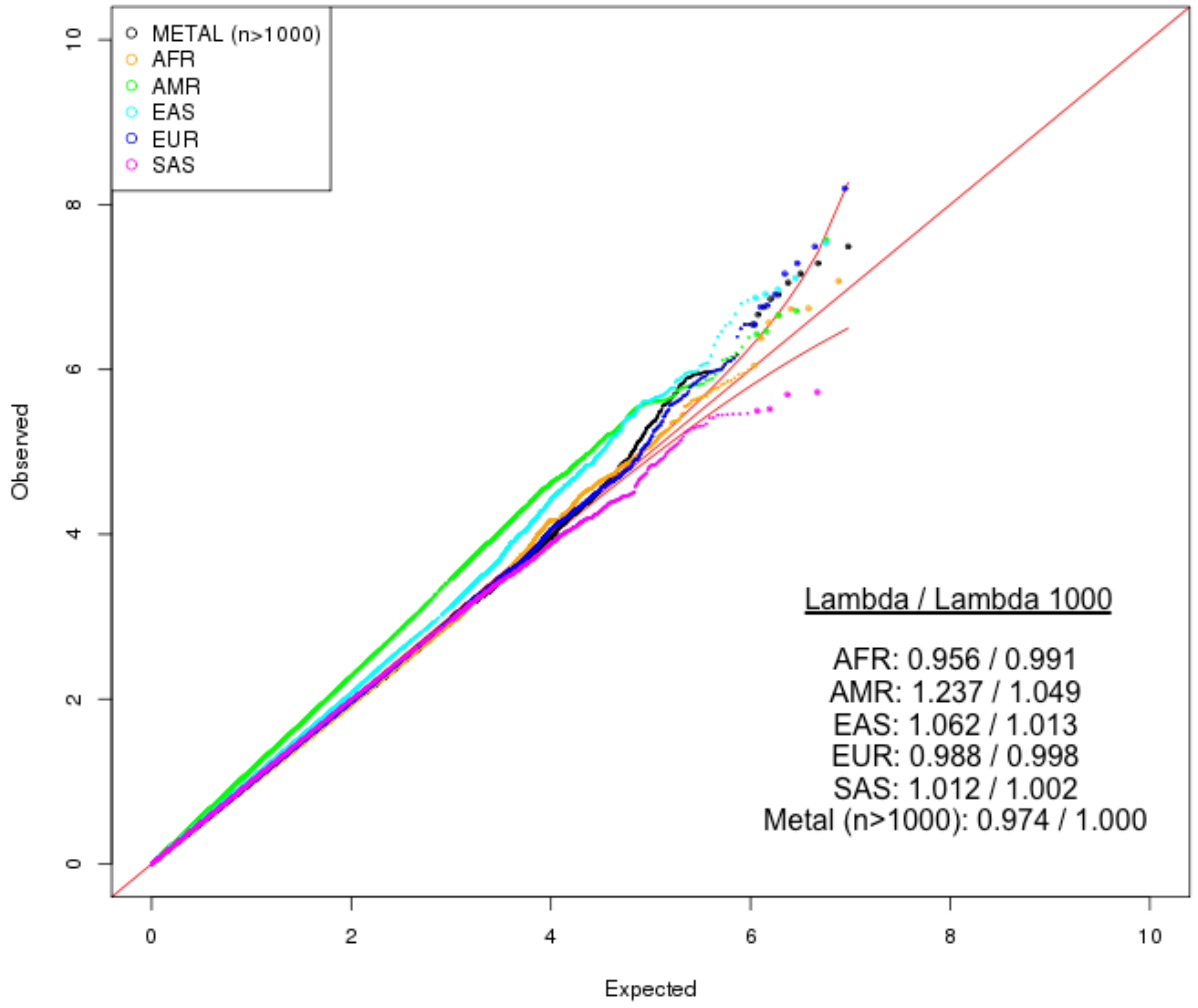

Figure 5.8. QQ plot of genome-wide association within-ancestry and meta-analysis results for conformity motives.

Table 5.2. Genomic annotation for loci with three or more SNPs reaching a suggestive level of association (*p* < 5e-05) with drinking motives.

CHR	Position	# SNPs	Min. *p*	Min. *q*	Max N	Annotated Gene	Nearby Genes (50 Mb)
				Social			
6	31310455	6	9.63E-07	1.00	4820	*HLA-B*	*MICA*
9	14731449	6	3.24E-06	1.00	4820	*None*	*CER1, FREM1, ZDHHC21*
				Enhancement			
1	112417478	5	5.08E-07	0.99	4509	*KCND3*	*None*
2	216881876	3	8.24E-07	0.99	2537	*PECR*	*MREG*
3	**13613907**	**2**	**2.97E-08**	**0.18**	**2537**	***FBLN2***	***BC152379, BC152380***
4	183429633	3	1.02E-06	0.99	2537	*TENM3*	*U2*
15	62852728	9	2.13E-06	1.00	4821	*None*	*None*
				Coping			
1	222247975	6	4.70E-06	0.53	4814	*None*	*None*
3	173989091	14	3.52E-06	0.53	4814	*NLGN1*	*None*
3	174044991	5	4.62E-06	0.53	4814	*None*	*NLGN1*
4	81172733	3	5.70E-08	0.11	2533	*None*	*FGF5, PRDM8*
5	16879933	5	2.97E-07	0.21	3580	*MYO10*	*None*
5	29512223	7	1.82E-07	0.16	2533	*None*	*None*
5	29542248	3	4.86E-06	0.53	2533	*None*	*None*
5	**29559845**	**15**	**6.90E-09**	**0.06**	**2533**	***None***	***None***
6	136944842	13	5.93E-06	0.53	2533	*5S_rRNA, MAP3K5*	*CRISPR_DR35*
7	42461966	21	5.87E-07	0.24	4053	*None*	*None*
10	16559589	3	8.11E-06	0.60	3580	*C1QL3*	*PTER, U2*
11	10525919	18	1.19E-06	0.28	4814	*AMPD3, DQ582265, JB137816, MTRNR2L8, RNF141*	*LYVE1, MRVI1, MRVI1-AS1,*

					Conformity		
2	16205386	4	3.23E-06	0.40	4817	*None*	*None*
2	46018826	4	5.16E-08	0.21	2536	*PRKCE*	*None*
2	71780906	3	4.69E-06	0.46	2536	*DYSF*	*None*
2	219263458	6	1.89E-06	0.34	3580	*CTDSP1*	*BC038211, C2orf62, MIR26B, SLC11A1, USP37, VIL1*
3	178665472	3	2.22E-06	0.36	4817	*None*	*None*
3	178708826	3	4.21E-06	0.45	4817	*None*	*ZMAT3*
5	34478736	5	2.16E-07	0.25	3580	*None*	*None*
7	4655524	40	8.87E-08	0.21	4817	*None*	*FOXK1*
7	114728738	3	6.69E-07	0.29	4817	*BC022431*	*None*
12	10495595	14	5.90E-06	0.51	4817	*None*	*AK096314, KLRC4, KLRC4-KLRK1, KLRD1, KLRK1*
12	**120744291**	**2**	**3.23E-08**	**0.21**	**2536**	***SIRT4***	***MSI1, PLA2G1B, PXN***
18	49162801	7	7.97E-07	0.29	4817	*None*	*None*

In addition to the meta-analysis results, we examined individual results from the larger African and European ancestry subgroups. These identified additional genome-wide significant loci for coping motives in peaks on chromosome 9 (Intergenic, 25kb from *GRIN3A*) and 15 (*LOC390617* pseudogene) and a lone significant SNP on chromosome 7 (intergenic). For enhancement motives there was also a strongly suggestive peak just below the genome-wide significance threshold, in the *PECR* (peroxisomal trans-2-enoyl-CoA reductase) gene on chromosome 2. The corresponding Manhattan plots for these results are shown in Figures 5.9 and 5.10.

Regional association plots for each of the genome-wide significant loci identified in the European or meta-analysis results were created using LocusZoom (Pruim et al., 2010) and are displayed in Figures 5.11 – 5.16. These figures show a zoomed in plot of the association *p* values at each locus and the LD structure of SNPs in the region (according to information from the 1000 Genomes EUR reference panel). The two genome-wide significant SNPs in the *FBLN* gene in the meta-analysis of enhancement motives showed no association enrichment for SNPs in high LD with the lead SNPs, suggesting these were likely spurious associations. All other loci had reasonable patterns of signal enrichment for SNPs in high LD in the regions. We also examined functional annotation of these top association results using the software FUMA (Watanabe, Taskesen, van Bochoven, & Posthuma, 2017). Genome-wide significant SNPs from each analysis and variants in LD (R^2 > .60) with these lead SNPs were annotated to genic regions. This annotation showed that all SNPs in the implicated loci described above were in intergenic or intronic regions except for 5 SNPs in the *GRIN3A* region for coping motives that were located in the 5'UTR.

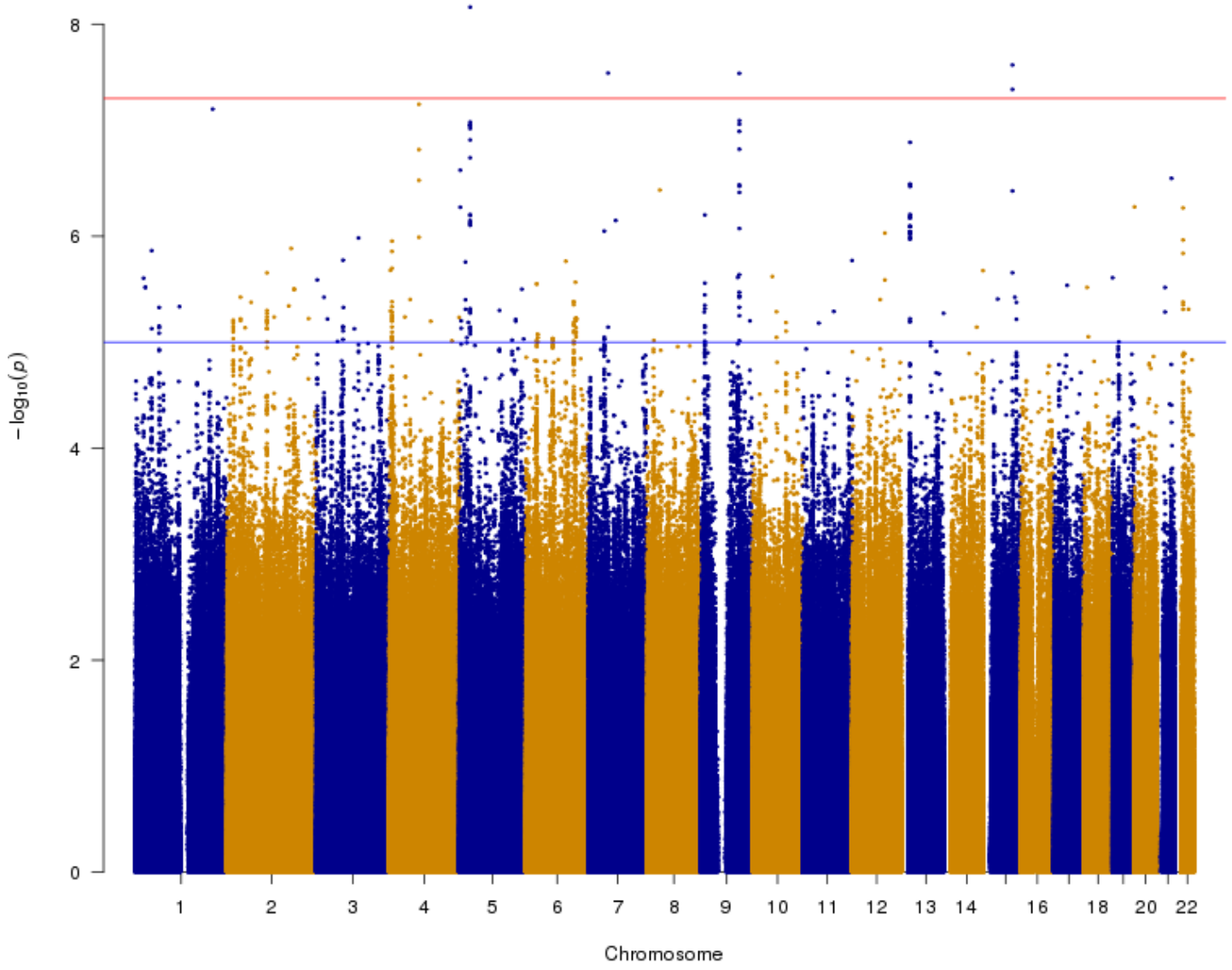

Figure 5.9. Manhattan plot of genome-wide association analysis results for coping drinking motives in Europeans.

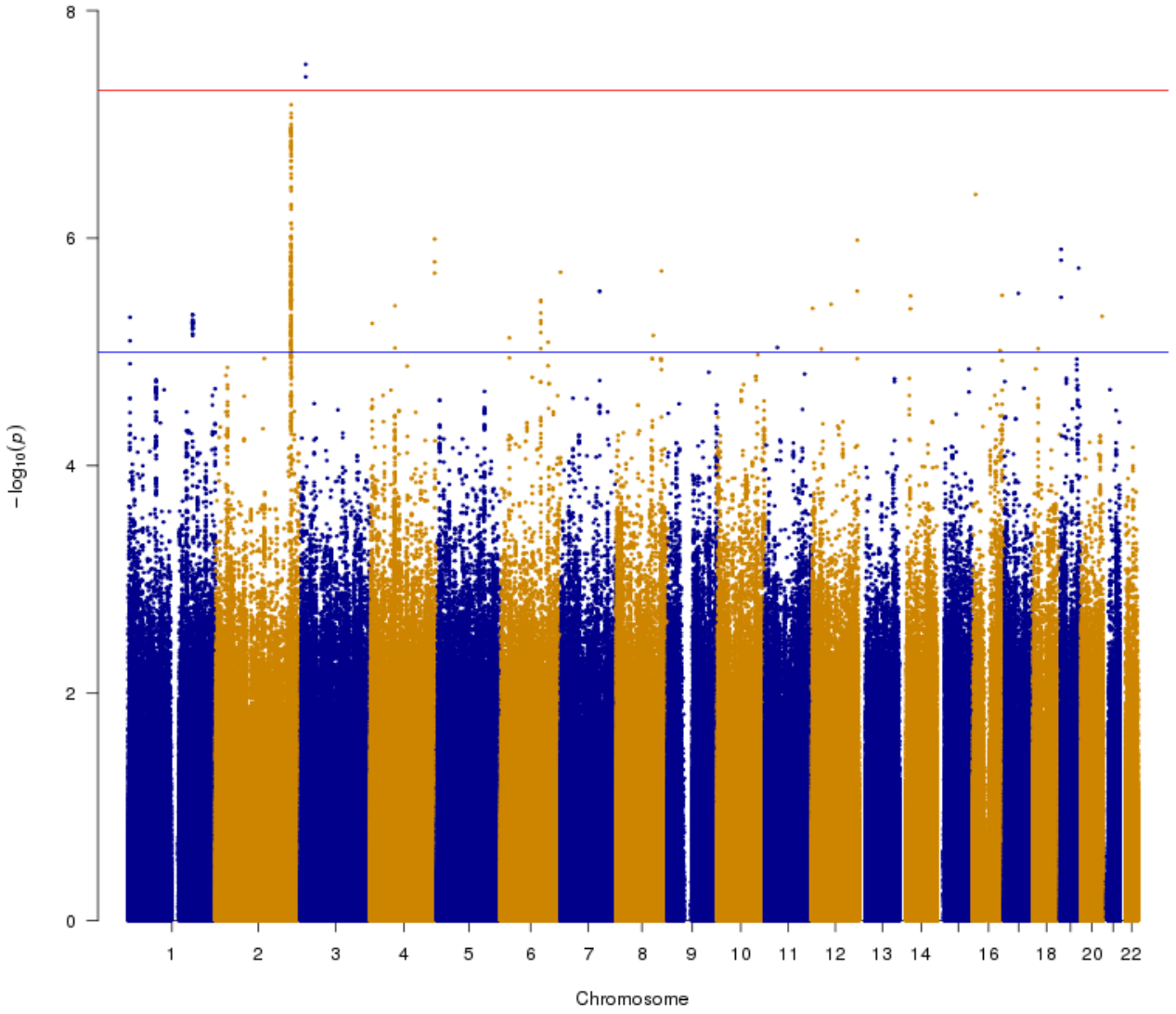

Figure 5.10. Manhattan plot of genome-wide association analysis results for enhancement drinking motives in Europeans.

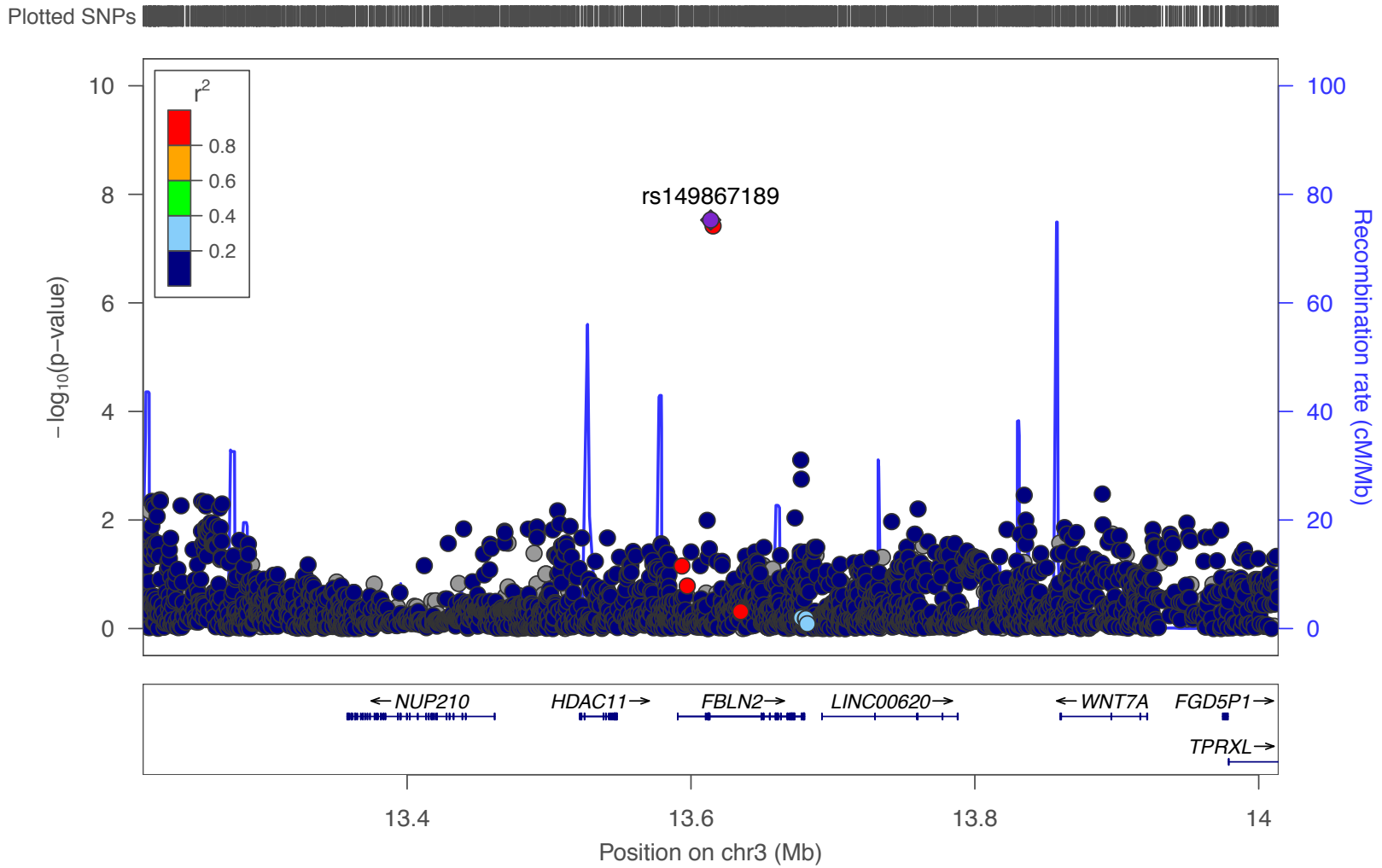

Figure 5.11. Regional association plot of –log(*p*) values in the *FBLN2* gene region for enhancement motives in the cross-ancestry GWAS meta-analysis.

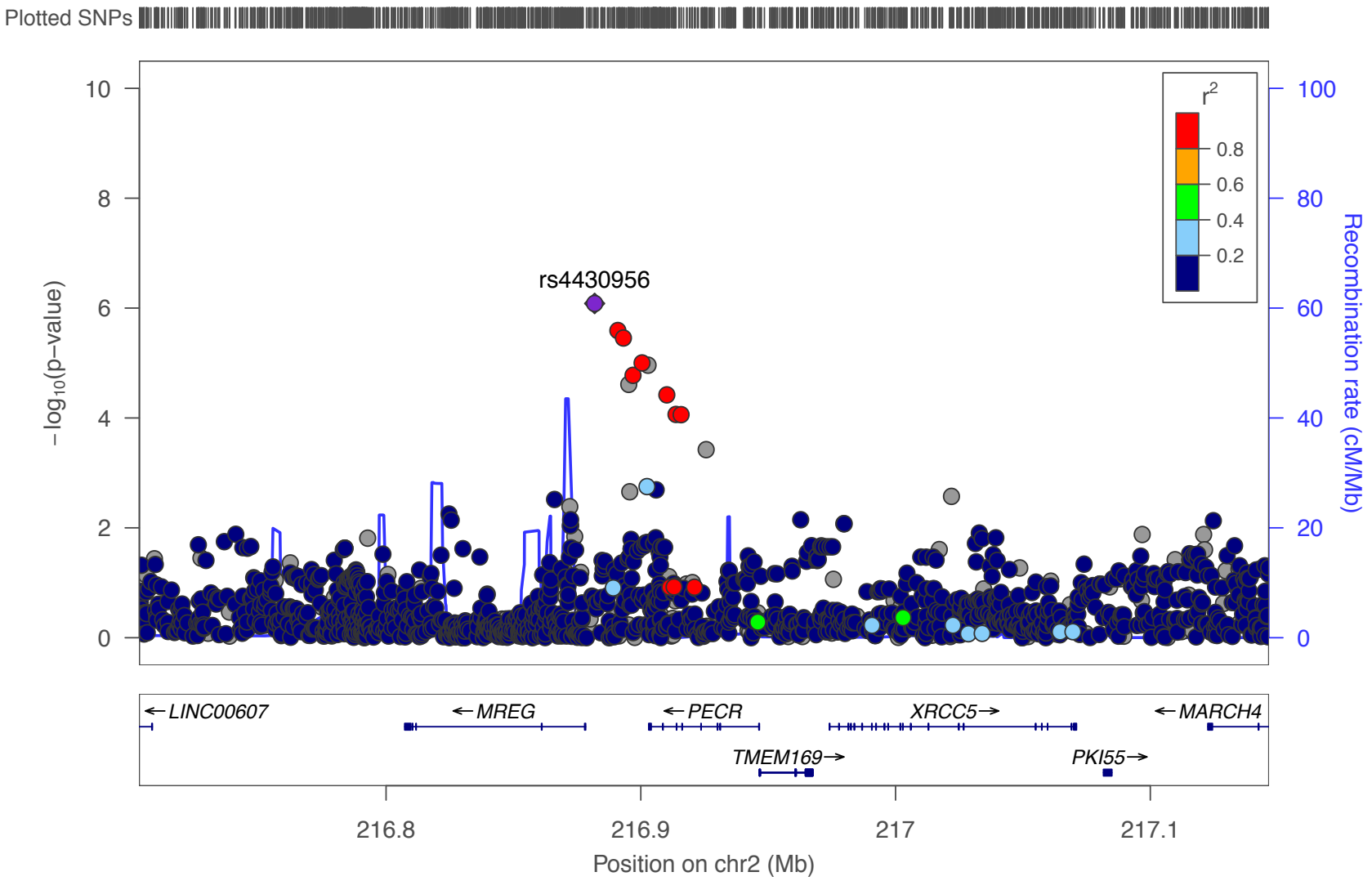

Figure 5.12. Regional association plot of –log(*p*) values in the *PECR* gene region for enhancement motives in the European ancestry GWAS.

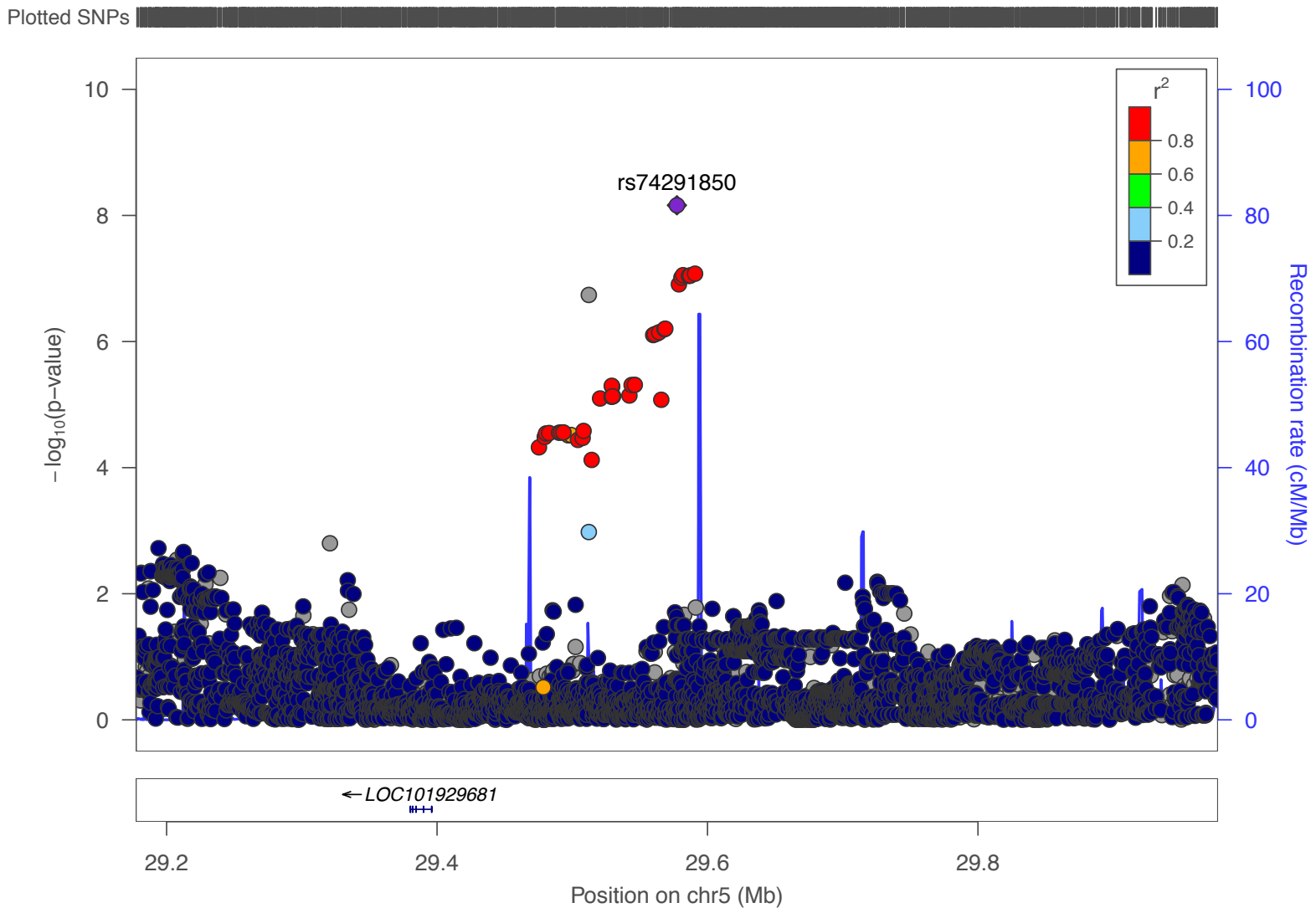

Figure 5.13. Regional association plot of –log(*p*) values in the chromosome 5 29.2-29.8Mb region for coping motives in the cross-ancestry meta-analysis.

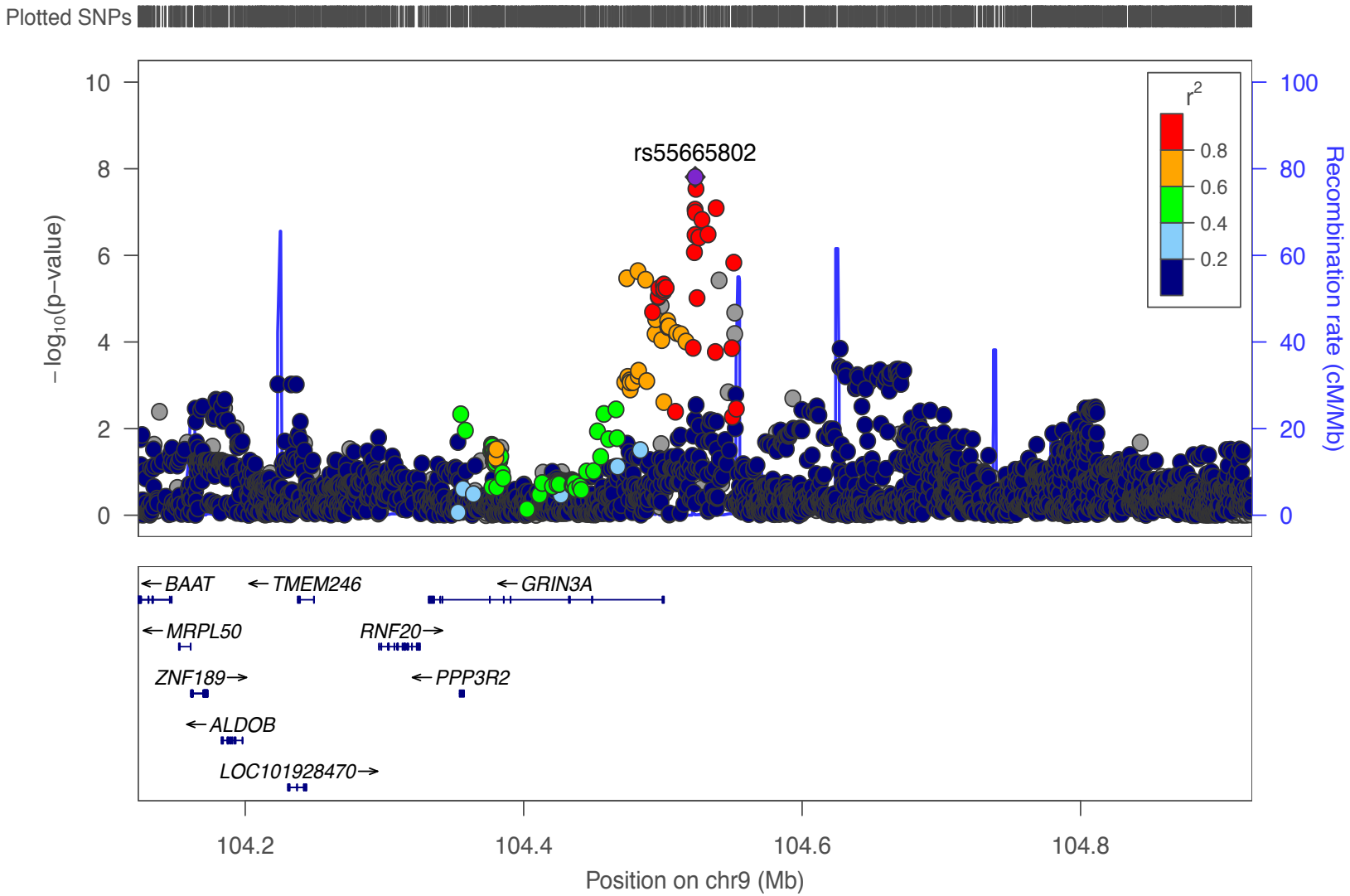

Figure 5.14. Regional association plot of –log(*p*) values in the *GRIN3A* gene region for coping motives in the European ancestry GWAS.

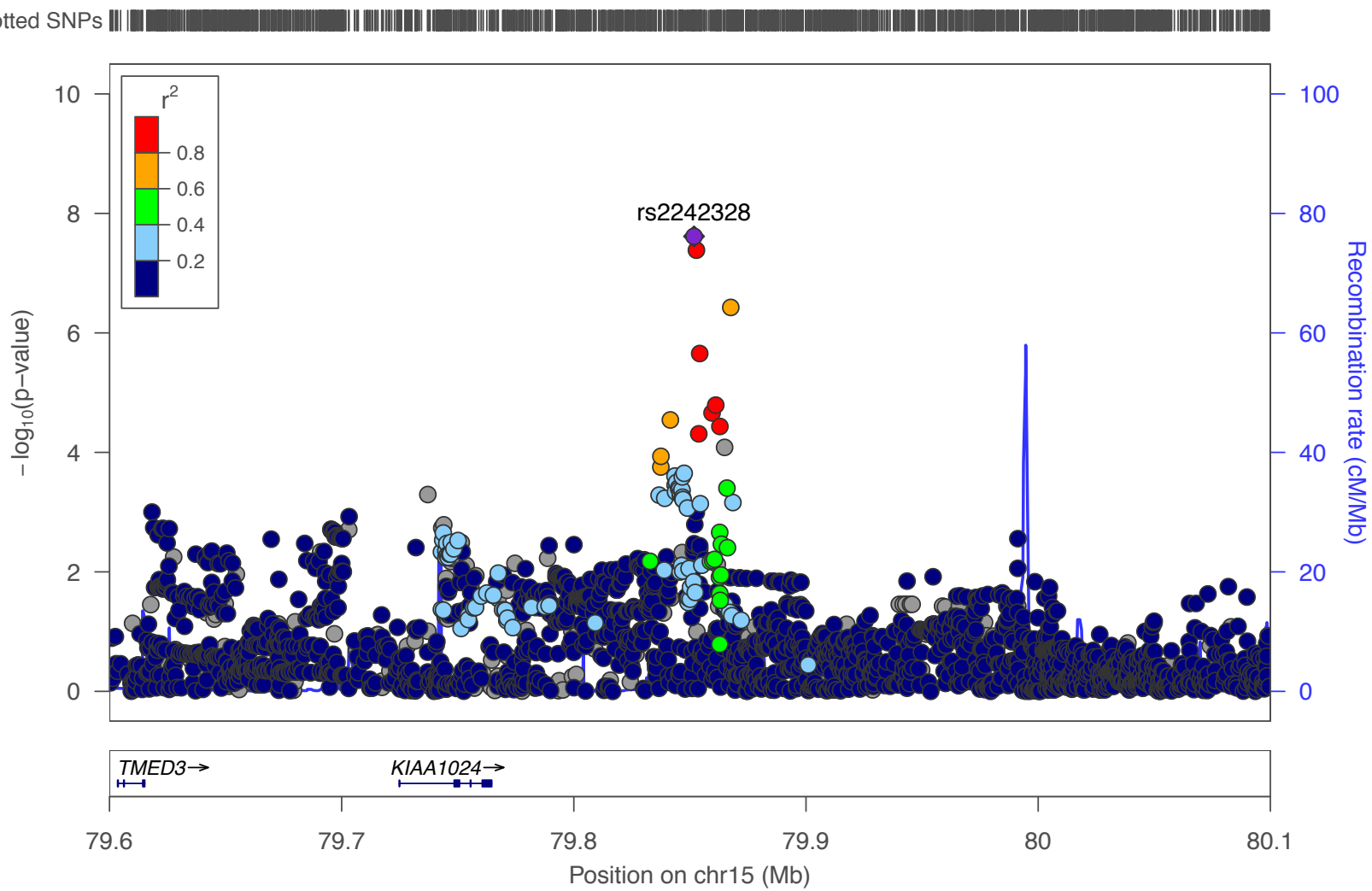

Figure 5.15. Regional association plot of –log(*p*) values in the *LOC390617* region for coping motives in the European ancestry GWAS.

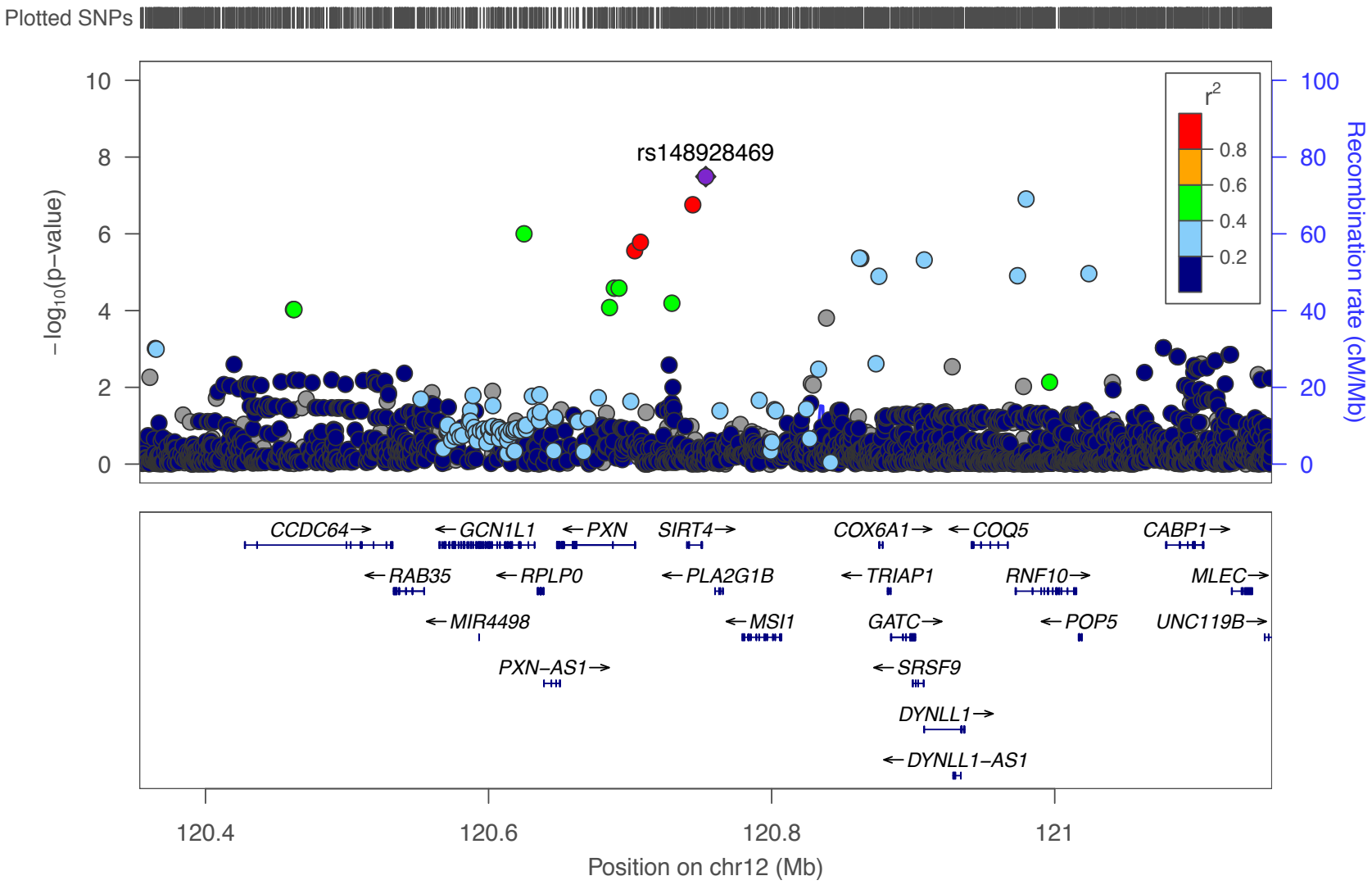

Figure 5.16. Regional association plot of –log(*p*) values in the chromosome *SIRT1* gene region for conformity motives in the cross-ancestry meta-analysis.

Top results from the gene-based association analyses are presented in Table 5.3. We use a Bonferroni corrected *p* value of .05/15,228 genes = 3.28e-06 to adjust for multiple testing of the number of genes that were included in the analyses. By this criterion, one gene, *PTER* (phosphotriesterase-related) was significantly enriched for association with coping motives. This association was primarily driven by the AFR (p = .0002) and EUR (p = .002) subsamples. This gene is highly expressed in the brain and kidney (Fagerberg et al., 2014) and has been previously associated with obesity (Meyre et al., 2009).

Top results from the pathway-based association tests are presented in Table 5.4. Using a Bonferroni corrected *p* value of .05/7,158 gene sets = 6.98e-06, there was no evidence for significant enrichment in any of the specified gene sets for any of the four drinking motives.

Table 5.3. Top results of gene-based enrichment meta-analysis tests for association with drinking motives.

Gene	CHR	# SNPs	Z	P	Gene Description
Social					
ARGLU1	13	13-18	4.28	9.4E-06	arginine and glutamate rich 1
GRIN2B	12	495-1198	4.16	1.6E-05	glutamate ionotropic receptor NMDA type subunit 2B
DHRS12	13	27-69	-3.37	3.8E-04	dehydrogenase/reductase 12
NACAD	7	16-25	3.24	6.1E-04	NAC alpha domain containing
PTPN13	4	59-500	3.21	6.5E-04	protein tyrosine phosphatase, non-receptor type 13
Enhancement					
DGKD	2	90-234	-3.73	9.6E-05	diacylglycerol kinase delta
ATP13A3	3	52-103	3.47	2.6E-04	ATPase 13A3
RCCD1	15	16-23	3.39	3.5E-04	RCC1 domain containing 1
SUMO3	21	21-44	3.34	4.2E-04	small ubiquitin-like modifier 3
IRF6	1	44-57	3.34	4.2E-04	interferon regulatory factor 6
Coping					
PTER	**10**	**164-253**	**4.56**	**2.5E-06**	**phosphotriesterase related**
BABAM1	19	25-38	4.29	9.1E-06	BRISC and BRCA1 A complex member 1
GPATCH1	19	59-124	4.16	1.6E-05	G-patch domain containing 1
PLEK	2	106-153	4.03	2.8E-05	pleckstrin
CCDC141	2	234-584	3.82	6.7E-05	coiled-coil domain containing 141
Conformity					
ACER1	19	27-128	4.26	1.0E-05	alkaline ceramidase 1
MED25	19	20-41	4.18	1.5E-05	mediator complex subunit 25
CAMK4	5	366-652	3.81	7.1E-05	calcium/calmodulin dependent protein kinase IV
DPRX	19	10-17	3.74	9.2E-05	divergent-paired related homeobox
OTOGL	12	147-357	3.60	1.6E-04	otogelin like

Note: Bonferroni corrected p value of .05/15,228 genes = 3.28e-06. Bolded values are significant.

Table 5.4. Top results of pathway-based enrichment meta-analysis tests for association with drinking motives.

Gene Set	# Genes	Z	P
Social			
GO_LOCALIZATION_WITHIN_MEMBRANE	116	3.81	6.87E-05
GO_POSITIVE_REGULATION_OF_ANTIGEN_PROCESSING_AND_PRESENTATION	15	-3.75	8.84E-05
GO_TELENCEPHALON_GLIAL_CELL_MIGRATION	16	-3.52	2.13E-04
REACTOME_CLASS_I_MHC_MEDIATED_ANTIGEN_PROCESSING_PRESENTATION	224	3.46	2.73E-04
GO_CARTILAGE_DEVELOPMENT_INVOLVED_IN_ENDOCHONDRAL_BONE_MORPHOGENESIS	19	-3.44	2.90E-04
Enhancement			
BIOCARTA_EIF_PATHWAY	13	4.00	3.19E-05
GO_POSITIVE_REGULATION_OF_OXIDOREDUCTASE_ACTIVITY	42	3.99	3.26E-05
BIOCARTA_EIF4_PATHWAY	24	3.95	3.90E-05
GO_MITOCHONDRIAL_DNA_METABOLIC_PROCESS	14	-3.65	1.33E-04
GO_ANAPHASE_PROMOTING_COMPLEX	22	3.62	1.46E-04
Coping			
GO_NADPH_BINDING	13	3.84	6.22E-05
GO_INVADOPODIUM	10	3.79	7.60E-05
GO_STRUCTURAL_MOLECULE_ACTIVITY	672	-3.70	1.08E-04
GO_PHOTORECEPTOR_ACTIVITY	11	3.65	1.29E-04
GO_PROTEIN_KINASE_C_BINDING	49	3.60	1.61E-04
Conformity			
GO_PYRIMIDINE_CONTAINING_COMPOUND_SALVAGE	10	3.89	5.03E-05
GO_RESPONSE_TO_OXYGEN_CONTAINING_COMPOUND	1328	3.83	6.48E-05
KEGG_STEROID_BIOSYNTHESIS	15	-3.70	1.08E-04
GO_NUCLEOBASE_CONTAINING_SMALL_MOLECULE_METABOLIC_PROCESS	500	3.59	1.63E-04
GO_NEURON_PROJECTION_EXTENSION_INVOLVED_IN_NEURON_PROJECTION_GUIDANCE	12	-3.32	4.53E-04

Note: Bonferroni corrected p value of .05/7,158 gene sets = 6.98e-06

Finally, we investigated the potential genetic overlap between the four drinking motives. Bivariate GCTA was first applied to each pair of drinking motives in each ancestry subset. However, all analyses failed to converge in the smaller groups (AMR, EAS, SAS), as did several in the AFR and EUR groups. For those that did converge, the genetic correlation between virtually all pairs of motives was between 0.75 and 1.00, but the standard errors were extremely large and these estimates were not differentiable from zero. Such results indicate a lack of statistical power to gain traction on the estimates, which is a common occurrence in mixed model analyses and particularly GCTA (Yang et al., 2011). To supplement these inconclusive findings, we carried out a parallel investigation of cross-trait genetic correlation using LD score regression with the summary statistics from the ancestry-specific GWAS described above. However, this method works by parsing apart the inflation in the SNP association (chi-square) statistics that is due to polygenic effects versus population stratification, and the SNP-based association statistics for drinking motives were underinflated to begin with. The models estimated a genetic correlation of 0.92 between coping and conformity motives in Europeans (p = .44), but the association signal was too small to estimate heritability or genetic covariance between all other pairs of motives in all ancestry groups using this method.

IV. Summary and Discussion

This investigation into the genetic etiology of drinking motives in college students identified some promising but largely inconclusive results. We found heritability estimates using measured genome-wide variants that were on par with estimates of latent heritability from twin models, with an indication that positive reinforcement motives had slightly higher heritability (16-22%) than negative

reinforcement motive (14-16%). However, these estimates were not significantly different from zero in our sample. In genetic association testing, several loci were identified with suggestive or marginally significant effects, although the results of the gene-based and pathway-based analyses showed little evidence of enrichment at the aggregate levels that should have greater power to detect associations in this size of sample. We were largely unable to carry out successful testing to identify whether different types of drinking motives have a shared or distinct genetic basis.

A few promising results from these analyses are still worth considering. First, we found a suggestive association with enhancement motives in the *PECR* gene in Europeans. Although not quite reaching the threshold of genome-wide significance, this signal showed a clear peak with enrichment of association signal in a large number of SNPs within a single locus, which bolsters confidence that it is a true effect. This gene is highly expressed in the liver and has been previously implicated in a GWAS of early onset alcohol dependence (Treutlein et al., 2009). Such evidence is consistent with the hypothesized connection between enhancement motives and an externalizing pathway/subtype of alcohol dependence characterized by early age of onset and stronger genetic influences (e.g. Cloninger et al., 1988). There was also evidence for a genome-wide significant association of the *SIRT4* gene with conformity motives. This gene is a close relative of the *SIRT1* gene that has been recently implicated in the genetic etiology of major depression (CONVERGE Consortium, 2016) and may suggest a common predisposition shared between internalizing psychopathology and this negative reinforcement motive. However, there is also a large number of other genes in the region that could be driving the identified association effect.

We conclude that our findings at this stage thus provide only modest insight into the biology underlying drinking motives and their potential genetic pathways to alcohol misuse. This is perhaps unsurprising given trends in gene identification efforts for complex, and particularly psychiatric/behavioral traits. The emerging landscape of the field indicates that early gene identification successes were likely false positives (Dick et al., 2015), and that tens if not hundreds of thousands of samples may be needed before credible results may be found. Although it was theorized that endophenotypes should be less genetically complex and thus require fewer samples to achieve comparable statistical power, early enthusiasm has been tempered by evidence that even plausible biological endophenotypes likely do not have a simple underlying genetic architecture (Flint & Munafo, 2007). Huge samples and even further phenotypic refinement are likely still necessary to achieve tangible successes in understanding the genetic etiology of endophenotypes and intermediate phenotypes on the path to behaviors and disorders. However, the potential insights that such phenotypes can provide into the mechanisms underlying complex outcomes underscores their value for study with larger samples and more powerful study designs in the future.

Chapter 6. The Internalizing Pathway and Alcohol Misuse

I. Specific Aim

In this final analytic chapter, attention is turned to focus on the internalizing domain of psychopathology as a means to understand alcohol misuse. Some previous research has indicated that the comorbidity between alcohol dependence/misuse and internalizing psychopathology is not primarily due to shared genetic etiology (Edwards et al., 2011a; Kendler et al., 2011; Kendler et al., 2003). This relationship is less clear for depression, for which other studies have found a genetic overlap with alcohol misuse (Prescott, Aggen, & Kendler, 2000), especially in adolescence (Edwards et al., 2011b). There is prospective evidence that pre-existing internalizing psychopathology increases one's risk for subsequent alcohol misuse (Merikangas et al., 1998; Swendsen et al., 2010; Swendsen et al., 1998). Although the direction of effect is less clear for depression, which often has an onset after that of AUD in comorbid cases, anxiety disorders tend to precede alcohol problems (de Graaf, ten Have, Tuithof, & van Dorsselaer, 2013; Swendsen et al., 2010; Swendsen et al., 1998). There is a particularly strong prospective link with alcohol misuse for some anxiety disorders like social anxiety disorder (Lepine & Pelissolo, 1998).

The anxiolytic and mood-enhancing properties of alcohol consumption have prompted many to propose a causal phenotypic relationship by which internalizing psychopathology (and particularly anxiety) contributes to the subsequent development of alcohol misuse. A causal pathway also fits succinctly into theoretical models that emphasize the use of alcohol as a means of dampening the stress

response, particularly among individuals prone to anxiety (Ham & Hope, 2003; Levenson, Sher, Grossman, Newman, & Newlin, 1980). However, this type of relationship is exceedingly difficult to tease apart in human studies, even when using longitudinal statistical models like the cross-lagged panel design employed in Chapter 4. There are many opportunities for causal effects to be missed if the timing of assessment does not match the timing of a developmental relationship between constructs or if they are clouded by reciprocal causal effects.

Although a true random experimental design will likely never be plausible for these kinds of research questions, there are a few experiments of nature that allow for credible inference of causal associations. One such design is Mendelian randomization (Haycock et al., 2016; Smith & Ebrahim, 2003), a strategy that leverages the genetic property of random segregation of alleles during gametogenesis to infer that the genes influencing one phenotype should not be associated with another, distinct phenotype, unless it is through a phenotypic causal pathway. Using genes as instrumental variables limits any potential for confounding and reverse causality, since DNA variants are essentially immutable. This method requires certain assumptions, most importantly that there is no genetic pleiotropy – meaning that the genes for phenotype A do not have any direct genetic effect on phenotype B. This is often a difficult criterion to meet for psychiatric disorders, as the biological impacts of genes involved in brain functioning are poorly understood and likely far-reaching. However, given the availability of candidate genes whose biological function is understood and which can be plausibly assumed to directly affect one but not another phenotype, this strategy has been successfully employed in a number of studies investigating psychiatric and physical health outcomes (Burgess, Timpson, Ebrahim, & Davey Smith, 2015).

A prerequisite for applying Mendelian randomization to test causal relationships between predictors such as internalizing psychopathology and alcohol misuse outcomes is that there must be credible genetic variants known to be associated with the putative causal phenotypes. For internalizing psychopathology, this is hardly the case. Although decades of genetic epidemiology studies have demonstrated that genetic factors contribute to 30-50% of the liability to developing anxiety and depression (Hettema, Neale, & Kendler, 2001; Hettema, Prescott, Myers, Neale, & Kendler, 2005; Shimada-Sugimoto, Otowa, & Hettema, 2015), few specific genetic variants underlying this moderate heritability have been uncovered. As reviewed by Shimada-Sugimoto et al. (2015) and Dunn et al. (2015), molecular genetic approaches such as candidate gene and genome-wide association studies (GWAS) have been attempted for anxiety disorders (ADs) and major depressive disorder (MDD). These have largely revealed no significant associations, or associations that are inconsistent or nonreplicable.

A recent meta-analysis by the Anxiety NeuroGenetics STudy (ANGST) Consortium sought to improve upon these earlier attempts by combining participant data across nine samples and analyzing a joint measure of anxiety that combined all of the primary ADs (Otowa et al., 2016). Previous evidence has shown that there is substantial overlap in the genetic influences on each distinct disorder (Hettema et al., 2005; Middeldorp, Cath, Van Dyck, & Boomsma, 2005), likely representing shared biological pathways for fear circuitry and threat response systems (Craske et al., 2009), and combining these disorders can thus increase power to detect genetic influences that are common across them. The meta-analysis utilized two phenotypes, a quantitative factor score that captured symptoms from all disorders (FS), and a binary case-control status representing a diagnosis of any versus none

of the disorders (CC). In doing so, they identified one genome-wide significant association for each phenotype: for FS, the *CAMKMT* gene on chromosome 2p21, encoding the calmodulin-lysine *N*-methyltransferase, and for CC, an uncharacterized non-coding RNA, *LOC152225*, located on chromosome 3q12.3.

Similar success has come recently for major depression, but through a different strategy. The CONVERGE Consortium (CONVERGE Consortium, 2015) sought to improve its statistical power by conducting a case-control association analysis in a narrowly defined sample (female-only sample of Han Chinese descent) to reduce heterogeneity, and used a refined phenotype (severe, recurrent MDD) to increase the expected effect sizes. In doing so, they also identified two genome-wide significant associations in the *SIRT1* and *LHPP* genes.

Although these results were replicated within the respective studies, the samples are limited to individuals of European or Han Chinese ancestry, respectively, and primarily older adults. Evidence suggests that the heritability of these disorders nearly double from adolescence to adulthood (Bergen, Gardner, & Kendler, 2007), and it is not known whether the same or different genes contribute to this heritability at different ages. In addition, virtually all molecular genetic research (and twin research, for that matter) has been conducted with samples of European ancestry and it is unknown whether the same genetic variants underlie genetic liability for disorders across ancestral groups despite known differences the prevalence of between ethnic and cultural groups (Asnaani, Richey, Dimaite, Hinton, & Hofmann, 2010; Marques, Robinaugh, LeBlanc, & Hinton, 2011).

The aim of the present analyses was thus to identify genetic variants for use in future models to test the potential causal relationship between internalizing psychopathology and alcohol misuse, either by replicating variants identified in the

previous studies or uncovering new association in this multi-ancestry college sample. We focus on anxiety as a primary outcome due to its stronger *a priori* likelihood of a causal association with alcohol misuse and availability of suitable phenotypes for association testing in S4S.

II. Methods

The analyses in this chapter use genotypic data and self-report measures collected in the freshman and sophomore spring surveys of S4S. After filtering based on missingness for genotypic and phenotypic data, we had an analytic sample size of n = 5,179 individuals.

Included in the survey were two screening items for each of the disorders of generalized anxiety disorder (GAD) (excess worry/worry felt out of control), social anxiety disorder (SAD) (embarrassment one of worst fears/nervous around people and avoided social activities), and specific phobia (PHO) (extreme fear of certain things/fear interfered with life), and one screening item each for panic disorder (PAN) (sudden attacks of uncontrollable fear or anxiety with somatic symptoms) and agoraphobia (AGO) (avoided places for fear of such attacks). Participants were asked to report with a binary response whether or not they had experienced each symptom for a period of one month or longer. The timeframe for the questions was a) ever in their lifetime (at the spring of freshman year), or b) in the past year since the previous survey (at the spring of sophomore year). The maximum endorsement across the two years was calculated for each item to index cumulative lifetime symptom experience. Finally, for disorders with two screening items (GAD, SAD, and PHO), the items were summed to create an ordinal 0/1/2 severity index that would

roughly correspond to control, sub-threshold, and case status for each disorder. PAN and AGO items were necessarily binary indicators of their respective disorders.

Replicating the methods of the ANGST meta-analysis (Otowa et al., 2016), two phenotypes were created from these items to index the latent genetic risk for anxiety shared across disorders. First, we calculated an "any anxiety disorder" categorical case/control status (CC), treating individuals endorsing the highest level of severity for any disorder as a case and those endorsing no symptoms for any disorder as a "supernormal" control, and excluding those reporting sub-threshold symptoms. Second, we created a quantitative anxiety factor score to capture the weighted contribution of each disorder to the latent anxiety phenotype. After exploratory factor analysis (EFA) confirmed a single factor structure underlying the covariance between disorder screening items, we conducted confirmatory factor analysis (CFA) using the OpenMx package (Boker et al., 2011) for R version 3.2.1 (R Core Team, 2015) and computed factor scores for each participant using two-stage, full information maximum likelihood estimation, which accounts for the binary/ordinal response structure of the items (Estabrook & Neale, 2013). The factor models were tested for measurement invariance across sex and genetic ancestry group (see "Genotyping" section below); we found that the factor loadings but not thresholds (i.e. endorsement rates) could be constrained across sexes while neither could be constrained across ancestry groups without a significance decrease in model fit. We therefore included sex as a covariate in all analyses and created the factor scores separately within each ancestry group.

Within ancestry groups, additional quality control steps were taken to remove related individuals and SNPs with low minor allele count (MAC; < 40) and violations of Hardy-Weinberg equilibrium ($p < 10^{-6}$). After quality control filters were applied, we

had a sample size of 3,883 and 4,832 for the CC and FS genetic analyses, respectively. We used the same genetic analysis pipeline as described for the drinking motives in the previous chapter: genome-wide complex trait analysis (GCTA) to estimate the trait heritability (h^2_{SNP}), GWAS using SNPTEST for individual variant associations, and gene-based/pathway-based enrichment testing using MAGMA. We also estimated heritability and genetic covariance between the two phenotypes with LD score regression. Again, all analyses were conducted separately by ancestry superpopulation and meta-analyzed.

We additionally tested for replication of the aggregate genomic associations identified in the ANGST meta-analyses using polygenic risk scores (PRS) created within S4S based on SNP association weights from the ANGST GWAS to predict their respective CC/FS phenotypes in S4S. These analyses were conducted using PRSice (Euesden, Lewis, & O'Reilly, 2015), a software which automates implementation of the *score* procedure in PLINK 1.9 (Chang et al., 2015) and optimization of the set of SNPs included in the PRS to improve scores' predictive ability. A list of independent (R^2 < .10) SNPs common to both studies and meeting filtering criteria in both (INFO > .9, MAF > .01, non-ambiguous) was first created, and then PRSice was run to filter SNPs based on linkage disequilibrium and create a PRS for each individual in the sample. The PRS represents a sum for each person of the number of "risk" alleles they possess with each allele weighted by its association strength in the discovery (ANGST) GWAS. PRSice varies the set of SNPs that are included in the score by filtering by higher or lower GWAS *p* value, and selects a final score based on the strongest prediction of the phenotype of interest in a linear model, with covariates as described above. We used this same method to create within-sample polygenic risk scores as well, using the GWAS results from the larger

S4S European subset to create scores in the other S4S ancestry groups. These within-sample PRS analyses test for aggregate genetic associations identified by the GWAS while eliminating some of the methodological variance between ANGST and S4S that might reduce cross-sample replication.

III. Results

Endorsement rates for the anxiety disorder screening items are shown in Table 6.1. Endorsement rates were high, particularly for the GAD and SAD items. Overall, 44% of the sample met the highest threshold for at least one disorder and were considered cases for the "any disorder" phenotype, while 23% reported no symptoms of any disorder and were considered controls, and 33% of participants reporting subthreshold symptoms for one or more disorder were excluded from the analysis. For the anxiety factor score phenotype, there was a clear single latent factor underlying all five disorders, with factor loadings of 0.61 to 0.90.

Table 6.1. Endorsement of lifetime screening criteria for five primary anxiety disorders in a sample of college students.

Endorsement level	**GAD** *N (%)*	**SAD** *N (%)*	**PAN** *N (%)*	**AGO** *N (%)*	**PHO** *N (%)*
0	1901 (37)	2185 (42)	3974 (77)	4401 (86)	3954 (77)
1	1083 (21)	1333 (26)	1156 (23)	728 (14)	663 (13)
2	2195 (42)	1654 (32)			539 (10)

Note: Items with three levels are the composite of two binary symptom criteria. GAD = generalized anxiety disorder; SAD = social anxiety disorder; PAN = panic disorder; AGO = agoraphobia; PHO = specific phobia.

SNP-based heritability from the GCTA meta-analysis was estimated at 0% for CC and 3% for FS. Neither estimate was significantly differentiable from zero. Using LD score regression in the EUR ancestry group, the heritability estimates were 31.5% (p = .24) for CC and 24.1% (p = .16) for FS, with a genetic correlation of 1.01 (p = .18). This estimate is outside the rational bounds for a correlation, indicative of

an untrustworthy result due to large standard errors. The association statistics in other ancestry groups were underinflated and could not be examined with LD score regression.

Results from the GWAS meta-analyses are displayed in Figures 6.1 and 6.2. Although no locus reached the threshold of genome-wide significance for either phenotype in the meta-analyses, there were several suggestive association peaks ($p < 5x10^{-5}$). Top results are shown in Table 6.2, listing association peaks in which 3 or more SNPs with suggestive associations were clustered in the same 20kB region (i.e. less likely to be spurious lone signals). There are notable peaks for CC on chromosomes 1 and 4 and for FS on chromosomes 5 and 14. The peaks in each of these loci were in intronic or intergenic regions and had no obvious regulatory functions based on information from the ENCODE tracks of the UCSC Genome Browser (Rosenbloom et al., 2013). The top SNP from each of the ANGST CC/FS meta-analyses did not replicate in this sample (*p*'s > .20).

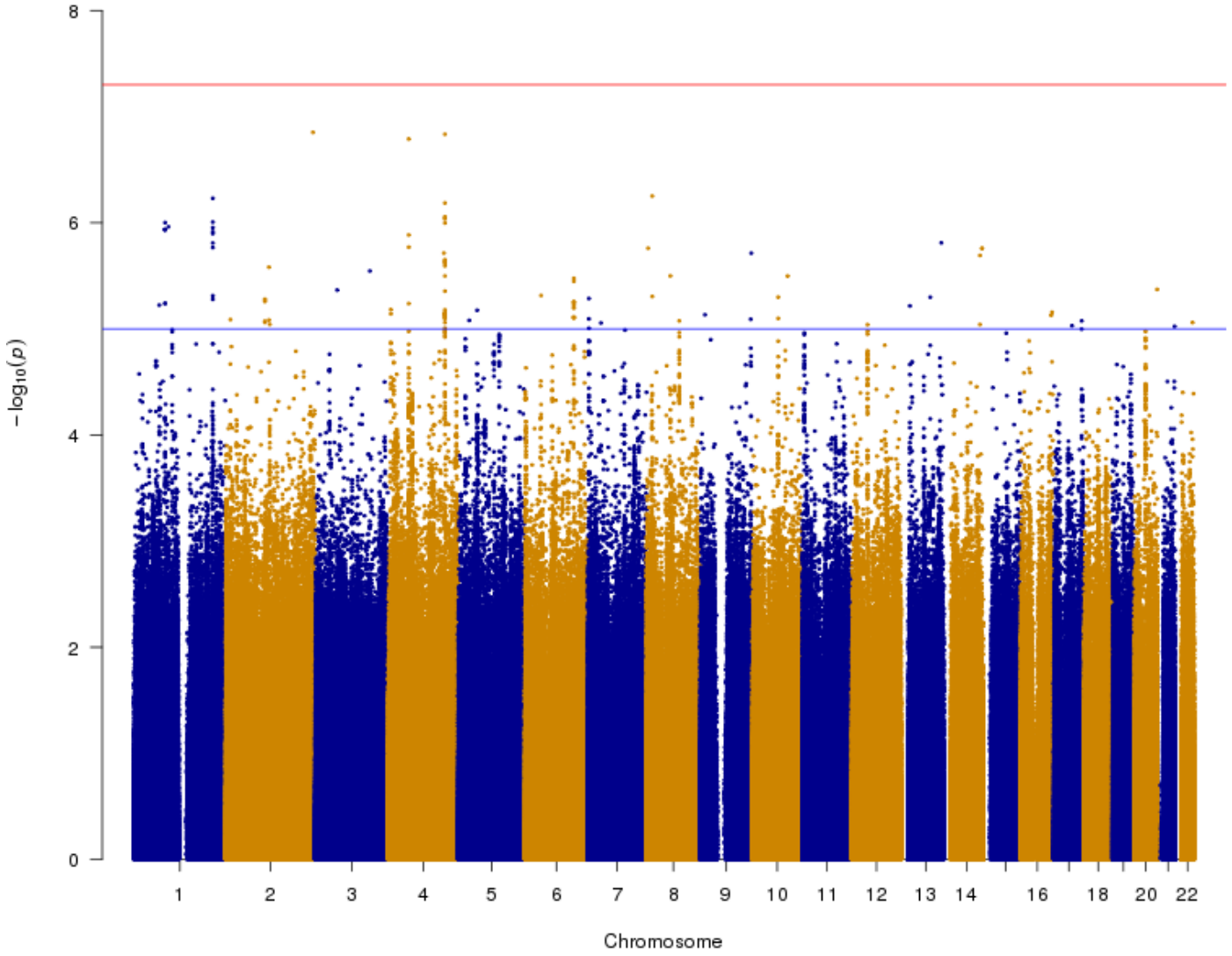

Figure 6.1. Genome-wide association meta-analysis results for anxiety disorder case-control (CC) status in a sample of college students from five genetic ancestry populations.

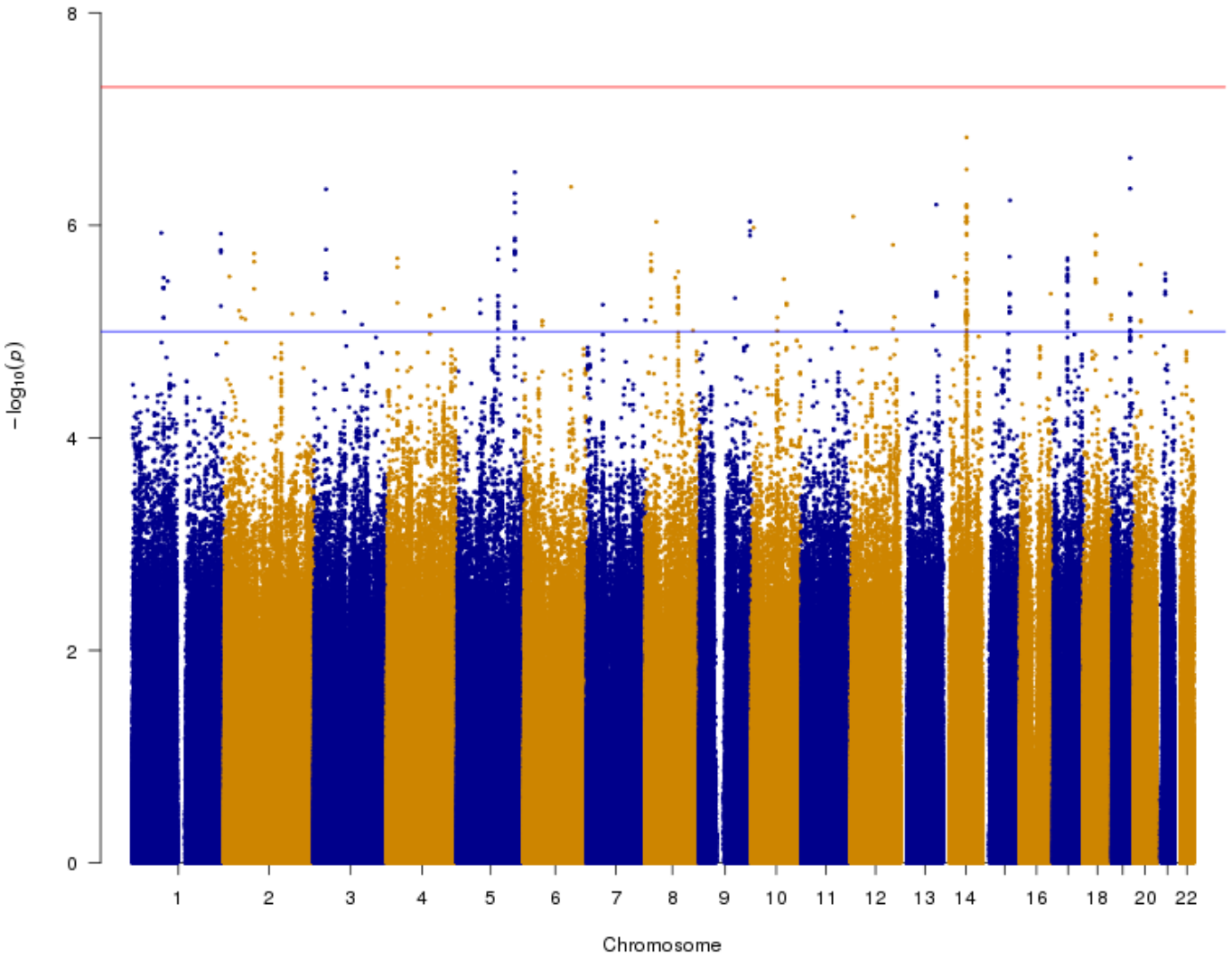

Figure 6.2. Genome-wide association meta-analysis results for an anxiety disorder factor score (FS) in a sample of college students from five genetic ancestry populations.

Table 6.2. Loci of top association peaks from a meta-analysis of genome-wide association scans for anxiety disorder phenotypes in college students from five genetic ancestry populations.

ANY ANXIETY DISORDER CASE-CONTROL STATUS						
CHR	**BP**	**# SNPs**	**Min. *p***	**Min *q***	**Max N**	**Annotated Genes (within 50kB)**
1	213994303	9	5.88E-07	0.61	3883	*AK092251*
2	106241202	5	5.28E-06	0.76	1919	*LOC285000*
4	55801962	3	1.63E-07^	0.53	3203	None
4	154303033	5	3.18E-06	0.75	3116	*MND1, TRIM2*
4	154329842	28	1.47E-07	0.53	3116	*KIAA0922, MND1*
6	132726450	14*	3.34E-06	0.76	3883	*MOXD1, STX7*
7	2650076	3	5.18E-06	0.76	3508	*IQCE, TTYH3*
ANXIETY DISORDER FACTOR SCORE						
CHR	**BP**	**# SNPs**	**Min. *p***	**Min *q***	**Max N**	**Annotated Genes (within 50kB)**
1	240009037	5	1.20E-06	0.47	3984	*CHRM3*
5	62399928	3*	5.02E-06	0.52	4832	None
5	110781178	10*	1.64E-06	0.47	4832	*CAMK4, STARD4, STARD4-AS1*
5	156262999	16	5.03E-07	0.47	1152	*PPP1R2P3, TIMD4*
8	15376049	7	1.87E-06	0.47	4832	*TUSC3*
8	89136847	13*	2.73E-06	0.47	4832	*MMP16*
9	137717379	4*	9.22E-07	0.47	4832	*COL5A1, LOC101448202, MIR3689A, MIR3689B, MIR3689C, MIR3689D1, MIR3689D2, MIR3689E, MIR3689F*
14	64905976	4*	4.08E-06	0.52	4832	*AKAP5, MTHFD1, ZBTB25*
14	64956317	75*	1.49E-07	0.47	4832	*AK055910, AKAP5, HSPA2, MTHFD1, PPP1R36, ZBTB1, ZBTB25*
14	65113224	3*	1.87E-06	0.47	4832	None
15	74223556	8	1.97E-06	0.47	1152	*LOXL1, LOXL1-AS1, PML, STOML1, TBC1D21*
17	38786451	5*	6.37E-06	0.52	4832	*KRT222, SMARCE1*
17	38807957	14*	2.04E-06	0.47	4832	*KRT222, KRT24, SMARCE1*
18	33838620	8	1.23E-06	0.47	1152	*FHOD3, MOCOS*

19	49530502	8	2.33E-07	0.47	3984	*CGB, CGB1, CGB2, CGB5, CGB7, CGB8, GYS1, KCNA7, LHB, LOC101059948, MIR324, NTF4, RUVBL2, SNAR-G1, SNAR-G2, SNRNP70*
21	22947402	6	2.85E-06	0.47	4832	*NCAM2*

*Note: Peaks represent loci in which 3 or more SNPs in a 20kB window reached a suggestive level of association ($p < 5x10^{-5}$). Sample sizes vary by locus because not all variants were analyzed in all ancestry groups due to ancestry-specific filtering for minor allele frequency. CHR = chromosome; BP = base pair position. ^Locus reached genome-wide significance in European ancestry subset alone; *all SNPs in locus had the same direction of association across all five ancestry groups.*

For CC, one locus in an intergenic region on chromosome 4 had an association peak with three SNPs reaching genome-wide significance in the EUR subset (p = 2.2x10^{-8} to 1.3x10^{-8}, Figure 6.3). These three SNPs (rs73234251, rs10014134, and rs904132) were clustered in a 219bp region located in a peak of activity of H3K4Me1, according to the ENCODE tracks of the UCSC Genome Browser (Rosenbloom et al., 2013). H3K4Me1 is a histone modification mark associated with enhancer activity (Aday, Zhu, Lakshmanan, Wang, & Lawson, 2011). There was no evidence of genomic inflation in the meta-analysis results or European subset (Table 6.3), although there was some under/overinflation in the smaller ancestry groups.

Table 6.3. Genomic inflation values for genome-wide association analyses and meta-analyses for two anxiety-related phenotypes.

Analytic Sample	# Individuals	λ	λ_{1000}
ANY ANXIETY DISORDER CASE-CONTROL STATUS			
Meta-analysis (n>1000)	varies by SNP	0.985	1.000
AFR	892	0.992	0.991
AMR	392	0.882	0.698
EAS	375	1.006	1.016
EUR	1919	1.000	1.000
SAS	305	1.067	1.219
ANXIETY DISORDER FACTOR SCORE			
Meta-analysis (n>1000)	varies by SNP	0.995	1.000
AFR	1152	1.003	1.003
AMR	470	0.887	0.759
EAS	467	1.000	1.001
EUR	2362	0.998	0.999
SAS	381	0.881	0.689

Note: Lambda values for the meta-analyses were calculated only for SNPs passing quality control filters in a subsample or combination of subsamples with a total of 1000 or more individuals. AFR = African, AMR = American, EAS = East Asian, EUR = European, SAS = South Asian.

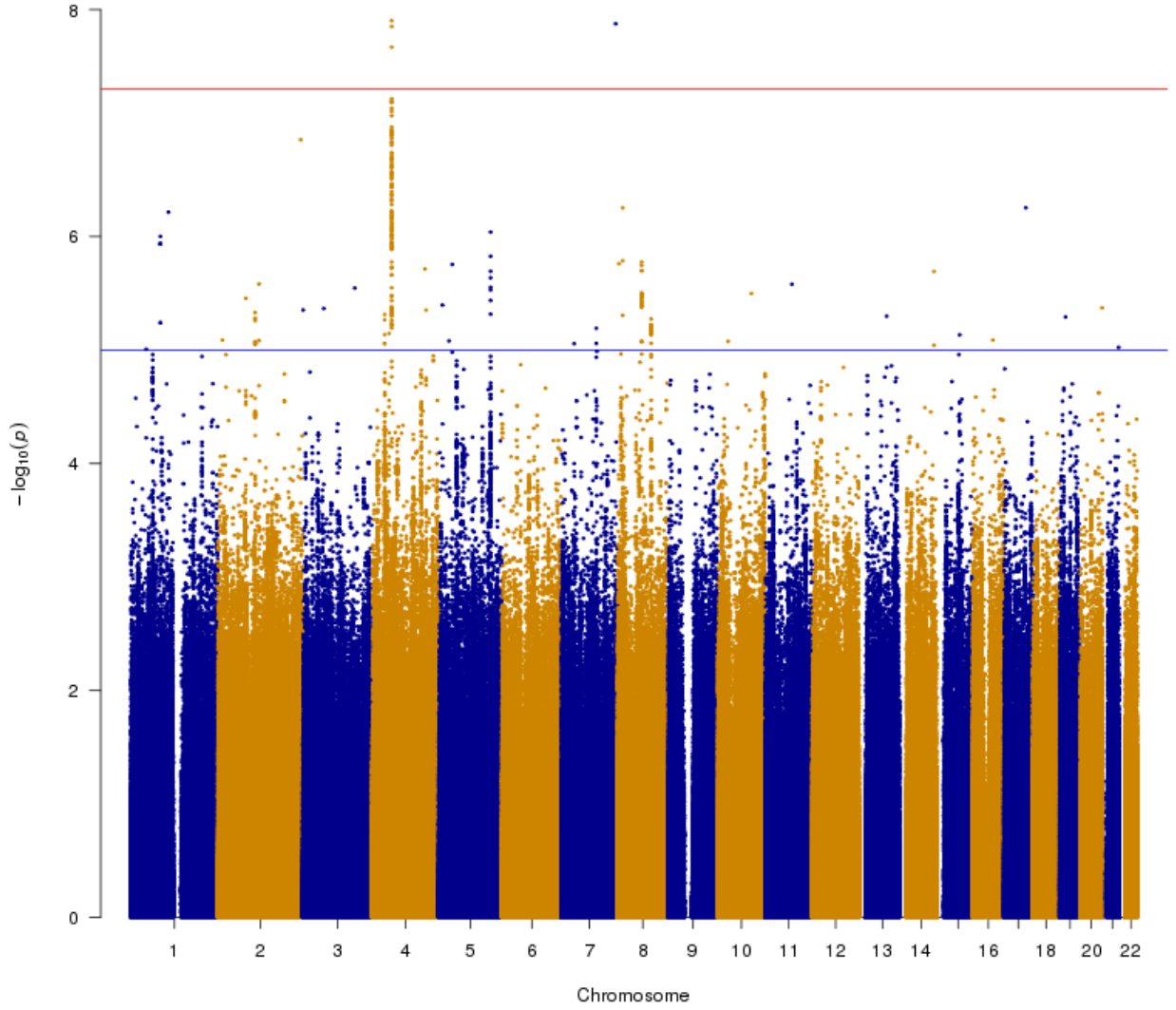

Figure 6.3. Genome-wide association results for anxiety disorder case-control (CC) status in the European subset (*n* = 1919) of a sample of college students.

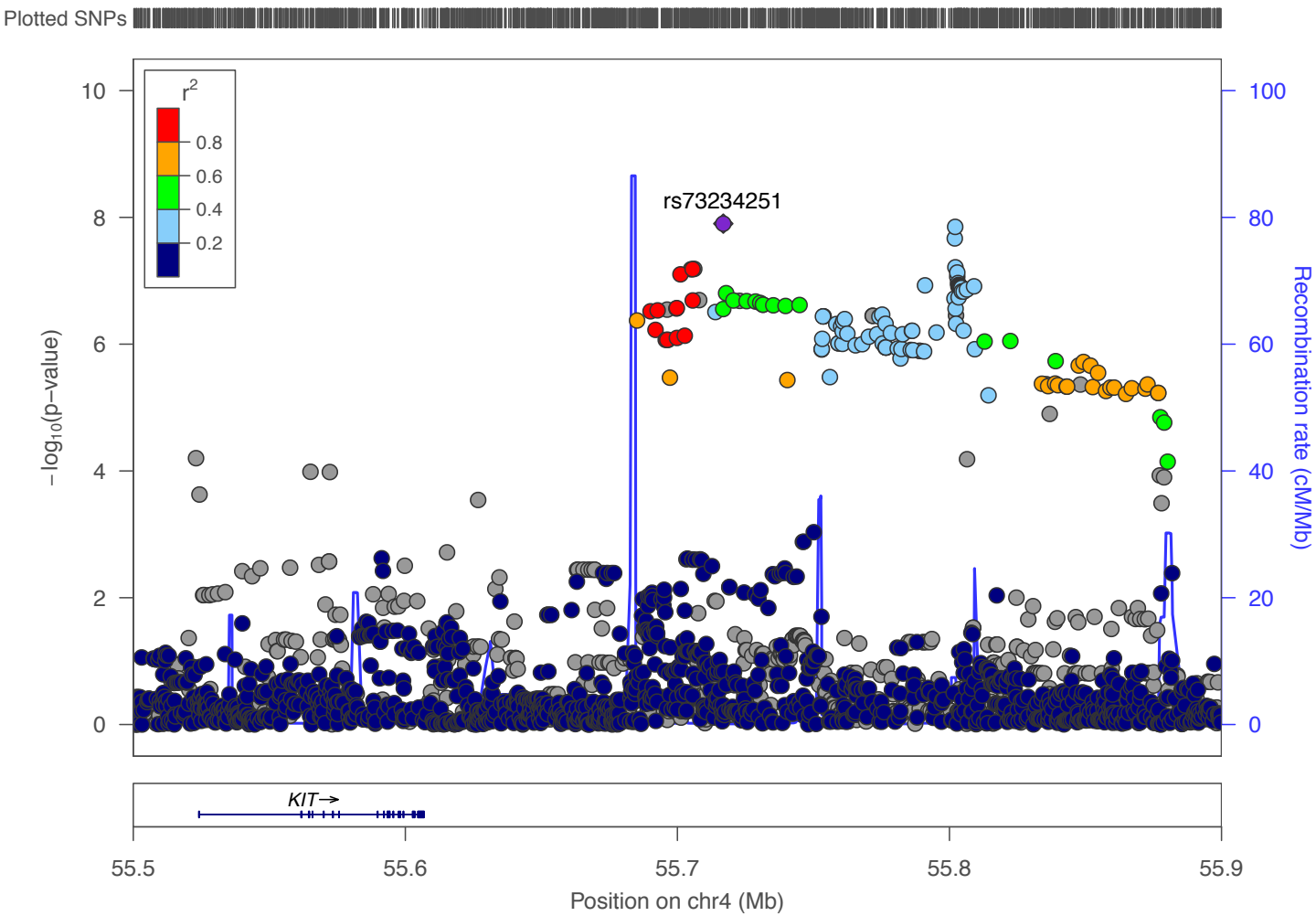

Figure 6.4. Regional plot of –log(*p*) values of association with case-control status in Europeans in a genome-wide significant locus on chromosome 4.

Results from the SNP-level GWAS presented above were used in gene-based association tests to assess the effects of individual SNPs at an aggregate gene level. QC-passing SNPs in each ancestry subset were mapped to up to 17,567 genic locations defined in the human reference assembly GRCh37 (hg19). Genes were filtered to exclude those with fewer than 10 SNPs in order to avoid spurious results driven by small sampling, leaving 14,635 tested genes and a corresponding Bonferroni-corrected significance level of 3.4×10^{-6}. As shown in Table 6.4, the *HLA-DQB2* gene located in the major histocompatibility complex on chromosome 6 was significantly associated with the FS phenotype. Notably, the genes *ZBTB1* and *ZBTB25*, both in a region on chromosome 14 with the strongest association signal in the FS GWAS, were also in the top results although they did not reach the statistical significance threshold. Within ancestry groups, the *HTR4* gene on chromosome 5 surpassed the genome-wide significance threshold in the EUR subpopulation ($p = 5.9 \times 10^{-7}$). The *CAMKMT* gene identified in the ANGST meta-analysis showed no evidence for association in any ancestry group (p's = .11 - .81). No genes were significant in the CC gene-based analyses, and no gene sets approached significance in the pathway enrichment meta-analyses.

Table 6.4. Top results from a meta-analysis of gene-based association results for an anxiety disorder case-control status (CC) or factor score (FS).

Gene Symbol	CHR	Min #SNPs	Max #SNPs	Z	Meta *p*	Gene Description
			CC			
PVALB	22	59	99	4.23	1.15E-05	parvalbumin
IQCE	7	166	384	3.91	4.64E-05	IQ motif containing E
OTOGL	12	217	627	3.86	5.78E-05	otogelin like
MEIOC	17	17	38	3.73	9.56E-05	meiosis specific with coiled-coil domain
HAO2	1	42	94	3.63	0.0001	hydroxyacid oxidase 2
			FS			
HLA-DQB2	**6**	**50**	**69**	**4.70**	**1.28E-06**	**major histocompatibility complex, class II, DQ beta 2**
ZBTB1	14	55	92	4.19	1.43E-05	zinc finger and BTB domain containing 1
FXYD5	19	43	66	-3.75	8.88E-05	FXYD domain containing ion transport regulator 5
ZBTB25	14	73	160	3.67	0.0001	zinc finger and BTB domain containing 25
HLA-DQA2	6	66	99	3.57	0.0002	major histocompatibility complex, class II, DQ alpha 2

Note: Bonferroni significance threshold for testing 14,635 genes is p < 3.4x10

Results from PRS analyses using association weights from the ANGST meta-analyses to predict their corresponding phenotypes in S4S are displayed in Table 6.5. In each ancestry group, the best PRS prediction came from including SNPs with relatively low GWAS *p* values in the scores (<0.016 to < 0.196); however, even these optimized scores showed little prediction of the anxiety phenotypes. There was a modest association between ANGST-weighted PRSs and the anxiety outcomes in the EAS ancestry subgroup – for CC, for example, accounting for 4.6% of the variance – but this was not evident in the larger European subset whose genetic ancestry is most similar to the discovery sample. Within the S4S sample, there was little evidence of PRSs based on the EUR GWAS results predicting anxiety outcomes in the other ancestry groups (meta-analysis *p*'s = .20 and .87).

Table 6.5. Logistic and linear regression results for prediction of anxiety-related traits using polygenic risk scores based on genome-wide association results from the ANGST consortium meta-analysis.

Ancestry Group	*p* Threshold	Beta	*p*	R^2
ANY ANXIETY DISORDER CASE-CONTROL STATUS				
AFR	0.064	-5.367	0.113	0.004
AMR	0.019	-3.966	0.179	0.006
EAS	0.196	36.367	4.7E-04	0.046
EUR	0.067	-3.385	0.181	0.001
SAS	0.016	5.613	0.068	0.013
Meta-Analysis	--	*-1.100*	*0.449*	*0.006*
ANXIETY DISORDER FACTOR SCORE				
AFR	0.024	-6.155	0.034	0.004
AMR	0.046	-6.036	0.395	0.002
EAS	0.029	14.584	0.014	0.013
EUR	0.080	-6.282	0.098	0.001
SAS	0.068	14.964	0.095	0.006
Meta-Analysis	--	*-2.738*	*0.172*	*0.004*

Note: p Threshold indicates the threshold of association p value in the ANGST meta-analysis below which genetic variants were selected for inclusion in the polygenic scores. AFR = African, AMR = American, EAS = East Asian, EUR = European, SAS = South Asian.

IV. Summary and Discussion

In the analyses in this chapter, we found that anxiety disorder phenotypes assessed by brief, web-based screening items did not have robust evidence for DNA-based heritability but nevertheless identified a few genes and genomic regions that may be involved in the etiology of ADs. Further, we found that genetic association results from a previous large meta-analysis with similar phenotypes did not replicate in this sample, and that ancestry may be an important consideration in the etiology of ADs.

Three SNPs with genome-wide significant associations for anxiety disorder case-control status were found in the European subset of the sample. These SNPs have no known functional significance, and the association peak is located in a large intergenic region on chromosome 4. It is unclear from the existing molecular evidence how genetic

variation in this region might be impacting anxiety phenotypes, but its location outside of a protein-coding region, and enrichment for a histone mark, suggest a regulatory role.

At the aggregate level, genes *HTR4* and *HLA-DQB2* also had a significant association with the anxiety factor score. *HTR4* codes for the serotonin receptor 4 and has been previously implicated in major depression (Madsen et al., 2014) and, in a few model organism studies, anxiety-like behaviors (Holmes, 2008). Genes in the serotonin system have received much attention in genetic epidemiology studies of mood and anxiety disorders, although there has been some controversy over the reported associations. Most criticisms stem from the use of pre-selected variants in candidate gene study designs (Fabbri, Marsano, & Serretti, 2013); our findings in a hypothesis-free genome-wide scan provides stronger evidence that this system may indeed be important to anxiety-related outcomes.

The *HLA-DQB2* gene forms part of the major histocompatibility complex, a set of genes centrally involved in immune system functioning and implicated in both autoimmune (Matzaraki, Kumar, Wijmenga, & Zhernakova, 2017) and psychiatric disorders such as schizophrenia (Mokhtari & Lachman, 2016). Similarly, the ANGST consortium also found genetic overlap between schizophrenia/bipolar disorder and anxiety disorder phenotypes. In addition, suggestive association results at both the variant and gene levels were found in two co-localized zinc finger and BTB domain containing genes, *ZBTB1* and *ZBTB25*, which have broad regulatory functions and are also implicated in immune system functioning (Punwani et al., 2012). These findings add to the larger literature suggesting that genetic risk is broadly shared across psychiatric disorders (Bulik-Sullivan et al., 2015), and that the molecular etiology of

psychiatric disorders is likely to stem more from DNA changes impacting the regulation of gene expression rather than protein changes with direct functional consequences (Maurano et al., 2012). Although not conclusive, these results point to several genomic loci that may be prioritized for further research.

Although several results identified in the genetic analyses of anxiety presented here may be informative for understanding the etiology of anxiety disorders, it is unlikely that they can be directly used in Mendelian randomization models to test causal associations between anxiety and alcohol misuse at this stage. Neurotransmitter systems like serotonin are likely to be involved in a broad array of behavioral outcomes and their potential pleiotropic effects cannot be discounted. Similarly, genes involved in immune system functioning (particularly ones that have already been implicated in other psychiatric disorders like schizophrenia) most likely have wide-ranging effects on human health and behavior. Several studies have already documented the common and substantial overlap in genetic effects between numerous psychiatric disorders, psychological traits, and other health-related phenotypes (Bulik-Sullivan et al., 2015; Docherty et al., 2017; Krapohl et al., 2016). Regulatory variants have also been shown to be enriched in psychiatric/behavioral disorders (Gusev et al., 2014) including alcohol misuse (Edwards et al., 2015). Further research is needed to investigate the specificity versus pleiotropy in functional consequences of the genes identified in these analyses before they may be able to aid in understanding the etiology of alcohol misuse.

Chapter 7: Discussion and Conclusions

I. Summary of Findings

This dissertation has encompassed a broad investigation into the theoretical internalizing and externalizing pathways to alcohol misuse and the mechanisms that might underlie them. Alcohol misuse, including heavy consumption and associated functional impairments, is one of the nation's top public health concerns (Office of the Surgeon General, 2016) and it is a particularly harmful and prevalent among college students (White & Hingson, 2014). Despite decades of research, however, the specific genetic influences on alcohol misuse remain largely unknown (Hart & Kranzler, 2015), while numerous but sometimes seemingly contradictory environmental and psychosocial influences have been identified (Stone et al., 2012). These findings are likely a consequence of phenotypic and genetic heterogeneity of alcohol misuse, whereby distinct underlying etiologies lead to the same outcome. Several typologies have been proposed that support the existence of distinct etiological pathways marked by the existence of different internalizing (anxiety, depression) and externalizing (antisocial behavior, impulsivity, sensation-seeking) traits. Here we have examined the validity of such pathways in college students, the mechanisms by which they might unfold across development, and the potential for negative and positive reinforcement drinking motives to serve as intermediate phenotypes involved in such distinct

etiological pathways. This investigation involved the use of multiple statistical and molecular methods to take a broad, integrative perspective on this topic.

In Chapter 3, statistical modeling was conducted to empirically characterize distinct patterns of internalizing and externalizing psychopathology alongside alcohol misuse. We found that the proposed internalizing and alcohol misuse outcomes each formed a single coherent latent dimension, although the externalizing measures were separable into antisocial behavior, impulsivity, urgency, and reward-sensitivity dimensions. Growth mixture models indicated relatively stable patterns in these domains throughout college, with those individuals starting with the highest levels of each measure in freshman year continuing to have the highest levels throughout the rest of college. When examining these domains as trait-like measures, in addition to other personality traits, latent profile analysis indicated that these domains separated clearly into an internalizing, externalizing, and low risk group, with the internalizing group having moderately increased risk of AUD symptoms but not binge drinking, and the externalizing group having extremely high levels of both AUD symptoms and binge drinking. Drinking motives mapped well onto these latent classes with the externalizing class having higher levels of all four motives and the internalizing class having higher risk particularly for negative reinforcement motives.

In Chapter 4, we explored the epidemiology of drinking motives and their longitudinal relationships with alcohol misuse and internalizing and externalizing psychopathology across college. Drinking motives were found to be relatively stable, with correlations of 0.40-0.56 at adjacent time points (and somewhat more decay with larger intervals). Some previously identified risk factors for alcohol misuse, like peer

deviance and parental autonomy granting, were robust predictors of nearly all types of motives, while others, like trauma exposure, were specifically related to higher levels of negative reinforcement motives. In addition, cross-lagged models implicated a causal role of social and enhancement motives leading to increases in alcohol misuse, with less evidence of this directional association for conformity motives and binge drinking, and no significant evidence for coping motives and either alcohol misuse outcome. The direction of this effect appeared to attenuate or reverse at the Y3S wave, indicative of a possible developmental shift in these relationships. The relationship between drinking motives and internalizing/externalizing measures appeared to be largely non-causal.

In Chapter 5, we explored the genetic etiology of drinking motives, in which modest but not significant heritability was attributable to variants in measured common SNPs, and a few suggestive loci were found to be associated with enhancement, coping, and conformity motives. Of interest were the *FBLN2* and *PECR* genes for enhancement motives, several intergenic loci for coping motives, and the *SIRT1* gene for conformity motives. Gene-based enrichment testing also implicated the *PTER* gene for coping motives. However, the association effects were underinflated and the sample seems to be underpowered for conclusive investigation into the genetic etiology of drinking motives and the potential for shared or distinct genetic influences across different motive types.

In Chapter 6, we investigated the genetic etiology of anxiety disorder symptoms to set the stage for future studies of the mechanism driving the internalizing pathway to alcohol misuse. We found several promising genes implicated by these association analyses, including *HLA-DQB2* and *HTR4*; however, these genes are not likely to viable

candidates to use in testing for causal associations between anxiety and alcohol misuse unless future functional work can exclude the possibility of their pleiotropic effects.

II. Implications

Validity of internalizing and externalizing pathways

Our results provide empirical support for the existence of distinct internalizing and externalizing pathways to alcohol misuse, as described by typologies such as those proposed by Cloninger et al. (1988) and Babor et al. (1992). Mixture models showed a clear separation between latent classes based on measures of externalizing and especially internalizing symptoms and traits, and these groups had very different patterns of endorsement of the two alcohol misuse domains of AUD symptoms and binge drinking frequency. However, it is important to consider both the quantitative and qualitative aspects of the distinction between such classes. While the classes differed from each other substantially on internalizing and externalizing measures, they also differed from the low risk class in both domains, such that the “externalizing” class still had relatively higher risk of anxiety/depression symptoms while the “internalizing” class had relatively elevated levels of illicit drug use and antisocial behavior.

Indeed, antisocial behavior did not relate as clearly to the externalizing domain as expected in either the factor analyses or growth mixture models. Some evidence has indicated that the externalizing domain is in fact a mixture of two dimensions: disruptive/antisocial behavior versus impulsivity/sensation-seeking (Ingole et al., 2015). Our findings suggest that it is the sensation-seeking or positive valence dimension that particularly separates the internalizing and externalizing pathways relevant to alcohol

misuse, as the sensation seeking and extraversion personality traits were unique in showing a qualitative distinction between latent classes such that the externalizing class had higher levels and the internalizing class had lower levels relative to the low risk class (rather than a quantitative low/medium/high ordering of differences between the low/internalizing/externalizing classes). Further, a dimension of stress reactivity appears to be important in this distinction, as the traits of high neuroticism, high negative urgency and low resilience especially characterized the internalizing class relative to both the externalizing and low risk classes. The latent classes showed distinct patterns of association with alcohol misuse and drinking motives such that the externalizing class has high levels of all motives and all forms of alcohol misuse, while the risk for the internalizing class is increased specifically for negative reinforcement motives and AUD symptoms but not binge drinking.

The existence of such internalizing and externalizing pathways is consistent with other empirical studies of alcohol use disorder (Hildebrandt et al., 2017; Sintov et al., 2010) as well as other addictive behaviors such as gambling (Gupta et al., 2013; Savage, Slutske, & Martin, 2014). These studies have indicated an “internalizing” class that has moderately increased levels of the addictive behavior and a unique personality profile of neuroticism and stress reactivity. They have also found an “externalizing” or “high risk” class with particularly high levels of addictive behavior and a broad elevation in risk for a range of internalizing and externalizing symptoms/disorders. When specific aspects of the addictive behavior were assessed, distinctions also emerged between “internalizing”, “externalizing”, and“high risk” classes (Savage et al., 2014). Likewise, in this study, we have found that the internalizing class had higher levels only of AUD

symptoms with no difference from the low risk class in binge drinking frequency, while the externalizing class had high levels of both. Several previous studies have indicated that internalizing psychopathology, particularly social anxiety disorder, is linked to higher rates of AUD but not drinking frequency/quantity, or even linked to lower rates of consumption (Savage et al., 2016; Schry & White, 2013). Such evidence suggests that the alcohol misuse dimensions of heavy consumption and problems/negative consequences are related but separate endpoints to which internalizing versus externalizing pathways may lead.

An important note should be reiterated that although internalizing and externalizing pathways may have distinct mechanisms and somewhat distinct endpoints, both may still be involved in the etiology of alcohol misuse for a given individual. Such pathways may occur at separate developmental stages or may concurrently influence alcohol use behaviors. Our results, in the context of the existing evidence and theories, suggest that the externalizing pathway represents a broad predisposition towards a variety of reward seeking behaviors including multiple aspects of alcohol misuse and drinking motives, and which may be more strongly genetically influenced (Cloninger et al., 1988; Sintov et al., 2010). It is possible that this pathway leads to the development of both internalizing and externalizing psychopathology, with a direct and broadly shared risk for externalizing psychopathology and an indirect risk for internalizing psychopathology that develops as a consequence of prolonged substance misuse. Such a hypothesis is supported by the effect of enhancement motives predicting later internalizing symptoms seen in the cross-lagged models in Chapter 4 and is consistent with the broad risk for psychopathology seen in the “externalizing”

classes of the latent class analysis studies described above. Conversely, our findings and theoretical models indicate that the internalizing pathway represents a subset of the risk for alcohol misuse development, which is specifically linked to negative affect and stress reactivity and may come about as an environmentally-triggered response to stressors. The consistency of such pathways with positive and negative valence neurobiological systems and evolutionary theory suggest it is very likely that these two pathways have distinct genetic and environmental etiologies, even if they may not represent truly separate subtypes of individuals.

Drinking motives as intermediate phenotypes

This set of analyses has also provided novel evidence that positive and negative reinforcement drinking motives map onto internalizing and externalizing pathways to alcohol misuse at both a phenotypic and genetic level, and thus may be useful intermediate phenotypes to understand its etiology. As seen in previous studies (Adams et al., 2012; Mezquita et al., 2010), positive and negative reinforcement motives were differentially associated with externalizing (sensation seeking, polysubstance use) versus internalizing (anxiety, depression, neuroticism) symptoms and traits. Mirroring the findings for the internalizing/externalizing latent classes, positive and negative reinforcement motives also had differential associations with alcohol misuse outcomes, with coping motives being most strongly linked to AUD and enhancement motives to drinking heaviness. The external social and conformity motives tended to have similar but weaker patterns of association as their respective valence motives from the internal dimension, consistent with previous research indicating that these are less robust predictors of alcohol use and misuse (Kuntsche et al., 2005).

Drinking motives appeared to map well onto the patterns of internalizing and externalizing traits identified in the mixture models. The externalizing class demonstrated elevated levels of all four types of drinking motives, while the internalizing class had specifically heightened levels of negative reinforcement motives, closely mirroring the broad/externalizing vs. specific/internalizing pathways discussed in the previous section. Similarly, models of environmental predictors indicated that some risk and protective factors that have been robustly associated with alcohol misuse, such as peer deviance and parenting, broadly predict higher levels of all four types of drinking motives, while others, like stressful life events, are associated with a specific increase in risk for negative reinforcement motives. These results indicate that drinking motives are well-suited to index the internalizing and externalizing pathways to misuse. The role of motivations in shaping behavior via approach/avoidance drives provides a compelling reason to believe that motives are involved in the mechanisms influencing alcohol use behaviors, and thus are useful for understanding the etiology of such pathways.

Even further, evidence from the cross-lagged models points to a causal role of drinking motives, particularly positive reinforcement motives, in the development of alcohol misuse in college students. This lends credibility to their role as intermediate/endophenotypes for alcohol misuse, and also points to drinking motives as potentially worthwhile targets for prevention, intervention, and treatment efforts. We have begun here to unravel the genetic and environmental etiology of drinking motives; however, additional research is needed to focus on these phenotypes themselves, and particularly on their genetic underpinnings, in order to understand what insights they may provide into the etiology of alcohol misuse.

III. Limitations

There are several important limitations that should be considered when interpreting the global findings of these analyses. First, all measures were assessed via self-report, which may result in social desirability biases especially when concerning stigmatized outcomes like mental health, and potentially illegal behaviors like (underage) substance use. Although the confidentiality of the survey and the ability of participants to complete the assessment in private and online may have encouraged honest answers, lack of supervision might have also resulted in inaccurate responding due to lack of engagement or misunderstanding of the questions.

Statistical power is also an important issue for the genetic analyses, as the results from GCTA, LDSC, and GWAS indicated that we were underpowered to detect true genetic associations. Using the Genetic Power Calculator (Purcell, Cherny, & Sham, 2003) we estimated that we had 1%, 30%, and 94% power, respectively, for SNPs accounting for 0.2%, 0.5%, and 1.0% of the phenotypic variance with our drinking motives GWAS meta-analysis sample size of n = 4,855, and 6%, 60%, and 99% power for the same effect sizes in the gene-based analyses (which require less stringent multiple testing correction). However, even these small effect sizes might be overestimates of the true effect sizes for highly complex traits, and the meta-analysis estimates are comprised of much smaller samples for which random chance can substantially impact the association estimates. This means that the results of all of our genetic analyses should be viewed with caution; however, the fact that genes or gene families previously implicated in psychiatric outcomes were found bolsters confidence in our results.

Additionally, though the sample was small by emerging standards for genetic association testing, it was quite large in comparison with most statistical models employed in psychological research. This means power is less of a limiting factor in the interpretation of linear and structural equation models at the phenotypic level; however, conversely, it means that statistically significant associations may be found when effect sizes are very small and not necessarily of clinical or substantive importance. For example, positive reinforcement drinking motives were modestly higher in the internalizing latent class relative to the low risk class (mean difference of 0.10 – 0.14 units on a 4-unit scale), but these differences were highly statistically significant despite the means of the distribution appearing to be identical (Figure 3.2). The interpretation of meaningful results from the analyses should be considered in addition to simple inference from statistical significance levels.

Finally, we emphasize that many of our conclusions about the role of drinking motives in intermediate internalizing/externalizing pathways is largely speculative, based on theory, plausible psychobiological systems, and patterns of association with other observed traits. Therefore although we believe they are promising as endophenotypes for gene discovery for alcohol misuse via these distinct mechanisms, this remains only a hypothesis until the specific genes influencing drinking motives can be identified and tested in mediational models.

IV. Conclusions and Future Directions

This project provides converging support for the existence of distinct but not completely separate internalizing and externalizing pathways to alcohol misuse and for drinking motives as intermediate mechanisms underlying these pathways. These

different pathways provide a platform from which to launch and refine future research into the etiology of alcohol misuse. Like many other complex psychiatric and behavioral outcomes, our current understanding of alcohol misuse, both from a genotypic and phenotypic perspective, remains plagued by challenges such as etiological heterogeneity and difficulties in conclusively defining the phenotype of interest for study. The notion of what alcohol misuse really is has been changed time and again throughout history, and will almost certainly change again. However, improvements in our understanding of what causes alcohol misuse and how to change it can reciprocally influence our definition of what alcohol misuse is (Kendler, 2009), until at some point in the future we reach an understanding that reflects the true nature of reality.

Although they themselves remain complex outcomes, perhaps intractably so at this stage, endophenotypes and intermediate phenotypes can aid these research efforts by providing insight into the mechanisms by which causal factors, both genetic and environmental, unfold across developmental pathways to influence complex outcomes like alcohol misuse. The cost of such consequences to health, society, and humanity demands that we pursue such understanding with all of the tools available, and apply the knowledge gained towards prevention, intervention, and treatment efforts. Future research is needed to investigate the origins of developmental pathways to alcohol misuse, how their intermediate processes change across the full range of development, and how such mechanisms can be modified to change alcohol misuse outcomes. Investigation of the etiology of drinking motives and their role in underlying internalizing and externalizing pathways to alcohol misuse is a promising next step towards this goal.

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List of References

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Vita

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