The Relationship Between Genetic Risk for Alcohol Use Disorders and Antisocial Personality Disorder, and other Complex Traits

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Declaration

I, Wenqianglong Li, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: Wenqianglong Li Date: 1st August 2022

Acknowledgements

I'd like to express heartfelt gratitude to my parents for their unwavering support, love, and understanding. I left for the UK almost nine years ago and haven't seen them for three years due to Covid-19. Their incessant support is instrumental to my achievements. I appreciate their help in exploring my interests and the liberty to choose my path. My mum deserves special mention for her limitless emotional backing and daily prayers for me. I thank my uncle’s family for their care, and my sister Jingjing. This work is dedicated to my relatives who have always wished me well.

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雪莱曾经写到，唯有你的光辉，能像漫过山岭的薄雾。希望所有我爱的和爱我的人，在生命的困境中，挣扎时都能看到光辉。也希望我们能在风光和开心时，不忘记，” where your treasure is, your heart will be also”.

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Wenqianglong Li wrote the paper and did the data analysis and preparation, Johan Thygesen prepared the data, Andrew McQuillin and Nicholas Bass designed and offered suggestion in writing and data analysis, data was collected by the rest of co-authors, and all of the co-authors offered advice in writing.
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Abstract

Introduction

Alcohol use disorder (AUD) is a prevalent psychiatric disease, driven by both environmental and genetic factors. To deepen our understanding, this research aims to identify replicable novel loci, understand associations between AUD genetic risk variants and brain morphology, and explore shared genetic risk variants between AUD and comorbid psychiatric diseases such as antisocial personality disorder (ASPD).

Methodology

We first improved the identification of AUD risk loci using AUD scores as a quantitative variable, while examining the influence of the regression model applying a quasi-Poisson distribution. This accounted for the skewed distribution of AUD criterion scores within genome-wide association studies (GWAS) populations towards more severe cases. To explore the potential risk genetic AUD and alcohol consumption (AC) pose on brain morphology, we utilised the largest available GWAS results of AUD and AC along with genetic and imaging data from the UK Biobank. We investigated the impact of AUD polygenic risk scores (AUD-PRS) and alcohol consumption polygenic risk scores (AC-PRS) on brain volumes in individuals consuming less than 14 alcohol units per week. Lastly, we conducted the largest meta-analytic GWAS of ASPD to date, considering individuals diagnosed with AUD to explore shared genetic risk variants.

Results

Our innovative GWAS data analysis approach revealed that the quantitative trait analysis adjusting for the criterion score distribution

provided the greatest precision. Imaging genetics identified negative associations between AUD-PRS and brain/grey matter volumes in cortical and subcortical regions in individuals consuming less than 14 alcohol units per week. A significant chromosome 15 variant (rs9806493) association was found in the GWAS of ASPD in individuals diagnosed with AUD (Z score = -5.501, $p = 3.77 \times 10^{-8}$). The phenome-wide analysis identified associations between *SLCO3A1* and educational attainment, depression, alcohol dependence, and lifetime number of sexual partners. PRS analysis revealed positive correlations between ASPD and smoking, ADHD, depression traits, and post-traumatic stress disorder, but negative correlations with alcohol intake frequency, reproductive traits, and level of educational attainment.

Conclusion

Our findings suggest that novel GWAS methods, adjusted for the distribution of quantitative traits, could enhance the identification of disease genetic risk variants. Alcohol consumption alone may not account for brain abnormalities resulting from alcohol's neurotoxic effects; AUD genetic risk factors could be predispositional. With AUD samples exhibiting ASPD symptoms, we provide evidence of an association of ASPD risk with *SLCO3A1* and shed light on the genetic architecture and pleiotropic associations of ASPD.

Impact Statement

This PhD thesis potentially impacts psychiatric disease genetics, especially alcohol use disorders and antisocial personality disorder. AUD, a leading cause of morbidity and mortality, likely stems from genetic elements contributing to its etiology and comorbidity with other psychiatric disorders, including ASPD. Current research aims to identify more AUD-associated genetic risk loci and their connection to comorbidity.

While augmenting sample sizes in genome-wide association studies (GWAS) is a common strategy, refining phenotypes to quantitative forms could also have the potential to enhance power for risk loci detection. Additionally, the choice of regression models might influence GWAS and post-GWAS results. Our study evaluates the impact of using quantitative versus binary phenotypes and different regression models in AUD patients and healthy controls, providing guidance for phenotype and regression model selection in future studies.

Each year, countless GWAS results are generated, illuminating associations between genetic variants and phenotypes while also offering insights through downstream analyses. Recently, large-scale projects such as UK Biobank have provided brain imaging-derived phenotypes, with about 40,000 participants' brain phenotypes currently released. Assessing the impact of disease-related genetic risk loci on brain phenotypes like brain volumes can aid GWAS result interpretation. Our imaging genetic study analysed the influence of alcohol consumption and AUD genetic risk variants on brain volumes in "healthy

drinkers." Our findings suggest that genetic risks of AUD might already associate with brain volume reduction in individuals consuming less than 14 units of alcohol weekly. This evidence could guide public health recommendations.

Concrete genome-wide significant results for ASPD remain elusive. In our ASPD GWAS, we utilised a cohort clinically diagnosed with AUD, for which ASPD diagnostic criteria data was also available. This approach minimized potential confounding of AUD genetic risk and alcohol exposure. A genome-wide significant SNP was identified, and the genetic correlation of ASPD diagnostic criteria with other complex traits was estimated. Our findings underscore the value of consistent measures of ASPD diagnostic criteria for detecting risk loci.

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List of abbreviations

Alcohol Consumption Polygenic Risk Scores (AC-PRS)

Adolescent Brain Cognitive Development (ABCD) Study

African-American (AA)

Alcohol Consumption (AC)

Alcohol Dehydrogenase (*ADH*)

Alcohol Dependence (AD)

Alcohol Dependence Syndrome (ADS)

Alcohol Use Disorder (AUD)

Alcohol Use Disorders Identification Test (AUDIT)

Aldehyde Dehydrogenase 2 (*ALDH2*)

Antisocial Personality And Behaviour (ASB)

Antisocial Personality Disorder (ASPD)

Attention Deficit Hyperactivity Disorder (ADHD)

AUD Polygenic Risk Scores (AUD-PRS)

Catechol O-Methyltransferase (*COMT*) Gene

Cis-Expression Quantitative Trait Loci (Cis-eQTL)

Conduct Disorder (CD)

Diagnostic And Statistical Manual Of Mental Disorders Of The American Psychiatric Association 4th Edition (DSM-IV)

DRD2 Gene (Dopamine D_2 Receptor)

European Collection Of Cell Cultures (ECACC)

Expression And Splicing Quantitative Trait Loci (Eqtls And Sqtls)

Genome-Wide Association Studies (GWAS)

Genotype-Tissue Expression (Gtex)

Hardy–Weinberg Equilibrium (HWE)

International Classification Of Diseases, 10th Revision (ICD-10)

LINC00951 (Long Intergenic Non-Protein Coding RNA 951)

Linkage Disequilibrium (LD)

Million Veteran Program (MVP)

Minor Allele Frequency (MAF)

Monoamine Oxidase A (*MAOA*) Gene

Post-Traumatic Stress Disorder (PTSD)

Principal Component Analysis (PCA)
Problematic Alcohol Use (PAU)
Prostaglandins (PG)
Schedule For Affective Disorders And Schizophrenia (SADS-L)
Semi-Structured Assessment For Drug Dependence And Alcoholism (SSADDA)
Semi-Structured Assessment For The Genetics Of Alcoholism (SSAGA-II)
Serotonin Transporter Promoter (*5-HTT*) Gene
Solute Carrier Organic Anion Transporter Family Member 3A1 (*SLCO3A1*) Gene
The 5th Edition Of The DSM Manual (DSM-5)
The Functional Mapping And Annotation Of Genome-Wide Association Studies (FUMA)
UK Biobank (UKB)
UK Office For National Statistics (ONS)
United Kingdom (UK)
United States (US)
World Health Organization (WHO)

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Chapter 1 INTRODUCTION

Alcohol drinking is socially accepted in many countries. In 2017, 29.2 million people aged 16 and over in the UK consumed alcohol, and in 2021, there were 9,641 alcohol-related deaths, representing a 7.4% increase from 2020 and a significant 27.4% rise compared to 2019, resulting in a record high in the data series, with a rate of 14.8 deaths per 100,000 people (Office of National Statistics (ONS), 2021). Although some people drink alcohol without experiencing harmful effects, the number of people who experience excessive and harmful alcohol use is growing, and alcohol misuse is now a leading cause of morbidity and mortality (Esser et al., 2020; R. K. Walters et al., 2018; White et al., 2020). According to the World Health Organisation (WHO), currently, alcohol consumption contributes to three million deaths each year globally and 5.1% overall experience the harmful effects of alcohol. Alcohol dependence (AD) not only causes physical, social and psychological harmful effects, but also coexists with other psychiatric disorders including depression (Tseng et al., 2017), anxiety (Mocanu & Wood, 2022), post-traumatic stress disorder (Suh & Ressler, 2018), and antisocial personality disorder (Moody et al., 2016). It has been suggested that AD may share common biological mechanisms with antisocial personality disorder (ASPD). For example, Malone et al. (2004) reported a moderate genetic influence on adult antisocial behaviour and alcohol dependence, suggesting they might share susceptibility genes.

Genome-wide association studies (GWAS) aim to identify common genetic risk variants associated with disease risk (Visscher et al., 2012).

They have been informative in psychiatric disorders such as schizophrenia (Ripke et al., 2014), and researchers have also conducted GWAS with large sample sizes to understand AD's complex polygenic architecture and how it is genetically related to other psychiatric disorders (Walters et al., 2018). However, to date, there is no GWAS with a large sample size investigating the common genetic risk variants that might potentially underlie AD and ASPD.

1. Alcohol Dependence and Alcohol Use Disorder

1.1 Definitions & Diagnoses

Harmful alcohol use has been labelled in different terms such as "alcohol abuse", "alcohol dependence", "heavy drinking", and "problem drinking". The WHO International Classification of Diseases, 10th Revision (ICD-10) describes it as a pattern of substance use which causes damage (i.e., physical and mental) to health along with adverse social consequences (WHO, 1992). The ICD-10 developed diagnostic guidelines for "dependence syndrome" which is known by another name "alcohol dependence" as it is more precise and reliably defined using the criteria (National Institute for Health and Care Excellence, 2011). A similar diagnostic term of "alcohol dependence" is described in the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM) 4th edition(APA, 2000). Under these diagnostic criteria, individuals with three or more of the "dependence" items within a 12-month period would receive an AD diagnosis.

Both ICD-10 and DSM-IV use questionnaire responses to define the relationship with alcohol and to identify spectral differences in severity.

The ICD-10 and DSM-IV diagnostic criteria for AD overlap to a large degree so they can be used interchangeably for research purposes but there is considerable discordance in the classification of harmful use and alcohol abuse (Stickel et al., 2017). The 5th edition of the DSM manual (DSM-5) has integrated alcohol abuse and alcohol dependence into a single category Alcohol Use Disorder (AUD). Under this diagnostic criteria, AUD symptoms of this condition include characteristics that were labelled alcohol abuse or alcohol dependence (see Appendix 1 for AUD symptoms). It defines AUD as a spectrum of problematic use with clinical impairments that contains more than 11 criteria. Shifting the terms "abuse" and "dependence" to "alcohol use disorder" reflects the fact that the terminology we used in the past may stigmatise people who have alcohol use disorder. For example, the term "alcohol use disorder" is more accurate to use, while "abuse" may refer to intentional behaviour which implies a personal failure rather than a disorder. The 10th edition of the ICD is currently undergoing revision and it is likely that its criteria will mirror closely those of the DSM-5.

For this PhD thesis, I used the term "alcohol use disorder" to be consistent with the newest clinical diagnostic criteria from DSM-5 even for studies that were conducted before 2013 which used the term "alcohol abuse or dependence symptoms" according to DSM-IV.

Table 1 The DSM-IV & DSM-5 diagnostic classifications of alcohol dependence/alcohol use disorder

Classification	DSM-IV	DSM-5	ICD-10
Disorder Name	Alcohol Dependence	Alcohol Use Disorder (AUD)	Harmful Use (F10.1), Dependence Syndrome (F10.2), Alcohol-Induced Mental/Behavioral Disorders, and more
Criteria (Main)	Tolerance	Impaired Control	Harmful use of alcohol, Alcohol dependence syndrome, Alcohol-induced mental/behavioral disorders
	Withdrawal	Social Impairment	Withdrawal state, Intoxication
	Drinking in larger amounts	Risky Use	Other alcohol-induced mental/behavioral disorders
	Unsuccessful efforts to quit/control	Pharmacological criteria (tolerance and withdrawal)	
	Continued use despite harm	Time spent on activities related to alcohol	
	Loss of important social activities	Craving	
Key Differences	Separate "Alcohol Dependence" and "Alcohol Abuse" categories.	Combines into a single "Alcohol Use Disorder" (AUD) category with severity specifiers.	Includes multiple categories: Harmful Use, Dependence Syndrome, and more.
	Lacks explicit "Social Impairment" and "Craving" criteria.	Includes "Social Impairment" and "Craving" criteria.	Provides comprehensive categories for different aspects of alcohol-related issues.
	No "Pharmacological Criteria" category; Tolerance and Withdrawal criteria are separate.	Incorporates Tolerance and Withdrawal under "Pharmacological criteria."	

1.2 Epidemiology

1.2.1 Prevalence

39% of pupils (aged from 11 to 15) reported drinking alcohol in the Survey “Drug use, smoking and drinking among young people in England in 2013” (Fuller & Hawkins, 2014). The number increased to 44% in 2016 (NHS Digital, 2017). The 2017 UK National Statistics showed that 57% of their survey respondents consumed alcohol, which equates to 29.2 million people in the population (National Statistics, 2017). Among those who consume alcohol, the latest Psychiatric Morbidity Survey showed that 16.6%, 1.9%, and 1.2% of adults drank at hazardous, harmful, and dependent levels, respectively (McManus S, Bebbington P, Jenkins R, 2016). More recent studies have suggested that alcohol consumption patterns changed during the COVID-19 lockdowns. Researchers found an increase in alcohol consumption, which was associated with mental disorders (Calina et al., 2021; Ramalho, 2020). For example, in the United States, a cross-sectional online survey of 993 participants showed an increase in alcohol consumption in April 2020 compared to February 2020, with a higher number of cases involving binge drinking (Barbosa et al., 2021). The Office of National Statistics (ONS) (2021) noted that alcohol-specific deaths, primarily caused by alcoholic liver disease, have surged significantly since the COVID-19 pandemic began, likely due to increased alcohol consumption, particularly among those who were already heavy drinkers before the pandemic.

Alcohol-related harm continues to pose a significant public health issue in England. According to Local Alcohol Profiles for England: Short Statistical Commentary (March 2023), in 2021, the data revealed 20,970 deaths related to alcohol (i.e., 38.5 per 100,000 population). Furthermore, there were 342,795 hospital admissions wholly attributed

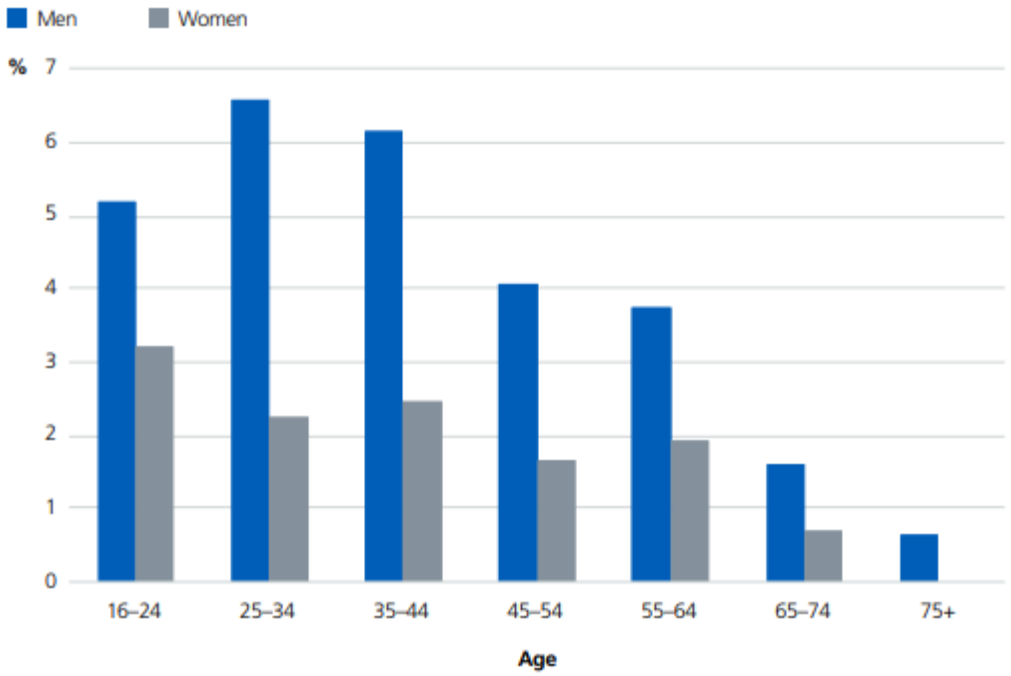
to alcohol in the same period, representing a rate of 626 per 100,000. Notably, men had twice the number of hospital admissions compared to women. In total, there were 948,312 alcohol-related hospital admissions under the broad definition where alcohol was either the primary cause for hospital admission or a contributing factor in a secondary diagnosis, corresponding to a rate of 1,734 per 100,000 population. Again, the number of such admissions was higher in men, with women accounting for approximately one-third of the total. Narrowly defined alcohol-related admissions where the primary cause for hospital admission was linked to alcohol totalled 270,774, equating to a rate of 494 per 100,000 population. Men had 1.8 times the number of admissions compared to women. The broad measure provides an overview of the full impact of alcohol on hospital admissions and the burden it places on the NHS, while the narrow measure specifically estimates the number of hospital admissions primarily due to alcohol consumption, offering insights into trends in alcohol-related hospital admissions. These figures underscore the significant burden of alcohol-related harm in England and emphasize the need for targeted interventions and comprehensive strategies to address this public health challenge.

For harmful drinking or probable alcohol dependence, figure 1 shows the percentage of adults who fit these criteria plotted by age and sex. They were all scored over 16 (i.e., presented more than 16 items in the questionnaire) in the Alcohol Use Disorders Identification Test (AUDIT). There is a lack of reliable data on the prevalence of AUD as the population surveys in the UK do not contain questionnaires that offer a diagnosis of AUD. Therefore, the estimate from the Psychiatric Morbidity Survey is the most reliable survey, which used AUDIT to measure alcohol-use disorders (i.e., a measure used by WHO).

In the United States (US), according to the 2017 National Survey on Drug Use and Health, 15.1 million (6.2%) adults have alcohol use disorder including 9.8 million men and 5.3 million women (Substance Abuse and Mental Health Services Administration [SAMHSA], 2016).

In 2016, 283 million people were estimated to have AUD (Glantz et al., 2020). Recently, a cross-nationally study that summarised results from the 29 world mental health surveys carried out in 27 countries or regions between 2001 and 2015 showed that mean lifetime prevalence was 8.6% for AUD, and 2.2% for 12-month prevalence of AUD (Glantz et al., 2020). The lifetime prevalence of AUD across the country (non-abstainers) was 10.7%.

Figure 1: Harmful drinking or probable alcohol dependence (AUDIT score of 16 and more)



Picture resource from Mental Health and Wellbeing in England: Adult Psychiatric Morbidity Survey 2014 (McManus S, Bebbington P, Jenkins R, 2016)

1.2.2 Social Problems

Over and above-causing problems at a personal level, alcohol drinking has been implicated in domestic violence, problems with parenting, and abuse. According to the report from (National Institute for Health and Care Excellence, 2011), more than 1 million children are impacted because of their parents' alcohol misuse and over half of child protection cases implicate alcohol misuse.

1.2.3 Criminality

According to the UK Office for National Statistics (ONS) data on alcohol-related incidents report, in 2016-2017, 12.4% of theft offences, 20.6% of criminal damage, and 21.5% of hate crimes were related to alcohol use. Moreover, more than 30% of sexual assault cases were under the influence of alcohol. Singleton, N., Meltzer (1998) showed that nearly two-thirds of male prisoners and more than one-third of female prisoners were harmful drinkers in a psychiatric morbidity among prisoners report.

1.3 Aetiology

1.3.1 Psychological and environmental factors

Social psychologists propose that the risk of developing AUD can be partly explained by social learning theory. The theory examines the impact of socialisation and the influence of people's close group members which are family and friends (Akers, 1973). Using a social learning approach, Watkins (2016) investigated prescription drug misuse among 84 undergraduate college students. He revealed that peer influences are related to a greater likelihood of drug and alcohol misuse, which is consistent with literature that showed alcohol-using peers is the most common factor in alcohol use behaviour (Hawkins et al., 1997;

Linden, 1993). Furthermore, Trucco et al., (2011) examined why relationships with deviant peers are correlated with alcohol use in 371 adolescents. The results showed that high levels of peer delinquency can predict the use of alcohol which in turn can predict initiation of alcohol use. They suggested that reinforcement and modelling of alcohol use might be vital mechanisms for alcohol use by adolescents. However, their study is limited to early adolescence, which might not be generalised beyond the age of the sample. In general, social learning theory has been confirmed in the case of adolescent drug and alcohol use (Durkin et al., 2005; Ford, 2008).

Harmful drinking use and dependence are closely associated with adverse life events, which are also frequently identified in AUD cases. Stressful life events including childhood abuse, sexual abuse, and neglect, can elicit harmful drinking. For example, Clark et al., (1997) investigated the relationships between adolescent alcohol use disorders and traumas and adverse life events among 132 adolescents with alcohol dependence, 51 adolescents with alcohol abuse, and 73 adolescents for the control group. The results showed that adolescents with AUD were 6 to 12 times more likely to have physical abuse experience and 18 to 21 times more likely to experience sexual abuse. Other adverse life events including legal difficulties and close friends/family members dying were also more common in the AUD group.

When discussing environmental factors in the context of AUD, it is essential to consider the potential interplay between genes and the environment. Gene-environment interplay refers to the dynamic interaction between genetic factors and environmental influences,

shaping an individual's behaviour and susceptibility to certain conditions (D. M. Dick & Kendler, 2012; Rende, 2011). It encompasses different forms, including gene-environment correlation (rGE) and gene-environment interaction (GxE).

Behavioural geneticists have long recognised the significance of gene-environment correlation (rGE), a phenomenon whereby individuals' exposure to specific environments can imbue these environments with heritability (Jaffee & Price, 2007). In essence, an individual's genetic makeup influences their likelihood of encountering particular environments or experiences. There are three primary types of gene-environment correlation:

1. Passive rGE: this form entails the association between a child's inherited genotype from their parents and the environment in which they are raised. For instance, parents with a history of antisocial behaviour have an increased risk of child abuse, implying that maltreatment serves as an indicator of genetic susceptibility rather than a direct cause of children's behavioural issues (Malone et al., 2004; Rhee & Waldman, 2002).
2. Evocative (or reactive) rGE: this type involves the association between an individual's genetically influenced behaviour and the reactions of those in their environment to that behaviour. For example, the link between marital conflict and depression may be attributed to the tensions that arise when interacting with a depressed spouse, rather than marital conflict being the direct cause of depression.
3. Active (selective) rGE: active rGE pertains to the association between an individual's genetically influenced traits or behaviours and the environmental niches they proactively choose (Jaffee & Price,

2007). For instance, individuals with extroverted personalities tend to actively select social environments that align with their outgoing traits, while those who are shy and withdrawn may opt for different social settings.

There are limited studies that examined rGE in AUD. Dick et al., (2006) delved into how GABRA2 interacts with marital status about the development of alcohol dependence in 1900 participants from the high-risk Collaborative Study of the Genetics of Alcoholism (COGA) sample. This study revealed that both GABRA2 and marital status independently contribute to the development of alcohol dependence. Moreover, individuals with the high-risk GABRA2 genotype exhibited decreased likelihoods of marriage and increased risks of divorce, influenced in part by their personality traits. This investigation offers insights into potential gene-environment correlation and interaction involving GABRA2, marital status, and alcohol dependence, although similar studies have been conspicuously absent since then.

Gene-environment interaction occurs when the effect of genetic variations on an individual's susceptibility to AUD is influenced by the presence or absence of certain environmental factors. For example, the findings from gene-by-environment twin studies indicate consistent patterns of gene-environment interaction effects (Dick & Kendler, 2012). These studies suggest that environments with higher levels of social control, such as increased parental monitoring and stable neighbourhoods, tend to diminish the influence of genetic factors on alcohol use. On the other hand, environments characterized by deviant peers and greater alcohol availability allow genetic predispositions to

have a stronger impact. Research focusing on specific genes has primarily explored the role of stress as a moderator of genetic effects. There is a disconnect between these two bodies of literature, highlighting the need for integration and a broader understanding of the multiple variables that influence alcohol use, including beliefs about alcohol, self-esteem, school attitudes, parental expectancies, family disruption, and more (Donovan & Molina, 2011). Incorporating these various factors and environmental influences into theoretical models will be crucial for comprehensively understanding the mechanisms of gene-environment interaction effects on alcohol use and related problems (Van Der Zwaluw & Engels, 2009).

2. Antisocial Personality Disorder

2.1 Definitions & Diagnoses

Antisocial personality disorder (ASPD) is described as impulsive, deceitful, irresponsible, aggressive and criminal behaviour. These personality traits begin in childhood or early adolescence and continue into adulthood (APA, 2000). The DSM-IV characterises ASPD as a persistent pattern of disregard for and violation of the rights of others starting from childhood (NICE & Excellence, 2014). DSM-5 definition of ASPD is similar to the previous version, which describes symptoms including a lack of empathy, impulsive and aggressive behaviour, and disregard of others' rights (Adanty et al., 2022).

A diagnosis of ASPD includes symptoms of conduct disorder (CD) in childhood and difficulties in the interpersonal, behavioural, cognitive and mood domains in adulthood (APA, 2000). It requires that three

symptoms of CD be present since age 15 years and three symptoms of ASPD since age 18 years (see Appendix 1 for ASPD symptoms). However, there is disagreement on the diagnostic criteria for ASPD. For example, the diagnostic criteria have been criticised for focusing more on criminality and its limitations include excessive comorbidity and considerable heterogeneity within diagnostic categories (Ferguson, 2010; Few et al., 2015; Widiger et al., 2009). Therefore, some researchers have used different antisocial behaviour measurements including conduct disorder assessment, an antisocial process screening, and rule-breaking behaviour reported by teachers and parents to measure and understand a broad spectrum of antisocial behaviour (Burt, 2009; Ferguson, 2010; Tielbeek et al., 2017). Attempts have also been made to break ASPD into constituent domains. For example, some studies focus on the motivation in ASPD while others have measured specific antisocial behaviours such as aggression and stealing.

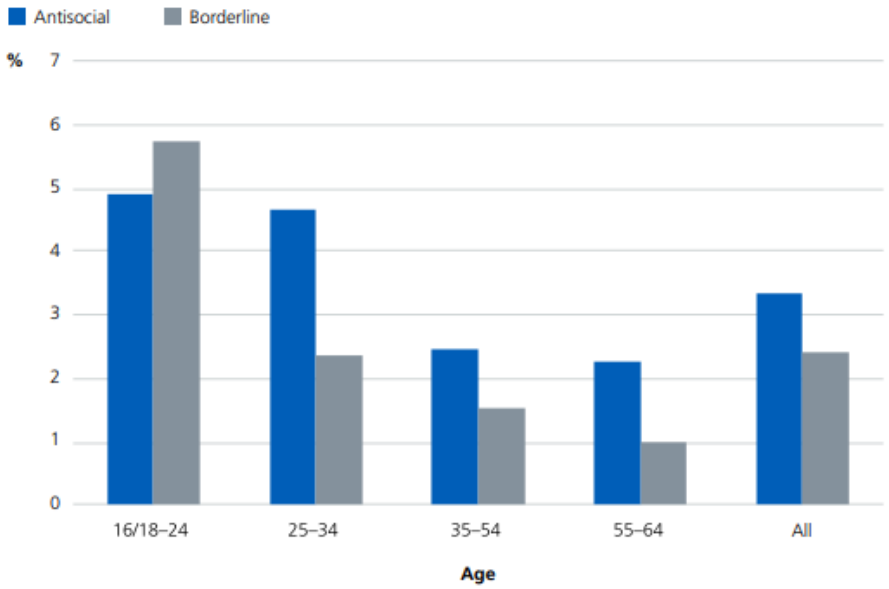
2.2 Epidemiology

2.2.1 Prevalence

The lifetime prevalence estimates of ASPD vary considerably across different studies, from 1% to 6.8% in men and from 0.2% to 1% in women (Coid et al., 2006; C. L. Dick et al., 1994; NICE & Excellence, 2014; Torgersen et al., 2001). Different estimates were due to varied diagnostic classification systems, assessment methods and different geographical locations including urban and rural areas. However, the higher prevalence in men is a consistent finding (Glenn et al., 2013; NICE & Excellence, 2014). According to the latest UK Psychiatric Morbidity Survey in which a self-completion structured clinical interview for DSM-IV ASPD was used, 3.3% of people aged 18-64 screened positive for ASPD where 4.9% were men and

1.8% were women (McManus S, Bebbington P, Jenkins R, 2016). Figure 2 shows the percentage of positive ASPD, and borderline personality disorder screened in the 2014 Psychiatric Morbidity Survey.

Figure 2: Screen positive for antisocial and borderline personality disorder



Picture resource from Mental Health and Wellbeing in England: Adult Psychiatric Morbidity Survey 2014 (McManus S, Bebbington P, Jenkins R, 2016).

ASPD has been found more common in prison environments (NICE & Excellence, 2014). In a systematic review that included 23000 prisoners, Fazel & Danesh (2002) showed that 47% of men and 21% of women had ASPD. Their results presented that prisoner was ten times more likely to have ASPD than the general population. More recently, a similar estimate of the prevalence of ASPD in prison samples has been reported by Glenn et al. (2013) where they found rates of ASPD to be 47% in males and 21% in females. In the UK, in an early Psychiatric Morbidity Among Prisoners report, the statistics showed 63% of males

and 31% of females have been identified with ASPD (Singleton, N., Meltzer, 1998).

There is significant comorbidity existing between ASPD and other psychiatric disorders. For example, research showed that ASPD has high rate of comorbidity with depression (Fu et al., 2002), anxiety (Goodwin & Hamilton, 2003), bipolar disorder (Glenn et al., 2013), schizotypal personality disorder (Raine, 2005), attention-deficit/hyperactivity disorder (Storebø & Simonsen, 2016), and substance abuse (Brook et al., 2016; Moody et al., 2016; Ogloff et al., 2015). More importantly, it has been shown by Swanson et al. (1994) that individuals with ASPD have increased the prevalence of having other psychiatric disorders. At least, over 90% of individuals diagnosed with ASPD have one other psychiatric condition. Among these ASPD comorbid psychiatric disorders, substance use disorder has been largely reported in many samples. For example, Chavez et al. (2010) reported that more than half (54.76%) with ASPD abusing cannabis and 30.95% with ASPD having amphetamines abuse. And more than 70% of patients in a rural psychiatric facility with ASPD abused alcohol. Sylvers et al. (2011) showed that heavy episodic drinking was significantly correlated with ASPD.

2.2.2 Social Problems

It is also well recognised that ASPD is associated with substantial harm to personal and family at societal levels (NICE & Excellence, 2014). People with ASPD regularly grow up in families with poor parenting including witnessing parental conflicts and harsh parenting. One of the results of such parenting is children's difficult behaviour, which in turn leads to substance misuse and school absence. Therefore, low educational attainment has continuously been reported associated with ASPD

(Tielbeek et al., 2017). Later, these difficulties lead to joblessness and poor relationships with partners. As a result, many individuals with ASPD end up in prison or die due to violent and reckless behaviour (NICE & Excellence, 2014).

2.2.3 Criminality

No doubt there is a strong association between ASPD and criminal behaviour as it is also one of the criteria of ASPD. However, research showed that gender affects criminal behaviour in ASPD. For example, early studies have revealed that nearly 90% of male felons still have ASPD at follow-up after 3 years, and only 33% of females engaged in criminal behaviour after 3 years (Guze, 1976; Martin et al., 1982). Moreover, ASPD is not just criminal behavioural, otherwise, all criminal offenders would be diagnosed with ASPD. As the prevalence of ASPD among prisoners has been indicated early, less than 50% of prisoners were shown with ASPD (Fazel & Danesh, 2002). Therefore, the relationship between ASPD and criminal behaviour is not very straightforward.

2.3 Aetiology

2.3.1 Environmental Factors

Early environmental factors might be closely related to the risk of the development of ASPD. For example, children who witness violence in the home or with ASPD parents increased risk of experiencing maltreatment (Glenn et al., 2013). Experiencing or witnessing intimate partner violence in childhood could later lead to intimate partner violence in adulthood suggested by Roberts et al. (2010). Parents who were diagnosed with ASPD have been suggested that might link to the risk of developing externalising and internalising problems in their children

(Berg-Nielsen & Wichström, 2012). The study showed that 13.2% of the variance in preschool children's behavioural problems can be explained by their parents' ASPD symptoms (Herndon & Iacono, 2005).
Researchers also conducted longitudinal studies to investigate ASPD environmental risk factors. From 120 low-income young adults, Shi et al. (2012) reported another longitudinal study that aimed to examine a number of components of the early childhood environment that could lead to ASPD in adulthood. Factors including childhood abuse, the severity of the abusive experience, early clinical risk, infant attachment security, disrupted mother-infant communication, childhood disorganisation, and behaviour problems in 5 and 7 years were examined. The data showed that the quality of early care, especially the parent-infant relationship during the first 18 months, could significantly predict ASPD symptoms and diagnosis in adulthood. Early clinical risk as another predictor can also be predictive of the features and diagnosis of ASPD. Moreover, disorganised-controlling patterns of interaction with parents at age 8 year was also a significant predictor of ASPD symptoms.

It is important to note that there could also be gene-environment interplay in the development of ASPD. Environmental factors could impact the expression of genes, change hormone and neurotransmitter levels, and eventually affect the structures and functions of the brain (Glenn et al., 2013). Moreover, it is crucial to recognise that environmental factors can exert their influence on ASPD development through biological pathways (Beauchaine et al., 2009). These factors can impact gene expression, alter hormone and neurotransmitter levels, and ultimately affect the structure and functioning of the brain. The interplay between genetic factors and environmental exposures can lead to long-term

alterations in the neurobiological systems involved in emotion regulation, impulse control, and social cognition, thereby increasing the risk of developing ASPD (Beauchaine et al., 2009).

There may also be gene-environment correlation at play. For example, when examining the impact of parenting styles as risk factors for ASPD, it is important to acknowledge two types of potential gene-environment interplay. Certain parenting styles associated with increased risk of ASPD may be a response to individuals with ASPD (this is known as ‘evocative’ gene-environment interplay – an environmental influence is elicited by a genetically predetermined trait such as ASPD). Secondly, the observed association between parenting style and ASPD in the child may simply reflect shared genetic factors underlying both the parenting style and ASPD (Rushing, 2009). In other words, genetic factors that contribute to ASPD in parents might also influence their parenting behaviours, creating a spurious association between parenting styles and the development of ASPD in their children (this is known as ‘passive' gene-environment correlation).

By considering gene-environment interplay and acknowledging potential genetic confounding, researchers can gain a more nuanced understanding of the environmental factors contributing to the development of ASPD. Additionally, recognising the impact of environmental factors on biological systems can provide insights into the underlying mechanisms through which these factors influence the expression of ASPD-related genes and affect brain function.

3. Genetic Epidemiology

3.1 Overview

Genetic Epidemiology is a field that investigates the role of genetic factors in the occurrence and distribution of diseases in populations (Burton et al., 2005). Various methods have been employed to study the genetic basis of diseases, including twin studies, genomic studies, and more advanced approaches like whole genome sequencing (Panoutsopoulou & Wheeler, 2018). Genomic methods have evolved, transitioning from candidate gene studies to genome-wide association studies (GWAS) and now incorporating whole genome sequencing techniques.

Twin studies have been instrumental in estimating the heritability of traits and diseases. They have several strengths that contribute to their usefulness in estimating heritability. First, they allow researchers to investigate the role of genetic factors by comparing the similarities between monozygotic (MZ) and dizygotic (DZ) twins (Boomsma et al., 2002). By assuming that MZ twins share 100% of their genetic material and DZ twins share, on average, 50%, the differences in concordance rates between the two types of twins can provide insights into the heritability of traits or diseases. This design effectively controls for shared environmental factors, as twins typically grow up in the same family and experience similar environments (Boomsma et al., 2002).

Moreover, twin studies offer the advantage of studying both genetic and environmental influences. By comparing the concordance rates of MZ and DZ twins, it is possible to estimate the contribution of genetic factors to phenotypic variation (Imamura et al., 2020). MZ twins share 100% of their

genetic material, while DZ twins share, on average, 50% of their genetic material, making them a valuable natural experiment for disentangling genetic and environmental influences.

In addition to assessing genetic factors, twin studies also shed light on environmental influences, including shared environmental factors and non-shared environmental factors (Sahu & Prasuna, 2016). Shared environmental factors encompass those aspects of the environment that contribute to the similarity between siblings or individuals who grow up in the same household. These factors include parenting style, socioeconomic status, family environment, and shared peer groups. The comparison of MZ and DZ twins allows researchers to estimate the role of shared environmental factors by assessing whether MZ twins are more similar to each other than DZ twins for a particular trait or behaviour (Matteson et al., 2013; Sahu & Prasuna, 2016).

Conversely, non-shared environmental factors come into play when examining the differences between siblings or individuals who share the same genetic background and family environment (Sahu & Prasuna, 2016). Non-shared environmental factors include unique life experiences, distinct parent-child interactions, random events, and the effects of birth order. These factors can result in differences in personality, interests, and behaviours among siblings or individuals raised in the same household (Beam et al., 2022; Sahu & Prasuna, 2016). The differences in concordance rates between MZ and DZ twins can also provide indications of the influence of non-shared environmental factors on the trait or disease being studied.

However, twin studies also have certain limitations that need to be considered. One major limitation is the assumption of equal environments for MZ and DZ twins (Boomsma et al., 2002). Although efforts are made to ensure similar environments for both types of twins, there may be subtle differences that could introduce bias into the estimates (Hagenbeek et al., 2023; Neale et al., 1994). For example, MZ twins may experience more similar treatment or parental expectations due to their physical resemblance, which could inflate the estimates of heritability (Neale et al., 1994).

Another major limitation is the inability to identify specific genes involved in the observed phenotypic variation (Sahu & Prasuna, 2016). Instead, twin studies provide a broad estimate of the overall genetic influence on a trait or behaviour. To gain a more precise understanding of the genetic basis, genomic studies are necessary, as they can pinpoint the specific genes contributing to the trait.

Furthermore, the results obtained from twin studies cannot be directly generalised to the broader population due to the absence of randomisation (Sahu & Prasuna, 2016). Twins are unique in their developmental environment as two fetuses grow simultaneously, and this distinction may influence the results. Despite their historical significance, twin studies continue to be based on assumptions made as far back as the 1920s, and some of these assumptions have been critiqued as being inherently flawed. As a result, findings from twin studies are sometimes misconstrued or exaggerated, both in the media and within scientific circles (Sahu & Prasuna, 2016).

Another limitation arises from the voluntary participation of twins in many twin registries (Craig et al., 2020; Sahu & Prasuna, 2016). This introduces volunteer bias, a specific type of selection bias, potentially skewing the sample towards the overrepresentation of identical twins and females. This overrepresentation can lead to an overestimation of the heritability of the trait or condition being studied.

Lastly, the use of twins alone in research designs does not allow for the simultaneous consideration of shared environmental influences and gene-environment interactions. This limitation can be addressed by incorporating additional siblings into the study design, providing a more comprehensive understanding of the complex interplay between genetics and the environment.

To address these limitations and provide more comprehensive insights, combining twin studies with other methodologies, such as molecular genetic approaches like GWAS or whole genome sequencing, can be beneficial. These approaches allow for the identification of specific genetic variants associated with traits or diseases, offering a more precise understanding of their genetic underpinnings and overcoming some of the limitations of twin studies (Friedman et al., 2021).

Genomic studies, particularly GWAS, have revolutionized the field by examining large numbers of common genetic variants across the genome to identify those that are associated with disease risk or traits (Korte & Farlow, 2013; Visscher et al., 2012). GWAS analyses a large number of single nucleotide polymorphisms (SNPs) (a single letter change in the genetic code) to identify associations between specific genetic markers and the phenotype of interest. It enables researchers to explore a vast

number of SNPs distributed throughout the genome. This comprehensive approach allows for the identification of novel genetic loci associated with diseases or traits, without relying on pre-existing hypotheses about specific genes or pathways (Gallagher & Chen-Plotkin, 2018).

GWAS studies require large samples in order to have adequate statistical power to identify common genetic variants associated with variation in disease risk, after adjustment for multiple testing (often upwards of 1 million tests) (Korte & Farlow, 2013). In GWAS, researchers commonly employ a standard p-value threshold for significance, which is typically set at 5×10^{-8} (Cano-Gamez & Trynka, 2020). This stringent threshold is established to mitigate the risk of false-positive findings when conducting millions of statistical tests. When a genetic variant reaches or surpasses this p-value threshold, it is considered statistically significant, suggesting a potential association with the trait or disease under investigation. GWAS also aim to replicate findings in independent populations, adding credibility and generalisability to the identified genetic associations. GWAS results have been used to develop polygenic risk scores, which combine the effects of multiple genetic variants identified to predict an individual's risk for a particular disease or trait (Gallagher & Chen-Plotkin, 2018).

Despite its advancements, GWAS has certain limitations that must be considered. One of the key challenges is the very low proportion of phenotypic variance explained by the identified genetic variants, commonly referred to as the "missing heritability" problem (Manolio et al., 2009; Young, 2019). The discovered variants often account for only a small fraction of the overall heritability (Bogardus, 2009), suggesting the

involvement of additional genetic factors, such as rare variants, gene-gene interactions, and gene-environment interactions.

Population specificity is another important consideration in GWAS (Wijmenga & Zhernakova, 2018). Genetic associations identified in one population may not be applicable to others due to variations in allele frequencies and genetic backgrounds. Replication in diverse populations is necessary to validate and understand the broader applicability of the identified genetic associations (Y. R. Li & Keating, 2014). However, the vast majority of GWAS have been undertaken in participants from a White European background.

GWAS primarily focuses on common genetic variants, potentially overlooking rare variants that may have larger effect sizes (Wray et al., 2011). To address this limitation, whole genome sequencing approaches are now being utilized to capture rare variants and structural variations that are not adequately captured by SNP arrays (Cirulli & Goldstein, 2010). Additionally, the sheer number of statistical tests conducted in GWAS raises the risk of false positive associations. Stringent statistical thresholds and independent replication efforts help mitigate this issue and ensure the reliability of the identified associations (Fadista et al., 2016).

Finally, GWAS associations, while informative, do not directly reveal the underlying biological mechanisms. Additional functional studies, such as gene expression analyses or functional genomics, are necessary to unravel the functional consequences of the identified genetic variants (Arslan, 2018; Gallagher & Chen-Plotkin, 2018; Korte & Farlow, 2013).

In light of the advancements of GWAS, it is important to critically evaluate the role of candidate gene studies. These studies, conducted before the widespread adoption of GWAS, focus on specific genes of interest based on prior knowledge or hypotheses regarding their involvement in a particular trait, disease, or biological process (Zhu & Zhao, 2007). However, candidate gene studies often suffered from limited sample sizes, high false positive rates, and a lack of robust replication (Farrell et al., 2015; Z. Li et al., 2014). Therefore, on the whole, their results have not been replicated in larger-scale GWAS analyses, rendering them of limited value in the current research landscape.

Genomic studies, particularly GWAS, have revolutionized genetic epidemiology by offering comprehensive genome-wide coverage and hypothesis-free exploration of genetic associations. Despite the limitations associated with explained variance, population specificity, rare variants, false positives, and the need for functional interpretation, GWAS has provided invaluable insights into the genetic architecture of complex traits and diseases. Continued advancements in genomics, including whole genome sequencing, will further enhance our understanding.

3.2 The Genetics of Alcohol Use Disorder

It is well known that AUD runs in families (Goldman et al., 2005; Verhulst et al., 2015). A number of twin and adoption studies of AUD have consistently proven the roles of genetic factors in the etiology of AUD since 1960. (Goldman et al., 2005) examined the heritability of AUD in a national survey of 9897 adult twin pairs and they found the heritability of AUD to be about 50%. A similar result was shown by (Verhulst et al., 2015) who published a meta-analysis that identified 12 twin (total number of

participants = 97, 252) and five adoption studies (total number of participants = 6,548) to estimate the heritability of AUD. The results showed the heritability of AUD was approximately 50%.

Genome-wide association studies (GWAS) which aim to identify common genetic risk variants have been proven informative in psychiatric disorders including schizophrenia (Ripke et al., 2014) and AUD (R. K. Walters et al., 2018b). These studies have provided valuable insights into the genetic basis of these disorders through the identification of common genetic risk variants (Visscher et al., 2012). GWAS findings have not only identified specific genetic risk variants but have also advanced our understanding of the genetic architecture underlying these disorders. They have revealed the polygenic nature of psychiatric disorders, indicating that multiple genetic variants across the genome collectively contribute to disease risk (Gallagher & Chen-Plotkin, 2018). These studies have also highlighted the importance of considering gene-gene interactions and gene-environment interactions in the development of these disorders.

The results of GWAS suggested that the genetic risk loci with most of small effect are contributing to the etiology of AUD. Numerous GWASs that aimed to detect AUD genetic risk variants have been reported in recent years. For example, studies have shown AUD genetic risk variants in alcohol dehydrogenase (*ADH*) gene cluster ADH1B, ADH1C, and aldehyde dehydrogenase 2 (*ALDH2*) (Goldman et al., 2005; D. Li et al., 2011, 2012; Luczak et al., 2006; R. K. Walters et al., 2018). These genes encode enzymes that catalyse alcohol metabolism. In general, the *ADH* proteins metabolise ethanol to acetaldehyde and then the *ALDH* protein converts it to acetate. It has been shown that two functional loci His47Arg in

ADH1B and Glu504Lys in *ALDH2* play important roles in the alcohol metabolism steps (Hurley & Edenberg, 2012). Arg48His SNP missense in *ADH1B* could influence the conversion of ethanol to acetaldehyde. The His48 allele confers high activity of ADH1B which helps to accelerate alcohol metabolism (Hurley & Edenberg, 2012; R. K. Walters et al., 2018). By blocking the translation of acetaldehyde to acetate, the Lys504 allele confers low activity of ALDH2 which also strongly influences alcohol metabolism (D. Li et al., 2012; Luczak et al., 2006). Therefore, both His48 and Lys504 alleles afford strong protective effects against the development of AUD (D. Li et al., 2012; Luczak et al., 2006). However, the results from GWAS of AUD offered inconsistent findings partially due to the complex genetic architecture of AUD, which tends to show high levels of polygenicity (Deak et al., 2019).

Researchers have been trying to increase the sample size in GWAS to detect more replicable genetic risk variants, reveal its polygenic architecture, and understand its relationship with other psychiatric disorders. For example, the substance use disorders working group of the Psychiatric Genomics Consortium published the largest GWAS on AUD containing nearly 15,000 AUD cases and more than 37,000 controls from European (EU) and African-American (AA) ancestries (R. K. Walters et al., 2018). Different *ADH1B* variants were found in the two ancestral groups and a genetic distinction between AUD and alcohol consumption was confirmed in this study. They also showed that the protective allele has much lower frequencies in EU and AA. Furthermore, a number of psychiatric outcomes including major depression and poor educational outcomes have been found that they were genetically correlated with AUD. Using an AUD identification test (AUDIT) from European ancestry, with a total number of 141,932

participants, an AUD GWAS meta-analysis study found 10 associated risk loci (Sanchez-Roige et al., 2019). While they successfully replicated previous genes including *ADH1B*, *ADH1C*, *KLB*, and *GCKR*, they also identified novel loci localised to genes *JCAD* and *SLC39A143*. With the increased size of AUD GWAS and including more ancestry groups, more risk loci have been found and replicated. Now, GWAS of AUD have been conducted in multiple populations including European, African, Latin American, and Asians (Bierut et al., 2012; Frank et al., 2012; Gelernter et al., 2014, 2018; Gelernter & Polimanti, 2021; Kranzler, Zhou, Kember, Smith, et al., 2019; Quillen et al., 2014; Sun et al., 2019; Treutlein et al., 2009; R. K. Walters et al., 2018; H. Zhou et al., 2022). Currently, the largest GWAS of problematic alcohol use (PAU), which is a proxy for AUD has identified 29 risk loci with 19 of them novel in European individuals (H. Zhou et al., 2020). In this study, the genetic correlations between PAU and other publicly available GWAS results have been estimated, which showed alcohol consumption, smoking, and lifetime cannabis use were positively correlated with PAU. It also showed that PAU Is positively genetically correlated with major depressive disorder, risk-taking behaviour, insomnia, and negatively correlated with cognitive traits and parents' age at death (H. Zhou et al., 2020).

3.2.1 AUD imaging genetic studies

Psychiatric imaging genetics and genomics studies using neuroimaging measures link genetic and epigenetic risk loci to human brain structures and functions, which offers us a way to examine and understand the neural mechanism that might be influenced by genetic risk variations (Bogdan et al., 2017). However, despite the potential of this field, the challenges also exist. For example, most imaging studies had small sample sizes compared with GWAS and even more modest effect sizes

due to polygenicity (Bogdan et al., 2017). Other concerns include a lack of replication, methodological differences, and unknow mechanisms that underlie genes, brain, and behaviour. Recently, the imaging genetic field has been growing with the help of large consortia projects that aim to increase sample size and develop and apply novel methods including polygenic risk scores and machine learning, which eventually can improve the quality of studies.

While there were a large number of studies published that were trying to understand the impact of alcohol on the brain and its underlying neural mechanism, only a limited number of AUD and alcohol consumption imaging genetics studies published yet. In alcohol consumption, one study found that the *BDNF* Val66Met variant mediates the association between functional putamen activation and alcohol intake in adolescents (Whelan et al., 2014). Ventral striatal activation during reward anticipation and feedback has been reduced in AUD patients (A. Heinz et al., 1998; Schumann et al., 2010). Moreover, a study found that reduced activation of the ventral striatum and other brain regions including the midbrain and prefrontal cortex at the age of 14 can successfully predict drug use at the age of 16. This finding has been replicated in another study that found reduced ventral striatal activation is associated with increased impulsivity in AUD patients. Furthermore, a variant in the gene Rasgrf2 has been reported to be associated with reduced activation of the ventral striatum in alcohol consumption (Schumann et al., 2011). Another polymorphism of *RASGRF2* was found to be associated with current and future binge drinking in the same cohort (Whelan et al., 2014). Researchers later reported that compared with Met carriers, during reward anticipation *BDNF* Val homozygotes showed lower putamen reactivity (Nees et al., 2015).

Researchers suggested that impulsivity might be implicated in patients with AUD and alcohol-related aggression behaviour (A. J. Heinz et al., 2011). Increased activation in the subthalamic nucleus was associated with the *PPM1G* gene, and with increased impulsiveness and alcohol consumption (Ruggeri et al., 2015).

Other imaging studies showed lower volume and cortical thickness of suctorial and cortical regions in AUD patients, suggesting that this is the consequence of alcohol consumption. However, evidence started to show that this might not be the whole story. For example, most recently, in substance naïve children (European ancestry) from the Adolescent Brain Cognitive Development (ABCD) study, researchers showed that polygenic risk scores for problematic alcohol use and alcohol consumption were associated with MRI-derived brain phenotypes (Hatoum et al., 2021). Reduced volumes of the left frontal pole and increased cortical thickness of the right supramarginal gyrus were associated with problematic alcohol use polygenetic risk scores. Therefore, brain-based associations with alcohol might not only be caused by the neurotoxic effects of alcohol.

3.3 The Genetics of Antisocial Personality Disorder

3.3.1 Twin Studies and linkage studies

A number of twin studies have suggested that approximately 50% of the variance is heritable for ASPD (Ferguson, 2010; Rhee & Waldman, 2002; Rosenström et al., 2017). For example, (Rosenström et al., 2017) used the 7 ASPD criteria from DSM-IV diagnosis in a population-based sample of 2794 Norwegian twins estimating heritability of 51%. Higher genetic influences (i.e., 69%) were reported in another twin study (Tuvblad et al., 2014).

Moreover, the heritability for antisocial behaviour (i.e., a broad spectrum of externalising behaviour) reported by (Krueger et al., 2002) was 80%. (Ferguson, 2010) presented a meta-analytic review of behavioural genetic etiological studies of antisocial personality and behaviour (ASB) that covered a range of personality traits that cause antisocial behaviour and behaviour itself. The results showed that 56% of the variance in ASB could be accounted for genetic influences, while shared and unique environmental factors including non-genetic biological influences such as trauma and non-family socialization processes could explain the remainder of the variance.

Researchers have looked at the families that were diagnosed with both AUD and ASPD, and they found that they had different allele patterns compared with patients who do not have AUD (Hill et al., 1999). More specifically, they had different risk loci of the *DRD2* gene (dopamine D_2 receptor), and these genetic risk variants were believed to account for drug-seeking behaviours which are often seen in AUD and ASPD (Blum et al., 1996).

3.3.2 ASPD GWAS

There are only a few GWASs that have investigated ASPD. (Tielbeek et al., 2012) reported the first GWAS of adult antisocial behaviour using a population-based sample (i.e., the Australian Twin Registry). No genome-wide significant results were reported, which is most likely explained by low statistical power to detect small genetic risk effects. (Rautiainen et al., 2016) conducted a GWAS in a prisoner cohort (370 ASPD cases and 5,859 controls) that met the DSM-IV diagnostic criteria of ASPD compared to the general population. Although no association results from the discovery and replication sub-cohort reached genome-

wide significance, (Rautiainen et al., 2016) showed the first genome-wide significant SNP in ASPD in the meta-analysis in which revealed rs4714329 ($p = 1.6 \times 10^{-9}$) on chromosome 6p21.2 that relate to the *LINC00951-LRFN2* gene region. Although the function of this gene region is not known, they pointed out that both genes are expressed in the frontal cortex. More recently, (Tielbeek et al., 2017) presented a GWAS of a quantitative measure of antisocial behaviour with larger sample sizes (i.e., 16 400 individuals in the discovery samples and 9381 individuals in the target samples). However, it did not replicate the result of chromosome 6p21.2 and no genome-wide significant results were found (Tielbeek et al., 2017). This might be attributable in part to measurement diversity, which covers conduct disorder, ASPD, and rule-breaking behaviour across children and adults. A GWAS with a consistent measure of ASPD with a large sample size could potentially ensure sufficient power to estimate common genetic risks of ASPD.

GWAS for AC, AUD, and ASPD.

Table 2 Summary of SNPs Identified in recent large AUD and ASPD GWAS

GWAS	study	years	population	phenotype	significant independent loci	variance explanined	SNP heritablity
Alcohol consumption & AUD	10.1038/mp.2017.153	2017	UK Biobank, *N*=112,117	self-reported alcohol consumption	14	1%	13%
	10.1176/appi.ajp.2018.18040369	2019	UK Biobank [N=121,604] and 23andMe [N=20,328]	AUDIT scores	15 (total AUDIT score) 8 (AUDIT-C) 5 (AUDIT-P)	NA	12% (AUDIT total score) 11% (AUDIT-C) 0.09 (AUDIT-P)
	https://doi.org/10.1038/s41467-019-09480-8	2019	Million Veteran Program sample (*N* = 274,424)	AUDIT-C & AUD diagnoses	5 (both traits) 8 (AUDIT-C) 5 (AUD)	NA	6.8% (AUDIT-C in EAs) 6.2% (AUDIT-C in AAs) 5.6% (AUD in Eas) 10% (AUD in AAs)
	10.1176/appi.ajp.2020.20091390	2021	the UK Biobank (N=5147,267), the Netherlands Twin Register (N=59,975), and the Avon Longitudinal Study of Parents and Children (N=53,582)	AUDIT scores	8 (AUDIT-C) 2 (AUDIT-P)	0.875% (AUDIT-C) 0.744% (AUDIT-P)	8% (AUDIT-C) 6% (AUDIT-P)
ASPD	10.1001/jamapsychiatry.2017.3069	2017	The discovery sample 16400, the target sample 9381	a broad range of antisocial measures, including aggressive and nonaggressive domains of antisocial behaviour	0	0.17% (sex combined) 0.18% (male)	5.2%
	10.1038/s41380-022-01793-3	2022	85,359 individuals	a broadly defined construct of antisocial behaviour	1	0.4% -3.9% depends on different cohorts	7.7%

4. PhD Aims and Hypotheses

The aims of my PhD research are threefold: to identify novel alcohol use disorder (AUD) loci, enhance understanding of how AUD genetic risk variants relate to brain morphology, and investigate the shared genetic risk variants potentially underlying AUD and its comorbidity with other psychiatric disorders, such as antisocial personality disorder (ASPD).

The first study, a genome-wide association study (GWAS) of AUD, utilised data gathered from the UCL Molecular Psychiatry Lab. We sought to enhance the detection of AUD risk loci by using a quantitative variable of AUD symptom scores. Additionally, we examined the influence of the regression models, which applied a quasi-Poisson distribution, considering the distribution of AUD criterion scores within GWAS populations might be skewed towards more severe manifestations.

In the second study, we aimed to investigate AUD's shared genetic risk variants with other psychiatric disorders. We conducted the most extensive meta-analytic GWAS of a comparable clinical measure of ASPD to date, focusing on individuals already diagnosed with AUD.

The final study strived to comprehend whether carrying the genetic risk of AUD and alcohol consumption (AC) could predispose individuals to certain brain morphology. Utilising the most comprehensive GWAS results of AUD and AC, we combined genetic and imaging data from the UK Biobank. The focus was on exploring the impact of AUD polygenic risk scores (AUD-PRS) and AC-PRS on brain volumes in individuals

consuming less than 14 alcohol units per week. My hypothesis was that a high PRS for AUD would correlate with specific brain structural variations compared to the low-risk group, even among individuals consuming fewer than 14 units per week.

5. Contributions and PhD training

In this work, I made several unique contributions to the thesis. For my PhD training, I completed online courses from platforms like edx and Coursera, focusing on biology, bioinformatics, R, and Python. Additionally, I actively participated in conferences (2018 & 2019), specifically the World Congress of Psychiatric Genetics (WCPG), where I presented my research findings. I also attended several summer and winter schools, including the SJTU-KCL Brain Facts International Summer School, Theoretical and Computational Neuroscience Summer School, neuromatch, and GWAS winter school from UCL.

Regarding my specific contributions to the projects, I performed various tasks. I manually inputted phenotypic data from papers into the computer (N > 3200), conducted phenotype data cleaning, X-chromosome genetic data imputation, and post-imputation quality control. I carried out all computational calculations and analyses. Additionally, I took the lead in writing the papers and presenting the results at conferences. My colleagues assisted in genetic and phenotypic data collection, and the UCL UKB team handled genetic data cleaning and quality control for the UK Biobank dataset. Throughout the research process, my supervisor provided valuable support and guidance, contributing to conceptualization, investigation, methodology, project administration, supervision, and review and editing.

Overall, my unique contributions involved data preparation, computational analysis, result dissemination, and active engagement in relevant academic events, while collaborating with colleagues and benefiting from my supervisor's guidance and expertise.

Chapter 2 THE INFLUENCE OF REGRESSION MODELS ON GENOME-WIDE ASSOCIATION STUDIES OF ALCOHOL USE DISORDER: A COMPARISON OF BINARY AND QUANTITATIVE ANALYSES

The work presented in this chapter has been published in Psychiatric Genetics Journal and please find the PDF in the appendix 2. Doi: 10.1097/YPG.0000000000000268

The influence of regression models on genome-wide association studies of alcohol dependence: a comparison of binary and quantitative analyses

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2.1 Alcohol use disorder GWAS

Alcohol use disorder (AUD) is a common disorder characterized by the excessive and compulsive use of alcohol, often resulting in physical, emotional and social harm; it poses significant problems for health and social agencies alike. AUD is a complex disorder which is influenced by both environmental and genetic factors (Stickel et al., 2017). Twin, family and adoption studies provide evidence for significant heritability of the alcohol dependence and alcohol misuse phenotypes. (Verhulst et al., 2015) undertook a meta-analysis of data from 12 twin and five adoption studies and provided an overall estimate of the heritability for alcohol use disorders of 49%. However, in an earlier meta-analysis of over 50

family, twin and adoption studies of alcohol misuse phenotypes (G. D. Walters, 2002) showed that there was significant heterogeneity across studies and provided a mean heritability estimate of 24%. (G. D. Walters, 2002) also showed that the heritability was much stronger in men with severe alcoholism/alcohol dependence and the heritability estimates were of the order of 30% to 36%.

While it is generally agreed that inheritance is polygenic (Goldman et al., 2005; Schork & Schork, 1998), identifying the genes involved and their relative contribution is difficult because of the considerable variations observed in population phenotypes, the design of studies, the type of data analysis, and because of a general failure to control for potential confounders such as co-morbid psychiatric conditions and co-occurring substance misuse (Ali et al., 2015; Hirschhorn et al., 2002)
Several genome-wide association studies (GWAS) of AUD have been undertaken many of which are based on collaborative studies in the USA (Hart & Kranzler, 2015; Kranzler, Zhou, Kember, Smith, et al., 2019; M. Liu et al., 2019; Stickel et al., 2017). The majority of the GWAS in AUD, undertaken to date, have failed to identify genome-wide significant associations. However, meta-analyses and studies in populations with greater phenotypic surety have identified genome-wide significant associations between genetic risk variants in the alcohol dehydrogenase (*ADH*) gene cluster on chromosome 4, which includes *ADH1B, ADH1C*, and aldehyde dehydrogenase 2 (*ALDH2*). These polymorphisms, which confer protection against problematic drinking, are significantly more prevalent in populations with East Asian ancestry (Bierut et al., 2012; D. Li et al., 2011, 2012). Other significant associations, appear to be specific to individual studies and have failed to replicate (Gelernter et al., 2014; Kranzler, Zhou, Kember, Smith, et al., 2019; M. Liu et al., 2019; R. K. Walters et al., 2018). This lack of

consistency and failure to replicate may reflect the fact that the studies undertaken to date were likely underpowered to detect variants with small effect sizes, particularly given the stringent genome-wide significance threshold.

Most of the published AUD GWAS have utilised a case-control design (Hart & Kranzler, 2015; Stickel et al., 2017). However, within the AUD phenotype there is considerable variability in the defining features and hence substantial uncertainty about which features might be inherited. Thus, for example, the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM) 4th edition (American Psychiatric Association, 2000) lists seven defining symptoms: tolerance, withdrawal, desire/failed efforts to control drinking, drinking more than intended, reduced/impaired activities, primacy of drinking, and drinking despite problems. Individuals who report three or more of these features within the same 12-month period are classified as having AUD. However, scores may range from 3 to 7 and it is clear that the contributing features may carry different weighting in relation to the clinical condition and its severity (Lane & Sher, 2015).

Thus, one approach to improving the identification of risk loci in AUD GWAS would be to create a quantitative variable based on the total number of DSM-IV criteria fulfilled. A score based on the DSM-IV, which is a proxy for the severity of AUD has been used as a quantitative variable in a number of studies (Gelernter et al., 2014; Heath et al., 2011; Kendler et al., 2011; Lai et al., 2019; Mcgue et al., 2013; J. Wang et al., 2013). However, the approach has not, to date, identified any novel associations at genome-wide significance or else novel findings that have been replicated. However, the distribution of the AUD criterion scores within GWAS

populations may vary considerably and these differences do not appear to have been taken into account to date. In the general population most AUD patients will experience fewer symptoms as severe cases are rare. AUD symptom counts will therefore not always follow a normal distribution, but may sometimes be closer to a quasi-Poisson distribution. There are currently no studies examining the impact of the assumptions made with regard to the distribution of quantitative symptom data and hence no studies which have examined the influence of regression models on the AUD GWAS data.

2.2 Aims

The aims of the present study were:

1. To compare the results of a classic case-control GWAS in AUD with those obtained using the DSM-IV criterion score as a quantitative trait.
2. To determine the proportion of the variance for AUD explained by AUD risk alleles utilising PRS analyses based on the case-control and quantitative trait analyses.

2.3 Methods

2.3.1 Participants

Individuals with AUD (n=742; 67 % men) were recruited from a variety of UK community and hospital-based services providing support and treatment for individuals with alcohol use disorders. The original study aimed to improve our understanding of the genetic architecture of alcohol dependence and its sequelae. Recruitment was through twenty NHS drug and alcohol services in England, Scotland and Wales. Recruitment was coordinated by the NIHR funded Mental Health

Research Network. The majority of research participants were being treated as outpatients with the remainder treated as inpatients on medical or detoxification wards.

It was funded by the Neuroscience Research Charitable Trust, Brain Damage Research Trust. Genotyping of the control samples was funded by the Stanley Center for Psychiatric Research at the Broad Institute. Genotyping of the alcohol dependence samples was funded by grants from the SysMedAlcoholism (01ZX1611B) and SysMedSUDs by the German Federal Ministry of Education and Research (BMBF) within the e: Med programme.

Participants were selected based on specific inclusion criteria, such as meeting diagnostic criteria for AUD, and consent to participate in the study. The recruitment process spanned several years, starting from 2000 and continuing until 2016. The diagnosis of AUD in the study was conducted by experienced nurses and doctors from an alcohol clinic in the UK. These healthcare professionals were trained to assess AUD using the Alcohol Dependence Syndrome section of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II) (Bucholz et al., 1994). This instrument incorporates the scoring items for the diagnosis of AUD delineated in the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) (American Psychiatric Association, 2000). All were of English, Scottish, Welsh or Irish descent with a maximum of one grandparent of non-British but Northern European ancestry; none of these individuals was related.

It is important to note that during the recruitment period, data collection measures were administered to capture relevant information regarding

AUD symptoms, demographics, family history, and other relevant variables. These measures were administered by the trained healthcare professionals mentioned above, who ensured standardized procedures for data collection and maintained confidentiality throughout the process.

Ancestrally matched healthy controls (n = 797) were recruited from London branches of the National Health Service (NHS) blood transfusion service, from family doctor clinics that were recruiting case participants and from among university students. Controls were screened for an absence of a personal or family history of mental illness or substance dependence. Participants consented for their clinical and genetic data to be used for medical research. Individuals were excluded if screening with the Schedule for Affective Disorders and Schizophrenia (SADS-L) (Endicott & Spitzer, 1978) revealed a lifetime history of neurosis, depression, bipolar disorder, schizophrenia or alcohol use disorders. DNA from a separate set of healthy controls of British ancestry (n = 454) was purchased from the European Collection of Cell Cultures (ECACC; Health Protection Agency Culture Collections, Salisbury, UK).

Please note that the information provided here is based on the available information and may need to be modified or expanded based on the specific details provided in the original thesis.

All procedures involving human subjects were approved by the NHS Metropolitan Multi-centre Research Ethics Committee (now the South Central - Hampshire A Research Ethics Committee) approval number MREC/03/11/090. All participants provided signed informed consent.

2.3.2 Genotyping, Imputation and Quality Control

Participants provided either blood or saliva samples for genomic DNA extraction. Genotyping of the DNA samples in the AUD cohort was performed at Life and Brain GmbH, Bonn, Germany, using the Illumina PsychArray. The DNA samples from the healthy controls were genotyped at the Broad Institute, MA, USA, using the same array. Quality control of the genotype data was performed in two stages, preimputation with more inclusive parameters aimed at retaining a maximal number of subjects, and postimputation with more stringent parameters aimed at obtaining a high-quality dataset. Pre-imputation quality control parameters were: individuals were excluded if they had incorrect gender assignment; excessive heterozygosity (in AUD samples and healthy controls: 3 SD > the mean); more than 10% of missing genotype data and evidence of relatedness. Data on SNPs which had a minor allele frequency (MAF) < 5% or deviated substantially from the Hardy–Weinberg equilibrium (HWE) ($P < 10^{-6}$) were excluded.

Imputation was undertaken using the Haplotype Reference Consortium (release 1.1) reference panel on the Sanger Imputation server (McCarthy et al., 2016). Prior to the upload of data, genotypes were prepared as instructed and checks were performed using the HRC-1000G-check-bim tool Version 4.2.3 (Rayner, 2015). A total of 393,270 SNPs with a MAF > 0.01 were uploaded for imputation (McCarthy et al., 2016). Prephasing was undertaken with EAGLE2 (Loh et al., 2016) and imputation was performed using the Positional Burrows-Wheeler Transform method (Durbin, 2014).

Postimputation quality control parameters used on the hard-called best-guess SNP genotypes. SNPs were included if they met the following criteria: call rates < 99%, HWE p-value < 1×10^{-5}, and MAF < 5%. All

quality control steps were performed in PLINK2 (Chang et al., 2015). A total of 2.1 million SNPs were available for analysis following this process.

2.3.3 Statistical Analysis

Data Processing

Participants with a DSM-IV AUD score of ≥ 3 were classified as alcohol dependent. The total number of positive responses to the DSM-IV listed criteria (3 to 7) was used to define the phenotype for the quantitative trait analyses (Fig 3 and Table 3). The study population was recruited from treatment centres across the UK so it was anticipated that the majority would have severe AUD according to criteria. In anticipation of a prominent right skew to the criterion scores the models using both Gaussian and quasi-Poisson distribution were assumed.

Association tests

A standardised genetic relationship matrix was first estimated from the genotype data using the genome-wide efficient mixed-model association (GEMMA) package in R (X. Zhou & Stephens, 2012). The genetic relationship matrix was then used in a generalized linear mixed model with the glmmkin function in the GMMAT to fit three models for the binary AUD and quantitative ASD criterion score.

Model 1 (Binomial): used the binary AUD phenotype data and assumed a Bernoulli distribution.

Model 2 (Gaussian): used a fitted linear mixed model for the quantitative AUD criterion scores.

Model 3 (quasi-Poisson): used the same quantitative AUD criterion scores as Model 2 but to fit the likely right-skewed distribution of the criterion scores using a quasi-Poisson regression.

Individual GWASs were performed using a generalised linear mixed model implemented in the GMMAT package with imputed best-guess genotypes with sex, and the first 10 principal components as covariates (Chen et al., 2016).

Effect sizes and p-values for the top nine independent SNPs from the three individual GWASs, were generated using Wald tests. All analyses were performed using R version 3.5.1 (R Core Team 2019, 2019).

Statistical power

To determine the smallest effect size detectable in a chapter, a power calculation was performed using a genetic power calculator (Purcell et al., 2003). The power calculation indicated that a minimum of 1272 cases is needed to detect a small effect size (0.2) with setting an alpha level of 5×10^{-8} for adjustment for multiple testing (180 cases for effect size 0.98 with alpha 0.01; 359 cases for effect size 0.9 with alpha 0.001).

Expression and splicing quantitative trait loci (eQTLs and sQTLs)

The GTEx V8 database was interrogated to identify correlations between genotype and gene expression levels (Carithers et al., 2015) for the top GWAS hits.

Gene-based association and enrichment analyses

The Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) package was used to explore gene prioritization, gene expression, and gene-based analysis (Watanabe et al., 2017).

Polygenic risk scores

Polygenic risk score (PRS) analyses were performed to determine the proportion of the variance for AUD that could be explained by the AUD risk alleles utilising the data from the binary and the quantitative trait analyses. The software package PRSice was used to estimate the PRS at a range of nine p-value thresholds (Euesden et al., 2015). For clumping, the linkage disequilibrium (LD) threshold was set to an R2 of 0.1 and a distance of 250kb. Sex and the first 10 PCAs were used as the covariates. Summary statistics for AUD GWAS were from the largest GWAS (N= 202, 004) for AUD to date (Kranzler, Zhou, Kember, Smith, et al., 2019).

2.4 Results

Data were available for 739 (67% men) participants who fulfilled DSM-IV criteria for alcohol dependence and 1253 (35% men) healthy controls. The AUD criterion scores ranged from 3 to 7 but with a prominent right-skew in the distribution (Figure 3 and Table 3).

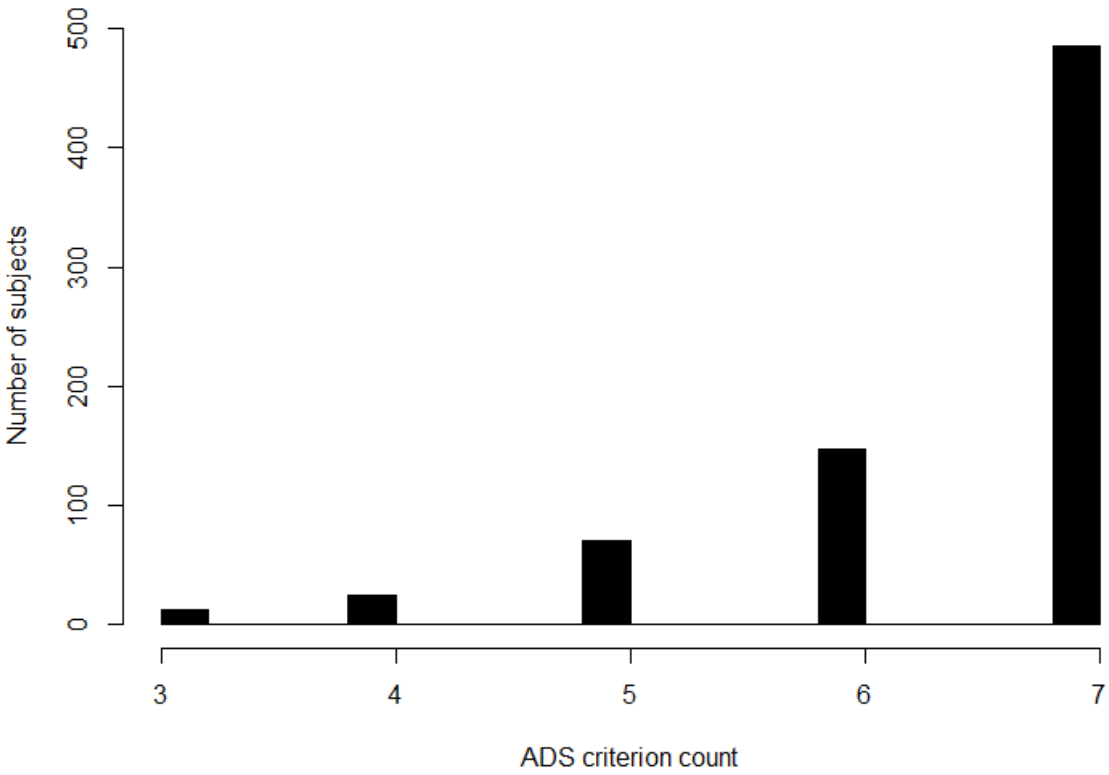


Figure 3 Number of positive responses to the listed criteria for classification of alcohol dependence using DSM-IV used to define phenotype in Models 2 and 3

Table 3 Distribution of AUD criterion in DSM-IV AUD cases with demographic

	AUD individuals
Gender	67%
Mean Age	33 (male & female)
Tolerance	77%
Failed efforts to control drinking	76%
Drinking more than intended	78%
Reduced activities	86%
Primacy of drinking	80%
Drinking despite problems	88%
Withdrawal	92%

2.4.1 GWAS

No associations were identified in the individual GWAS which were significant at the genome-wide level ($p = 5 \times 10^{-8}$, Table 4). The top associated SNP in the GWAS utilising a binary diagnosis of AUD (Model 1) was rs34361428, located in the alcohol dehydrogenase (*ADH*) cluster on chromosome 4 ($p = 8.58 \times 10^{-7}$, beta = 0.48). This was also the top associated SNP identified in the GWAS based on the DSM-IV criterion scores although the levels of significance were lower in both (Model 2: $p = 1.50 \times 10^{-6}$, beta = 0.62; Model 3: $p = 8.13 \times 10^{-6}$, beta = 0.24). (Manhattan and QQ plots for the GWAS analyses are shown in Figures 4 - 6)

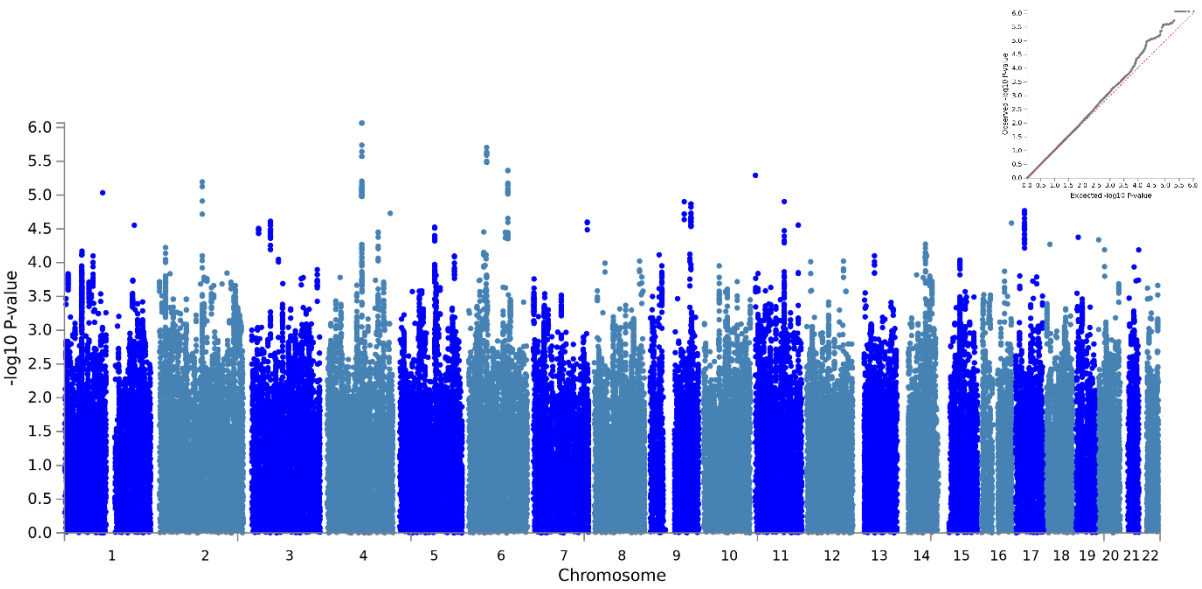


Figure 4 GWAS results of Binomial model Manhattan and QQ plots

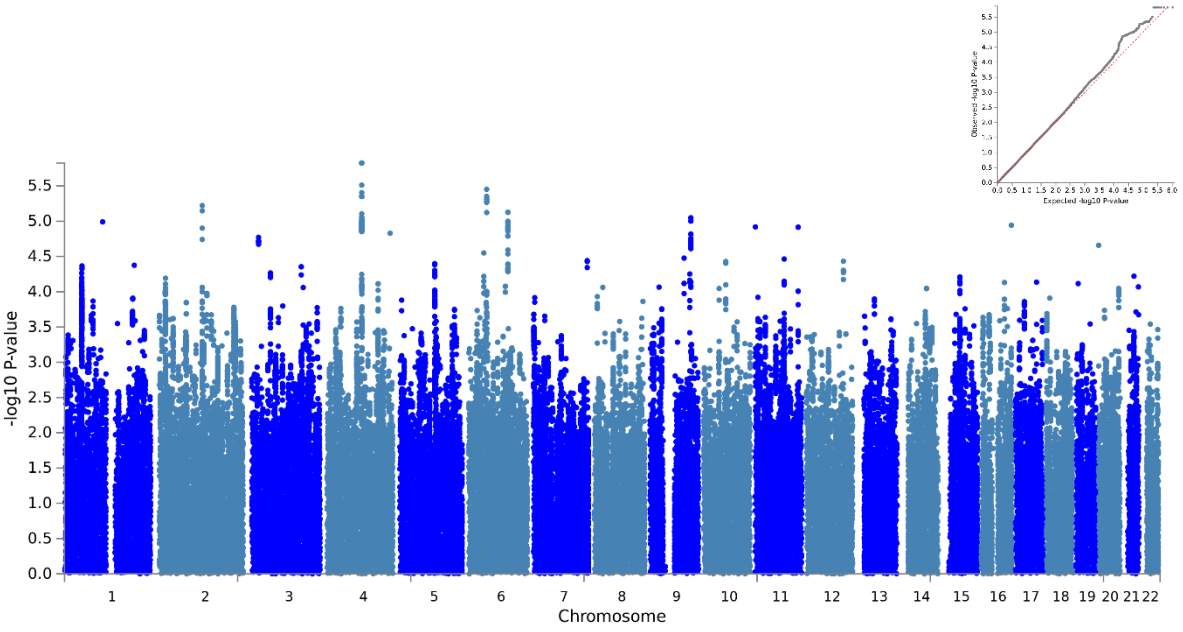


Figure 5 GWAS results of Gaussian model Manhattan and QQ plots

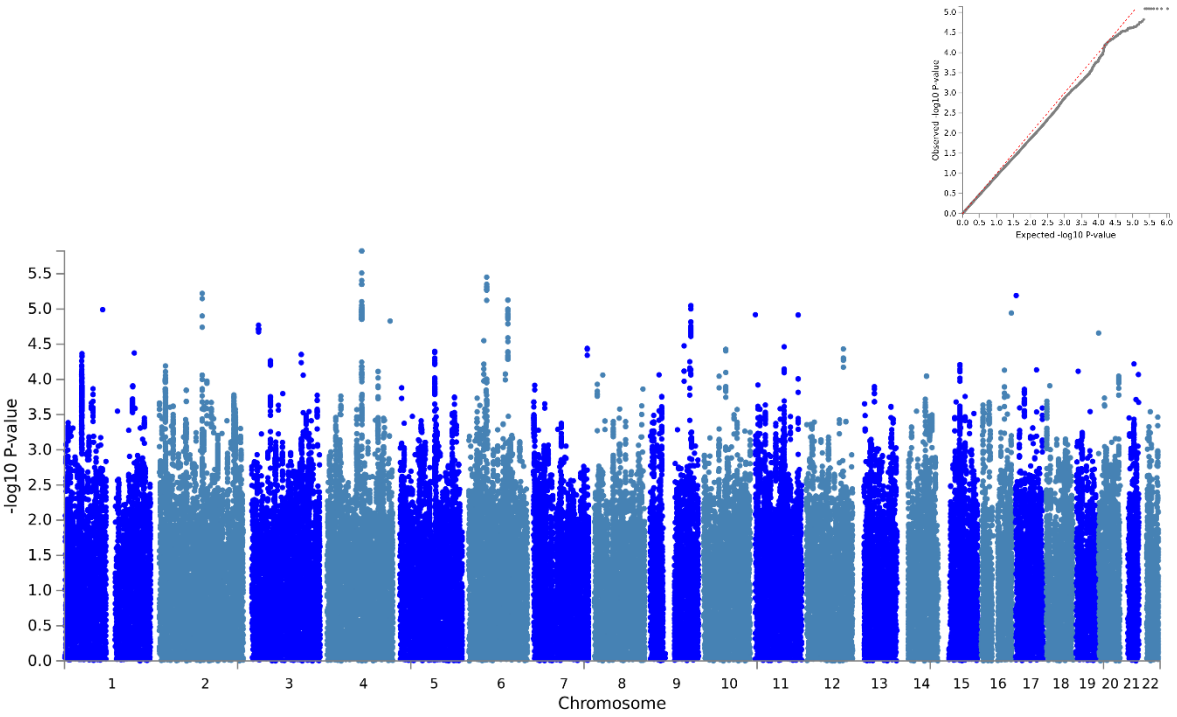


Figure 6 GWAS results of quasi-Poisson model Manhattan and QQ plot

Table 4 Association results for the lead markers in the GWAS of alcohol use disorder, by model

CHR	SNP ID	A1	A2	eQTL Gene Symbol	BETA(SE)			P-value			EAF
					Binomial	Gaussian	quasi-Poisson	Binomial	Gaussian	quasi-Poisson	
4	rs34361428	G	C	*ADH1A*, *ADH1B*, *ADH1C*, *METAP1*	0.48 (0.1)	0.62 (0.13)	0.24 (0.05)	$8.58x10^{-7}$	$1.50x10^{-6}$	$8.13x10^{-6}$	20%
6	rs11950	G	A	-	0.52 (0.11)	0.68 (0.15)	0.26 (0.06)	$2.48x10^{-6}$	$4.44x10^{-6}$	$2.20x10^{-5}$	14%
6	rs2294231	T	C	*MARCKS*	-0.33 (0.07)	-0.42 (0.09)	-0.18 (0.04)	$4.34x10^{-6}$	$7.46x10^{-6}$	$2.53x10^{-5}$	58%
11	rs11245976	A	G	-	0.4 (0.09)	0.51 (0.12)	0.19 (0.05)	$5.10x10^{-6}$	$1.20x10^{-5}$	$6.96x10^{-5}$	22%
2	rs1519302	C	T	-	0.36 (0.08)	0.47 (0.1)	0.19 (0.04)	$6.39x10^{-6}$	$5.99x10^{-6}$	$1.53x10^{-5}$	31%
4	rs1442493	G	A	*ADH4*, *ADH1C*, *EIF4E*	-0.42 (0.09)	-0.53 (0.12)	-0.23 (0.06)	$6.69x10^{-6}$	$8.81x10^{-6}$	$4.60x10^{-5}$	85%
1	rs6693815	T	C	*GSTM3*, *GSTM4*, *EPS8L3*	0.40 (0.09)	0.53 (0.12)	0.21 (0.05)	$9.23x10^{-6}$	$1.02x10^{-5}$	$3.66x10^{-5}$	24%
9	rs10817822	G	A	-	0.38 (0.09)	0.51 (0.11)	0.21 (0.05)	$1.36x10^{-5}$	$8.96x10^{-6}$	$2.41x10^{-5}$	24%
16	rs9646303	G	A	*FBXO31*, *ZCCHC14*	-0.45 (0.11)	-0.63 (0.14)	-0.24 (0.06)	$2.60x10^{-5}$	$1.14x10^{-5}$	$3.07x10^{-5}$	14%

The loci shown represent the top FUMA independent signals. GTEx eQTL data for each independent SNP was listed in eQTL Gene Symbol.

CHR: chromosome, A1: effect allele, A2: other allele, EAF: effect allele frequency, eQTLs: expression quantitative trait loci

2.4.2 Expression and splicing quantitative trait loci (eQTLs and sQTLs)

Based on information provided by the GTEx V8 database, rs34361428, the top identified SNP associated with AUD is a significant eQTL for *ADH1B* ($p = 6.5 \times 10^{-17}$, normalised effect size (NES) = -0.27); carriage of the C allele is associated with increased expression of *ADH1B*. Of interest, from GTEx V8, rs34361428 is also a significant eQTL for *ADH1C* ($p = 3.6 \times 10^{-12}$, NES = -0.22), *ADH1A* ($p = 1.4 \times 10^{-11}$, NES = -0.33), and *METAP1* (p = 0.0002, NES = -0.065) but at these loci the C allele is associated with decreased expression. Additionally, single-tissue sQTLs data from GTEx V8 show that rs34361428 is a significant sQTL for *ADH1C* ($p = 8.6 \times 10^{-7}$, NES = -0.54); expression is influenced by the C allele but varies by tissue; carriage of the C allele is associated with decreased *ADH1C* expression in the liver.

2.4.3 Gene-based test, pathway, and enrichment analyses

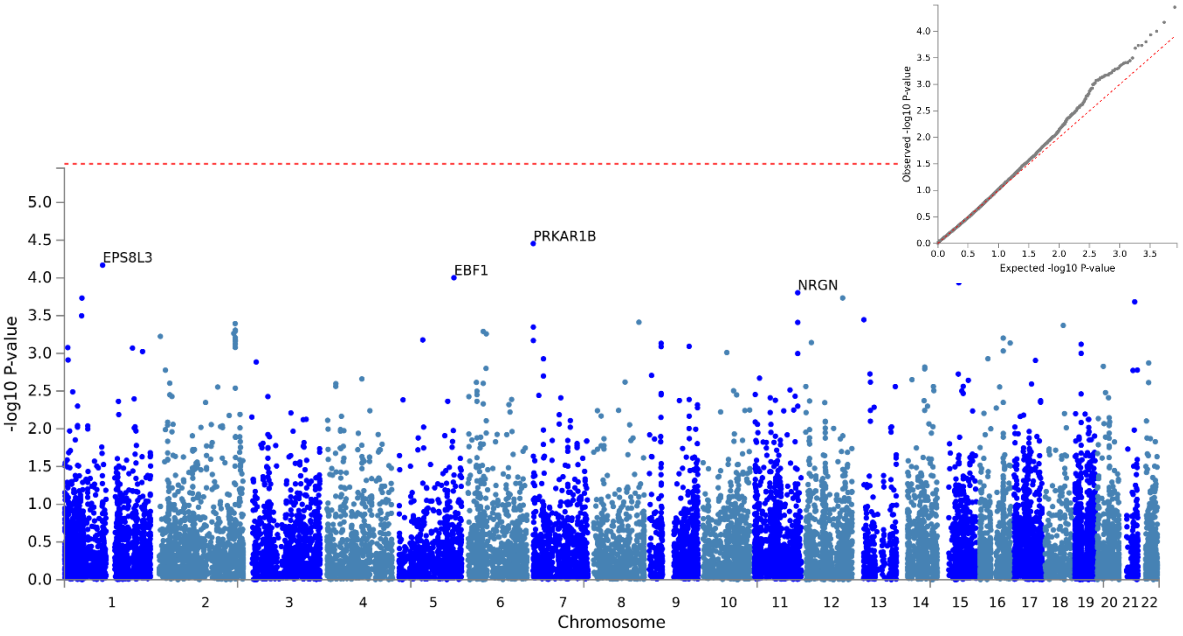
MAGMA gene-based tests showed no significant gene prioritization and gene expression. Likewise, MAGMA gene-set analysis revealed no significant gene set related to AUD in our data after correcting for multiple testing (table 5 A - C). MAGMA tissue expression analysis also showed no significant enrichment for any tissue types (Figures 7 - 9).

Table 5 (A-C) MAGMA gene-set analysis

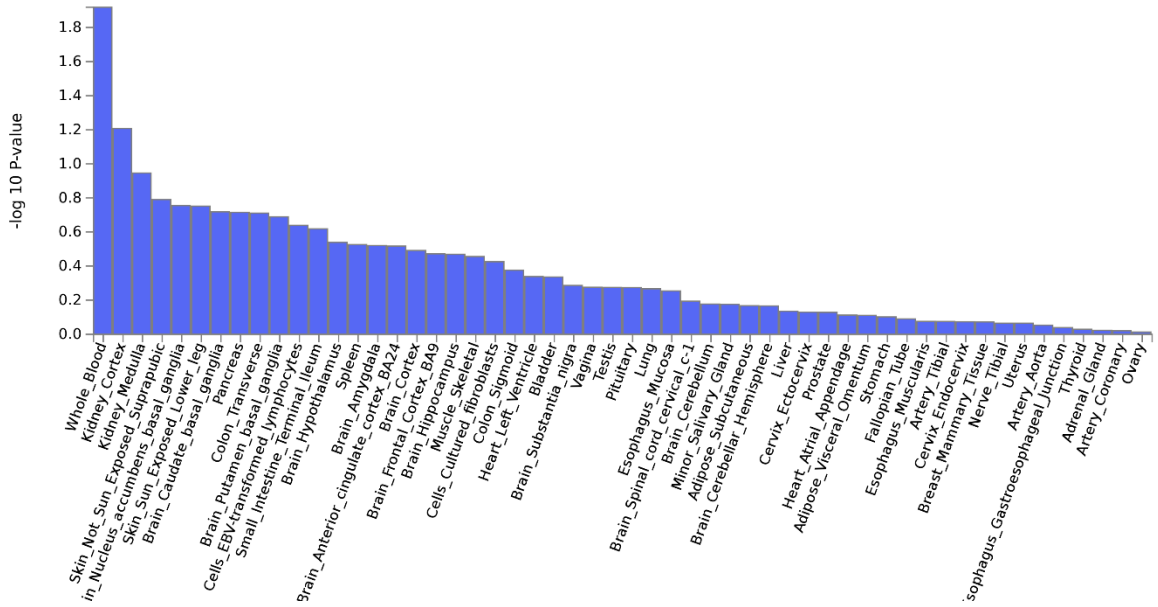
Table A: MAGMA Gene-Set Analysis						
Model 1 (Binomial)						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
prostate gland growth	9	1.195	0.028	0.303	3.97E-05	0.614
muscle cell differentiation	290	0.191	0.025	0.051	8.86E-05	1
matzuk male reproduction sertoli	20	0.693	0.024	0.187	1.05E-04	1
axon extension	98	0.324	0.025	0.087	1.07E-04	1
positive regulation of immunoglobulin secretion	7	1.177	0.024	0.320	1.21E-04	1
riggi ewing sarcoma progenitor dn	168	0.245	0.025	0.067	1.39E-04	1
yagi aml survival	98	0.313	0.024	0.087	1.67E-04	1
ballif developmental disability p16 p12 deletion	13	0.810	0.023	0.231	2.34E-04	1
developmental cell growth	187	0.227	0.024	0.065	2.37E-04	1

Table B: MAGMA Gene-Set Analysis						
Model 2 (Gaussian)						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
prostate gland growth	9	1.195	0.028	0.303	3.97E-05	0.614
muscle cell differentiation	290	0.191	0.025	0.051	8.86E-05	1
matzuk male reproduction sertoli	20	0.693	0.024	0.187	1.05E-04	1
axon extension	98	0.324	0.025	0.087	1.07E-04	1
positive regulation of immunoglobulin secretion	7	1.177	0.024	0.320	1.21E-04	1
riggi ewing sarcoma progenitor dn	168	0.245	0.025	0.067	1.39E-04	1
yagi aml survival	98	0.313	0.024	0.087	1.67E-04	1
ballif developmental disability p16 p12 deletion	13	0.810	0.023	0.231	2.34E-04	1
developmental cell growth	187	0.227	0.024	0.065	2.37E-04	1

Table C: MAGMA Gene-Set Anslysis						
Model 3 (quasi-Poisson)						
Gene Set	N genes	Beta	Beta STD	SE	P	Pbon
matzuk male reproduction sertoli	20	0.715	0.025	0.187	6.46E-05	0.998
prostate gland growth	9	1.155	0.027	0.302	6.73E-05	1
axon extension	98	0.328	0.025	0.087	8.57E-05	1
positive regulation of immunoglobulin secretion	7	1.141	0.024	0.320	1.82E-04	1
cell body membrane	27	0.599	0.024	0.170	2.11E-04	1
biocarta srcrptp pathway	11	0.963	0.025	0.274	2.22E-04	1
burton adipogenesis 5	93	0.305	0.023	0.089	2.89E-04	1
growth	804	0.106	0.023	0.031	3.54E-04	1
regulation of developmental growth	270	0.177	0.023	0.053	4.06E-04	1

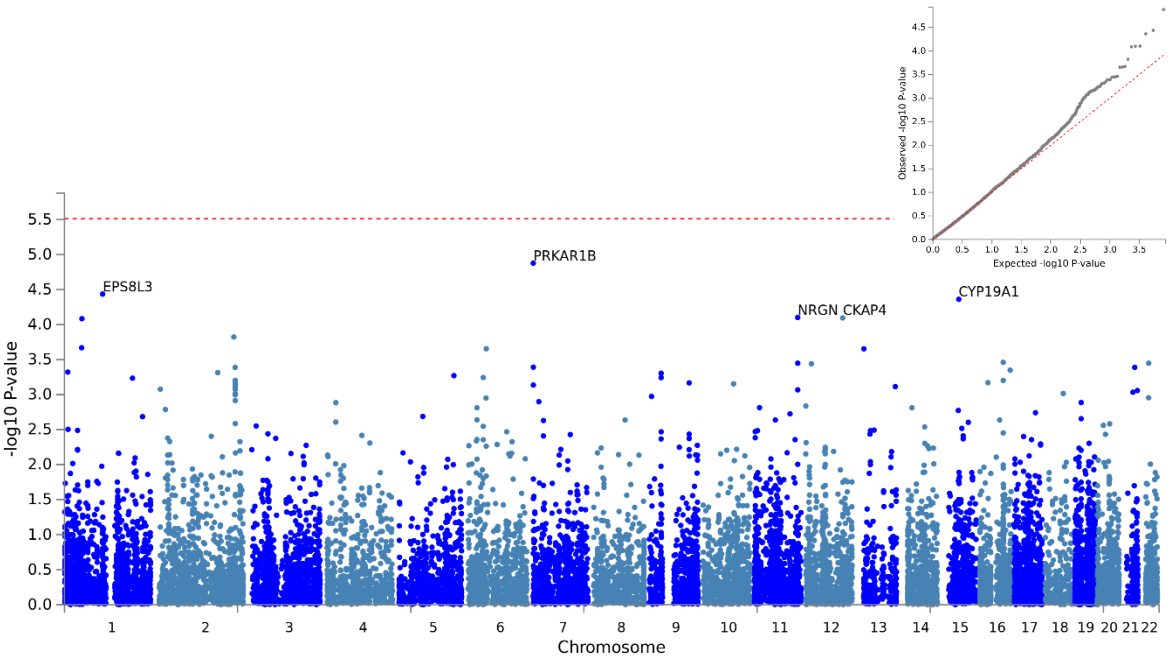


A (Manhattan and QQ plots for gene-based test)

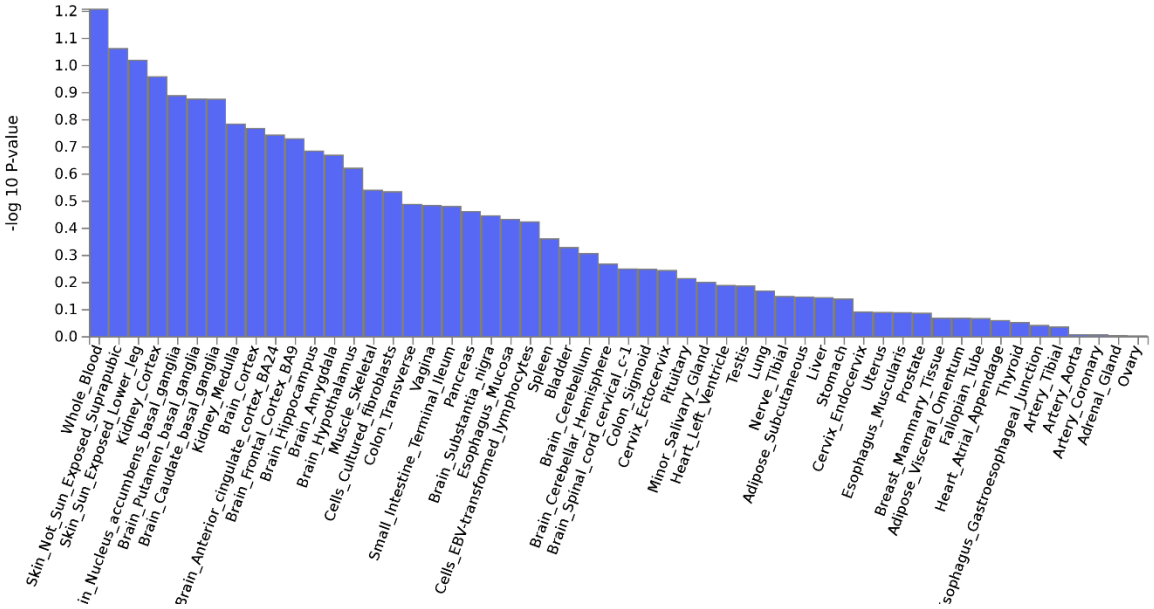


B (tissue expression analysis)

Figure 7 MAGMA gene-based and tissue expression analysis for Model 1 (Binomial)

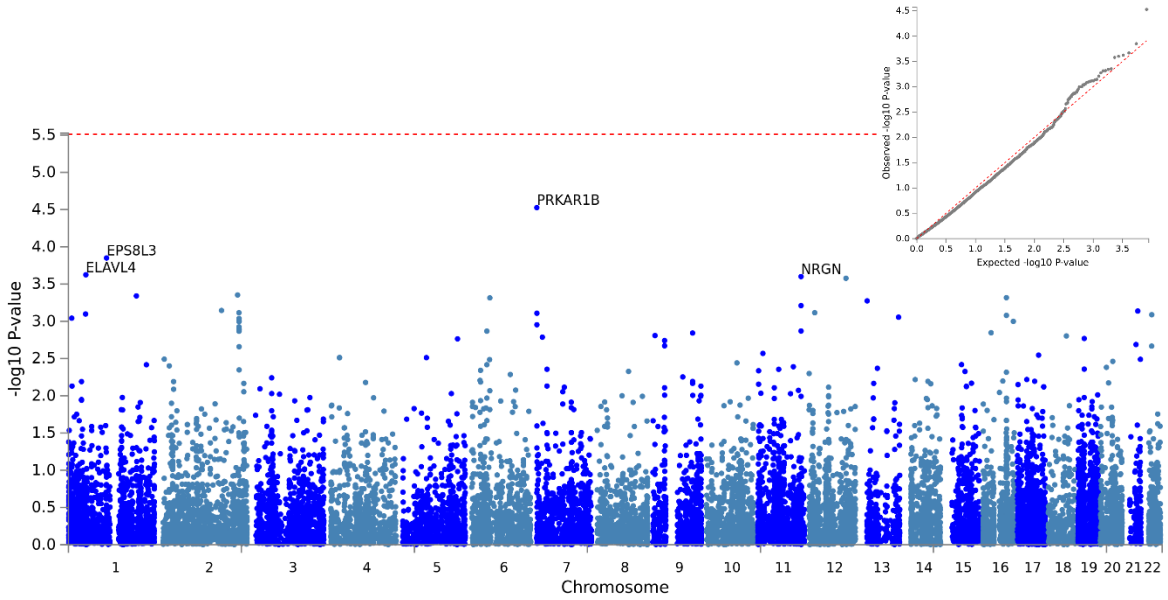


A (Manhattan and QQ plots for gene-based test)

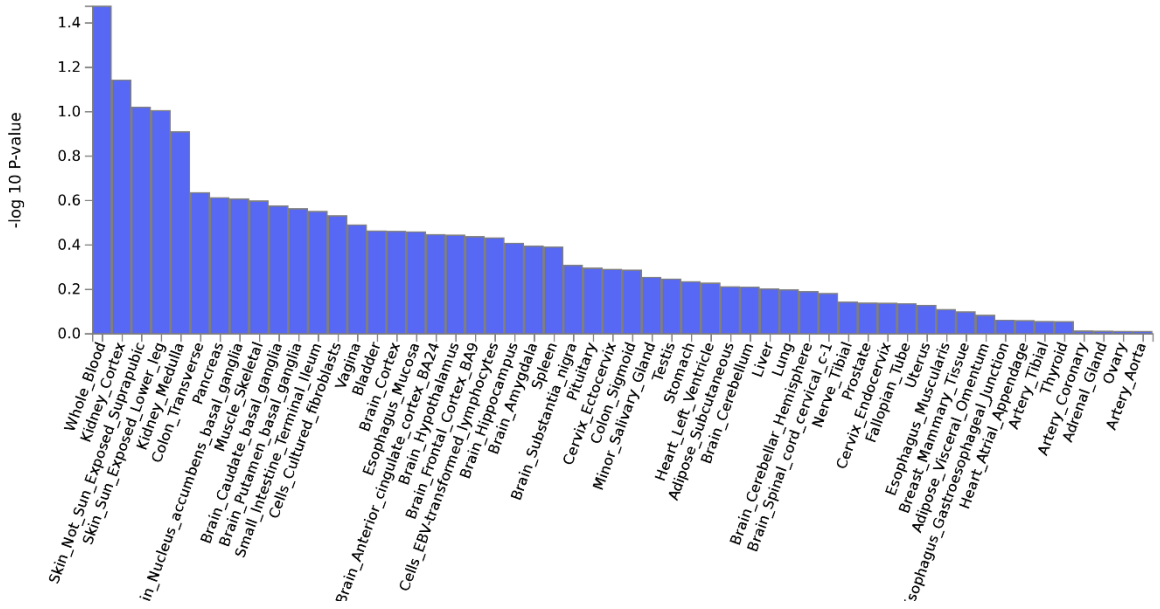


B (tissue expression analysis)

Figure 8 MAGMA gene-based and tissue expression analysis for Model 2 (Gaussian)



A (Manhattan and QQ plots for gene-based test)



B (tissue expression analysis)

Figure 9 MAGMA gene-based and tissue expression analysis for Model 3 (quasi-Poisson)

2.4.4 Polygenic risk scores (PRS) analysis

The PRS generated from publicly available AUD GWASs were tested in our sample in the three individual GWAS. The PRSs explained a proportion of the variance for AUD at all p-value thresholds. At a threshold of 0.5 the binary model explained 3.59% of the variance ($p = 0.026$) for AUD and the quantitative model upwards of 2.64% of the variance ($p = 3.9 \times 10^{-15}$) (Table 6). The PRS based on the binary model seemed to explain more of the variance in AUD than the quantitative models, but the difference was not significant ($p = 0.23$).

Table 6 Results of application of polygenic risk scores to the alcohol use disorder GWAS data generated using the binary and quantitative models

	PRS - Threshold	Number of SNPs	R^2		p-value		Coefficient		Standard Error	
			binary	quantitative	binary	quantitative	binary	quantitative	binary	quantitative
AUD	$5x10^{-8}$	14	0.63%	0.39%	0.245	$2.7x10^{-3}$	0.140	0.157	0.120	0.052
	$1x10^{-6}$	25	0.79%	0.50%	0.220	$6.5x10^{-4}$	0.158	0.182	0.129	0.053
	$1x10^{-5}$	61	1.13%	0.77%	0.102	$2.3x10^{-5}$	0.203	0.247	0.124	0.058
	0.0001	230	1.85%	1.24%	0.026	$8.2x10^{-8}$	0.274	0.332	0.123	0.062
	0.001	1170	1.92%	1.28%	0.015	$4.9x10^{-8}$	0.285	0.349	0.117	0.064
	0.01	8077	2.41%	1.79%	0.007	$1.0x10^{-10}$	0.298	0.387	0.111	0.060
	0.05	32963	2.60%	2.02%	0.019	$7.3x10^{-12}$	0.326	0.431	0.139	0.063
	0.5	235586	3.59%	2.64%	0.022	$3.9x10^{-15}$	0.400	0.511	0.175	0.064
	1	392311	3.56%	2.62%	0.026	$5.3x10^{-15}$	0.395	0.505	0.177	0.064

AUD: Alcohol use disorder;

PRS – threshold: p-value threshold;

Number of SNPs: number of SNPs included in two models;

R^2: Variance explained by the PRS;

Coefficient: regression coefficient of two models.

2.5 Discussion

Classical GWAS approaches in AUD have failed to identify consistently replicable loci with the exception of protective variants within the alcohol metabolizing genes, notably *ADH1B*, and to a lesser degree, *ADH1C* but these explain only a small proportion of the associated risk (Hart & Kranzler, 2015; Stickel et al., 2017). Thus, as with other complex traits, it is likely that a large number of causal risk variants contribute to the development of AUD but that individually they have comparatively small effect sizes (Manolio et al., 2009). This coupled with the stringent correction for the large number of SNPs tested in GWAS, means that very large samples are necessary to reliably detect the associated loci.

One additional challenge to the identification of novel loci contributing to the risk of developing AUD may be the underlying heterogeneity of the condition. The majority of the GWAS undertaken to date have used a binary case-control definition for the AUD phenotype. Another strategy which might improve the detection of variants would be to use the more granular approach of defining quantitative traits. The utilisation of a quantitative approach, as opposed to a binary score, holds substantial advantages in genetic studies. This approach has been recognised for its potential to enhance statistical power, generate unbiased polygenic risk scores, and uncover quantitative mechanisms underlying complex traits and disorders (Plomin et al., 2009). Plomin et al. (2009) examined the disconnection between the dichotomous classification of disorders and the underlying polygenic liabilities, stating that common disorders' genetic foundations lie within the quantitative extremes of continuous distributions of genetic risk. This perspective highlights the importance of moving beyond simplistic binary classifications and embracing a

quantitative framework to capture the complex nature of genetic influences.

The adoption of a quantitative variable based on the total number of DSM-IV criteria fulfilled represents one possible approach to capture the genetic contributions to a disorder quantitatively (Heath et al., 2011; Sanchez-Roige et al., 2019). By quantifying the number of criteria met, researchers can better capture the continuum of risk within a population, accounting for the subtle variations and graduations in severity. This approach recognises that genetic risk factors are not solely confined to the presence or absence of a disorder but exist along a spectrum, reflecting the influence of multiple genetic variants and their cumulative effects. Moreover, incorporating a quantitative approach facilitates the exploration of gene-environment interplay in the etiology of disorders (Chanda et al., 2009; McAllister et al., 2017). The complex interplay between genetic factors and environmental exposures contributes significantly to the manifestation and development of psychiatric disorders. Quantitative measures provide more power to assess gene-environment interactions, allowing researchers to examine how genetic risk factors may amplify or mitigate the impact of environmental factors on disorder risk. This integrative approach can shed light on the interplay between genetic and environmental factors and provide a more comprehensive understanding of the underlying mechanisms.

For AUD, one approach would be to create a quantitative variable based on the total number of DSM-IV criteria fulfilled. Although multiple combinations of criteria and study characteristics may result in a similar criterion count, this proxy for AUD severity has been successfully employed in previous studies although did not result in the identification

of any additional findings at genome-wide significance (Heath et al., 2011; Kendler et al., 2011; Mcgue et al., 2013; J. Wang et al., 2013).
(Gelernter et al., 2014) included DSM-IV AUD criterion counts in their GWAS of AUD to increase statistical power and to correct for co-occurring dependences. They reported association with the ADH cluster (*ADH1B* and *ADH1C*), and other novel loci that included *MTIF2*, *CCDC88A*, *PDLIM5*, and *LOC100507053*. However, the results were not replicated in two later larger meta-analyses (Kranzler, Zhou, Kember, Smith, et al., 2019; R. K. Walters et al., 2018). (Lai et al., 2019) have recently undertaken a GWAS in AUD based on DSM-IV criteria counts in European and African Americans and reported an association with a SNP in *ADH1B* at genome-wide significance. In addition, they reported four novel loci associated with individual DSM-IV criteria. However, apart from the ADH1B locus, only one SNP on chromosome 8 replicated in an independent dataset and meta-analysis ($P = 3.71 \times 10^{-9}$) and the only gene near this region, FAM84B, does not appear to be related to any neuropsychiatric disorder. Nevertheless, while significant increases in sample size can potentially overcome the heterogeneity in ADS, the study of quantitative traits could provide a more detailed picture of how genetic risk variants influence the disorder. Such quantitative approaches have also been applied to the analysis of alcohol use disorders identification test (AUDIT) data on alcohol use and these have produced robust findings. Interestingly the findings from the analysis of AUDIT data and data on AUD have provided evidence for some important differences in the liability for the two traits (Sanchez-Roige et al., 2019).

One consideration that needs to be taken into account when adopting a quantitative approach in GWAS is that the phenotypic data used in the

analysis may not be normally distributed. Thus, the GWAS employing the DSM-IV criterion scores, as a surrogate for disease severity, was undertaken using both a linear regression model, based on the assumption that the sores would be normally distributed, and a quasi-Poisson regression model based on an assumption that the data would show a prominent right skew, which indeed it did.

All three GWAS produced similar results although the quasi-Poisson regression model provided the results with the smallest standard errors. No associations were identified which were significant at the genome-wide level. The top associated SNP, which was consistent across all three GWAS, was rs34361428, which is located in the *ADH* cluster on chromosome 4. This SNP is an eQTL for *ADH1B, ADH1C, ADH1A*, and *METAP1* and its association with AUD has been reported previously (Goldman et al., 2005; D. Li et al., 2011; Luczak et al., 2006; R. K. Walters et al., 2018; Zuo et al., 2012). *METAP1* (methionyl aminopeptidase 1) is a protein-coding gene and its biological pathways are related to the metabolism of fat-soluble vitamins. The C allele of rs34361428 is associated with reduced expression of *METAP1*. This association has been reported previously but specifically in African Americans classified as having alcohol use disorder (Gelernter et al., 2014; Kranzler, Zhou, Kember, Smith, et al., 2019).

There were no significant differences in the variance estimates provided by the binary and quantitative despite the fact that the quantitative PRS offered more precise estimates.

This study had two clear limitations: first the relatively small numbers and second the highly selected nature of both the cases and the controls. Thus, the cases were treatment-seeking chronic alcohol users,

the majority of whom had the most severe AUD phenotype. In contrast, the majority of the controls were healthy blood donors or screened controls with no past or current history of excess alcohol use. The inclusion of a cohort of non-dependent heavy drinkers and a general population sample would have provided a better representation of the drinking spectrum. The strength of this study is its novel approach to the analysis of the GWAS data which took into account the distribution of the phenotypic trait of interest.

Chapter 3 GWAS OF ANTISOCIAL PERSONALITY DISORDER DIAGNOSTIC CRITERIA PROVIDES EVIDENCE FOR SHARED RISK FACTORS ACROSS DISORDERS

3.1 Genetics of antisocial personality disorder

Antisocial personality disorder (ASPD) is characterized by impulsive, irresponsible and often criminal behaviour. People with ASPD tend to be manipulative, deceitful, reckless, and lacking in empathy. These traits often arise in childhood or early adolescence and continue into adulthood (APA, 2000). ASPD is associated with adverse outcomes not only for the person living with the disorder, but also for their families and friends and the wider society (NICE & Excellence, 2014). The prevalence estimates of ASPD vary considerably, from 1% to 6.8% in men and from 0.2% to 1% in women (Coid et al., 2006; Torgersen et al., 2001), with the higher prevalence in men being a consistent finding (Glenn et al., 2013). The Diagnostic and Statistical Manual of Mental Disorders (4th edition, DSM-IV) diagnostic criteria for ASPD include signs and symptoms of conduct disorder in childhood and difficulties with cognition, affectivity, interpersonal functioning and impulse control in adulthood (APA, 2000).

Epidemiological studies indicate that both genetic and environmental factors influence the development of ASPD. Ferguson (2010)undertook a meta-analytical review of antisocial personality and behaviour that covered a range of personality traits and antisocial behaviour itself. The results showed that 56% of the variance in antisocial personality and

behaviour could be explained by genetic influences, while shared and unique environmental factors, including non-genetic biological factors such as trauma and non-family socialization, explained the remainder.

Genome-wide association studies (GWAS) have been used to identify common genetic risk variants in psychiatric disorders (Ripke et al., 2014). To date, three GWASs have been undertaken to identify associations with antisocial behaviour including ASPD. Tielbeek et al. (2012)reported the first GWAS of adult antisocial behaviour using a community sample of twin pairs registered with the Australian Twin Registry and their families comprising 4,816 individuals from 2,227 independent families; the definition of adult antisocial behaviour was based largely on DSM-IV criteria. No genome-wide significance for association with antisocial behaviour was identified. Rautiainen et al. (2016)conducted a discovery GWAS in 370 Finish criminal offenders who fulfilled DSM-IV criteria for a diagnosis of ASPD and 5850 general population controls. None of the associations reached genome-wide significance in this analysis; however, eight suggestive variants associated with ASPD originated in the vicinity of *HLA-DRA* on chromosome 6. These SNPs were genotyped in a replication cohort of 173 offenders and 3766 controls and the results from both cohorts were meta-analysed. One SNP, rs4714329, on chromosome 6p21.2 close to *LINC00951* (Long Intergenic Non-Protein Coding RNA 951) was associated with ASPD at genome-wide significance ($p = 1.6 \times 10^{-9}$) in the meta-analysed data. More recently, Tielbeek et al. (2021) reported a genome-wide significant association with SNPs at the *FOXP2* locus and a broadly defined antisocial behaviour phenotype.

ASPD displays considerable comorbidity with other psychiatric diseases (Abram et al., 2015; Tielbeek, Vink, et al., 2018). Thus individuals with ASPD have been shown to be at high risk for substance use disorders such as alcohol dependence (Bahlmann et al., 2002; Yoshino et al., 2000); depression (Moody et al., 2016), attention deficit hyperactivity disorder (ADHD) (Anney et al., 2009; Instanes et al., 2016), anxiety disorder (Brook et al., 2016; Galbraith et al., 2014), post-traumatic stress disorder (PTSD) (Goodwin & Hamilton, 2003), and schizophrenia (Schiffer et al., 2017; Sedgwick et al., 2017). The rate of alcohol use disorder in people with ASPD is particularly high at an estimated 76.7% (Guy et al., 2018). Moreover, data from two consecutively collected cohorts of prisoners has shown that alcohol dependence syndrome (ADS) shows high comorbidity with ASPD, suggesting that there may be common biological risk mechanisms (Bahlmann et al., 2002; Yoshino et al., 2000). Malone et al. (2004) found a significant and moderate genetic influence on adult antisocial behaviour and ADS at ages 17, 20, and 24 years in a cross-sectional twin study. Moreover, Malone et al. (2004) showed, using cross-twin cross-trait correlations, that the covariation of antisocial behaviour and ADS was due to genetic factors, and that both disorders have a common genetic vulnerability, suggesting they might share susceptibility genes. Tielbeek et al. (2018) assessed the relationships between antisocial behaviour risk and substance use disorder risk and identified significant genetic correlations with cannabis use and smoking but not with alcohol consumption. However, the nature of any common genetic susceptibility has yet to be discovered.

The aim of the present study was to use genome-wide data to examine the genetic architecture of ASPD symptoms and to identify potential genetic risk factors. By using a cohort of people clinically diagnosed with

alcohol dependence syndrome for whom there was also data for ASPD diagnostic criteria, we reduced the potential confounding of the genetic risk for alcohol dependence syndrome and environmental exposure to alcohol. We also estimated the genetic correlation of ASPD diagnostic criteria with other complex traits.

3.2 Methods

3.2.1 Participants

The UCL cohort: 644 participants were recruited from a variety of UK community and hospital-based services providing support and treatment for individuals with alcohol dependence. The UCL cohort used in Chapter 3 consisted of a subset of individuals from the UCL cohort in Chapter 2 who had ASPD phenotype data. Therefore, for detailed information on sample characteristics and recruitment, the reader is referred back to the methods section in Chapter 2.

All participants had received a clinical diagnosis of alcohol dependence according to ICD-10 (F10.2). A clinical diagnosis of alcohol dependence was confirmed by clinicians and trained researchers using the Alcohol Dependence Syndrome section of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II) (Bucholz et al., 1994). The assessment allowed the diagnosis of ASPD as a DSM-IV binary trait but also allowed for the generation of a quantitative ASPD criterion score (APA, 2000). All participants were of English, Scottish, Welsh, or Irish descent with a maximum of one grandparent of non-British (but still Western European) ancestry; none of the individuals was related. Approval for the study was obtained from the NHS Metropolitan Multi-centre Research Ethics Committee (now the South Central - Hampshire

A Research Ethics Committee) approval number MREC/03/11/090. All participants provided signed informed consent.

The Yale-Penn cohort: participants were recruited as part of the Yale-Penn study of the genetic bases of drug and alcohol dependence, as described elsewhere (Gelernter et al., 2014). The subjects were interviewed using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) (Pierucci-Lagha et al., 2005). Lifetime psychiatric and substance use disorders were diagnosed based on DSM-IV criteria. A European American (EA) subset of subjects from the Yale-Penn dataset was included in the current study. These included 1,081 from Yale-Penn Phase 1, 1,029 from Yale-Penn Phase 2, and 463 from Yale-Penn Phase 3. The study was approved by the Institutional Review Boards at the sampling sites and written informed consent was obtained from all study participants. Certificates of confidentiality were issued by the National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism.

3.2.2 Phenotypes

The DSM-IV criteria for ASPD include A: evidence of conduct disorder (15 criteria) with onset before age 15; B: evidence of ASPD (9 criteria) that is a pervasive pattern of disregard for, and violation of, the rights of others occurring since age 15 years. (Appendix 1 for the ASPD diagnostic criteria). A count of fulfilled ASPD diagnostic criteria rather than ASPD diagnosis was used as the phenotype to maximise the informativeness of the data. For each participant positive criteria were summed. Participants were excluded if their behaviour occurred during schizophrenia/manic episodes.

3.2.3 Genotyping and Quality Control

Genome-wide genotyping of genomic DNA from the UCL cohort was undertaken using the Illumina PsychArray. Genomic DNA from the Yale-Penn cohort underwent genotyping in three phases using the Illumina HumanOmni1-Quad array (phase 1), the Illumina HumanCore Exome array (phase 2), the Illumina Multi-Ethnic Genotyping array (phase 3), and each phase was analysed separately. Details of the genotyping, pre-imputation quality control and imputation are provided below.

Genotyping, Imputation and Quality Control in UCL sample

Participants provided either blood or saliva samples for genomic DNA extraction. Genotyping of the DNA samples in the alcohol dependence cohort was performed at Life and Brain GmbH, Bonn, Germany, using the Illumina PsychArray. Quality control of the genotype data was performed in two stages, pre-imputation with more inclusive parameters aimed at retaining a maximal number of subjects, and post-imputation with more stringent parameters aimed at obtaining a high-quality data set. Pre-imputation quality control parameters were: individuals were excluded if they had incorrect gender assignment; excessive heterozygosity (3 standard deviations > the mean); more than 10% of missing genotype data and evidence of relatedness. Data on SNPs which had a minor allele frequency (MAF) < 5% and/or deviated substantially from the Hardy-Weinberg equilibrium (HWE) ($p < 10^{-6}$) were excluded.

Imputation was undertaken using the Haplotype Reference Consortium (release 1.1) reference panel on the Sanger Imputation server (McCarthy et al., 2016). Genotypes were prepared as instructed and checks were performed using the HRC-1000G-check-bim tool Version

4.2.3 (Rayner, 2015) prior to the upload of data. A total of 393,270 SNPs with a MAF > 0.01 were uploaded for imputation (McCarthy et al., 2016). Pre-phasing was undertaken with EAGLE2 (Loh et al., 2016) and imputation was performed using the with Positional Burrows-Wheeler Transform (Durbin, 2014) method.

Post imputation quality control (QC) parameters were used on the hard-called best-guess SNP genotypes. SNPs were included if they met the following criteria: INFO scores > 0.8, call rates > 95%, HWE p-value > 1 x 10^{-5}, and MAF > 1%. All QC steps were performed in PLINK2 (Chang et al., 2015). A total of 5.8 million SNPs were available for association analysis following this process.

Genotyping, Imputation and Quality Control in Yale-Penn sample

The Yale-Penn (YP) phase 1 sample was genotyped using the HumanOmni1-Quad array (Illumina) containing approximately 988,000 SNPs. The YP phase 2 sample was genotyped using the HumanCore Exome array (Illumina) containing approximately 266,000 exomic SNPs and approximately 240,000 tagging SNPs for genome-wide imputation. The YP phase 3 sample was genotyped using the Multi-Ethnic Genotyping Array (Illumina) containing approximately 1.7 million SNPs. Standard pre-imputation quality control included the removal of individuals and SNPs with call rates less than 98% and filtering out SNPs with a minor allele frequency less than 1%. To verify and correct the misclassification of self-reported race, we performed principal component (PC) analysis on SNPs common (pruning by linkage disequilibrium of $r^2 > 0.2$) to each of the 3 individual genotyping arrays and the 1000 Genome phase 3 reference panels (Devuyst, 2015) using EIGENSOFT (Patterson et al., 2006; Price et al., 2006). The first 10 PCs

were used to cluster the participants into African-American and European-American groups and remove outliers from the 2 groups. A second PC analysis within groups was conducted, and the first 10 PCs were used to correct for population stratification. Imputation was performed using Minimac3 (Das et al., 2016) implemented in the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html) and the Haplotype Reference Consortium reference panel (McCarthy et al., 2016). After imputation, SNPs with a Hardy-Weinberg equilibrium $P < 10^{-6}$, a minor allele frequency less than 1%, or imputation INFO < 0.8 were excluded from downstream analysis, resulting in 6,701,741, 5,223,230, and 6,570,984 SNPs been analysed in Yale-Penn 1, Yale-Penn 2, and Yale-Penn 3, respectively.

3.2.4 Statistical Analysis

Association tests

GWAS analyses of the data generated in the four cohorts were conducted separately on imputed best-guess genotypes using a linear regression model with a quantitative scale of ASPD diagnostic criteria as the phenotype, and sex, age, and the first 10 principal components as covariates. The analyses in the UCL data were performed in PLINK2 (Chang et al., 2015), while the separate analyses of the three Yale-Penn data sets (which include relatives) were performed in GEMMA-v0.98.1(X. Zhou & Stephens, 2012). Analysis of chromosome X data was performed using XWAS (version 3.0) with male genotypes on the X chromosome were coded as 0/2 (Gao et al., 2015).

Statistical power

To determine the smallest effect size detectable in a chapter using a genetic power calculator (Purcell et al., 2003), a power calculation can be performed. The power calculation indicated that a minimum of 4,284 cases is needed to detect a small effect size (0.2) with an alpha level of 5×10^{-8} after adjustment for multiple testing (1,209 cases are needed for alpha with 0.001 for effect size 0.9).

Meta-analysis of four cohorts

Sample size weighted meta-analysis of the four ASPD GWAS data sets including chromosome x (N= 3,217) was performed using METAL (Willer et al., 2010).

Fine mapping

LocusZoom was used to make local association plots by uploading the meta-analysis summary statistics (Teslovich et al., 2010). Hg19/1000 Genomes Nov 2014 EUR was used for the background LD structure.

Gene-based test, pathway, and enrichment analyses

Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) software was used to explore gene prioritization, gene expression, and pathway process enrichment with meta-analysis summary statistics as input. FUMA implemented Bonferroni correction ($P_{bon} < 0.05$) to correct for multiple testing (Watanabe et al., 2017).

Phenome-wide association analysis (PheWAS)

To examine whether any of the top hits and related genes identified in the present study are associated with other complex traits, PheWAS

plots were created by exploring the 4,756 GWAS summary stats available on the GWAS ATLAS platform (Watanabe et al., 2019). All GWASs and related genes were used in the analysis. SNPs with P values < 0.05 were used in the PheWAS SNP plot. P-values were adjusted for multiple comparisons using Bonferroni correction.

Polygenic risk scores

Polygenic risk score (PRS) analyses were calculated to test whether risk alleles for a variety of psychiatric and behavioural traits correlated with genetic risk variants of the ASPD diagnostic criteria scores in the UCL and Yale Penn samples. PRSice2 was used to estimate the best-fit PRS at a range of P-value thresholds (Choi et al., 2020). The linkage disequilibrium (LD) threshold was set to an R^2 of 0.1 and a distance of 250 kb.

The meta-analysis of PRS results from UCL and Yale-Penn was conducted using metagen in the meta package in R (Balduzzi et al., 2019). The FDR method was used to correct for multiple comparisons.

Summary statistics of complex traits and psychiatric disorders were downloaded from publicly available resources (Psychiatric Genomics Consortium [PGC]: https://www.med.unc.edu/pgc/results-and-downloads and the GWAS ATLAS: http://atlas.ctglab.nl). The summary statistics for a GWAS of coronary artery disease were also included to act as a negative control for the PRS analyses (Nikpay et al., 2015).

3.3 Results

A total of 3,217 individuals with a lifetime history of AUD and an ASPD diagnostic criterion score were included in the study (Table 7). There

were no significant differences in the sex distribution of age between the UCL and Yale-Penn cohorts, but the UCL cohort was less severely affected ($p < 0.01$). (Table 7).

Table 7 Demographics and total ASPD diagnostic criteria counts for GWAS cohorts.

Sample (n)	Sex (% men)	Age (Mean [range])		ASPD diagnostic criteria score (Mean)	
		Men	Women	Men	Women
UCL (644)	67	44 (19-74)	45 (22-69)	4	2
Yale-Penn Phase 1 (1,081)	63	40 (16-71)	39 (16-69)	7	6
Yale-Penn Phase 2 (1,029)	67	40 (18-76)	39 (19-80)	7	6
Yale-Penn Phase 3 (463)	68	40 (17-73)	38(18-75)	7	5
Total	63	41	40	7	5

3.3.1 GWAS of the four cohorts and meta-analysis

Results from individual cohorts:

We found a genome-wide significant locus on chromosome 14q.13.1 (rs142893681, $p = 3.19 \times 10^{-8}$), in the vicinity of the *SNX6* (sorting nexin 6) gene in the UCL cohort GWAS. A genome-wide significant locus on chromosome 18q11.2 (rs59381075, 1.98×10^{-8}), near the *ZNF521* (Zinc Finger Protein 521) gene was found in the Yale-Penn Phase 1 GWAS. No genome-wide significant associations were identified in the Yale-Penn Phases 2 and 3 cohorts (Table 8 – 11) or on chromosome X.

Results from meta-analyses:

The meta-analysis of the four individual GWAS of ASPD criteria scores identified a genome-wide significant association on chromosome 15q.26.1 (rs9806493 $p = 3.77 \times 10^{-8}$) (Table 12 and Figure 10). Two additional independent SNPs showed suggestive evidence of association, rs10186418 ($p = 2.79 \times 10^{-7}$) and rs11682196 ($p = 3.69 \times 10^{-7}$). The two SNPs, that were identified at genome-wide significance (rs142893681 and rs59381075) ($P < 5 \times 10^{-8}$) in the UCL and Yale-Penn Phase 1 individual GWASs respectively, were not significant in the meta-analysis. No significant results were found from the meta-analysis of the X chromosome data.

3.3.2 Fine mapping and expression quantitative trait loci

rs9806493 is located 7.5 kb downstream from the Solute Carrier Organic Anion Transporter Family Member 3A1 (*SLCO3A1*) gene (Figure 11). All of the available SNPs in linkage disequilibrium with rs9806493 ($R^2 > 0.2$) map to a region towards the 3' end of *SLCO3A1*. rs9806493 is identified as a peripheral blood cis-expression quantitative trait loci (cis-eQTL) for *SLCO3A1* (Z-score = -9.33, $p = 1.09 \times 10^{-20}$) in the eQTLGen database (Võsa et al., 2018). It is also identified as an eQTL for *SLCO3A1* (regression slope = -0.035, $p = 1.01 \times 10^{-4}$) in the QTL maps from the PsychEncode project (Gandal et al., 2018). However, it is not identified as an eQTL for *SLCO3A1* in the Genotype-Tissue Expression (GTEx) database (version 8.0) (Consortium, 2020).

3.3.3 Gene-based tests, pathway, and enrichment analyses

MAGMA gene-based, gene-set tests, and tissue expression analysis of the individual UCL and the Yale-Penn GWAS did not identify any statistically significant findings. MAGMA gene-based and tissue expression analyses of the data from the GWAS meta-analysis showed

no evidence for association with ASPD. MAGMA gene-set tests of the meta-analysis data provided nominal evidence for the association of several gene-sets with ASPD. However, none of these survived Bonferroni correction for multiple testing (Tables 13 – 17).

Table 8 UCL ASPD GWAS top independent SNPs results

Top independent SNPs								
uniqID	**rsID**	**chr**	**pos**	**P-value**	**nSNPs**	**nGWAS SNPs**	**gene**	**annot**
14:35124173:A:T	rs142893681	14	35124173	3.19E-08	3	2	ENSG00000129515	intergenic
11:117786899:C:T	rs117268885	11	117786899	2.02E-07	1	1	ENSG00000137747	intronic
12:25928463:C:T	rs113750817	12	25928463	5.20E-07	8	7	ENSG00000152936	intergenic
14:91263703:A:G	rs148612718	14	91263703	5.98E-07	35	27	ENSG00000165914	intronic
13:60392332:A:G	rs73208955	13	60392332	9.47E-07	131	115	ENSG00000139734	intronic
8:66715687:C:T	rs73242920	8	66715687	1.02E-06	48	35	ENSG00000205268	intronic
6:111354634:C:T	rs151221356	6	111354634	1.02E-06	4	3	ENSG00000197498	intergenic
7:2966713:C:T	rs34855232	7	2966713	1.53E-06	2	2	ENSG00000198286	intronic
4:1169813:G:T	rs878323	4	1169813	1.65E-06	23	13	ENSG00000159674	intronic
15:92711091:C:T	rs7172018	15	92711091	2.14E-06	11	8	ENSG00000258761	ncRNA_intronic

Notes (These abbreviations are also used in tables 7 - 9):
r^2 threshold to define independent significant SNPs ≥ 0.6, No: Index of independent significant SNPs
GenomicLocus: Index of assigned genomic locus matched with "GenomicRiskLoci.txt". Multiple independent lead SNPs can be assigned to the same genomic locus.
uniqID: Unique ID of SNPs consists of chr: position: allele1: allele2 where alleles are alphabetically ordered.
rsID: rsID of the SNP, chr: chromosome, pos: position on hg19, p: P-value (from the input file).
nSNPs: Number of SNPs which are in LD of the independent significant SNP given r2, including non-GWAS-tagged SNPs (which are extracted from the reference panel).
nGWASSNPs: Number of GWAS-tagged SNPs which are in LD of the ind. sig. SNP given r2.
annot: annotation

Table 9 Yale-Penn phase 1 ASPD GWAS top independent SNPs results

Top independent SNPs								
uniqID	**rsID**	**chr**	**pos**	**P-value**	**nSNPs**	**nGWA SSNPs**	**gene**	**annot**
18:22607819:A:G	rs59381075	18	22607819	1.98E-08	21	18	ENSG00000229248	intergenic
18:22653562:A:C	rs11876226	18	22653562	4.23E-07	14	11	ENSG00000198795	intronic
3:82487066:C:G	rs113693054	3	82487066	6.73E-07	2	2	ENSG00000239440	ncRNA_intronic
2:230322749:A:G	rs111293120	2	230322749	1.14E-06	9	6	ENSG00000187957	intronic
8:123835375:A:G	rs79188580	8	123835375	2.06E-06	1	1	ENSG00000178764	intronic
1:35046633:C:T	rs10733012	1	35046633	2.65E-06	5	4	ENSG00000270241	intergenic
1:173117889:C:T	rs1234304	1	173117889	2.65E-06	4	1	ENSG00000230849	intergenic
3:106624294:C:T	rs1517605	3	106624294	2.82E-06	6	4	ENSG00000242759	ncRNA_intronic
14:27714822:C:T	rs10873462	14	27714822	3.84E-06	16	12	ENSG00000258081	intergenic
8:79108379:C:T	rs73690332	8	79108379	6.48E-06	12	12	ENSG00000254001	intergenic

Table 10 Yale-Penn phase 2 ASPD GWAS top independent SNPs results

Top independent SNPs								
uniqID	**rsID**	**chr**	**pos**	**P-value**	**nSNPs**	**nGWAS SNPs**	**gene**	**annot**
2:127580764:A:C	rs62158471	2	127580764	3.02E-06	25	21	ENSG00000206963	intergenic
4:82124238:C:T	rs28371390	4	82124238	3.15E-06	94	62	ENSG00000138669	intronic
1:113863848:C:T	rs12754133	1	113863848	6.37E-06	16	13	ENSG00000236887	intergenic
7:9786617:A:G	rs10239571	7	9786617	6.80E-06	63	39	ENSG00000234710	ncRNA_intronic
12:33642169:A:C	rs74593552	12	33642169	8.14E-06	30	14	ENSG00000256070	intergenic

Table 11 Yale-Penn phase 3 ASPD GWAS top independent SNPs results

Top independent SNPs								
uniqID	**rsID**	**chr**	**pos**	**P-value**	**nSNPs**	**nGWAS SNPs**	**gene**	**annot**
8:115900897:A:G	rs148982374	8	115900897	3.86E-07	12	6	ENSG00000253756	intergenic
10:87962048:A:G	rs11201938	10	87962048	4.15E-07	29	23	ENSG00000182771	intronic
6:23337597:A:G	rs74316667	6	23337597	2.19E-06	4	1	ENSG00000235743	upstream
4:96702035:A:G	rs72684036	4	96702035	2.87E-06	1	1	ENSG00000242936	intergenic
8:62342597:C:G	rs7821162	8	62342597	2.93E-06	53	44	ENSG00000177182	intronic
14:84619290:C:T	rs77546191	14	84619290	2.95E-06	11	10	ENSG00000258532	intergenic
5:162689149:A:C	rs60143408	5	162689149	3.69E-06	4	2	ENSG00000250061	ncRNA_intronic
13:98755800:C:T	rs285039	13	98755800	3.75E-06	28	26	ENSG00000065150	intergenic
13:70825366:A:T	rs9542248	13	70825366	3.87E-06	8	7	ENSG00000230223	intergenic
12:47650459:A:G	rs7137397	12	47650459	4.01E-06	1	1	ENSG00000257925	ncRNA_intronic

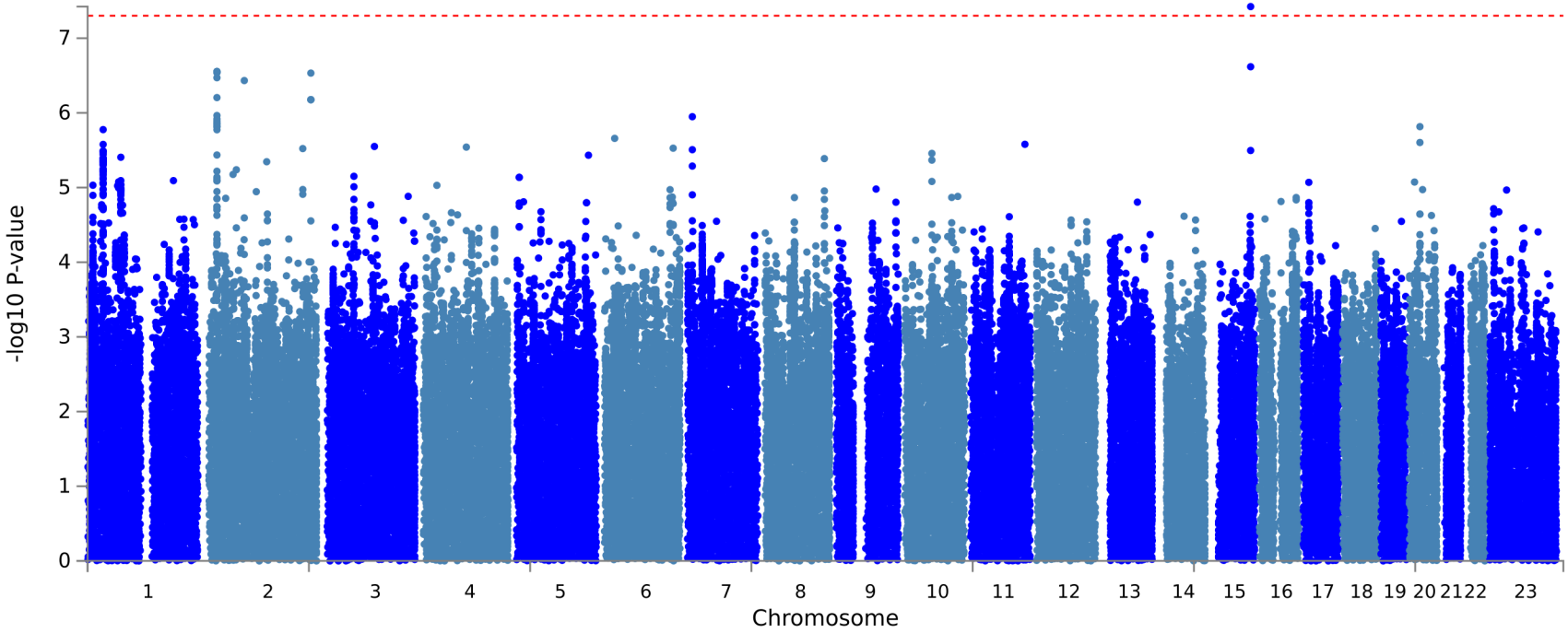


Figure 10. GWAS Meta-analysis of quantitative ASPD symptoms in the UCL and Yale-Penn samples.

The positions of the autosomal and chromosome X SNPs are shown on the X axis and the -log10 of the significance values from the meta-analysis of quantitative ASPD symptoms in the UCL and Yale-Penn samples is shown on the Y axis. Genome-wide significance ($P = 5x\ 10^{-8}$) is shown by the dotted red line.

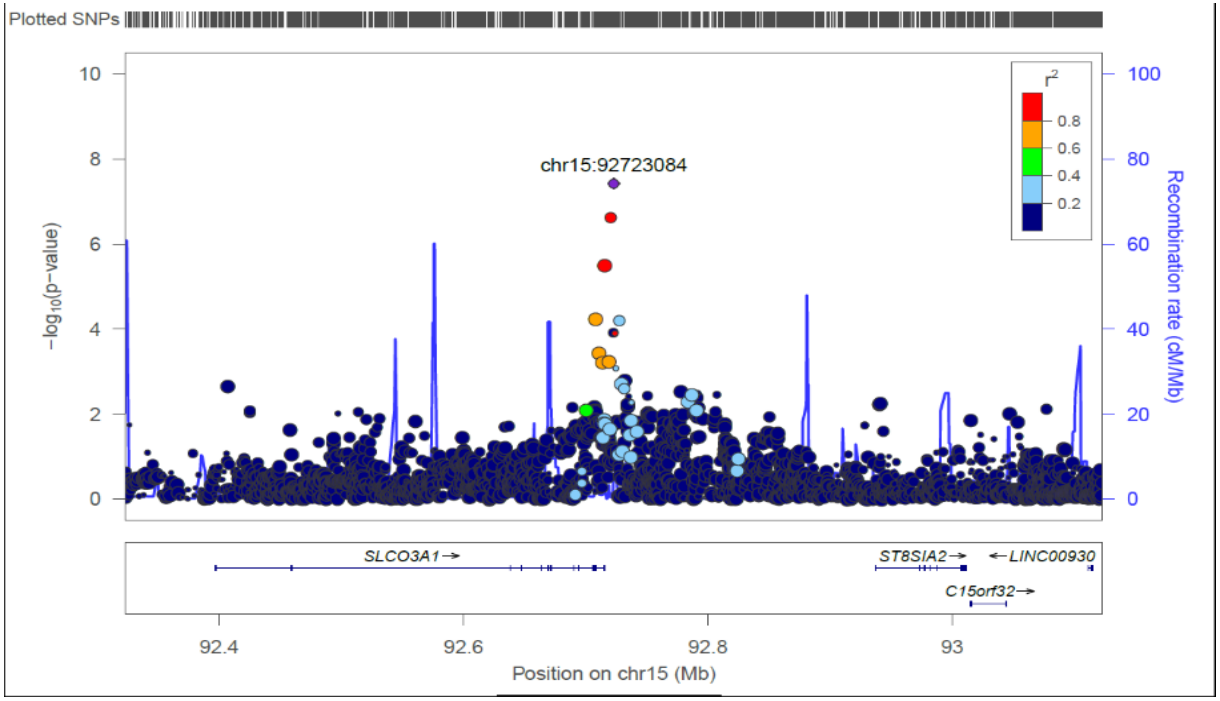


Figure 11. Regional locus plot of the association findings with rs9806493 close to the *SLCO3A1* gene in the meta-analysis of ASPD symptoms

Table 12 Top independent variants associated with ASPD in the meta-analysis of the GWAS data

CHR	SNP ID*	Effect Allele	Other Allele	Gene	Weight	Z-score	P-value	Effect Allele Frequency (%)	Direction
15	**rs9806493**	**C**	**T**	***SLCO3A1***	**1673**	**-5.501**	**3.77×10^{-8}**	**47.1**	**--??**
2	rs10186418	A	G	*KCNS3*	3197	5.137	2.79×10^{-7}	86.8	++++
2	rs11682196	C	A	*CTNNA2*	3157	-5.084	3.69×10^{-7}	86.9	----
7	rs967758	C	T	*Y_RNA*	2153	-4.868	1.13×10^{-6}	20.2	--?-
20	rs6076184	T	C	*RP5-1100I6.1*	2181	4.807	1.53×10^{-6}	5.3	++?+
1	rs6691165	C	A	*MIR552*	3175	-4.789	1.68×10^{-6}	45.2	----

Abbreviations: CHR: chromosome. SNP: Single Nucleotide Polymorphism Direction: - for negative, + for positive and ? for missing in the UCL, Yale-Penn Phases 1, 2, and 3 samples respectively. The SNP marked in bold text reached a genome wide level of significance in the meta-analysis. *Only SNPs that were present in both UCL and one or more Yale-Penn samples are shown.

Table 13 MAGMA Gene set analysis results

UCL						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
protein localization to non motile cilium	12	0.790	0.020	0.214	1.08E-04	1
takao response to uvb radiation up	76	0.314	0.020	0.085	1.11E-04	1
negative regulation of rho protein signal transduction	22	0.645	0.022	0.178	1.47E-04	1
browne hcmv infection 20hr dn	97	0.293	0.021	0.081	1.55E-04	1
foster kdm1a targets dn	196	0.196	0.020	0.055	1.73E-04	1
protein localization to photoreceptor outer segment	8	0.918	0.019	0.266	2.82E-04	1
norepinephrine biosynthetic process	5	1.403	0.023	0.416	3.69E-04	1
regulation of sodium ion transmembrane transporter activity	48	0.380	0.019	0.113	3.86E-04	1
organ or tissue specific immune response	30	0.446	0.018	0.134	4.43E-04	1
mcclung delta fosb targets 2wk	45	0.404	0.020	0.123	5.18E-04	1

Table 14 MAGMA Gene set analysis results

Yale-Penn phase 1						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
regulation of axon guidance	41	0.512	0.024	0.126	2.57E-05	0.397
reactome insertion of tail anchored proteins into the endoplasmic reticulum membrane	19	0.665	0.021	0.165	2.85E-05	0.440
reactome sema3a pak dependent axon repulsion	14	0.893	0.025	0.229	4.73E-05	0.733
kegg axon guidance	123	0.284	0.023	0.076	9.12E-05	1
fad binding	29	0.490	0.019	0.138	1.91E-04	1
reactome sema3a plexin repulsion signaling by inhibiting integrin adhesion	13	0.782	0.021	0.222	2.17E-04	1
reactome vitamin c ascorbate metabolism	8	1.123	0.023	0.324	2.66E-04	1
reactome semaphorin interactions	61	0.338	0.019	0.100	3.78E-04	1
protein insertion into er membrane	5	0.883	0.014	0.265	4.40E-04	1
gazda diamond blackfan anemia myeloid dn	36	0.446	0.020	0.135	4.75E-04	1

Table 15 MAGMA Gene set analysis results

Yale-Penn phase 2						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
regulation of adenylate cyclase activating adrenergic receptor signaling pathway involved in heart process	3	2.010	0.026	0.455	4.97E-06	0.077
negative regulation of adenylate cyclase activating adrenergic receptor signaling pathway	3	1.913	0.025	0.455	1.31E-05	0.202
phosphatidylinositol 3 kinase regulatory subunit binding	9	1.032	0.023	0.256	2.71E-05	0.420
carbohydrate catabolic process	168	0.231	0.022	0.064	1.59E-04	1
alonso metastasis emt dn	5	1.500	0.025	0.417	1.60E-04	1
g protein coupled acetylcholine receptor signaling pathway	17	0.706	0.022	0.206	3.05E-04	1
reactome formation of atp by chemiosmotic coupling	15	0.559	0.016	0.164	3.23E-04	1
mhc class I receptor activity	7	0.986	0.020	0.290	3.47E-04	1
protein modification by small protein conjugation	806	0.096	0.020	0.029	4.03E-04	1
positive regulation of fertilization	14	0.757	0.021	0.228	4.58E-04	1

Table 16 MAGMA Gene set analysis results

Yale-Penn phase 3						
Gene Set	N genes	Beta	Beta STD	SE	P	P_{bon}
protein phosphatase activator activity	12	1.041	0.027	0.260	3.13E-05	0.485
phosphatase activator activity	15	0.900	0.026	0.229	4.30E-05	0.665
regulation of vitamin d receptor signaling pathway	6	1.775	0.032	0.460	5.75E-05	0.890
lei hoxc8 targets up	11	0.947	0.023	0.250	7.56E-05	1
negative regulation of vascular endothelial cell proliferation	4	1.493	0.022	0.395	7.85E-05	1
biocarta mitr pathway	5	1.449	0.024	0.408	1.94E-04	1
wakabayashi adipogenesis pparg bound 36hr	29	0.510	0.020	0.147	2.68E-04	1
sa g1 and s phases	15	0.691	0.020	0.202	3.17E-04	1
coreceptor activity involved in wnt signaling pathway planar cell polarity pathway	5	1.191	0.020	0.354	3.85E-04	1
zhu cmv all dn	112	0.252	0.020	0.077	5.61E-04	1

Table 17 MAGMA Gene set analysis results

UCL and Yale-Penn phase 1, 2, 3						
Gene Set	N genes	Beta	Beta STD	SE	P	Pbon
regulation of nad p h oxidase activity	11	1.005	0.024	0.231	6.84E-06	0.106
positive regulation of nad p hoxidase activity	7	1.159	0.022	0.287	2.71E-05	0.420
pid er nongenomic pathway	39	0.452	0.021	0.119	7.56E-05	1
insulin receptor signaling pathway	129	0.250	0.021	0.067	1.02E-04	1
amp binding	11	0.958	0.023	0.258	1.04E-04	1
response to insulin	251	0.171	0.020	0.049	2.29E-04	1
determination of digestive tract left right asymmetry	6	1.258	0.023	0.373	3.69E-04	1
mrna 3 end processing	92	0.226	0.016	0.069	4.77E-04	1
cytoplasmic pattern recognition receptor signaling pathway in response to virus	24	0.449	0.016	0.137	5.06E-04	1
kyng environmental stress response not by 4nqo in old	11	0.671	0.016	0.205	5.16E-04	1

3.3.4 Phenome-wide association analysis (PheWAS)

PheWAS analyses using the GWAS Atlas platform were performed to examine secondary phenotypes associated with rs9806493 and the *SLCO3A1* gene. rs9806493 was associated with the reproductive (and risk-taking) trait: "number of sexual partners" (Bonferroni corrected p = 0.00049; Figure 12). The *SLCO3A1* gene was associated with 43 different traits after Bonferroni correction, including educational attainment ($p = 9.28 \times 10^{-8}$), body mass index (BMI) ($p = 5.56 \times 10^{-7}$), broad depression ($p = 6.06 \times 10^{-7}$), seeing a doctor for nerves, anxiety, tension or depression ($p = 2.42 \times 10^{-6}$), alcohol dependence ($p = 1.66 \times 10^{-5}$), and depression ($p = 1.13 \times 10^{-4}$; Table 18; Figure 13).

3.3.5 Polygenic Risk Score (PRS) Analysis

PRS analyses were performed to investigate the genetic correlation between major psychiatric disorders and other complex behavioural traits with ASPD diagnostic criterion scores (Figure 14, Table 19 & 20). The analyses were performed using publicly available GWAS summary statistics downloaded from the PGC or the GWAS ATLAS.

The meta-analysis of PRSs results for educational attainment, alcohol intake frequency, and the reproductive traits "age at first live birth in women and men" were negatively correlated with higher ASPD criterion scores (P_{FDR} corrected < 0.05). The PRS for whether a subject had ever smoked, had depression including major depressive disorder, and two sub clinical depressive traits were positively correlated with the number of ASPD diagnostic criteria (P_{FDR} corrected < 0.05).

The PRS for post-traumatic stress disorder did not show a consistent direction of effect in the UCL sample but was positively correlated with

ASPD diagnostic criteria in the Yale-Penn sample (Table 19). None of the results from the PRS analyses with schizophrenia, anxiety, aggression, or coronary artery disease survived correction for multiple testing in the meta-analysis.

Table 18 Top significant trait associations in the SLCO3A1 Phenome-wide association analysis

Trait	Domain	Reference	P-values	Participants (n)
Educational attainment	Environment	Okbay et al (2016)	9.28×10^{-8}	328,917
Broad depression	Psychiatric	Howard et al (2018)	6.06×10^{-7}	322,580
Educational attainment	Environment	Lee et al (2018)	8.40×10^{-7}	766,345
Seen doctor (GP) for nerves, anxiety, tension or depression	Psychiatric	Watanabe et al (2019)	2.42×10^{-6}	383,771
Alcohol dependence	Psychiatric	Wang et al (2013)	1.66×10^{-5}	2,322
Lifetime number of sexual partners	Reproduction	Watanabe et al (2019)	4.52×10^{-5}	316,569
Depression	Psychiatric	Howard et al (2019)	1.13×10^{-4}	500,199

A total of 436 GWAS were included in the PheWas., the Bonferroni corrected P-value threshold is 1.15×10^{-4}. PMID PubMed identifier

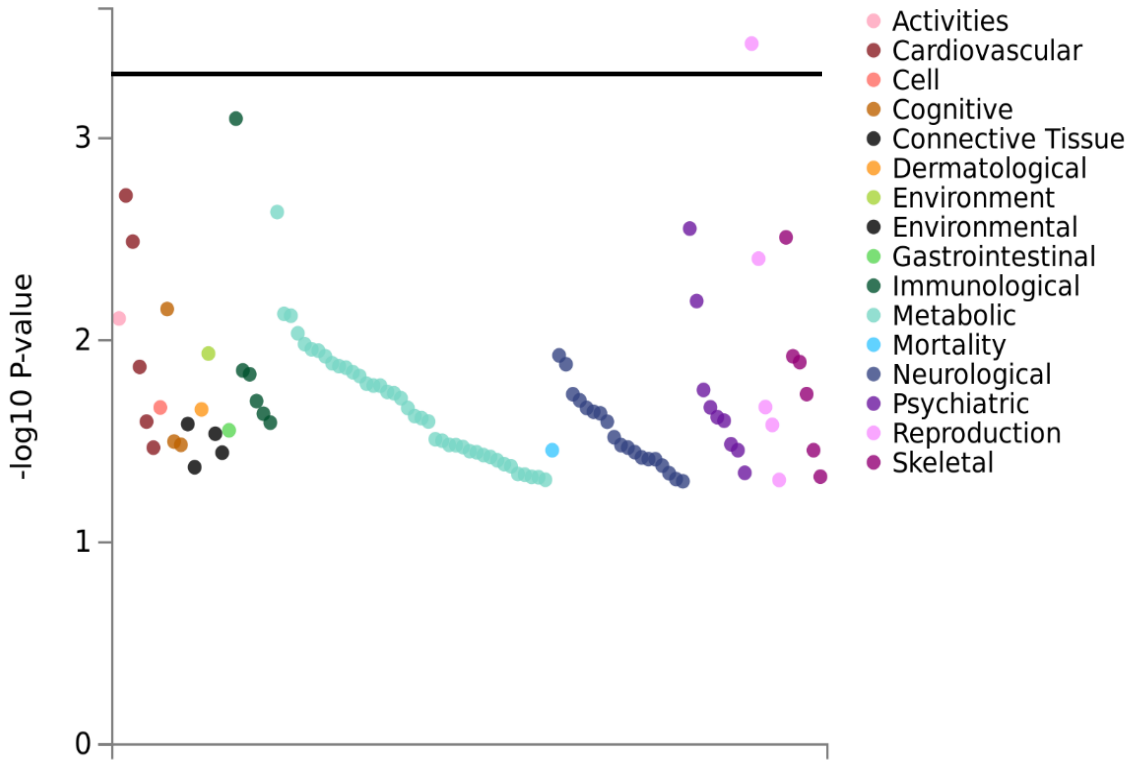


Figure 12: rs9806493 PheWAS plot

Phenome-wide association analysis for rs9806493 from 103 GWASs. The results are sorted by domain and P-value. The Bonferroni corrected P-value threshold is 4.85×10^{-4} (horizontal black line).

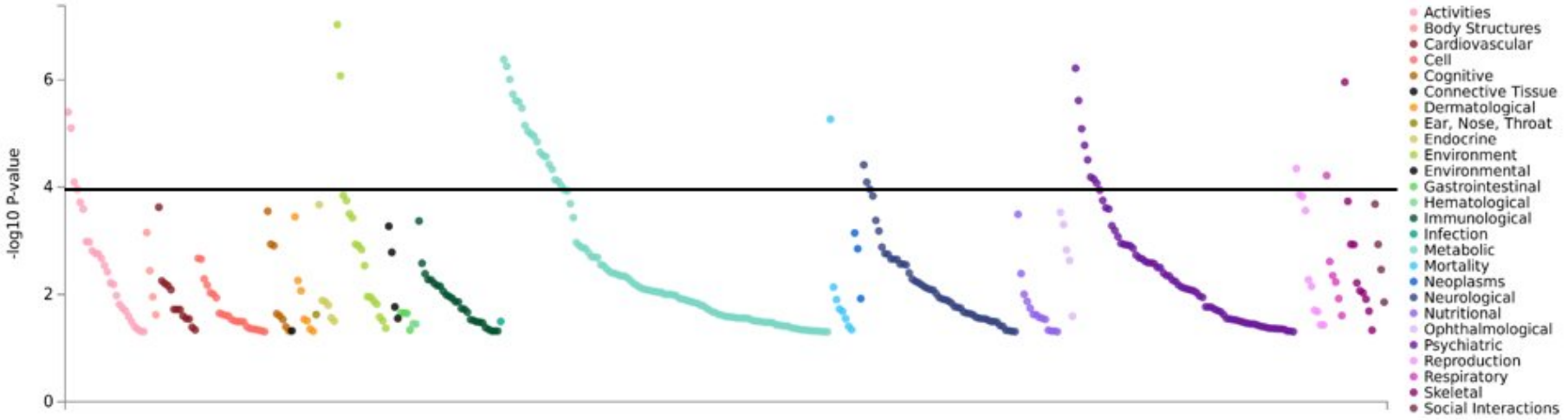


Figure 13 *SLCO3A1* gene PheWAS plot

Phenome-wide association analysis for the *SLCO3A1* gene from 436 GWASs.

The results were sorted by domain and p-value. The Bonferroni corrected P-value threshold is 1.15×10^{-4} (horizontal black line).

Table 19 PRS analysis in the UCL, Yale-Penn ASPD cohorts, and the meta-analysis results.

Directions	Traits	Best P value threshold		total R^2	P-values		P-values (meta-analysis)
		UCL	Yale-Penn	total	UCL	Yale-Penn	
Negative correlation	age at first live birth (F)	0.5	$1x10^{-5}$	0.63%	0.038	> 0.05	0.044
	age at first live birth (M)	$1x10^{-5}$	1	0.87%	0.015	0.01	0.018
	age at last birth (F)	$1x10^{-5}$	1	0.44%	> 0.05	0.014	> 0.05
	age of completion of full-time education	1	0.5	1.19%	0.004	0.006	$9.4 x10^{-4}$
	alcohol intake frequency	0.001	1	0.62%	0.043	0.039	0.039
Positive correlation	ever smoked	0.05	0.01	1.30%	0.002	$5.8x10^{-4}$	$9.4x10^{-4}$
	MDD	$1x10^{-4}$	0.05	1.20%	0.004	0.036	0.03
	depressive symptoms	0.05	0.5	1%	0.009	0.036	0.006
	seen doctor for nerves, anxiety, tension or depression	1	$1x10^{-4}$	1.96%	$1.7x10^{-4}$	> 0.05	$1.6x10^{-3}$
	PTSD	0.05	$1x10^{-5}$	0.67%	> 0.05	$1.0x10^{-9}$	$9.4x10^{-4}$

P-values for the meta-analysis of PRS were corrected for multiple comparisons using the FDR method.

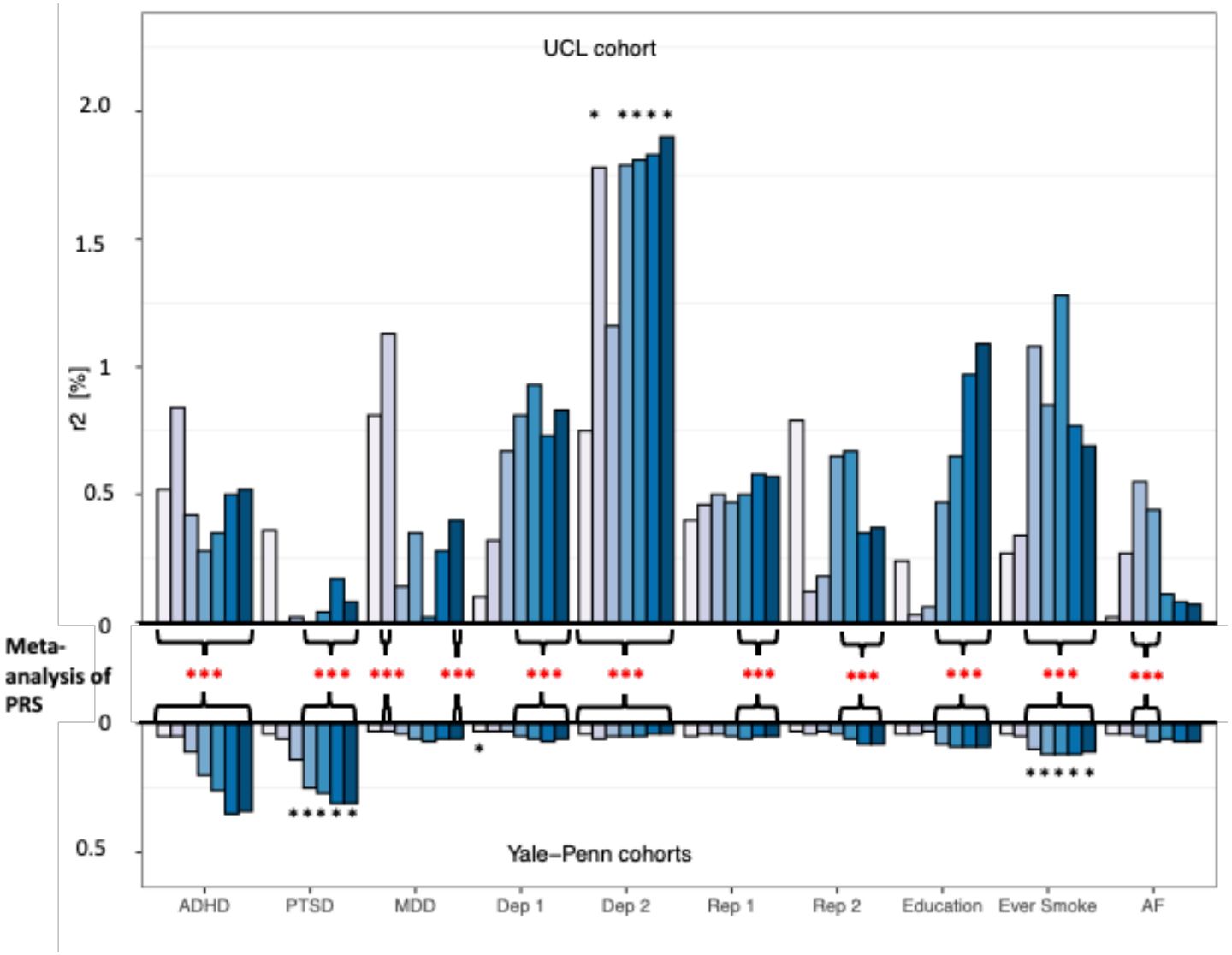


Figure 14 PRS analysis in the UCL and Yale-Penn ASPD samples

Figure 14 legend

PRS results are shown at different P-value thresholds for each trait along with the percentage of variance explained by the PRS (Nagelkerke's R^2) for ten traits in the UCL and Yale-Penn cohorts. *Comparisons significant after FDR correction in the individual PRS analyses (black *) or in the PRS meta-analysis (red *).

Abbreviations: ADHD: attention deficit hyperactivity disorder; PTSD: post-traumatic stress disorder; MDD: major depression disorder; Dep 1: depressive symptoms; Dep 2: seen doctor for nerves, anxiety, tension or depression; Rep 1: age at first live birth (female); Rep 2: age at first birth (male); Education: age completed full time education; Ever Smoke: Whether a participant had ever smoked a cigarette; AF: alcohol frequency.

Table 20 Top significant trait associations in the SLCO3A1 Phenome-wide association analysis

A		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573						n = 644				n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
100_SMK: Light smokers, at least 100 smokes in lifetime	5.00E-08	3	0.12	0.16	0.04%	0.45	2	0.03	0.19	0.00%	0.87	0.49	0.59
	1.00E-07	3	0.12	0.16	0.04%	0.45	na	na	na	na	na	na	na
	1.00E-06	9	0.17	0.21	0.04%	0.4	9	0.02	0.18	0.00%	0.91	0.52	0.6
	1.00E-05	32	0.00	0.26	0.03%	0.99	43	-0.07	0.19	0.02%	0.72	0.77	0.82
	1.00E-04	199	-0.05	0.25	0.03%	0.83	265	-0.03	0.18	0.00%	0.85	0.78	0.82
	0.001	1063	0.34	0.23	0.05%	0.15	1685	-0.15	0.18	0.08%	0.43	0.79	0.82
	0.01	6404	0.44	0.22	0.06%	**0.05**	11424	-0.11	0.19	0.05%	0.56	0.41	0.53
	0.05	21896	0.38	0.24	0.05%	0.11	42006	-0.06	0.19	0.02%	0.73	0.48	0.58
	0.5	108381	0.94	0.29	0.11%	**1.10E-03**	242337	0.01	0.20	0.00%	0.95	0.058	0.12
	1	146232	0.96	0.29	0.11%	**9.80E-04**	352617	0.05	0.20	0.01%	0.82	**0.041**	0.1
ADHD: attention deficit hyperactivity disorder	5.00E-08	11	0.18	0.27	0.04%	0.49	11	0.46	0.18	0.86%	**0.012**	**0.013**	**0.044**
	1.00E-07	15	0.20	0.25	0.04%	0.43	15	0.52	0.18	1.10%	**0.004**	**0.0053**	**0.024**
	1.00E-06	34	0.30	0.23	0.05%	0.19	33	0.49	0.18	1.00%	**0.006**	**0.0029**	**0.016**
	1.00E-05	93	0.42	0.26	0.05%	0.1	101	0.36	0.18	0.52%	0.051	**0.011**	**0.041**
	1.00E-04	303	0.36	0.23	0.05%	0.13	365	0.48	0.19	0.84%	**0.013**	**0.0038**	**0.019**
	0.001	1320	0.75	0.24	0.11%	**1.80E-03**	1690	0.34	0.20	0.42%	0.078	**8.00E-04**	**5.30E-03**
	0.01	6324	1.10	0.23	0.20%	**2.10E-06**	8287	0.27	0.18	0.28%	0.15	**1.00E-04**	**9.40E-04**
	0.05	19451	1.34	0.24	0.26%	**3.40E-08**	26354	0.30	0.19	0.35%	0.11	**1.00E-04**	**9.40E-04**
	0.5	87713	1.56	0.24	0.35%	**7.10E-11**	123131	0.36	0.19	0.50%	0.054	**1.00E-04**	**9.40E-04**
	1	118006	1.53	0.24	0.34%	**1.40E-10**	166867	0.37	0.19	0.52%	0.051	**1.00E-04**	**9.40E-04**

B		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573						n = 644				n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
age_first_birth: age at first live birth (female)	5.00E-08	20	-0.16	0.22	0.04%	0.46	30	-0.22	0.18	0.22%	0.21	0.15	0.25
	1.00E-07	26	-0.26	0.22	0.04%	0.23	37	-0.27	0.18	0.30%	0.14	0.055	0.12
	1.00E-06	61	-0.23	0.22	0.04%	0.28	86	-0.25	0.18	0.25%	0.18	0.084	0.16
	1.00E-05	179	-0.43	0.26	0.05%	0.09	261	-0.30	0.18	0.40%	0.087	**0.018**	0.057
	1.00E-04	595	-0.25	0.24	0.04%	0.3	886	-0.34	0.18	0.46%	0.066	**0.037**	0.095
	0.001	2225	-0.30	0.30	0.04%	0.32	3555	-0.39	0.20	0.50%	0.055	**0.031**	0.086
	0.01	9567	-0.46	0.35	0.05%	0.19	16856	-0.41	0.22	0.47%	0.062	**0.023**	0.066
	0.05	27034	-0.61	0.37	0.06%	0.1	52777	-0.43	0.23	0.50%	0.055	**0.013**	**0.044**
	0.5	110561	-0.53	0.38	0.05%	0.16	259063	-0.49	0.24	0.58%	**0.038**	**0.012**	**0.044**
	1	145994	-0.55	0.38	0.05%	0.14	373772	-0.48	0.24	0.57%	**0.04**	**0.012**	**0.044**
age_first_birth_M: age at first birth (male)	5.00E-08	na	na	na	na	na	na	na	na	na	na	na	na
	1.00E-07	na	na	na	na	na	na	na	na	na	na	na	na
	1.00E-06	2	-0.09	0.13	0.04%	0.5	2	-0.21	0.18	0.18%	0.25	0.218	0.32
	1.00E-05	7	0.11	0.20	0.03%	0.6	8	-0.42	0.17	0.79%	**0.015**	0.13	0.22
	1.00E-04	72	0.22	0.20	0.04%	0.28	76	-0.17	0.18	0.12%	0.34	0.995	0.99
	0.001	511	0.09	0.22	0.03%	0.69	587	-0.20	0.17	0.18%	0.25	0.519	0.6
	0.01	3567	-0.19	0.21	0.04%	0.38	4009	-0.39	0.18	0.65%	**0.029**	**0.025**	0.07
	0.05	12868	-0.47	0.24	0.06%	0.055	14293	-0.40	0.18	0.67%	**0.027**	**0.0034**	**0.018**
	0.5	66329	-0.53	0.22	0.08%	**0.016**	74861	-0.28	0.18	0.35%	0.11	**0.006**	**0.027**
	1	88371	-0.59	0.23	0.08%	**0.01**	101054	-0.29	0.18	0.37%	0.1	**0.004**	**0.019**

C		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573						n = 644				n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
age_last_birth: age at last live birth (female)	5.00E-08	4	-0.18	0.16	0.04%	0.26	7	-0.15	0.19	0.09%	0.41	0.16	0.26
	1.00E-07	8	-0.12	0.19	0.04%	0.53	11	-0.25	0.19	0.23%	0.19	0.17	0.27
	1.00E-06	18	-0.14	0.21	0.04%	0.51	25	-0.22	0.19	0.18%	0.25	0.19	0.29
	1.00E-05	55	-0.20	0.21	0.04%	0.36	82	-0.30	0.19	0.36%	0.1	0.067	0.14
	1.00E-04	250	-0.35	0.24	0.05%	0.15	351	-0.19	0.20	0.13%	0.34	0.1	0.19
	0.001	1330	-0.19	0.27	0.04%	0.48	2091	-0.13	0.18	0.07%	0.47	0.32	0.45
	0.01	7351	-0.41	0.24	0.06%	0.081	12901	-0.17	0.19	0.11%	0.367	0.073	0.15
	0.05	23437	-0.67	0.30	0.07%	**0.027**	46009	-0.27	0.19	0.27%	0.157	**0.018**	0.057
	0.5	108961	-0.74	0.32	0.07%	**0.022**	254786	-0.07	0.19	0.02%	0.706	0.13	0.22
	1	145932	-0.79	0.32	0.08%	**0.014**	373500	-0.08	0.19	0.02%	0.698	0.11	0.2
Aggression	5.00E-08	na	na	na	na	na	na	na	na	na	na	na	na
	1.00E-07	1	0.01	0.09	0.03%	0.95	1	-0.33	0.19	0.44%	0.07	0.16	0.26
	1.00E-06	1	0.01	0.09	0.03%	0.95	na	na	na	na	na	na	na
	1.00E-05	5	0.10	0.19	0.03%	0.58	4	0.09	0.18	0.04%	0.6	0.45	0.56
	1.00E-04	33	-0.03	0.23	0.03%	0.89	32	0.16	0.19	0.10%	0.38	0.56	0.64
	0.001	299	0.09	0.23	0.03%	0.71	339	0.06	0.19	0.01%	0.76	0.64	0.7
	0.01	2356	0.13	0.24	0.03%	0.6	2783	0.08	0.19	0.02%	0.67	0.52	0.6
	0.05	9024	0.08	0.23	0.03%	0.73	10559	0.10	0.18	0.05%	0.56	0.5	0.6
	0.5	48762	0.09	0.21	0.03%	0.68	56683	0.25	0.17	0.29%	0.14	0.16	0.26
	1	66552	0.05	0.21	0.03%	0.82	77172	0.24	0.17	0.27%	0.16	0.21	0.3

D		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573						n = 644				n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
alc_freq: alcohol intake frequency	5.00E-08	93	0.06	0.30	0.03%	0.85	109	-0.08	0.18	0.03%	0.67	0.79	0.82
	1.00E-07	109	0.00	0.30	0.03%	0.99	122	-0.04	0.18	0.01%	0.84	0.87	0.88
	1.00E-06	199	-0.10	0.29	0.03%	0.74	220	0.09	0.18	0.04%	0.59	0.78	0.82
	1.00E-05	437	-0.34	0.28	0.04%	0.24	514	-0.06	0.18	0.02%	0.72	0.35	0.48
	1.00E-04	1073	-0.27	0.30	0.04%	0.36	1366	-0.26	0.19	0.27%	0.16	0.094	0.18
	0.001	3297	-0.43	0.28	0.05%	0.12	5101	-0.43	0.21	0.55%	**0.043**	**0.01**	**0.04**
	0.01	11844	-0.62	0.32	0.07%	0.055	22069	-0.43	0.24	0.44%	0.072	**0.01**	**0.039**
	0.05	30389	-0.54	0.32	0.06%	0.088	65187	-0.28	0.30	0.11%	0.36	0.066	0.14
	0.5	112604	-0.70	0.34	0.07%	**0.04**	321275	-0.24	0.32	0.08%	0.45	0.051	0.11
	1	146086	-0.70	0.34	0.07%	**0.039**	482496	-0.22	0.31	0.07%	0.49	0.056	0.12
Anxiety	5.00E-08	1	-0.06	0.10	0.03%	0.57	1	0.08	0.18	0.03%	0.65	0.79	0.82
	1.00E-07	1	-0.06	0.10	0.03%	0.57	na	na	na	na	na	na	na
	1.00E-06	3	-0.41	0.16	0.08%	**0.01**	na	na	na	na	na	na	na
	1.00E-05	12	-0.46	0.25	0.06%	0.07	11	0.19	0.18	0.14%	0.31	0.8	0.83
	1.00E-04	80	-0.36	0.26	0.05%	0.16	115	0.07	0.18	0.02%	0.69	0.62	0.69
	0.001	584	-0.22	0.22	0.04%	0.33	773	-0.03	0.18	0.00%	0.88	0.47	0.57
	0.01	4347	-0.15	0.24	0.04%	0.54	5520	-0.13	0.19	0.06%	0.49	0.36	0.48
	0.05	15760	-0.12	0.23	0.03%	0.62	19538	0.03	0.19	0.00%	0.86	0.86	0.88
	0.5	67641	-0.04	0.23	0.03%	0.85	84878	-0.10	0.19	0.04%	0.58	0.59	0.66
	1	84445	-0.02	0.22	0.03%	0.92	107376	-0.12	0.19	0.05%	0.54	0.59	0.67

E		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573					n = 644					n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
cad: coronary artery disease	5.00E-08	48	-0.02	0.29	0.03%	0.94	57	-0.41	0.19	0.63%	**0.03**	0.064	0.14
	1.00E-07	50	-0.02	0.26	0.03%	0.93	60	-0.39	0.19	0.58%	**0.04**	0.084	0.16
	1.00E-06	73	-0.08	0.26	0.03%	0.75	90	-0.38	0.19	0.55%	**0.04**	0.069	0.14
	1.00E-05	128	-0.06	0.27	0.03%	0.82	155	-0.29	0.19	0.32%	0.12	0.16	0.26
	1.00E-04	295	0.11	0.26	0.03%	0.66	356	-0.24	0.19	0.23%	0.19	0.42	0.53
	0.001	932	0.17	0.26	0.04%	0.52	1259	-0.31	0.19	0.37%	0.1	0.34	0.46
	0.01	4549	0.19	0.23	0.04%	0.41	7107	-0.29	0.20	0.27%	0.16	0.62	0.69
	0.05	15446	0.25	0.23	0.04%	0.26	26170	0.01	0.20	0.00%	0.95	0.43	0.55
	0.5	79860	0.52	0.27	0.06%	0.05	153109	0.21	0.19	0.17%	0.27	**0.043**	0.1
	1	105785	0.58	0.27	0.07%	**0.03**	214689	0.21	0.19	0.17%	0.26	**0.032**	0.086
dep_sym: depression symptoms	5.00E-08	8	-0.59	0.22	0.09%	**0.01**	10	0.25	0.19	0.24%	0.19	0.42	0.54
	1.00E-07	12	-0.26	0.20	0.05%	0.2	15	0.32	0.20	0.33%	0.12	0.84	0.85
	1.00E-06	46	-0.16	0.22	0.04%	0.46	52	0.48	0.21	0.72%	**0.021**	0.25	0.35
	1.00E-05	139	-0.01	0.26	0.03%	0.98	165	0.17	0.19	0.10%	0.38	0.49	0.59
	1.00E-04	460	0.11	0.25	0.03%	0.66	611	0.29	0.19	0.32%	0.13	0.14	0.23
	0.001	1834	0.09	0.23	0.03%	0.71	2847	0.42	0.19	0.67%	**0.026**	**0.049**	0.11
	0.01	8196	0.38	0.24	0.05%	0.11	15297	0.46	0.19	0.81%	**0.014**	**0.0036**	**0.018**
	0.05	24761	0.53	0.26	0.06%	**0.044**	54573	0.50	0.19	0.93%	**0.0088**	**0.0009**	**0.0057**
	0.5	110115	0.47	0.22	0.07%	**0.036**	329241	0.45	0.19	0.73%	**0.021**	**0.0017**	**0.0099**
	1	145896	0.45	0.22	0.06%	**0.041**	515825	0.48	0.19	0.83%	**0.013**	**0.0013**	**0.0079**

F		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573					n = 644					n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
eduYr: Age completed full time education	5.00E-08	10	-0.29	0.22	0.05%	0.18	10	-0.37	0.18	0.59%	**0.037**	**0.014**	**0.044**
	1.00E-07	11	-0.37	0.22	0.05%	0.09	12	-0.38	0.18	0.63%	**0.031**	**0.0062**	**0.027**
	1.00E-06	28	-0.20	0.22	0.04%	0.36	31	-0.38	0.20	0.49%	0.058	**0.043**	0.1
	1.00E-05	93	-0.23	0.24	0.04%	0.33	123	-0.27	0.20	0.24%	0.18	0.099	0.19
	1.00E-04	380	-0.18	0.23	0.04%	0.43	590	-0.09	0.19	0.03%	0.64	0.39	0.52
	0.001	1643	-0.10	0.26	0.03%	0.7	2826	-0.12	0.19	0.06%	0.52	0.46	0.57
	0.01	8075	-0.67	0.28	0.08%	**0.0166**	15565	-0.39	0.21	0.47%	0.062	**0.0034**	**0.018**
	0.05	24609	-0.69	0.26	0.09%	**0.0079**	52346	-0.51	0.23	0.65%	**0.029**	**7.00E-04**	**5.00E-03**
	0.5	109479	-0.71	0.26	0.09%	**0.0055**	288788	-0.64	0.24	0.97%	**0.0074**	**1.00E-04**	**9.40E-04**
	1	146235	-0.70	0.26	0.09%	**0.0063**	431910	-0.68	0.24	1.10%	**0.0044**	**1.00E-04**	**9.40E-04**
ever_smk: ever smoked	5.00E-08	53	0.13	0.22	0.03%	0.56	62	0.06	0.19	0.01%	0.77	0.5463	0.63
	1.00E-07	61	0.08	0.24	0.03%	0.73	73	0.14	0.18	0.08%	0.43	0.4043	0.53
	1.00E-06	138	-0.02	0.22	0.03%	0.91	145	0.07	0.19	0.02%	0.71	0.831	0.85
	1.00E-05	321	0.19	0.22	0.04%	0.4	345	0.28	0.20	0.27%	0.16	0.1086	0.2
	1.00E-04	856	0.36	0.22	0.05%	0.1	1065	0.31	0.20	0.34%	0.11	**0.0228**	0.066
	0.001	2737	0.80	0.27	0.10%	**2.70E-03**	3986	0.55	0.20	1.10%	**4.80E-03**	**1.00E-04**	**9.40E-04**
	0.01	10275	1.06	0.31	0.12%	**5.80E-04**	18555	0.51	0.20	0.85%	**1.20E-02**	**1.00E-04**	**9.40E-04**
	0.05	27702	1.03	0.31	0.12%	**7.10E-04**	59356	0.70	0.23	1.30%	**2.00E-03**	**1.00E-04**	**9.40E-04**
	0.5	110805	1.00	0.31	0.12%	**1.00E-03**	317553	0.58	0.24	0.77%	**1.70E-02**	**1.00E-04**	**9.40E-04**
	1	146021	0.97	0.30	0.11%	**1.40E-03**	482566	0.55	0.24	0.69%	**2.40E-02**	**2.00E-04**	**1.60E-03**

G		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573					n = 644					n = up to 3,217	
	Pt	**N_{SNP}**	**beta**	**se**	**r2**	p	**N_{SNP}**	**Coefficient**	**se**	**R2**	P	**p**	**p_adjust (FDR)**
MDD: major depressive disorder	5.00E-08	20	0.04	0.25	0.03%	0.86	11	0.29	0.19	0.32%	0.12	0.18	0.28
	1.00E-07	21	0.08	0.25	0.04%	0.75	12	0.32	0.19	0.39%	0.09	0.12	0.22
	1.00E-06	30	0.05	0.26	0.03%	0.83	29	0.31	0.19	0.38%	0.1	0.14	0.23
	1.00E-05	69	0.12	0.32	0.03%	0.69	93	0.44	0.18	0.81%	**0.015**	**0.02**	0.061
	1.00E-04	224	0.11	0.29	0.03%	0.69	391	0.51	0.18	1.10%	**0.0037**	**0.0072**	**0.03**
	0.001	928	0.15	0.24	0.04%	0.54	1937	0.18	0.18	0.14%	0.31	0.24	0.34
	0.01	4663	0.44	0.25	0.06%	0.079	11812	0.29	0.18	0.35%	0.107	**0.019**	0.06
	0.05	14354	0.52	0.25	0.07%	**0.036**	43001	0.06	0.18	0.02%	0.727	0.13	0.22
	0.5	63707	0.54	0.29	0.06%	0.061	251040	0.28	0.19	0.28%	0.153	**0.03**	0.071
	1	83862	0.55	0.29	0.06%	0.056	377859	0.33	0.19	0.40%	0.088	**0.013**	**0.044**
PTSD: post-traumatic stress disorder	5.00E-08	na	na	na	na	na	na	na	na	na	na	na	na
	1.00E-07	na	na	na	na	na	na	na	na	na	na	na	na
	1.00E-06	1	0.44	0.19	0.07%	**0.018**	1	0.08	0.17	0.03%	0.625	0.05	0.11
	1.00E-05	9	0.25	0.22	0.04%	0.27	18	0.31	0.19	0.36%	0.1	**0.05**	0.11
	1.00E-04	98	0.47	0.24	0.06%	0.051	135	0.02	0.19	0.00%	0.919	0.19	0.29
	0.001	695	0.80	0.22	0.14%	**2.10E-04**	1104	-0.07	0.19	0.02%	0.7	**0.033**	0.086
	0.01	5515	1.22	0.23	0.25%	**7.40E-08**	8700	-0.03	0.19	0.00%	0.88	**7.00E-04**	**5.00E-03**
	0.05	20529	1.22	0.22	0.27%	**1.70E-08**	34209	0.11	0.20	0.04%	0.59	**1.00E-04**	**9.40E-04**
	0.5	108287	1.25	0.20	0.31%	**1.00E-09**	221179	0.21	0.19	0.17%	0.26	**1.00E-04**	**9.40E-04**
	1	145722	1.25	0.20	0.31%	**1.00E-09**	338308	0.18	0.24	0.08%	0.46	**1.00E-04**	**9.40E-04**

H		YALE-Penn phase 1, 2, 3 meta-analysis					UCL					PRS meta-analysis	
		n = 2573						n = 644				n = up to 3,217	
	Pt	N_{SNP}	beta	se	r2	p	N_{SNP}	Coefficient	se	R2	P	p	p_adjust (FDR)
Schizophrenia	5.00E-08	136	0.02	0.26	0.03%	0.93	134	0.19	0.20	0.12%	0.34	0.37	0.49
	1.00E-07	156	0.03	0.25	0.03%	0.92	157	0.20	0.20	0.13%	0.33	0.37	0.49
	1.00E-06	240	0.10	0.25	0.03%	0.68	267	0.12	0.20	0.05%	0.54	**0.045**	0.11
	1.00E-05	483	0.04	0.25	0.03%	0.86	594	0.24	0.19	0.21%	0.21	0.34	0.46
	1.00E-04	1098	0.09	0.24	0.03%	0.7	1445	0.25	0.19	0.23%	0.19	0.25	0.35
	0.001	2945	0.13	0.24	0.04%	0.59	4545	0.26	0.19	0.27%	0.16	0.18	0.28
	0.01	9525	0.44	0.31	0.05%	0.16	18988	0.29	0.19	0.32%	0.13	0.12	0.22
	0.05	23307	0.51	0.36	0.05%	0.16	56407	0.27	0.20	0.24%	0.19	0.2	0.3
	0.5	85477	0.64	0.38	0.06%	0.095	331432	-0.03	0.18	0.01%	0.85	0.63	0.7
	1	110561	0.63	0.39	0.06%	0.11	541916	-0.19	0.16	0.19%	0.24	0.79	0.82
see_doc: seen doctor for nerves, anxiety, tension or depression	5.00E-08	36	0.15	0.31	0.03%	0.62	36	0.25	0.17	0.31%	0.13	0.12	0.21
	1.00E-07	42	0.15	0.30	0.03%	0.63	43	0.27	0.17	0.34%	0.11	0.1	0.19
	1.00E-06	83	0.16	0.32	0.03%	0.61	92	0.35	0.17	0.60%	**0.036**	**0.036**	0.093
	1.00E-05	205	0.32	0.32	0.04%	0.31	225	0.38	0.16	0.75%	**0.019**	**0.01**	**0.04**
	1.00E-04	630	0.44	0.24	0.06%	0.065	777	0.67	0.18	1.80%	**0.00027**	**1.00E-04**	**9.40E-04**
	0.001	2103	0.42	0.25	0.05%	0.1	2993	0.54	0.18	1.20%	**0.0033**	**8.00E-04**	**5.30E-03**
	0.01	8726	0.39	0.24	0.05%	0.11	16022	0.65	0.18	1.80%	**0.00026**	**1.00E-04**	**9.40E-04**
	0.05	25346	0.38	0.24	0.05%	0.12	54232	0.67	0.18	1.80%	**0.00024**	**1.00E-04**	**9.40E-04**
	0.5	109805	0.30	0.26	0.04%	0.26	313146	0.71	0.19	1.80%	**0.00022**	**2.00E-04**	**1.60E-03**
	1	146273	0.28	0.27	0.04%	0.3	482861	0.73	0.19	1.90%	**0.00017**	**2.00E-04**	**1.6E-03**

r^2: Variance explained by the PRS
Coefficient: Regression coefficient of the model
se: standard error
P-adjust $_{FDR}$: p-value corrected using FDR method

Note: PRS results sources		
	GWAS summary	websites
100_SMK: Light smokers, at least 100 smokes in lifetime	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3261
ADHD: attention deficit hyperactivity disorder	PMID: 20732625	https://www.med.unc.edu/pgc/data-index/
age_first_birth: age at first live birth (female)	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3343
age_first_birth_M: age at first birth (male)	PMID: 27798627	https://atlas.ctglab.nl/traitDB/49
age_last_birth: age at last live birth (female)	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3344
alc_freq: alcohol intake frequency	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3261
cad: coronary artery disease	PMID: 26343387	https://atlas.ctglab.nl/traitDB/108
dep_sym: depression symptoms	PMID: 29942085	https://atlas.ctglab.nl/traitDB/3796
eduYr: Age completed full time education	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3203
ever_smk: ever smoked	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3425
mdd: major depressive disorder	PMID: 29700475	https://www.med.unc.edu/pgc/download-results/mdd/
ptsd: post-traumatic stress disorder	PMID: 28439101	https://www.med.unc.edu/pgc/download-results/ptsd/
scz: schizophrenia	PMID: 25056061	https://www.med.unc.edu/pgc/download-results/scz/
see_doc: seen doctor for nerves, anxiety, tension or depression	PMID: 31427789	https://atlas.ctglab.nl/traitDB/3301

3.4 Discussion

The present study investigated the genetic architecture of ASPD criteria in the context of AUD and is to the best of our knowledge the largest meta-analytic GWAS of comparable clinical measure of ASPD undertaken to date. Our GWAS meta-analysis identified a novel genome-wide significant signal with rs9806493 on chromosome 15q26.1 close to the *SLCO3A1* gene. This marker is supported by additional SNPs in linkage disequilibrium with the main finding that did not reach genome-wide significance. In the PRS analysis, genetic correlations that survived correction for multiple testing were identified with genetic risk variants for many complex behavioural traits and psychiatric disorders including education attainment, smoking, alcohol intake frequency, reproductive behaviours, depression, PTSD, and ADHD.

Two of three eQTL databases indicate that rs9806493 is an eQTL for *SLCO3A1*. The effect allele in our study (rs9806493:C) is associated with decreased expression of *SLCO3A1*. In our PheWas analysis, the ASPD risk allele rs9806493:C showed evidence for association with having had a greater number of sexual partners albeit not at genome-wide levels of significance (Karlsson Linnér et al., 2019). *SLCO3A1* has also been shown to mediate the transport of Na (+)-independent of organic anions and hormones including thyroxine and vasopressin, the cyclic oligopeptides BQ-123 (endothelin receptor antagonist), and prostaglandins (PG) E1 and E2 (Huber et al., 2007; Tamai et al., 2000). GTEx data show that *SLCO3A1* is strongly expressed in the spinal cord, substantia nigra, hippocampus, hypothalamus, anterior cingulate cortex, and frontal cortex. *SLCO3A1* is widely expressed in many cells in the brain including pericytes, cells that are integral to the blood brain-barrier, and therefore this protein is likely to have a role in the transport of

organic anions across the blood-brain barrier (Sweeney et al., 2019). Two splice isoforms of human *SLCO3A1* show differences in localisation with the major isoform being expressed in the basolateral plasma membrane of the choroid plexus and the grey matter of the frontal cortex, whereas the minor isoform was expressed in the apical pole of epithelial cells of the choroid plexus and white matter of the frontal cortex (Huber et al., 2007).

The C allele of rs9806493, a risk variant in our GWAS, decreases the expression of *SLCO3A1* which is predicted to lead to decreased uptake of hormones including PGE1, PGE2, T4, and vasopressin. Increased levels of PGE1, PGE2, and T4 have been reported in ASPD. For example, thyroid hormones have been suggested to influence the development of aggression in antisocial ASPD patients (Evrensel et al., 2016). In that study, as T3 and T4 levels increased, the aggression scores in ASPD patients also increased (Evrensel et al., 2016). An early study that investigated prostaglandins in alcoholic and ASPD patients found that concentrations of PGE1 and PGE2 were higher in ASPD patients than male controls (Evrensel et al., 2016). Vasopressin influences social responses including empathy and ASPD is associated with deficiencies in affective empathy (Virkkunen et al., 1987). However, the exact impact of vasopressin on ASPD and/or antisocial behaviour remains unclear. Taken together, we present evidence that genetic variation in the *SLCO3A1* gene may confer risk for ASPD via altered hormone levels.

ASPD shows considerable comorbidity with other psychiatric diseases (Abram et al., 2015; Tielbeek, Vink, et al., 2018). Our polygenic risk score analysis suggested that common genetic loci underlie risk for

ASPD and other complex traits including smoking, alcohol use frequency, PTSD, ADHD, reproductive traits, and educational attainment. These findings were consistent with previous genetic correlation study of antisocial behaviours, which showed that antisocial behaviour is significantly correlated with lifetime cannabis use and cigarettes smoked per day (Tielbeek, Vink, et al., 2018). Tielbeek, Vink, et al. (2018) examined the genetic correlations of antisocial behaviour and life-history traits. They found that genetic risks of antisocial behaviour are positively correlated with higher reproductive traits and negatively correlated with delayed reproductive traits. This is consistent with our results that showed ASPD risk is negatively correlated with age at the first live birth (in females) and age at first birth (in males; Figure 14 & 15). The evidence from our PRS analysis provides further support that ASPD is a highly polygenic disorder that shares genetic risk loci with other psychiatric and neurodevelopmental disorders.

Figure 15 Polygenic risk score relationships among ASPD and other complex traits

The direction of correlations between ASPD PRS and the PRS for other complex traits are shown. Yellow arrows indicate a positive correlation; blue arrows indicate a negative correlation. ADHD: attention deficit hyperactivity disorder, PTSD: post-traumatic stress disorder, depression traits include: MDD (major depression disorder), depression symptoms and seen doctor for nerves, anxiety, tension or depression, reproduction traits include: age at first birth (female), age at first birth (male). See Supplementary Figure 3 for GWAS ATLAS genetic correlation results.

Limitations

The power of this study was restricted by sample size despite the use of the use of much larger samples than previous studies. Moreover, this study only used subjects of European ancestry which limits the generalizability of the findings. The results from this study need replication in larger cohorts.

Conclusion

Our study revealed a genome-wide significant association with ASPD criterion score that implicates the *SLCO3A1* gene, which may play a role in the risk for ASPD by regulating hormone levels. This study also provided evidence that ASPD is a polygenic disorder that shares risk with other complex traits. Our study showed that the use of a consistent measure of ASPD diagnostic criteria is a useful approach to detect risk loci.

Chapter 4 POLYGENIC RISKS FOR ALCOHOL CONSUMPTION, ALCOHOL USE DISORDER, AND BRAIN VOLUMES IN LOW RISK DRINKING INDIVIDUALS FROM UK BIOBANK

4.1 Alcohol use disorder imaging genetics research

Alcohol is a toxic and psychoactive substance; it is still a large part of the social landscape in most countries. In the United Kingdom, there was an 18.6% increase in deaths due to alcohol-specific causes in 2020 compared with 2019 (Office of National Statistics (ONS), 2021). Alcohol consumption contributes to almost three million global deaths every year as well as to significant problems with healthcare and economic costs. Alcohol use disorder (AUD) is a common psychiatric condition described as a pattern of substance use that causes physical, cognitive, and social functioning damage along with adverse social consequences (Edition, 2013).

Numerous neuroimaging studies have suggested that individuals with chronic heavy alcohol use and/or AUD showed brain structures and functions abnormalities (Beresford et al., 2006; Cardenas et al., 2005; Meyerhoff et al., 2004; Park et al., 2007; Sullivan & Pfefferbaum, 2019; Topiwala & Ebmeier, 2018). A wide range of abnormal brain structures including frontal, diencephalic hippocampal, and cerebellar structures are associated with chronic heavy alcohol use (Sullivan & Pfefferbaum, 2019). These results from small-sample neuroimaging studies are now confirmed by large neuroimaging consortium data. For example, a recent UK Biobank (UKB) study that

examined the relationship between alcohol consumption and grey and white matter volumes from 36,678 healthy individuals showed a negative association between alcohol consumption and brain macrostructure and microstructure (Daviet et al., 2022). Even for individuals who consume an average of one to two daily alcohol units, the negative associations still exist. However, similar studies are lacking for AUD imaging studies. While evidence suggested brain abnormalities may be caused by chronic alcohol use, speculations have been also raised that genetic predisposing risk factors for alcohol involvement may also contribute to brain structural reductions (Baranger et al., 2020; Hatoum et al., 2021; Robert et al., 2020).

Early functional neuroimaging and imaging genetics findings of alcohol have focused on the influences of polymorphisms on alcohol cue processing (Fauth-Bühler & Kiefer, 2016). Studies examined candidate genes including genes that encode proteins of opioidergic, GABAergic, and brain-derived neurotrophic factor, suggesting these genes influence alcohol-cue-induced brain activity in mesocorticolimbic areas (Fauth-Bühler & Kiefer, 2016). Nowadays researchers have leveraged data from large neuroimaging consortiums and biobanks (e.g., UK Biobank) to increase statistical power to test genomic associations with magnetic resonance imaging (MRI)-derived brain phenotypes in various diseases including bipolar, major depression, schizophrenia, and addiction (Mascarell Maričić et al., 2020; Medland et al., 2022; Miller et al., 2016; Neilson et al., 2019; Reus et al., 2017). However, only a few studies have investigated the impact of genetic risk factors of alcohol consumption and AUD on brain structural reductions. Using data from ABCD, researchers examined the influences of polygenic risk scores (PRS) for alcohol use and AUD on brain structure phenotypes (Hatoum et al.,

2021). The results showed no brain regions were associated with alcohol consumption PRS. AUD-PRS, on the other hand, was associated with reduced left frontal pole grey matter volume and greater right supramarginal gyrus cortical thickness. Another study that also utilised ABCD data tested whether substance use behaviours including alcoholic drinks per week PRS predicted brain imaging phenotypes (Rabinowitz et al., 2022). They first showed that there was a negative genetic correlation between alcohol use and average cortical thickness. Moreover, they also showed that a higher alcohol use PRS predicted greater postcentral gyrus surface area and cortical surface area. The exact mechanisms underlying these associations are still unclear.

The emerging evidence of associations between alcohol consumption and AUD PRSs and brain morphology suggested that predispositional genetic risk factors may partially contribute to alcohol-related differences in brain phenotypes. Leveraging the largest available GWAS summary data of alcohol consumption and alcohol use disorder, and using UK Biobank imaging data, we aimed to estimate associations between alcohol related PRS and brain volumes in cortical and subcortical regions among people who drink less than 14 units per week (i.e., ‘healthy drinkers’).

Including only 'healthy drinkers' in a sample selection strategy has both advantages and disadvantages. One advantage is that focusing on 'healthy drinkers' allows for a more specific investigation of the effects of moderate alcohol consumption on the outcome of interest. By excluding non/never drinkers and heavy drinkers, the sample is more homogeneous, reducing potential confounding factors and providing clearer insights into the potential benefits or risks associated with

moderate alcohol consumption. Potential confounders in a genetic association study investigating the effects of moderate alcohol consumption on a specific outcome can include factors like smoking status (P. Liu et al., 2017) and dietary patterns (Kokubo et al., 2019), age, and gender. For instance, genetic variants associated with these confounding factors may lead to misleading conclusions if not properly controlled in the study. Therefore, researchers must identify and account for these confounders to ensure that observed genetic associations accurately reflect the relationship between genetic variants and the outcome of interest.

On the other hand, there are also disadvantages to this approach. By excluding non/never drinkers, the sample may not represent the entire population, limiting the generalisability of the findings. Non/never drinkers may have different characteristics or health profiles that could influence the outcome of interest, and excluding them may introduce selection bias.

4.2 Methods

4.2.1 Participants

This study included participants from the UK Biobank (www.ukbiobank.ac.uk), which is a biomedical database that contains half a million UK individuals who were recruited aged between 40 and 69 years old from 2006 to 2010 (Bycroft et al., 2018). Participants with T1-weighted Imaging data were first identified and extracted for later selection. We excluded participants who never drank and had missing data in imaging and alcohol frequency (i.e., prefer not to answer).

Following these exclusions, there were 19395 individuals available at the time of the analyses. Participants were also excluded if they were diagnosed (or self-report) with alcohol dependence syndrome and if they drank more than 14 units (i.e., the recommended weekly limit, which is equivalent to 6 pints of beer or 10 small glasses of wine) in a week (Rosenberg et al., 2018).

Only participants who have European ancestry were included in the study. Related participants with kinship coefficient > 0.15 were also removed from the genetic analysis. Following exclusions, this study included 8689 participants who passed quality control by the UK Biobank Imaging group.

All participants provided consent information to participate in the UK Biobank. Ethical approval for the UK Biobank study was granted by the North-West Research Ethics Committee (ref06/MREC08/65), and participants who decided to withdraw their consent after providing their sample for genetic analysis were excluded from the study.

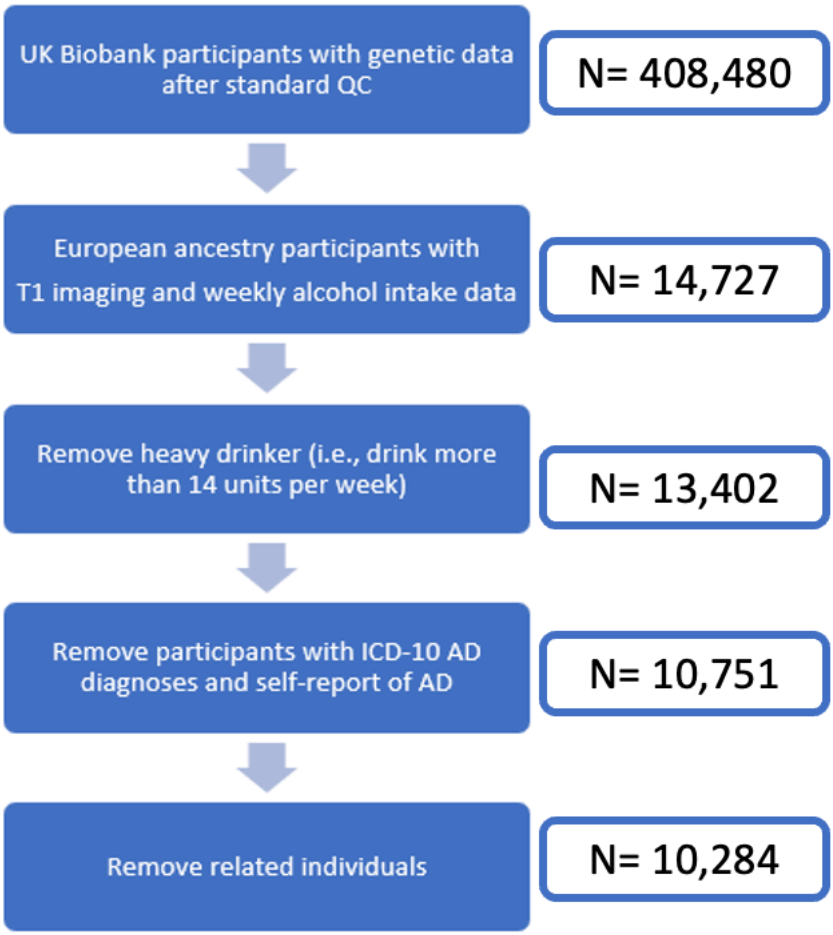


Figure 16. Overview of healthy drinking participants selected from UKB

4.2.2 Brain volumes

The UK Biobank commissioned three identical imaging centres in Cheadle, Reading and Newcastle with the same acquisition protocols (Miller et al., 2016). Siemens Skyra 3T scanner with a standard Siemens 32-channel head coil was used to acquire MRI data (Miller et al., 2016). We downloaded and processed T1-weighted brain volumetric data for 8689 participants. The brain volume variables we processed were obtained from the image-derived phenotypes released by the UK Biobank team. More information about brain volumes and grey matter measures can be found here (Alfaro-Almagro et al., 2018). Extreme

values defined as ±2.5 standard deviations from the group mean were excluded before the PRS calculations.

4.2.3 Genotyping and Polygenic risk scores computation

The UK Biobank contains genotypes for 488,377 participants. Two similar arrays were used to perform genotyping. The Applied Biosystems UK BiLEVE Axiom Array by Affymetrix (807,411 probes) was used initially for 49,950 participants, and 438,427 participants were then genotyped using Applied Biosystems UK Biobank Axiom Array (820,967 probes). The two arrays share over 95% of common maker content. More details about the UK Biobank phenotype, genetic data collection methods, quality control, and imputation have been described previously by the Wellcome Trust Centre for Human Genetics (Bycroft et al., 2018). We downloaded the imputed genotype data in March 2018. Post-imputation quality control was performed locally with the following steps: variants were removed if minor allele frequency < 1%, Fisher information score < 0.3, and missing call rate greater than or equal to 5%. Individuals were excluded if a mismatch between submitted gender and inferred gender, more than 10 putative third-degree relatives in the kinship table, with more than 10% missingness, and if they were identified as outliers in heterozygosity and missing rates.

We calculated alcohol consumption (4,384,455 SNPs) and alcohol use disorder (5,933,416 SNPs) PRS separately using summary statistics from the largest alcohol consumption and alcohol use disorder GWAS study at the time (Kranzler, Zhou, Kember, Smith, et al., 2019). In their study, the Million Veteran Program (MVP) biobank data were used where phenotype data were collected using questionnaires and the

electronic health record, and the blood samples were obtained for genetic analysis. The alcohol consumption phenotype data were collected from 2007 to 2017 using AUDIT-C scores which comprise 3 questions that measure typical quantity, frequency of drinking and frequency of heavy or binge drinking. The AUDIT-C scores and AUD diagnostic codes were obtained from the electronic health record. 200,680 participants with European ancestry were included in the alcohol consumption GWAS, and 202,004 (34,658 cases; 167,346 controls) participants were involved in the alcohol use disorder GWAS.

To calculate PRS, we first checked and removed summary statistics SNPs if the imputation info scores less than 0.9. SNPs were then pruned using $r^2 = 0.1$ and a physical distance threshold of 250kb. Sex, age, UK Biobank assessment centre, genotype measurement batch, weekly alcohol drink units, and genetic principal components 1-20 were included as covariates in the calculation of the scores. We used a range of p-thresholds (5e-08,1e-04,5e-04,1e-3,5e-3,0.01,0.05,0.1,0.5,1) to capture PRS maximally. The AC-PRS and AUD-PRS were constructed using PRSice2 (Choi & O'Reilly, 2019).

4.2.4 Analyses

We tested the association of AC-PRS and AUD-PRS with total grey matter, white matter, and total grey + white matter; with the grey matter volume of 140 cortical and subcortical regions; and with the volume of 15 subcortical regions. The regression analyses used the brain volumes as the outcome variables and PRS as the predictor, after including the same covariates described above. False Discovery Rate corrections based on Benjamini–Hochberg were conducted separately for the 3

global brain measures, 140 grey matter regions, and 15 subcortical volumes. Furthermore, in the sensitivity analysis, we also tested the influence of weekly drink units on brain volumes, we conducted correlation analyses between weekly drink units and all brain regions.

4.3 Results

4.3.1 Alcohol consumption PRS associations with brain volumes

After exclusions 8689 participants were included in the analyses (Table 21). The average age of participants was 63 years old (age range 45-80) and 58% of them were females. As they were all exposed to alcohol, the average of weekly alcohol drink unit is 7.4.

No association was found between alcohol consumption PRS and subcortical volumes after FDR correction. Likewise, alcohol consumption PRS did not show any significant association with any global brain volumes and individual cortical and subcortical grey matter volumes after FDR correction.

Table 21 Participant characteristics

	Sex	N	age (M and SD)	weekly alcohol drink units (M and SD)
All participants	Male	7910	63 (7.5)	16 (12.4)
	Female	7218	61 (7)	9 (7)
	total	15128	62 (7)	13 (11)
Heavy drinkers (removed)	Male	3776	62 (7)	25 (12.3)
	Female	1313	61 (7)	20 (8.4)
	Total	5089	62 (7)	22 (10)
Healthy drinkers	Male	3670	64 (7.5)	8.3 (3.5)
	Female	5019	62 (7.1)	6.8 (3.4)
	Total	8689	63 (7)	7.4 (3.5)

N: number; M: mean; SD: standard deviation. Low-risk drinking: no more than 14 units alcohol per week; Heavy drinkers: alcohol intake over 14 units per week

4.3.2 Alcohol use disorder PRS association with subcortical brain volumes

Alcohol use disorder PRS results showed all negative associations with the volume of accumbens (right: $\beta = -3.81$, $P_{FDR} = 0.011$), amygdala (left: $\beta = -8.83$, $P_{FDR} = 0.011$, and right: $\beta = -11.81$, $P_{FDR} = 0.006$), hippocampus (left: $\beta = -19.39$, $P_{FDR} = 0.006$, and right: $\beta = -21.11$, $P_{FDR} = 0.006$), thalamus (left: $\beta = -22.33$, $P_{FDR} = 0.027$, and right: $\beta = -23.97$, $P_{FDR} = 0.015$), and brain stem and 4th ventricle ($\beta = -109.66$, $P_{FDR} = 0.01$) after FDR correction (Table 22).

4.3.3 Alcohol use disorder PRS association with individual cortical and subcortical grey matter volumes

AUD PRS results also showed negative associations with the grey matter volume of right parahippocampal gyrus posterior division (β = -7.38, P_{FDR} = 0.04), right temporal fusiform cortex anterior division (β = -9.78, P_{FDR} = 0.04), right central opercular cortex (β = -25.99, P_{FDR} = 0.04), and left (β = -16.31, P_{FDR} = 0.04) and right hippocampus (β = -18.2, P_{FDR} = 0.04) (Table 23 and Figure 1).

Table 22 Significant associations of AUD-PRS with subcortical brain volumes.

Brain regions (volumes)	Best P value threshold	R^2	P-values	Coefficient	SD	Number of SNP	P_{FDR}
Accumbens (right)	0.01	0.10%	8.62×10^{-4}	-3.81	1.14	9625	0.011
Amygdala (left)	5×10^{-8}	0.13%	8.93×10^{-4}	-8.83	2.66	15	0.011
Amygdala (right)	0.005	0.17%	1.09×10^{-4}	-11.81	3.05	5665	0.006
Hippocampus (left)	0.005	0.15%	1.49×10^{-4}	-19.39	5.11	5665	0.006
Hippocampus (right)	0.01	0.16%	1.11×10^{-4}	-21.11	5.46	9625	0.006
Thalamus (left)	0.5	0.08%	4.6×10^{-3}	-22.33	7.88	169511	0.027
Thalamus (right)	0.5	0.10%	1.73×10^{-3}	-23.97	7.65	169511	0.015
Brain stem + 4^{th} ventricle	0.01	0.14%	4.46×10^{-4}	-109.66	31.22	9625	0.010

R^2: Variance explained by the PRS
Coefficient: Regression coefficient of the model
SD: standard deviation
P_{FDR}: p-value corrected using FDR method

Table 23 Significant associations of AUD-PRS with cortical and subcortical grey matter volumes.

Brain regions (grey matter volumes)	Best P value threshold	R^2	P-values	Coefficient	SD	Number of SNP	P_{FDR}
Parahippocampal gyrus posterior division (right)	0.5	0.16%	1.73×10^{-4}	-7.38	1.96	169511	0.040
Temporal fusiform cortex anterior division (right)	1×10^{-4}	0.18%	5.73×10^{-5}	-9.78	2.43	310	0.040
Central opercular cortex (right)	0.01	0.16%	1.01×10^{-4}	-25.99	6.68	9625	0.040
Hippocampus (left)	0.05	0.16%	1.55×10^{-4}	-16.31	4.31	33312	0.040
Hippocampus (right)	0.05	0.18%	4.81×10^{-5}	-18.20	4.48	33312	0.040

R^2: Variance explained by the PRS
Coefficient: Regression coefficient of the model
SD: standard deviation
P_{FDR}: p-value corrected using FDR method

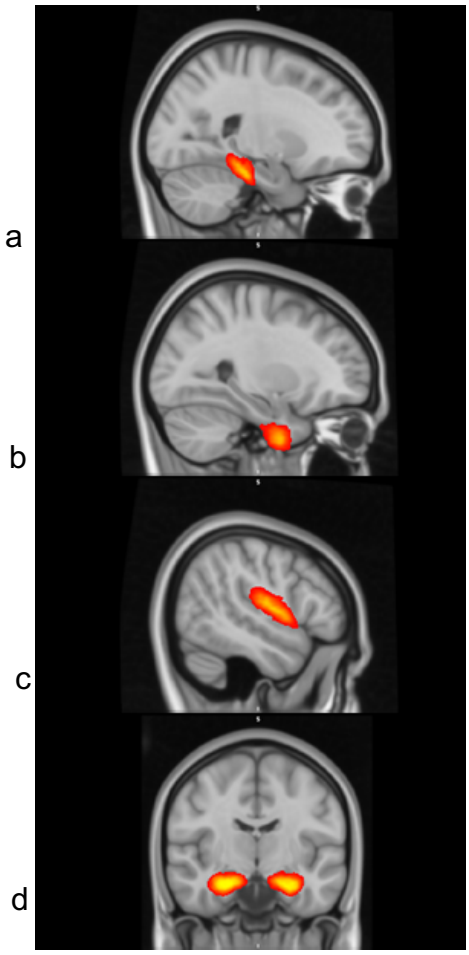


Figure 17 AUD-PRS associated with less grey matter volumes in cortical and subcortical regions

Red-yellow colour indicates the brain regions

a. Parahippocampal gyrus posterior division (right: β = -7.38, P_{FDR} = 0.04)

b. Temporal fusiform cortex anterior division (right: β = -9.78, P_{FDR} = 0.04)

c. Central opercular cortex (right: β = -25.99, P_{FDR} = 0.04)

d. Hippocampus (right: β = -18.2, PFDR = 0.04, and left: β = -16.31, P_{FDR} = 0.04)

4.4 Discussion

Among 8689 participants from the UK Biobank, the current study finds evidence that genetic risk to alcohol use disorder also contributes to brain matter volume differences in cortical and subcortical regions. Our results based on UKB data, shared with a previous imaging genetic study from ABCD data, suggested that alcohol drinking may not be the only reason behind brain abnormalities that appear to be caused by the neurotoxic effects of alcohol. More importantly, the current study showed that if people have a higher alcohol use disorder PRS, they may also have a higher risk for developing brain morphological changes, even for people who drink within the guidelines proposed by a healthcare system (e.g., the NHS).

The results showed that AUD-PRS were negatively associated with cortical and subcortical total brain matter and grey matter volumes. Firstly, AUD-PRS were negatively correlated with subcortical brain volumes in the right and left of the amygdala, hippocampus, thalamus, brainstem, and the 4th ventricle. Right accumbens were also found negatively associated with AUD-PRS. Previous imaging studies have shown that heavy drinkers and/or alcohol use disorder patients presented structural abnormalities in the amygdala (De Bellis et al., 2000; Gilpin et al., 2015), the hippocampus (de Bellis et al., 2000), the thalamus (De Bellis et al., 2005; Tuladhar & de Leeuw, 2019), and the brainstem (Bloomer et al., 2004; Siddiqui et al., 2021). The amygdala and hippocampus volume reduction has been suggested to be associated with alcohol craving and alcohol abuse relapse in alcohol dependence patients as both regions involved with the brain reward system. Thalamus shrinkage has been shown in AUD patients and alcoholics

with Korsakoff's syndrome (Segobin et al., 2019; Yang et al., 2016). Smaller size and potential cellular injury of the brainstem in chronic heavy alcohol drinkers have been showed by several imaging studies, which might suggest the acute brain injury in AUD patients and untreated chronic alcohol drinkers (Squeglia et al., 2014; X. Wang et al., 2020; Yang et al., 2016).

Secondly, we also found negative associations between grey matter volumes within specific brain regions and AUD-PRS, which were in the right parahippocampal gyrus posterior division, left and right hippocampus, right central opercular cortex, and right temporal fusiform cortex anterior division. This result is consistent with existing evidence that AUD patients and/or chronic alcohol users show atrophy in brain regions including the parahippocampal gyrus (Rolland, Dricot, Creupelandt, Maurage, & De Timary, 2020; Suzuki et al., 2010), the hippocampus (Suzuki et al., 2010), the central opercular cortex (Thayer et al., 2017), and the temporal fusiform cortex (Rolland, Dricot, Creupelandt, Maurage, & de Timary, 2020). The damage to the limbic system including the parahippocampal gyrus and the hippocampus due to alcohol have been suggested to be linked with alcohol-induced memory impairments and emotional changes (Suzuki et al., 2010). While the hippocampus is involved in learning and memory, and the parahippocampal gyrus is associated with episodic memory, contextual association and visuospatial processing (Courtney et al., 2015), the fusiform gyrus, on the other hand, has been suggested to be related to the memory of people and social language and behaviour (Rolland, Dricot, Creupelandt, Maurage, & de Timary, 2020). Moreover, a positive relationship between alcohol and failures to control over drinking has been shown in the central opercular cortex in a study that was trying to identify neurobiological phenotypes with AUD severity (Claus et al.,

2011). Another study also showed an increase activation in the central opercular cortex when presented with alcohol cue to alcohol-dependent participants (Courtney et al., 2015). Similar to our study using UK Biobank data, a study using data from ABCD showed problem alcohol drinking PRS were associated with lower frontal pole volume and thicker supramarginal gyrus cortex, although we did not replicate their results which might be the difference between participants from UK Biobank and ABCD. Combing these neuroimaging evidence together, the negative relationships between AUD-PRS and grey matter volumes in the brain regions mentioned above, suggest that carrying higher AUD genetic risk factors may be associated with predispositional risk in brain regions that are involved with various alcohol-induced memory impairments and a higher desire for alcohol.

Notably we found no associations between AC-PRS and any brain region volume. This might reflect the fact that, in order to remove as much of the influence of alcohol on the brain we could, we specifically chose a group of participants who were “healthy drinkers” which, according to the National Health Service in the United Kingdom, were people who drank less than 14 units of alcohol per week. However, a recent large imaging biobank study that used 36,678 healthy adults showed that there was no safe amount of alcohol consumption as even 1-2 units would have a negative impact on brain macrostructure and microstructure. It might be the influence of AC genetic risk factors was too small to be identified in the regression analysis. Therefore, a larger imaging genetic study is needed in the future to confirm our AUD-PRS results and to further explore the impact of AC-PRS on brain structures.

There are several limitations of this study. While this study allowed us to example the genetic risk of AC and AUD in “healthy drinkers”, and we were trying to reduce the influence of alcohol on brain structures in this study, it is not possible to accomplish as the effect of alcohol in “healthy drinkers” still existed. Due to the availability of the imaging data from the UK Biobank, we only performed the analysis of brain volumes and grey matter volumes. It will be important to explore the influence of genetic risk factors of AUD and AC on white matter integrity and whether there are impacts on brain structure trajectories. Our results were based on the largest GWAS of AUC and AC and imaging data at the time, therefore, more potential regions might be revealed when larger GWAS are performed and more individuals with brain imaging data from biobank are released in the future. Finally, the current study only utilised the data from European-ancestry participants as other ancestries GWAS and imaging data highly unlike to show any results considering they were underpower and the small effects sizes of the genetic risk factors. Therefore, increases in the genetic diversity in GWAS and imaging genetic studies could benefit many aspects of genetic studies.

Our study provides further evidence that genetic risk factors of AUD also contribute to the reduction of brain volumes even in people who drank alcohol less than 14 units a week. Our results suggested “healthy drinking” individuals who carry higher AUD genetic risks might already have higher risks for brain alterations compared with “healthy drinkers” who have lower AUD genetic risks.

Chapter 5 Final discussion

5.1 General discussion

Over the last two decades, researchers utilised high-density single nucleotide polymorphisms (SNP) arrays and DNA sequencing to present GWAS results that are designed to reveal the polygenic architecture of common diseases to identify consistent genetic risk variants. Researchers now are making efforts such as increasing sample size and adding more ancestral groups to better understand the genetic architectures and identify consistently replicable loci.

The majority of the GWAS undertaken to date have used a binary case-control definition for the AUD phenotype. Thus, one strategy which might improve the detection of variants would be using quantitative traits. Not only it could potentially enhance statistical power, produce unbiased PRSs, and identify quantitative mechanisms (Plomin et al., 2009), but also the study of quantitative traits could provide a more detailed picture of how genetic risk variants influence the disorder. Another consideration that needs to be considered when implementing a quantitative approach in GWAS is that the phenotypic data used in the analysis may not be normally distributed. Hence, applying a regression model that is designed for the distribution may be beneficial, in this case, a quasi-Poisson regression model based on an assumption that the data would show a prominent right skew was used. In the first study, we examined the influences of using binary and adjusted quantitative measures of AUD on GWAS outcomes and calculated polygenic risk scores (PRS). No associations were identified at genome-wide significance in any of the individual GWAS; results were comparable in all three. The top associated SNP (rs34361428) was located on the alcohol

dehydrogenase (*ADH*) gene cluster on chromosome 4, consistent with previous AUD GWAS. This SNP is an eQTL for *ADH1B*, *ADH1C*, *ADH1A*, and *METAP1* and its association with AUD has been reported previously (Goldman et al., 2005; D. Li et al., 2011; Luczak et al., 2006; R. K. Walters et al., 2018; Zuo et al., 2012). The C allele of rs34361428 is associated with reduced expression of *METAP1*. This association has been reported previously but specifically in African Americans classified as having alcohol use disorder (Gelernter et al., 2014; Kranzler, Zhou, Kember, Vickers Smith, et al., 2019). The quantitative trait analysis adjusted for the distribution of the criterion score and the associated PRS had the smallest standard errors and thus the greatest precision. Together, these results suggest that applying quantitative trait analysis in GWAS and using an applicable regression model might be beneficial.

The accumulating GWAS results continuously reveal AUD risk genes. On the other hand, global neuroimaging consortia such as UK Biobank started to release brain phenotypes from tens of thousands of people, which provides exclusive opportunity to validate and discover AUD related findings. Imaging genetics has been proven as a unique tool to assess the impact of SNP on brain structures and functions with the potential to recognise the influence on behaviour. Since the 2000s, researchers have increasingly used imaging genetics in the studies of psychiatric diseases such as schizophrenia (Arslan, 2018). We therefore designed an imaging genetic study to estimate associations between alcohol related PRS and brain volumes in cortical and subcortical regions among 8689 people who drink less than 14 units per week. Alcohol use disorder PRS results showed all negative associations with the volume of accumbens, amygdala (left and right), hippocampus (left and right), thalamus (left and right), brain stem, and 4th ventricle after

FDR correction. AUD PRS results also showed negative associations with the grey matter volume of the right parahippocampal gyrus posterior division, right temporal fusiform cortex anterior division, right central opercular cortex, and left and right hippocampus. The amygdala and hippocampus volume reduction has long been suggested to be associated with alcohol craving and alcohol abuse relapse in AUD patients as both regions involved with the brain reward system. However, no association was found between alcohol consumption PRS and subcortical and cortical brain volumes and grey matter volumes, which might be because we specifically chose a group of participants who were “healthy drinkers”. It might also be the influence of AC genetic risk factors was too small to be identified in the regression analysis. Taking this evidence together, the negative relationships between AUD-PRS and grey matter volumes in the brain regions mentioned above, suggest that carrying higher AUD genetic risk factors may be associated with predispositional risk in brain regions that are involved with various alcohol-induced memory impairments and a higher desire for alcohol.

AUD displays considerable comorbidity with other psychiatric diseases (Castillo-Carniglia et al., 2019). Studies suggested that mood and anxiety disorders, substance use disorders and thought disorders are higher in AUD patients than in the general population (Grant et al., 2004; Melchior et al., 2014; Sørensen et al., 2018). There are various potential mechanisms that could lead to AUD comorbidity, for example, the causal effect from other psychiatric disorders, shared genetic and environmental factors, and shared psychopathological characteristics (Castillo-Carniglia et al., 2019). Thus, understanding how AUD is comorbid with other psychiatric disorders is essential to determine applicable interventions to reduce the risk of comorbidity. Among other

psychiatric disorders, individuals with ASPD have been shown to be at high risk for substance use disorders such as alcohol dependence (Bahlmann et al., 2002; Yoshino et al., 2000). The lifetime AUD in people with ASPD is particularly high at an estimated 76.7% (Guy et al., 2018). To investigate the genetic architecture of ASPD criteria in the context of AUD, we conducted the largest meta-analytic GWAS of comparable clinical measures of ASPD undertaken to date. Our GWAS meta-analysis identified a novel genome-wide significant signal with rs9806493 on chromosome 15q26.1 close to the *SLCO3A1* gene. This marker is supported by additional SNPs in linkage disequilibrium with the main finding that did not reach genome-wide significance. In the PRS analysis, genetic correlations that survived correction for multiple testing were identified with genetic risk variants for many complex behavioural traits and psychiatric disorders including education attainment, smoking, alcohol intake frequency, reproductive behaviours, depression, PTSD, and ADHD.

Results indicated that rs9806493 is an eQTL for *SLCO3A1*, and the effect allele (C allele) is associated with decreased expression of *SLCO3A1*. Previous studies showed that *SLCO3A1* mediates the transport of $Na^{(+)}$-independent of organic anions and hormones including thyroxine and vasopressin, the cyclic oligopeptides BQ-123 (endothelin receptor antagonist), and prostaglandins (PG) E1 and E2 (Huber et al., 2007; Tamai et al., 2000). As the C allele of rs9806493 decreases the expression of *SLCO3A1,* it could lead to decreased uptake of hormones including PGE1, PGE2, T4, and vasopressin. Increased levels of PGE1, PGE2, and T4 have been reported in ASPD (Evrensel et al., 2016; Virkkunen et al., 1987).

Our polygenic risk score analysis suggested that common genetic loci underlie risk for ASPD and other complex traits including smoking, alcohol use frequency, PTSD, ADHD, reproductive traits, and educational attainment. The evidence from our PRS analysis provides further support that ASPD is a highly polygenic disorder that shares genetic risk loci with other psychiatric and neurodevelopmental disorders.

5.2 Limitations

First of all, the relatively small sample size restricted the power of my PhD studies. Further evidence could be obtained in larger GWAS using the quantitative traits analysis and adjusted regression models. Second the results need to be carefully interpreted considering the highly selected nature of participants in all three studies. For the first study, the inclusion of a cohort of non-dependent heavy drinkers and a general population sample would have provided a better representation of the drinking spectrum. While the imaging genetic study allowed us to examine the genetic risk of AC and AUD in “healthy drinkers”, we only performed the analysis of brain volumes and grey matter volumes. It will be important to explore the influence of genetic risk factors of AUD and AC on white matter integrity and whether there are impacts on brain structure trajectories. The ASPD GWAS along with the other two studies only utilised the data from European-ancestry participants as other ancestries GWAS and imaging data were highly unlike to show any results considering they were underpowered and the small effects sizes of the genetic risk factors. Therefore, increases in the genetic diversity in GWAS and imaging genetic studies could benefit many aspects of genetic studies.

5.3 Conclusion and future directions

The findings presented in this PhD thesis hold broader implications for the field of AUD research. The identification of novel AUD loci, the exploration of the association between genetic risk variants and brain morphology, and the investigation of shared genetic risk variants underlying AUD and its comorbidity with ASPD contribute to our understanding of the complex genetic architecture of AUD. These findings highlight the need for further investigations to validate and expand upon the results obtained.

To make more progress in this field, future directions should involve utilising quantitative traits analysis and adjusted regression models with larger sample sizes. These approaches will help generate more precise results in GWAS and post-GWAS analyses. Additionally, conducting meta-analyses of GWAS with diverse ancestral groups would be valuable. However, imaging genetic studies involving different ancestral groups have been limited due to the scarcity of available data. Collaborative efforts among large consortia should be encouraged to explore methods that can effectively investigate the relationships between genetic risk variants and brain phenotypes in individuals from different ancestral backgrounds.

Looking ahead, the field of AUD research would benefit from the integration of newer methods and technologies. Advancements in genomic sequencing techniques, such as whole genome sequencing, can facilitate the detection of rare variants with potentially larger effects on AUD risk. Incorporating multi-omics approaches, such as integrating genetic data with transcriptomics or epigenomics, can provide a more

comprehensive understanding of the biological mechanisms underlying AUD. Furthermore, leveraging advanced neuroimaging techniques, such as functional connectivity analyses or machine learning algorithms, can enhance our understanding of the complex interactions between genetic factors and brain function in the context of AUD.

In summary, the work presented in this thesis contributes to the broader field of AUD research by uncovering novel loci, exploring the association between genetic risk variants and brain morphology, and investigating shared genetic risk factors. Moving forward, future directions should involve larger sample sizes, meta-analyses with diverse ancestral groups, and the integration of advanced methods and technologies. To achieve these, comprehensive planning and resources are essential. For example, collaborating with research institutions, biobanks, and consortia, and engaging in robust participant recruitment efforts are key steps. Longitudinal studies and international collaborations can diversify the sample. Ensure effective data management, access to bioinformatics expertise, and validate findings through replication studies. These efforts will help propel the field towards a more comprehensive understanding of the genetic and neurobiological underpinnings of AUD and pave the way for the development of more effective prevention and treatment strategies.

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APPENDIX 1

UK-COGA FACE SHEET AND SSAGA-II-LITE HANDSCORE FOR DIAGNOSES

FACE SHEET v6.4

HOSPITAL NUMBER:					INSTITUTION:			
NAME:						TEL:		
ADDRESS:								
DOB:				OCCUPATION:		INTERVIEWER'S NAME:		
SEX (M=1, F=2)	1	2	ETHNICITY:			DATE OF ASSESSMENT:		
MARITAL STATUS:	1	2	3	4	5	(YEARS)	EDUCATION:	
	(MAR)	(WID)	(SEP)	(DIV)	(SIN)			

*CODES FOR SYMPTOMS/DIAGNOSES: 1=Absent, 5=Present, 9=Uncertain

PAGE #	ITEM#	DIAGNOSIS	DSM-3R			DSM-IV			ICD-10			Age of onset (y)
		Alcohol Dependence Syndrome	1	5	9	1	5	9	1	5	9	
		Depressive syndrome (''dirty'' depression)				1	5	9				
		Depression – ''clean''				1	5	9				
		Bipolar - 1				1	5	9				
		Bipolar - 2				1	5	9				
		Attention deficit/hyperactivity disorder (ADHD)				1	5	9				
		Antisocial personality disorder (ASPD)				1	5	9				
		Wernicke-Korsakoff Syndrome (WKS)				1	5	9				
		Only give clinical impression here (not from interview):										
		Cannabis Dependence				1	5	9				
		Cannabis Abuse				1	5	9				
		Cocaine Dependence				1	5	9				
		Cocaine Abuse				1	5	9				
		Stimulants Dependence				1	5	9				
		Stimulants Abuse				1	5	9				
		Sedative, Hypnotic/ Anxiolytic Dependence				1	5	9				
		Sedative, Hypnotic/ Anxiolytic Abuse				1	5	9				
		Opioid Dependence				1	5	9				
		Opioid Abuse				1	5	9				
		Nicotine Dependence				1	5	9				

Checklist:	
Informed Consent	
Ancestry	
Blood tubes, labelled	
Tallies A-E	
SSAGA-II Lite	
MMSE	
Blood stored/sent	

Interviewer comments, clinical impressions, concerns:

Medical history

B3 Has a doctor ever told you that you have (had):	NO	YES	YR DIAGNOSED
1 High blood pressure?	1	5	
2 Migraine headaches?	1	5	
3 A brain injury or concussion?	1	5	
4 Been unconscious for longer than 5 min?	1	5	
5 Epilepsy or have had a seizure?	1	5	
6 Meningitis or encephalitis?	1	5	
7 A stroke?	1	5	
8 Heart disease?	1	5	
9 Liver disease?	1	5	
10 Thyroid disease?	1	5	
11 Asthma?	1	5	
12 Diabetes?	1	5	
13 Cancer? **SPECIFY:**	1	5	
14 HIV/AIDS?	1	5	
15 A sexually transmitted disease?	1	5	
16 Cirrhosis of the liver?	1	5	
17 Any other?	1	5	

Medications

Please give names & doses of your current medications:

Wernicke-Korsakoff Diagnosis? Y/N

If clinically suspected/known, please specify criteria (from notes or patient):

1=Absent, 5=Present, 9=Uncertain

Wernicke's:	Confusion	1	5	9
	Nystagmus	1	5	9
	Ataxia	1	5	9
Korsakoff's:	Disorientation	1	5	9
	Memory impairment	1	5	9
	Confabulation	1	5	9
Family history for WKS?		1	5	9

<u>If WKS, ARBD or memory problems suspected, complete MMSE (other cognitive assessments may be appended).</u>

Ancestry Checklist

			Town/county of origin
Mother	Scottish		
	Welsh		
	English		
	Irish		
Mother's Mother	Scottish		
	Welsh		
	English		
	Irish		
	Other (specify)		
Mother's Father	Scottish		
	Welsh		
	English		
	Irish		
	Other (specify)		
Father	Scottish		
	Welsh		
	English		
	Irish		
Father's Mother	Scottish		
	Welsh		
	English		
	Irish		
	Other (specify)		

Father's Father	Scottish		
	Welsh		
	English		
	Irish		
	Other (specify)		

UK COGA Pedigree Collection Sheet

Participant Name: _____

Hospital/clinic ID Numer _____

Name of contact person _____

Address of contact _____

Telephone number of contact _____

Email of contact:

Use an arrow to denote proband (case sampled) and write diagnosis under each family member. Squares = males; circles = females

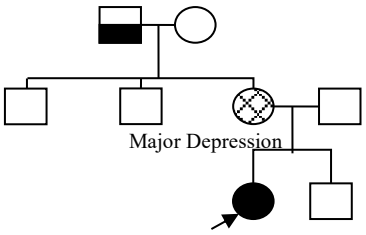
Codes

Alcohol Dependence Syndrome

Harmful drinking without dependence

Major Depression/bipolar/ADHA/Antisocial PD (Specify)

Example



SSAGA II 'Lite' Tally Sheet A,B,C – DSM-3R/IV/ICD-10 Alcohol Dependence

The following pages are "Tally Sheets" (tick sheets). These may be used on their own if you are short of time (minimum dataset) or informed by the SSAGA-II-Lite. Answers from the latter are used to calculate diagnoses. Clinical impression is also important and notes can be made on the front page or wherever appropriate.

Tally sheet	**Diagnosis**
A-B-C	Alcohol dependence syndrome (ICD-10, DSM-IIIR, DSM-IV)
I	Depression (DSM-IV)
D	Bipolar Disorder (DSM-IV)
CC	ADHD (DSM-IV)
M	Antisocial PD (DSM-IV)

<u>Instructions:</u> Minimum dataset: Mark E questions as respondent endorses them or from SSAGA-II Lite.

Stem question: "When you were drinking, did the following apply to you?"

SSAGA question		DSM-3R	- 4	ICD-10
				then mark these columns↓
E9D	Needed 50% more alcohol to get an effect			
E9I	Could drink 50% more alcohol before getting drunk			
E10	Wanted to stop or cut down 3+ times			
E10C	Tried but was unable to stop or cut down			
E12C	Drank more than intended, more days in row than intended, or when promised self wouldn't 3+ times			
E13B	Became drunk when didn't want to 3+ times			
E14B	Gave up or greatly reduced important activities to drink 3+ times or for 1 + month			
E15A	Drinking or recovering from effects left little time for anything else for 1+ month or 3+ times			
E16B (B.1)	Neglected responsibilities when binging 3+ times			
E21*	Mixed alcohol & medications/drugs when knew this was			**E21 or E29B only for ICD-10.*
E22B (B.1)	dangerous 3+ times Was drunk in situations where could have injured self 3+times			
E23B (B.1)	Arrested for drunk driving 3+ times Drinking and driving resulted in accident 3+ times			
E24B (B.1)	Drinking or being hung over often interfered with responsibilities			
E25	Alcohol caused accidental injuries 3+ times			
E29B* (B.1)				
E31B	Continued to drink knowing alcohol caused health problems			
E32	Continued to drink despite serious physical illness			
E33A	Continued to drink knowing alcohol caused emotional problems			
E26A.1	Drinking caused problems with family or friends 3+ times			
E26A.2	Lost friends because of drinking 3+ times			
E26A.3	Drinking caused problems with work/school 3+ times			
E27C	Drank knowing it caused problems with love relationships			
	Arrested/detained by police for drunk behaviour 3+ times			

E28B (B.1)				
E37D E38 E39	Experienced withdrawal symptoms (shakes, insomnia, anxiety, depressed/irritable, heart beating fast, nausea/vomiting, physically weak, headaches, see or hear things not there, fidgety/restless) Had fits or seizures from drinking Had the DT's from drinking	*2+ with-draw-al sx*	*2+ with-draw-al sx*	*3+ with-draw-al sx*
E37I E38C E39C	Drank to relieve or avoid withdrawal symptoms 3+ occasions Drank to relieve or avoid fits or seizures 3+ times Drank to relieve or avoid delirium tremens (the DT's) 3+ times			
Number of boxes in *each* column with marked E questions:				
Mark if number if boxes >= number here (=diagnosis made):		3	3	3
Mark face sheet = 5 for each diagnosis made or =1 if not made.				

Additional questions (minimum dataset only):

Q1. In a 24-hour period, what is the largest number of drinks (units) you have taken? _____

Q2. At what age did you first become dependent (addicted to) alcohol? _____

Q3. Have you noticed that you suffer hangovers less than others? _____

Q4. How many episodes of abstinence (when you were "clean") have you had? _____

Q5. What is the longest period of abstinence you have had? _____ (days, weeks, months)

Q6. How many inpatient detoxifications ("detoxes") have you had? ___ ;and outpatient (community/home)? ___

Q7. How many rehabilitation or continuing care placements ("rehabs") have you had? _____

Q8: When drinking dependently how may drinks (units) did you drink/ day? _____ [x9 =g alcohol_____]

SSAGA-II 'Lite' Tally Sheet I DSM-IV criteria for Depression

Instructions: SSAGA-II: Use codes from SSAGA-II Lite Section I (Depression)

Minimum dataset: Mark 'I' questions as respondent endorses them.

… Then mark here if any criterion endorsed in each box ↓

Stem : "Has there been a period in your life lasting at least 2 weeks when you (were)…"

Box	Criterion	SAGA II question	SAGA II code		Endorsed?
A	Felt depressed for 2+ weeks	I 12	1	5	
	Felt irritable for 2+ weeks	I 12B	1	5	
B	Lost interest in most things for 2+ weeks	I 12A	1	5	
	Less able to enjoy sex or other things	I 18	1	5	

Stem : "During this period of feeling depressed / irritable / loss of enjoyment for at least 2 weeks, did yo you…"

Box	Criterion	SAGA II question	SAGA II code		Endorsed?
C	Have a change in appetite	I 14A	1	5	
	Gain or lose weight	I 14 B	1	5	
D	Unable to fall asleep (for 1 hr+)	I15B	1	5	
	Trouble sleeping through the night	I15C	1	5	
	Waking up an hour earlier than usual	I15E	1	5	
	Slept more than usual	I15F	1	5	
E	Was fidgety or restless	I 16	1	5	
	Moved or talked slower	I 17	1	5	
F	Felt a loss of energy or more tired	I 19	1	5	
G	Felt excessively guilty or bad	I 20	1	5	
	Felt was a failure or worthless	I 21	1	5	
H	Had more difficulty than usual thinking, concentrating, or making decisions	I 22	1	5	
I	Thought about dying / wishing was dead	I 23	1	5	
	Made a suicide plan	I 23A	1	5	
	Attempted suicide	I 23B	1	5	
		Number of boxes endorsed:			

1	Is Box A or B endorsed?	Yes – go to 2	No – mark face sheet 1 for depression and next section
2	Is one+ Box C-I endorsed?	Yes – mark face sheet 5 for depression and go to 3	No – mark face sheet 1 for depression
3	Ever had a *clean* episode*?	Yes – mark face sheet 5 for clean and go to 4	No – go to 4
4	Ever had a *dirty* episode**?	Yes – mark face sheet 5 for dirty and cause, and go to next section	No – next section

These can be diagnostically challenging to work out with the participant:

Clean* episode = no regular drugs/alcohol, pregnancy/childbirth/miscarriage, change in medication, or severe physical illness during the **2 weeks before episode

***Dirty* episode = with regular use of drugs/alcohol,
pregnancy/childbirth/miscarriage,
a change in medication,
or severe physical illness during the **2 weeks** before episode (circle if present)

SSAGA II 'Lite' TALLY SHEET D: MANIA / Hypomania

Instructions: Circle items endorsed by respondent then check right-hand column instructions.

Minimum dataset: Ask questions here↓

	or SSAGA-II Lite codes here↓		*Result↓*
Box A: Have you ever had a period of time lasting 2 days or longer when you felt…			*Core criteria. Mark if endorses any.*
K1A hyper/elated/manic for 2+ days	1	5	
K1B unusually irritable for 2+ days	1	5	
K1G elated / irritable 2+ days …. Clearly different from your normal self?	1	5	
**** DO NOT COUNT RECOVERY FROM DEPRESSION OR INTOXICATION FROM DRUGS/ALCOHOL****			
Box B: During this period were you also…			*Number of symptoms.*
K1D .1 much more active than usual?	1	5	
K1D .2 much more talkative than usual?	1	5	*Mark here if K1A/K1G and 3+ symptoms.*
K1D .3 talking unusually fast or were your thoughts racing?	1	5	
K1D .4 feeling very special, gifted with special powers?	1	5	
K1D .5 needing much less sleep than usual?	1	5	
K1D .6 more easily distracted than usual?	1	5	*Mark here if K1B and 4+ symptoms*
K1D .7 doing reckless or foolish things (spending sprees, reckless driving, affairs)?	1	5	
Number of Symptoms from K1D.1-7 or SSAGA-II Lite Box K19:_____			
Box C: **Think about your most severe episode of feeling hyper/elated/irritable.** How long did it last?_____ (days)			*Duration. Mark here (MANIA) if 7+ days or hospitalized / incapacitated.*
Were you hospitalized or incapacitated (unable to work/school/function)?	1	5	
Think about your least severe episode. How long did it last? _____ (days)			*Mark here (HYPO-MANIA) if 4+ days, others noticed but <u>not</u> hospitalized / incapacitated.*
Did others notice a change in your functioning?	1	5	
Were you hospitalized or incapacitated (unable to work/ go to school/function)?	1	5	
Box D: 1. **During your most severe episode** did you see or hear things that other people could not see or hear (that is, hallucinations)?	1	5	*Psychotic features.*
Specify: _____			

2. **During your most severe episode** did have beliefs or ideas that you later found out not to be true (that is, delusions)? 1 5 Specify: _____ Did 1. or 2. above occur **after** onset of symptoms in Box A above? 1 5		*Mark here if endorsed and occurred after onset of abnormal mood in Box A.*
Box E: During the 2 weeks before any episode of feeling (hyper/elated/ irritable) began, did you… • drink at least 5 alcoholic drinks (units) 2 or more times a week? • receive ECT (shock therapy) or bright light therapy? • take any drugs for a high or intoxication daily or almost daily? • start or change the dose of prescription medications such as decongestants, steroids, or antidepressants? • have an episode of a serious physical illness like multiple sclerosis, AIDS, hyperthyroidism, lupus, Cushings, or encephalitis? Did you ever have an episode of feeling hyper/elated/irritable that lasted 2 days or longer, that did NOT follow excessive drinking, daily use of drugs, ECT, starting/changing medication, or a serious physical illness?	ANY? 1 5 ANY? 1 5	*Clean/Dirty* *If any in box E question 1-5 endorsed, mark face sheet here:* ***Bipolar dirty = 5****.* *If any clean episodes, mark here:* ***Bipolar clean=5.***

SAGA II Lite TALLY SHEET D: BIPOLAR / MANIA / Hypomania - continued

Onset: How old were you the first time you had a manic/hypomanic/mixed episode? (years) _____

How many separate episodes in your lifetime (with 2 months between episodes):

- **Mania:** where you were hyper/elated/irritable for at least a week or hospitalized _____
- **Hypomania:** where you were hyper/elated/irritable for at least 4 days but were <u>not</u> hospitalized _____
- **Mixed:** where you experienced both Mania **and** Depression nearly every day for 1+ week _____
- **Depressed:** for at least 2 weeks (see Depression Tally Sheet to confirm) _____

Rapid cycling: Have you ever switched back and forth quickly between feeling (hyper/elated/irritable) and feeling depressed?	NO….1	YES….5
A. Circle: Did that happen every few **hours,** every few **days**, or every few **weeks**?		
B. Did you ever have 4 or more episodes like this within a 12-month period?	NO….1	YES….5 = Rapid Cycling

<u>Diagnoses:</u> Mark below if criteria fulfilled…	
Mania = marks in Boxes **A, B, C** (Mania), and **Clean** **Mania** with psychotic features = Mania + mark in Box D **Bipolar 1** = at least one Manic/Mixed and one other mood disorder episode	**Hypomania** = marks in Boxes **A, B, C** (Hypomania) and **Clean** ** NOT Hypomanic if mark in Box D ** **Bipolar 2** = at least one hypomanic and one other (NOT Manic/Mixed) episode ➔ **Mark Face sheet Bipolar 2 = 5**

(manic/mixed/hypomanic/ depressed) → **Mark face sheet Bipolar 1 = 5**	

SAGA-II Lite Tally Sheet CC- DSM-IV CRITERIA: ATTENTION-DEFICIT / HYPERACTIVITY DISORDER

Instructions: Either code from SSAGA-II ADHD section, or ask minimum dataset stem questions, and code in result column.

	CRITERIA	SSAGA-II Lite QUESTION(S)	RESULT
A.	*Stem: "Let me ask you about what you were like in your first few years at school (from age 6-10). During this period, was there ever at least 6 months when the following applied to you…?"*		
A1.	**[Six (or more)** of the following symptoms of **inattention** have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:] **1=Absent 5=Present 9=Unclear↓**		
(a)	Often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities	CC1.2=5	1 5 9
(b)	Often has difficulty sustaining attention in tasks or play activities	CC1.5=5	1 5 9
(c)	Often does not seem to listen when spoken to directly	CC1.4=5	1 5 9
(d)	Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behavior or failure to understand instructions)	CC1.9=5	1 5 9

(e)	Often has difficulty organizing tasks and activities	CC1.8=5	1	5	9
(f)	Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)	CC1.7=5	1	5	9
(g)	Often loses things necessary for tasks or activities	CC1.1=5	1	5	9
(h)	Is often easily distracted by extraneous stimuli	CC1.6=5	1	5	9
(i)	Is often forgetful in daily activities	CC1.3=5	1	5	9

A2. **[Six (or more)** of the following symptoms of **hyperactivity-impulsivity** have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:]

(a)	Often fidgets with hands or feet or squirms in seat	CC6.4=5	1	5	9
(b)	Often leaves seat in classroom or in other situations in which remaining seated is expected	CC6.5=5	1	5	9
(c)		CC6.1=5	1	5	9

	Often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)				
(d)	Often has difficulty playing or engaging in leisure activities quietly	CC6.3=5	1	5	9
(e)	Is often “on the go” or often acts as if “driven by a motor”	CC6.2=5	1	5	9
(f)	Often talks excessively	CC6.6=5	1	5	9
(g)	Often blurts out answers before questions have been completed……	CC6.7=5	1	5	9
(h)	Often has difficulty awaiting turn	CC6.9=5	1	5	9
(i)	Often interrupts or intrudes on others	(CC6.8=5) OR (CC6.10=5)	1	5	9

IF SIX OR MORE 5s OR 9s IN A1 (a-i) OR IF SIX OR MORE 5s OR 9s IN A2 (a-i), GO TO B. OTHERS CODE 1 FOR ADHD ON FACE SHEET AND GO TO NEXT DIAGNOSIS.

B.	*Stem: Did these symptoms cause problems before age 7?* Some hyperactive-impulsive or inattentive symptoms that caused impairment were present before age 7 years	(CC3 AGE ONS <7) OR (CC8 AGE ONS <7)	1	5	9
	IF B=5 OR 9, GO TO C. **OTHERS CODE 1 FOR ADHD ON FACE SHEET AND GO TO NEXT DIAGNOSIS.**				
C.	*Stem: Did these symptoms cause problems in two/more places (school, home, other places)?* Some impairment from the symptoms is present in two or more settings	<u>ANY TWO POSITIVE IN</u>: [(CC2.a =5) OR (CC7.a =5)] [(CC2.b =5) OR (CC7.b =5)] [(CC2.c =5) OR (CC7.c=5)]	1	5	9
	IF C=5 OR 9, GO TO D. **OTHERS CODE 1 FOR ADHD ON FACE SHEET AND GO TO NEXT DIAGNOSIS.**				
D.	*Stem: Did these difficulties get you into serious trouble at school, home, work or other places?* There must be clear evidence of clinically significant impairment in social, academic, or occupational		1	5	9

	functioning	(CC2.d =5) OR (CC4=5) OR (CC5=5) OR (CC7.d=5) OR (CC9=5) OR (CC10=5)	

IF D=5 OR 9, GO TO E.
OTHERS CODE 1 FOR ADHD ON FACE SHEET AND GO TO NEXT DIAGNOSIS.

E.	The symptoms do not occur exclusively during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorder and are not better accounted for by another mental disorder (e.g., Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).	(Clinical judgement) If symptoms did occur during one of these disorders, do not code on face sheet and go to next section	
F.	Does this participant meet criteria for ADHD?		1 5 9

CRITERIA FOR 5: (SIX OR MORE 5'S IN A1.a-i)
OR (SIX OR MORE 5'S IN A2.a-i)
AND (B=5)
AND (C=5)
AND (D=5)

→CODE FACE SHEET FOR ADHD

SSAGA-II'Lite' Tally sheet M DSM-IV CRITERIA: ANTISOCIAL PERSONALITY DISORDER *Instructions: Either code from SSAGA-II ASP section in middle column, or ask minimum dataset stem questions. All questions coded 3 in SSAGA-II ASP section should be coded 9 here*

A. Current age at least 18…….		Y N
IF A=N, CODE Y FOR ASPD ON FACE SHEET AND END. IF A=Y, GO TO B.		
B. Evidence of Conduct Disorder with onset before age 15, as indicated by a history of **three or more** of the following: **CODE 5 ONLY IF AGE ONSET=14 OR LESS** *Minimum dataset stem : "Before you were 15 years old, would you say that you…"* **CD criteria** **SSAGA-II** **Lite codes ↓**		**Result:** 1= absent 5= present 9= unsure ↓
Often bullied, threatened, or intimidated others...	M9 = 5	1 5 9
Often initiated physical fights.....	M6 OR M6.B = 5 OR 6	1 5 9

Used a weapon that can cause serious physical harm to others (e.g., a bat, brick, broken bottle, knife, gun).....	M20 = 5 OR 6	1 5 9
Were physically cruel to people.....	M19 = 5 OR 6	1 5 9
Were physically cruel to animals	M10 = 5	1 5 9
Stole with confrontation of a victim (e.g., mugging, purse-snatching, extortion, armed robbery).....	M16 = 5 OR 6	1 5 9
Forced someone into sexual activity	M21 = 5 OR 6	1 5 9
Deliberately engaged in fire setting with the intention of causing serious damage.....	M17.A = 5	1 5 9
Deliberately destroyed other's property (other than by fire-setting).....	M18 = 5 OR 6	1 5 9
Broke into someone else's house, building, or car	M15 = 5 OR 6	1 5 9

.....		
Often lied to obtain goods or favors or to avoid obligations (i.e., “cons” others)	M11.A , M11.B1, OR M13 = 5 OR 6	1 5 9
Stole items of nontrivial value without confrontation of a victim (e.g., shoplifting, but without breaking and entering; forgery).....	M14, M14.B OR M14.D = 5 OR 6	1 5 9
Often stayed out at night despite parental prohibitions, beginning **before a**ge 13 years.....	(M4.A = 5 AND M4.B < 13) OR (M5.A = 5 AND M5.B < 13)	1 5 9
Ran away from home overnight at least twice while living in parental or parental surrogate home (or once without returning for a lengthy period)...	(M3.A = 4) AND [(M3.B =5) OR (M3.C = 1) OR (M3.C1 ≥7)]	1 5 9
Were often truant from school, beginning before age 13	(M1.A = 5) AND (M1.B <13)	1 5 9

IF FEWER THAN THREE 5s OR 9s IN B1-B15, CODE 1 FOR ASPD ON FACE SHEET. OTHERS GO TO C. * FOR C CODE 5 ONLY IF AGE ONSET=15 OR OLDER *****		
C. A pervasive pattern of disregard for and violation of the rights of others occurring since age 15 years, as indicated by three or more of the following (C1-C7): *Minimum dataset stem : "When you were 15 years or older, would you often…"* **ASPD Criterion** **SSAGA-II Lite codes↓** **1=Absent 5=Present 9=Unsure↓**		
C1. * Steal money or things from family or friends *Break into someone's home, car, or building *Steal money or property by using force or threatening *Set fires on purpose (in order to cause damage) *Damage property on purpose *Deliberately write bad cheques, receive/sell/buy stolen goods, sell drugs, run numbers (illegal gambling), get paid for having sex with someone, or find customers for male or female prostitutes?	ANY 5 IN: M14.F, M15.B, M16.B, M17.C, M18.D, M23.B OR M29.B=3+ OR THREE OR MORE 5s OR 6s IN: M14, M14.B, M14.D, M15, M16, M17.A, (M18 OR M18.C = 5), M21, M23.1, M23.2, M23.3, M23.4, M23.A, M29 (*Failure to conform to social norms / lawful behaviours by arrestable deed)*	1 5 9
C2.* Tell a lot of lies, lie to get out of trouble or	(M11.A, M11.B1, M13	

use an alias * Often cheat (on schoolwork, exams, work, taxes) * Enjoy conning people to get one over on them 3+ times?	OR M13.A = 5 OR 6) OR (M39.B = 5) (*Deceitfulness*)	1 5 9
C3. * Not provided financial support for your family when you were supposed to *Leave young children under 6 alone while you were out doing something else *Run out of money for food for the family because you had spent it on yourself or going out (2+ times)?	M25.E, M30, M30.A, OR M33 = 5 OR 6 *(Impulsivity or failure to plan ahead)*	1 5 9
C4. * Start fights 3+ times *Often hit or assault others *Injure someone on purpose *Use a weapon (not as part of work or to defend self/others) *Force someone into sexual activity?	<u>ANY 5 OR 6 IN:</u> M6, M6.B, M6.D, M21, M27 <u>OR THREE OR MORE 5s OR 6s IN:</u> M19, M20 *(Irritable and aggressive)*	1 5 9
C5. * Left young children under 6 at home alone * Had unprotected sex (without a condom) with someone you believed could give you a disease,	(M25.B, M37, M38, OR M38.A = 5 OR 6) OR (M28 = 5 OR 6 AND	1 5 9

or when you had a disease that could be spread that way * Often taken chances where you or someone else might get physically hurt (fireworks, guns, car racing, etc)?	M28.A=3+) *(Reckless disregard for safety of self or others)*	
C6. * Failed to pay debts or take care of financial responsibilities * Have often not provided financial support for your family when you were supposed to * Were frequently late for work, or without a job for 6 months or more in the last 5 years?	(M24, M25.A, M25.C, M25.D, OR M31 = 5 OR 6) OR (M32.A=1) *(Consistent irresponsibility in work behavior or financial obligations)*	1 5 9
C7. * Often ignored the feelings of others in order to do what you wanted * Often felt that others were to blame for your mistakes * Were never faithful to a partner for more than 1 year?	(M40 = 5 OR 6) OR (M42.A = 1) OR (M42.B=5) *(Lack of remorse, being indifferent to or rationalizing having hurt, mistreated, or stolen from another)*	1 5 9
D. (Clinical judgement) Occurrence of antisocial behavior **not** exclusively during		1 5 9

schizophrenia/manic episodes?	
E. Does this participant meet criteria for ASPD = 5?: **Criteria:** Age 18+ AND (3+ 5s in B1-B15) AND (3+ 5s in C1-C7)	1 5 9 Code face sheet ASPD