SUBSTANCE USE GENETICS

Uncovering genes and testing gene-environment interplay

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PREFACE

"Alles ist mit einander verbunden"

Odar, B. bo, & Friese, J. (2017). *Dark* [Television series]. Netflix.

CHAPTER 1

General introduction

Despite some decreases in the western world in recent years, substantial groups of individuals worldwide use tobacco, alcohol, and cannabis. Twenty percent of European and US individuals smoke on a regular basis (in the age group >14 years, WHO, 2016). Almost half of all people 15 years and older have consumed alcohol in the past year, with a worldwide average intake of 14 grams of alcohol per day (about one glass; WHO, 2018). More than a quarter of Europeans aged 15-64 have used cannabis in their lifetime. In the US, this rate is more than 50% for individuals older than 15 (EMCDDA, 2011; 2020).

Consumption of tobacco, alcohol, and cannabis has deleterious health effects. Smoking is among the lead preventable causes of mortality (World Health Organization, 2017). Despite increased awareness of the dangers of smoking and reductions in smoking prevalence in the past decades, smoking rates are still substantial and the associated disease burden is large (Reitsma et al., 2017). Health risks associated with alcohol use are less widely acknowledged among the public and in most countries alcohol use policies are less stringent. Still, the risks associated with alcohol use are substantial, with 5% of deaths worldwide in 2016 (indirectly) attributable to alcohol (WHO, 2018). Despite recent legalizations and (proclaimed) benefits (Pratt et al., 2019), cannabis has also been associated with substantial health risks, including risks for respiratory disease (National Academies of Sciences & Medicine, 2017) and negative effects on brain function and development (Batalla et al., 2013).

Beside these physical effects, there are important consequences of substance use for mental health. For all three substances, regular use can develop into substance abuse and addiction. It has been estimated that 9% of cannabis users, 23% of alcohol users, and an astounding 67% of tobacco users will develop dependence (Lopez-Quintero et al., 2011). There are high rates of comorbidity between different substance use disorders, as well as between substance use disorders and other mental health problems, including anxiety, depression, and psychosis (Lai, Cleary, Sitharthan, Hunt, & Dependence, 2015; Large, Sharma, Compton, Slade, & Nielssen, 2011). Even in the absence of abuse or dependence, substance use shows associations with mental health problems. The relationships seem to be bidirectional, meaning that substance use causally contributes to psychopathology and that psychiatric symptoms can lead to increased substance use. For instance, there is evidence that smoking increases chances for depression, anxiety, and insomnia (Boden, Fergusson, & Horwood, 2010; Pedersen & Von Soest, 2009; chapter 5), that alcohol abuse increases risk for depression (Boden & Fergusson, 2011), and that cannabis increases risk for psychosis, anxiety and depression (Degenhardt, Hall, & Lynskey, 2003; Pratt et al., 2019; Volkow et al., 2016).

Given the high prevalence and health risks associated with substance use, it is important to gain insight in its etiology. Beside a plethora of environmental risk factors, genetic predisposition strongly contributes to substance use behavior. **Part 1** of my PhD thesis focuses on identifying genetic risk factors for substance use. Information from gene identification studies can be leveraged to investigate more specific questions. **Part 2** of my thesis describes 4 studies that show how findings from genetic research can be used to answer important research questions. As an exciting example, Mendelian Randomization (MR) studies use genetic information to test causal relationships between traits, something that is not viable in observational designs.

Although the focus of my thesis lies on genetic risk factors for substance use, it is unlikely that such factors operate in a vacuum. That is, many environmental factors influence substance use behaviors, and these are likely to show interplay with genetic factors. The effect of genetic influences might in some cases depend on environmental exposures. Therefore, **part 3** of my thesis focuses on the interplay between environmental and genetic factors. Figure 1 gives an overview of the 3 components of my thesis with the corresponding chapter numbers. Below, each part of my thesis is further introduced.

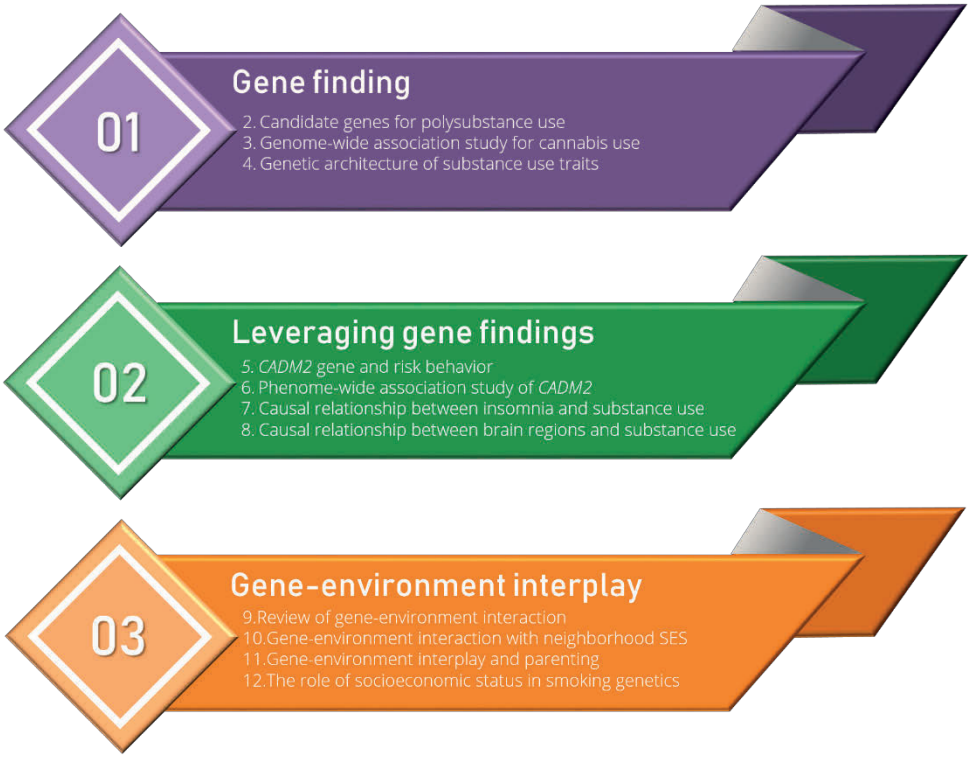

Figure 1. Thesis overview by sub theme
with corresponding chapter numbers

Part 1. Gene finding

Much research effort has been devoted to uncovering genetic factors underlying substance use. Traditionally, twin, adoption, and pedigree studies have been used to estimate what proportion of individual differences in a trait is due to genetic factors as compared to environmental factors. Such heritability research has provided crucial insights into the etiology of substance use, showing that genetic predisposition is an important contributor. For 'normative' substance use traits, such as initiation of use and quantity of use, genetic factors explain up to half of the differences between individuals, which lines up with the estimated 49% average heritability across all human traits (Polderman et al., 2015). For smoking, heritability estimates lie around 40-55% (Timberlake et al., 2006; Vink & Boomsma, 2011; Vink, Willemsen, & Boomsma, 2005). For alcohol use estimates range from 40-60% (Grant et al., 2009; Hansell et al., 2008; Lessov-Schlaggar et al., 2006). Cannabis use is estimated to be 40-48% heritable (Verweij et al., 2010). Heritability estimates seem to be generally higher for more 'problematic' substance use traits, such as abuse and dependence (Ducci & Goldman, 2012; Mbarek et al., 2015; Verweij et al., 2010; Vink et al., 2005; Walters, 2002). Another recurring finding is that the heritability increases as individuals mature, while the influence of the family environment on substance use behavior becomes less strong (Bergen, Gardner, & Kendler, 2007).

Although twin studies have proven invaluable to provide insight into the genetic contribution to substance use, they cannot uncover specific genetic variants and thus give no clues on the underlying biological mechanisms. Linkage studies have aimed to do this by studying co-segregation of genetic variants and traits in a family pedigree (Vink & Boomsma, 2002). This method performs well for identifying genetic variants linked to rare disorders, but has important limitations for studying more common traits. It has been largely abandoned in favor of other methods in the study of behavior. Candidate-gene research simply tests associations between substance use traits and a genetic variant that is hypothesized to be involved because of its biological function. For example, as the neurotransmitter dopamine is related to experiencing a substance's rewarding properties, decades of research have been devoted to finding links between dopamine regulating genes and substance use. The advantages of the candidate-gene design include the theoretical basis for biological mechanisms underlying behavior and the feasibility to study common traits, without requiring any complex pedigree data. However, candidate-gene study results have been mixed, with some studies showing clear associations whereas other did not (e.g., in smoking, Munafo, Clark, Johnstone, Murphy, & Walton, 2004). In other subject areas, as well, candidate-gene research has

been plagued by non-replication issues. This is likely mainly due to the use of small sample sizes to detect minuscule effects of single genetic variants, combined with under-publication of null-findings (e.g., Border et al., 2019; Johnson et al., 2017; Duncan & Keller, 2011). In order to circumvent such limitations, my first study investigated a pathway of multiple candidate-genes in two relatively large samples (N=2,500 and N=1,173). Investigating multiple variants in a pathway in these samples should increase the power to detect effects. We tested the association of use of tobacco, alcohol, and cannabis with a sum score of variants that had previously been associated with dopamine function and substance use (**chapter 2**). We did not detect an effect of this genetic risk score.

These findings added to a growing body of literature suggesting that candidate-gene research could not be rehabilitated by simply employing larger sample sizes. For one thing, it became more and more clear that the effects of genetic variants are even smaller than anticipated, requiring not simply large (i.e., thousands of individuals), but astronomical sample size (hundreds of thousands or even millions). Also, it appeared that the body of scientific knowledge hardly sufficed to select a good candidate-gene. In effect, it appeared that pre-selected candidate-genes did not have a higher chance to be implicated in the trait under investigation than some random other variant (Johnson et al., 2017). Fortunately, new methods had been developed that do not rely on preselection of any gene or variant, but scan the full genome (with millions of genetic variants) for any association with a trait in a hypothesis-free manner (Klein et al., 2005). Technical advances and the increasing availability of genome-wide data from large population samples have made this genome-wide association study (GWAS) approach a tremendous success, sparking an avalanche of new gene discoveries.

GWASs test the association between each measured genetic variant (generally a one-letter variation in the DNA sequence, called a single nucleotide polymorphism [SNP]) and an outcome trait. In **chapter 3**, we adopted this approach to discover genetic factors underlying lifetime cannabis use in a population sample of N=184,765 individuals. We found 8 SNPs and 35 genes that contributed to cannabis initiation, among them many that had not been implicated in cannabis or substance use before. Eleven per cent of the variation in the population in lifetime cannabis use could be explained by the genetic variants that were measured in this study (i.e., the SNP-heritability was estimated to be 11%). Only one of the proposed candidate-genes from the candidate gene study (chapter 2) was among the top findings in this GWAS, suggesting that, indeed, GWASs are now the method of choice for gene discovery studies. They can be used to generate new hypotheses on likely candidate-genes.

The 11% heritability that we found for lifetime cannabis use is substantially smaller than heritability that was estimated by twin research (about 40-50%, Verweij et al., 2010; Vink, Wolters, Neale, & Boomsma, 2010). This disparity between GWAS- and twin-based heritability is commonly observed and has been dubbed 'missing heritability' (Manolio et al., 2009). There is a plethora of possible reasons for this phenomenon. Although generally more powerful than traditional candidate-gene research, GWASs might still have limited power. Huge sample sizes are needed because of the small effect sizes and the multiple testing burden: as there are millions of variants scanned across the genome, millions of tests are conducted, each with their own margin of error. Other reasons might include that twin research has overestimated heritability, that rare variants (that have not been included in GWASs) will explain large amounts of variance, or that interactions between genes or between genes and environmental exposures mask the actual heritability. Another explanation lies in the nature of the traits we have been investigating using GWASs. The use of what I would like to call 'shallow phenotyping' could decrease our chances of detecting important associations. It results in more error and lower heritability estimates (Van Der Sluis, Verhage, Posthuma, & Dolan, 2010). Because genetic research requires such large sample sizes, collaborative data collection efforts tend to focus on including a broad range of measures that are of interest to many scientists, thereby limiting how much into depth each measure can go. For example, most databases include a question on if a person has ever used cannabis, but not on how much, how often, what kind of cannabis they used, or if they experience abuse or dependence symptoms. GWAS-heritability for one of the latter traits might turn out to be higher. Also, to reach the necessary sample sizes meta-analyses are conducted across studies that have used slightly different measures, increasing the measurement error and decreasing the GWAS-heritability. Finally, measures in general tend to be designed to capture a construct that exists as an abstraction, but does not necessarily constitute a demarcated trait in nature. Think for example of depression, a phenomenon that can be expressed in many different ways, but is often thought of as a single concept and is often analyzed as a binary diagnostic category.

Much can be gained in future years, when some or most of these limitations could be tackled. Behavior genetics is a dynamic, quickly developing field, where new techniques are constantly being presented to increase gene discovery and decrease the missing heritability. A new technique that came out during my PhD project is Genomic SEM, that can be used to follow-up on existing GWAS results (Grotzinger et al., 2018). By capitalizing on the genetic correlations between different substance use traits captured in the summary statistics from GWASs, this method is able to tell us how these traits cluster together, and what traits are more or less genetically independent from one another. Genomic SEM provides an interesting opportunity to use a more data-driven method to

study behavior, rather than approaching traits as demarcated natural entities. In **chapter 4** I use this technique to show that substance use traits cluster together per substance, but also that dependence on different kinds of substances has a common genetic architecture. Hence, we could identify genetic variants that are associated with addiction-proneness, rather than with the use of a specific substance. Also, the GWAS- heritability of the common genetic factors underlying substance use behavior was higher than the GWAS-heritability for the traits separately. Thus, techniques such as these can contribute to tackling the limitations of GWASs and discovering the missing heritability.

Over the course of Part 1 of my PhD project, I moved to increasingly powerful and sophisticated methods capitalizing on all available information to capture genetic variants associated with substance use. I abandoned candidate-gene research in favor of GWASs, and used multivariate approaches to extend the possibilities of GWASs to identify variants for overlapping and related traits. Using these methods I contributed to scientific knowledge on what variants and genes are important for substance use, providing insight into genetic architecture of related and distinct traits. Such gene discovery efforts lay the groundwork for a number of ingenious designs that can be used to answer new research questions. In part 2 and 3 of my thesis, I leveraged my own and others' gene findings to test causal relationships, to assess genetic relationships across a myriad of psycho-behavioral traits, and to create individual-level genetic risk scores.

Part 2. Leveraging gene findings

Research does not end, but rather begins, at discovering genetic variants for substance use. A profusion of techniques is continuing to be developed to use gene findings to investigate new questions. For example, in **chapter 5** we proposed a new take on the traditional candidate-gene approach, capitalizing both on the strengths of that design and GWAS techniques. Chapter 2 showed that preselected candidate-genes were not robust instruments to predict substance use. In our and other published GWASs, proposed candidate-genes are not strongly represented among the top hits, showing that they might not be the most important genetic predictors. Reversely, maybe genes uncovered by GWASs can be used as empirically based candidate-genes and tested in follow-up studies. One gene repeatedly found in risk behavior GWASs, including our lifetime cannabis use GWAS (chapter 3), is the *CADM2* gene (e.g., Liu et al., 2019; Strawbridge et al., 2018). In chapter 5 we tested the association between *CADM2* and a number of substance use and other risk behaviors, focusing on more than 4,000 variants in or near the gene (instead of one or a handful of variants, as was done in traditional candidate-gene studies). Rather than focusing on one (shallow) phenotype, we compared results for a wealth of different traits and trait clusters within the risk behavior spectrum. A big advantage was that we could use smaller samples than required in GWASs, as the multiple testing burden associated with scanning all variants in the genome was now reduced. We found associations with a range of substance use and risk behavior traits, and even found hints that the effect of *CADM2* on smoking and alcohol use was mediated by risk-taking proneness. I followed up on this work in **chapter 6** in which I examined if these *CADM2* associations were actually limited to the risk behavior spectrum by testing its association with a broad range of psycho-behavioral traits in a sizeable population sample. Indeed, it seemed that *CADM2* was involved in a number of other behaviors, as well, but effects were largest for the health behavior and substance use spectrum. These findings suggest (common) underlying biological mechanisms for these traits, that may involve CADM-mediated neural connectivity.

Another promising venue opened up by gene discovery findings is testing causal relationships between traits using a technique called Mendelian Randomization (MR). In observational research, it is unfeasible to test causal relationships due to the presence of confounding variables that are not randomly distributed in the population. Also, reverse causation can often not be ruled out. Using genetic variables as 'instruments' to measure a trait makes it possible to circumvent these limitations. The assumption is that genes are randomly distributed across the population, fixed at birth, and cannot be influenced by environmental confounders. The idea of MR is that if one finds a relationship between a

genetic instrumental variable for a trait with an outcome, it has to go via its relationship with the trait, rather than some other, non-measured variable. Thus, using genetic variants as instrumental variables enables us to make inferences about causal relationships between traits, something that is normally impossible in observational research. I made use of this Mendelian Randomization (MR) technique at several points in my PhD project.

MR can be used to test causal relationships between substance use and mental health traits, to see what causes what. In chapter 3 we used this method to show that liability to schizophrenia had a causal effect on cannabis use, but found only weak evidence for an effect of cannabis use risk on schizophrenia, a finding that stirred much media attention. In **chapter 7** I tested causal associations between different types of substance use and insomnia. I showed strong evidence that insomnia causes different smoking behaviors, alcohol dependence, and cannabis initiation. In the other direction, I found evidence only for an effect of smoking, such that being a current or ex-smoker increased risk for insomnia. These findings suggest that substance use should be targeted in insomnia therapies, with special attention for smoking, which could give rise to a vicious cycle with sleeping problems and smoking causing each other.

MR can also be used to answer more fundamental questions about the etiology of substance use. In **chapter 8** we tested if differences in subcortical brain region volumes led to substance use, or if substance use actually induces volume changes in these brain regions. We found that liability to alcohol dependence decreases amygdala and hippocampus volume, and smoking decreases pallidum and hippocampus volume. In the other direction, we hardly found evidence that subcortical brain structure volume led to substance use. These findings confirm the importance of targeting substance use to improve (mental) health.

Another way to leverage findings from gene discovery studies, perhaps the one used most often, is to create polygenic (risk) scores (PGSs). The idea is to use the results from a GWAS to create individual genetic risk profiles in a new sample. A GWAS provides a per-SNP estimate of the strength of association to a trait. Risk scores are then created by identifying the risk-alleles of these SNPs in a group of individuals and weighting them by the strength of association. In that way, each individual has a GWAS-based polygenic risk score that quantifies their genetic predisposition. For example, using the results from our cannabis GWAS, we created polygenic scores in a different sample and used these to predict rates of lifetime cannabis use. The applicability of PGSs is still limited, due to low heritability of the source GWAS and the measurement error that is also summed in the PGS. Still, on group level they can be used to answer interesting research questions. For

example, they can be used to test interplay between genetic risk and environmental circumstances.

In part 2 of my PhD thesis I showed how the results of gene finding studies can be used to answer new research questions. The advances that the GWAS-era has provided, enabled us to turn back to candidate-gene studies and to test causal associations using genetic variants as instrumental variables. In part 3 of my thesis, a different application of gene finding studies is presented. For the studies in this part I used GWAS-based polygenic risk scores to test gene-environment interplay.

Part 3. Gene-environment interplay

Environmental and genetic risk factors for substance use do not operate in isolation. For example, in the case of gene-environment interaction (GxE) the effect of genes depend on environmental characteristics. For example, it is possible that someone with a high genetic chance of becoming addicted to cannabis never gets exposed to it and does not start using, and thus never becomes addicted. Compare that to a person with a similar genetic risk who finds themselves in situations where everyone around them uses cannabis: the second person would be much more likely to start using and become dependent. Another example of gene-environment interplay is gene-environment correlation (rGE). This describes the phenomenon that someone's genetic risk for an outcome shows a relationship to an environmental characteristic. Such correlations can arise through a number of processes (Plomin, Defries, & Loehlin, 1977). Take the second person from the example above; perhaps it is no coincidence that they find themselves in a situation where they are exposed to many cannabis users. If their genetic risk for cannabis use also leads them to be more prone to risk taking, they might prefer more 'exciting' company or go clubbing more. This is referred to as 'active' rGE. In the case of 'reactive' rGE someone's genetic make-up elicits a response in the environment. For example, someone's genetic predisposition for alcohol dependence could become associated with social isolation if it contributes to behavior that is not socially accepted. In the parenting environment, rGE might also arise through overlap between parental and offspring genetic material ('passive' rGE). For example, if someone has a high genetic predisposition for smoking, it is likely that one or both parents have that as well and that they actually smoke. This could lead to a correlation between their own genetic risk for smoking and exposure to environmental smoke through parental smoking. This phenomenon has also been referred to as genetic nurture or dynastic effects. Since both genetic predisposition and parental smoking are risk factors for smoking, this could subsequently give rise to an interaction. These GxE and rGE processes can thus result in complex interrelationships between genetic and environmental risk factors, that can be hard to disentangle.

In the third part of my PhD I have focused on identifying such interplay effects in substance use. I started out conducting a systematic review on all polygenic GxE studies on substance use in **chapter 9**. I included studies that used a genetic measure that comprised more than a single variant, either using a composite of candidate-genes (as I did in chapter 2), or using GWAS-based polygenic scores. To assess study quality and the reliability of the result I designed an instrument to rate the included studies on a number of characteristics. In line with earlier observations, I found that polygenic candidate-gene

studies often lacked quality and power, even though many studies reported significant GxE findings. The studies using GWAS-based polygenic scores provided some evidence for GxE effects, mainly showing that genetic risk for substance use could be further augmented by environmental adversity. However, evidence was weak and many studies had important methodological shortcomings. Among these limitations was the neglect of rGE effects, an important oversight, as rGE could lead to spurious GxE findings. Based on my findings, I created a roadmap for future GxE research.

Following this roadmap, in **chapter 10** I aimed to increase the body of knowledge on GxE in substance use. I tested whether the chance that individuals with high genetic vulnerability for smoking, alcohol use, and cannabis use (according to their GWAS-based PGSs) would actually show high levels of substance use depended on the neighborhood they lived in. I tested 14 different GxE effects and found one significant effect, such that genetic risk for alcohol use led to more alcohol use when the neighborhood socioeconomic status was high. Although I could not control for rGE effects in the GxE analysis (as I recommended in the roadmap from study 9), I did test for rGE in separate analyses. I found some indications for rGE with covariates, but the effects were small and somewhat unexpected. Overall, the main, GxE, and rGE effects were inconclusive.

In **chapter 11** I designed a method that enabled me to test GxE while controlling for rGE, so that I could assess the relative contribution of such effects. I focused on more proximal (and perhaps more important) predictors of substance use: parenting characteristics during adolescence, around the age that most individuals start using substances. I again used PGS based on large GWASs for smoking, alcohol use, and cannabis initiation. The results showed a clear pattern of both GxE and rGE for smoking, but not for alcohol use or cannabis initiation. For smoking, it appeared that one's own genetic risk for smoking overlapped with parental risk factors, including low parental involvement, high parental substance use, and a low quality parent-child relationship. Furthermore, higher levels of these parental risk factors increased the chance that genetic risk actually led to smoking. Thus, I showed the feasibility of testing both rGE and GxE within the same model, and found convincing support that both effects contributed independently to smoking behavior.

Still, the overall image that appears from study 9-11 is that GxE effects are small and certainly not universally present for all types of environmental exposures and outcomes. One plausible explanation lies in the use of polygenic risk scores that are based on GWASs of the outcome trait. For example, to test interaction between parenting characteristics and genetic risk on smoking, I measured genetic risk with a PGS based on GWASs for smoking initiation and cigarettes smoked per day. The possible limitation of this

approach is that variants that are captured in this GWAS might increase chances for smoking per se, but not necessarily chances that someone will be vulnerable to environmental risk factors. There is a possibility that genetic susceptibility to environmental adversity is constituted by quite different variants than genetic susceptibility to the outcome itself (Fox & Beevers, 2016). Also, it may be that genetic risk for smoking is mediated by environmental factors, which would be indiscernible in a GWAS, that only picks up on main effects. In **chapter 12** I aimed to disentangle genetic effects on socioeconomic status (i.e., rGE with SES) from direct genetic effects to predict smoking status. I showed that the genetic profile for SES and smoking overlap, but that there are also specific, 'direct' genetic effects for smoking. The remaining heritability after partialling out rGE with SES was still substantial with 7% (as compared to 8% before subtracting SES). I further tested if direct genetic risk for smoking (without rGE) was more sensitive to picking up interaction effects with environmental SES. I did not find GxE with neighborhood-level SES, nor with individual-level SES as measured by educational attainment. For the latter variable, however, I did observe a pattern such that the smoking PGS only had an effect at high educational attainment, although the GxE effect did not survive correction for multiple testing. Follow-up research in larger samples is needed to corroborate this finding. For now, my findings seem to suggest once more that rGE effects are vital in the etiology of substance use, whereas GxE effects are more difficult to detect.

This thesis presents the scientific output of my journey through the field of behavior genetics. In part 1 I present research where I succeeded in capturing genetic risk factors for substance use. In part 2 I show how findings from such studies can be used to investigate exciting questions, including questions on causal relationships between traits. In part 3 I present my effort to design methods for testing gene-environment interplay, showing that environmental and genetic risk factors overlap and interact in complex ways. After reading this thesis, the reader should get an idea of the great versatility of genetic research in illuminating etiological processes underlying substance use.

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PART 1

GENE FINDING

CHAPTER 2

Hypodopaminergic polygenic risk for polysubstance use

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Abstract

Twin studies have shown substantial heritability for polysubstance use. Previous research has sought to pinpoint this genetic influence to variants in genes related to dopamine signaling, that are known to lower baseline dopamine levels (hypodopaminergic function). Candidate-gene studies often used single-gene designs and have yielded inconsistent results. Genome-wide association studies mainly include Single Nucleotide Polymorphisms (SNPs). In this study, a risk score was calculated based on both SNPs as well as Variable Number of Tandem Repeats (VNTRs).

Survey data on nicotine, alcohol, and cannabis use from two family samples were analysed (N=2435 and N=1173). Moderate and problematic polysubstance use were explored. A polygenic risk score was calculated by averaging the number of hypodopaminergic variants in three polymorphisms. Polysubstance use was regressed on this score with sex and age as covariates. Power was sufficient to detect small effect sizes (R2=0.4-0.8%).

The hypodopaminergic polygenic risk score (HPRS) was not related to polysubstance use in either sample. There were some indications for opposing effects of individual polymorphisms and separate substance use outcomes, and for an interaction of the polygenic risk score with education level. There were no effects of a score extended with extra polymorphisms, and there were no quadratic effects of the HPRS.

The HPRS did not predict polysubstance use. Several explanations for these findings were ruled out. Future research might employ more comprehensive genetic models, thereby including gene-environment interaction.

Introduction

In the Netherlands, about 25% of the population older than twelve is a current smoker, 77% is a current alcohol drinker, and 20% has used cannabis at least once (Centraal Bureau voor de Statistiek, 2016), which is largely in line with prevalence estimates from developed countries worldwide (World Health Organization, 2014, 2016a, 2016b). Factors that influence whether an individual (ab)uses a substance have been found to be shared across different substances and across moderate and problematic use patterns (e.g., Palmer et al., 2009).

Genetic predisposition may be such a shared vulnerability factor. Twin models show that the genetic factors underlying nicotine, alcohol, and cannabis use overlap to a large extent (Young et al., 2006). Molecular genetic studies show that variants associated with the use of one substance also show a relation with the use of other substances, at least when looking at the same stage of use. For example, there are substantial genetic correlations between smoking initiation and cannabis initiation, and between glasses of alcohol per week and number of cigarettes per day (Nivard et al., 2016). Likewise, genetic risk factors for smoking quantity predicted drinking quantity (Vink et al., 2014). Therefore, it is sensible to look at multiple substances concurrently when considering the etiology of substance use.

Dopamine-mediated vulnerability to substance use

Traditional candidate-gene studies have sought to pinpoint the genetic influence in substance use at dopamine-related genes. These genes are considered plausible candidates, because of the function of dopamine in the brain's reward system. Addictive substances enhance levels of dopamine, resulting in feelings of pleasure (e.g., Di Chiara and Imperato, 1988; Tanda et al., 1997). According to the reward deficiency hypothesis some individuals are more prone to substance use than others because of differences in dopamine function. It has been proposed that individuals with lower baseline dopamine levels are more easily 'bored' and will seek more stimulation in order to experience the rewarding effects of dopamine (Blum et al., 1996; Bowirrat and Oscar-Berman, 2005). Indeed, results from PET studies suggest lower dopamine receptor availability, receptor binding, and release in substance abusers than in controls (Hommer et al., 2006).

These lower homeostatic dopamine levels may be caused by variations in dopamine-related genes. For example, alleles related to deficient dopamine reception (such as TaqI A1 in DRD2) might lead to lower basal dopamine ('hypodopaminergic) function and thus to lower reward sensitivity, which might then elicit substance-seeking behavior (Blum et

al., 2016). Many similar hypodopaminergic polymorphisms have been implicated in substance use. Importantly, a large proportion of those polymorphisms are not Single Nucleotide Polymorphisms (SNPs) but Variable Number of Tandem Repeats (VNTRs). Whereas a SNP is a variation of only one nucleotide, VNTRs are variations in the length of a repeat sequence of larger units of DNA. Genome-Wide Association Studies (GWAS), which look for an association between genetic variation and a given phenotype, have as of yet not included VNTRs.

Hypodopaminergic polygenic risk score

As a reflection of dopamine-related genetic vulnerability to substance use, this study will use a genetic risk score for hypodopaminergic functioning. Variations in dopamine-related genes may lead to individual differences in basal dopamine levels, for example by influencing the number of dopamine receptors. These genes all have a small contribution, so considering them together is a more powerful method for identifying genetic risk than a single-gene approach. Although other recent studies have used polygenic risk scores in predicting substance use (e.g., Vink, et al., 2014), these generally did not include non-SNP variations.

Table 1. Overview of polymorphisms in dopamine-related genes included in the hypodopaminergic polygenic risk score.

Gene	**Polymorphism**	**Risk allele**	**Dopamine-related effect of risk allele**	**Representative literature examples**	
				supportive	**opposing**
DAT1 dopamine transporter gene	3' UTR 40-bp VNTR	10R	enhanced clearance	*Smoking:* Laucht et al. (2008); Herman et al. (2014) *Alcohol:* Schacht et al. (2013) *Drugs:* Stolf et al. (2014)	*Smoking:* Munafo et al. (2004) *Substance abuse:* Blum et al. (2013) *Polydrug use:* Conner, et al. (2010)
DRD2 dopamine receptor D2 gene	rs1800497 TaqIA SNP[a]	T	reduced D2 receptor density	*Smoking:* Munafo, et al. (2004) *Alcohol:* Smith et al. (2008) *Drugs:* Esposito-Smythers et al. (2009)	*Alcohol:* Hallikainen et al. (2003)
DRD4 dopamine receptor D4 gene	3rd exon 48-bp VNTR	long (>=7 repeats)	reduced receptor efficiency	*Addiction:* McGeary (2009) *Substance use:* Olsson et al. (2013)	*Addiction:* Comings et al. (1999)

A supportive research finding indicates that a positive relation was found between the risk allele and substance use; an opposing finding indicates a positive relation between the non-risk allele and substance use.

[a] This polymorphism was previously thought to lie in the DRD2 gene but is actually located in the ankyrin repeat and kinase domain containing 1 (ANKK1) gene next to DRD2.

To the best of our knowledge, only two studies have used a sum score of risk alleles for substance use combining both SNPs and VNTRs. One study looked at the number of alleles associated with hypodopaminergic function and found this number to be associated with the use of licit and illicit substances in adolescent males (Conner et al., 2010). In contrast, Davis and Loxton (2013) used a score of similar variants, but found that alleles associated with higher dopamine levels predicted behavioral and substance addiction. These conflicting results might be due to differences in outcome, the exact polymorphisms under study, and importantly, in the choice for which allele of each variant was considered as the risk allele.

For the current study, the literature was therefore carefully examined in order to make an informed prediction. Well-studied polymorphisms with a clear implication in dopamine function and substance use were included. Many studies showing a relation between these polymorphisms and substance use could be identified, of which representative examples are given in Table 1. However, conflicting studies were identified as well (see some examples in Table 1), underlining the need for more powerful tests of those associations. Most studies identified alleles associated with low dopamine function as conferring risk for substance use, which is in line with the reward deficiency hypothesis. Thus, for the current study, we counted the number of alleles associated with low dopamine for our genetic risk score. Although the number of selected polymorphisms is limited, previous polygenic risk score studies have

successfully predicted phenotypes using a score of only a few variants (Belsky et al., 2013; Brody et al., 2013; David et al., 2013; Davis and Loxton, 2013; Guo et al., 2015). Because heritability is likely to be overlapping for different substances, and because it appears from the literature that the same variants are implied, it is hypothesized that reward-related polymorphisms form a liability factor common to different substance use phenotypes. Thus, we predict that hypodopaminergic genetic risk predicts higher chances of having initiated use of multiple substances.

Material and methods

To test whether hypodopaminergic polygenic risk would predict polysubstance use, data of two independent family samples were utilized.

NTR sample

Participants

The first sample included participants from the Netherlands Twin Register (NTR), an ongoing longitudinal study of twins and their family members. A detailed description of study methods has been provided elsewhere (Willemsen et al., 2013). Data on substance use were collected between 1991 and 2014 in nine waves. For a subsample there were also data on SNPs and VNTRs (see Fig 1). Because of the family structure in the sample, age had a bimodal distribution, with a mean of M=22.1 (SD=3.0, N=1139) and M=47.1 (SD=11.2, N=1296). Females made up 62.8% of the sample.

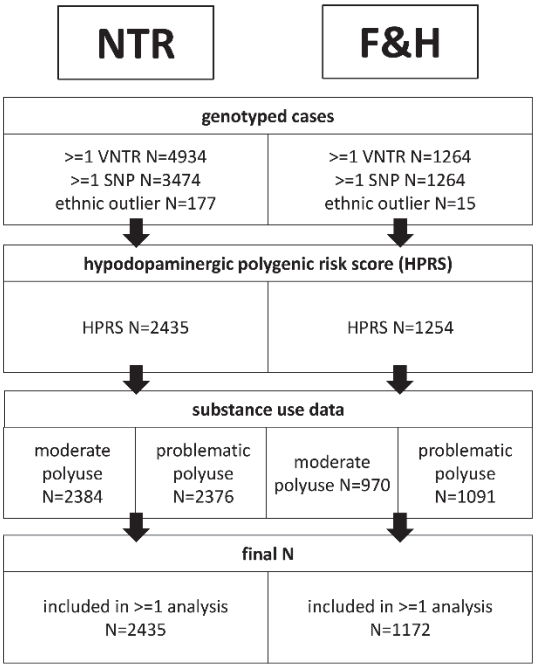

Figure 2. Data availability. Flowchart of data availability in the NTR (left) and F&H sample (right).

Genotype data

NTR participants have been genotyped for common SNPs and VNTRs using procedures described elsewhere (Huppertz et al., 2014; Willemsen et al., 2010). The hypodopaminergic polygenic risk score (HPRS) was the average number of risk alleles in the three variants. Individuals with more than one missing genotype were excluded (N=58). The formula for this procedure was:

$$HPRS = \frac{\sum(DAT1_{10R}, DRD2_{T},\ DRD4_{long})}{N_v}$$

where the numerator counts the risk alleles (0,1 or 2 per genetic variant) and the denominator Nv is the number of genotyped variants (minimal two). Thus, the HPRS reflects the mean number of risk alleles for each individual. The variants were not weighted by previously found effect sizes (as is common for polygenic risks score studies), since GWAS on which such weighting procedures are based have not included VNTRs. Principal components for genetic ancestry were not used to control for population stratification, as required data were not available for all NTR participants and no such data were available in the F&H sample. Non-Caucasian individuals were excluded from analysis (N=177) and individuals with no information on ethnicity (N=663) were kept in the analyses (results did not change when they were excluded; data not shown). The data met quality criteria for minor allele frequency (MAF>0.05), Hardy-Weinberg disequilibrium (H-W, threshold p>.001), and Mendelian errors (<.02 per variant).

F&H sample

Participants

The second sample included participants from the Dutch longitudinal Family & Health (F&H) study. Details on the F&H sample and procedures are provided elsewhere (e.g., Hiemstra et al., 2013; van der Vorst et al., 2005). The sample consisted of families of two children and both their parents (N=428 families). Survey data were collected between 2002 and 2009 in six yearly waves. DNA was collected for 1265 individuals between 2006 and 2007 via saliva sampling (see Fig 1).

Mean age was 19.0 (SD=0.8) years for the child cohort (N=621) and 49.8 (SD=3.7) years for the parent cohort (N=551). About half of the sample was female (49.4%).

Genotype data

Individual variants were genotyped using polymerase chain reaction (PCR; for details, see Hiemstra et al., 2014). The HPRS again comprised the mean number of risk alleles in the DAT1, DRD4, and DRD2 genes. Individuals born outside of Europe were excluded (N=15).

Persons with missing birth country were included (N=61; results did not change when these individuals were not considered; data not shown). The genotype variables withstood MAF (>.05) and Hardy-Weinberg quality control (p>.001).

Measures

Moderate substance use

Similar survey items were used to determine moderate substance use in both samples. Moderate polysubstance use was defined as having done at least two of the following: 1) smoked daily; 2) drank at least one glass of alcohol per day; 3) ever used cannabis (see Table 2). In the F&H sample, cannabis use was only measured in adolescents. No distinction was made between using substances concurrently or at different time points, so that an ex-smoker who currently drank alcohol was in the same category as someone who both drank and smoked currently. We expect that using such a composite will enhance power to detect effects, as it measures initiation of use of multiple substances, and will serve the purpose of capturing genetic variance common to different substance use phenotypes.

Table 2. Overview of aggregate measures included in the main and exploratory analyses, with corresponding cut-off points (if applicable) and descriptive statistics.

	NTR		**F&H**	
composits	**based on measures**	**Descriptives**	**based on measures**	**Descriptives**
HPRS mean number of risk alleles	*DAT1, DRD4, DRD2*	N=2435 M=0.81 SD=0.38	*DAT1, DRD4, DRD2*	N=1172 M=0.76 SD=0.34
eHPRS[b] mean number of risk alleles	*DAT1, DRD4, DRD2, DRD5, MAOA, OPRM1, COMT*	N=1771 M=1.01 SD=0.24	*DAT1, DRD4, DRD2, OPRM1*	N= 1122 M=1.02 SD=0.27
moderate poly use at least two substances used over time	>=1 cigarette per day[c] >=6 glasses alcohol per week & >= drinking a few times per week[d] ever use of cannabis	N=2384 prevalence= 37.2%	>=1 cigarette per day >=6 glasses alcohol per week & >= drinking a few times per week ever use of cannabis (for adolescents only)	N=970 prevalence= 62.6%
problematic poly use problematic use of at least two substances over time	FTND[a] score of >=6 CAGE score of >=2 regular cannabis use	N=2376 prevalence=2.4%	FTND score of >=6 RAPI score of >=8 regular cannabis use (for adolescents only)	N=1091 prevalence= 4.3%

education level[b]	low: medium vocational school, higher secondary school or lower high: higher vocational school/ university	N=2366 prevalence low=50.1%	low: medium vocational school, higher secondary school or lower high: higher vocational school/ university	N=1171 prevalence low=42.5%

[a] FTND= Fagerström Test of Nicotine Dependence; CAGE= alcohol problems questionnaire; RAPI= Rutgers Alcohol Problem Index; for details, see below.
[b] Explained in the exploratory analyses.
[c] Following definitions from Centraal Bureau voor de Statistiek (2016); [d] Following guidelines from Gezondheidsraad (2015). Drinking at least one glass per day corresponded to the answering category of drinking at least 6-10 glasses per week combined with drinking at least a few times per week.

Problematic substance use

For smoking, in both samples the Fagerström Test of Nicotine Dependence (FTND) was used. The 6-item FTND measures the degree of nicotine dependence (Heatherton et al., 1991). The Dutch version of this questionnaire has shown sufficient reliability and validity (Vink et al., 2005).

For drinking, the 4-item CAGE questionnaire was used in the NTR sample, and the short version of the Rutgers Alcohol Problems Index (RAPI) was used in the F&H sample. CAGE is an acronym for the four items in the questionnaire: feeling you need to Cut down on drinking; feeling Annoyed by people criticizing your drinking; feeling Guilty about drinking; and using alcohol as an Eye-opener to wake up in the morning (Mayfield et al., 1974). The Dutch version has shown sufficient quality (Aertgeerts et al., 2000). The common cut-off score of 2 has shown to yield good specificity and sensitivity (Buchsbaum et al., 1991). The RAPI is a longer instrument aimed at measuring problematic use, and includes items similar to the CAGE, such as 'was told by a friend, neighbor or relative to stop or cut down drinking' (White and Labouvie, 1989). The shortened 18-item version correlates highly with the original version, which has good measurement properties (White and Labouvie, 2000). Previous studies used a cut-off total score of >=15 for the 23-item version to classify persons as problematic users (Danielson et al., 2003; Thombs and Beck, 1994; Watt et al., 2006), which corresponds to a >=12 cut-off for the 18-item version. Although they are different instruments, the RAPI and CAGE show overlap (Myerholtz and Rosenberg, 1998).

In both samples problematic cannabis use was determined based on a question of the format 'Have you ever started using cannabis on a regular basis?'. In the F&H sample, this information was only available for adolescents. A person was considered a problematic polysubstance user when he/she had met at least two of the following criteria on at least

one measurement moment: 1) score above cut-off for the FTND; 2) score above cut-off for the CAGE/ RAPI; 3) regular use of cannabis.

Statistical analyses

A logistic generalized estimating equations (GEE) model was used to examine the relationship between the hypodopaminergic polygenic risk score and polysubstance use. GEE is a form of multilevel regression with the possibility to control for (family) clustering. Separate analyses were conducted for both outcome measures and for both samples. Age and sex were included as covariates in the analyses. Birth cohort (being a parent or a child in the family) correlated almost perfectly with age and was not included in the model. Continuous variables were centered on the sample mean.

Power

In Fig 2 power calculations are depicted for both samples. Effect sizes of individual genetic variants are commonly found to be between R2 = 0.1 to 1% (Ioannidis et al., 2006; So et al., 2011). In the current investigation, an R2 of 0.3-3% might thus be expected, since three variants were considered. As can be seen in Fig 2, power in the NTR sample is sufficient (80%) for an explained variance of approximately 0.4% or more. In the F&H sample, a larger effect size of 0.8% would be required to have an 80% chance of detecting the effect.

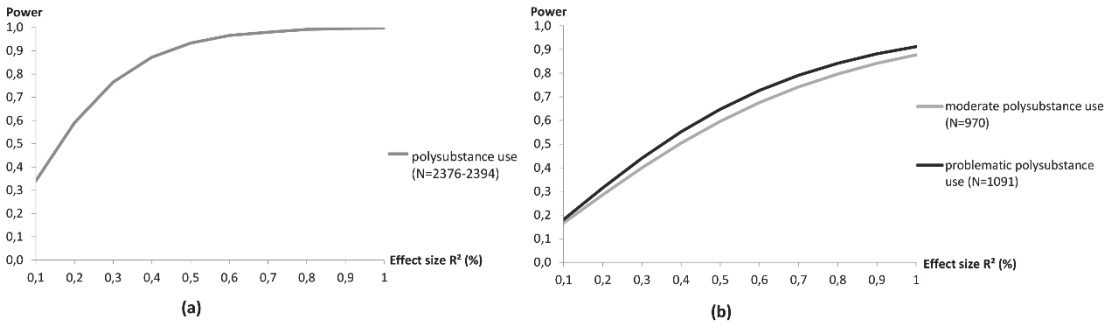

Figure 3. Power analysis. Power for main analysis in NTR (panel a) and F&H sample (panel b). In the NTR sample, sample sizes for both outcomes were very similar, so that power was estimated for both outcomes together. Estimations of effect sizes are in percentage of explained variance (R2). An α-level of .05 was used.

Results

Descriptive statistics for the predictor and outcome variables were given in Table 2. The HPRSs were normally distributed between 0 and 2 with a mean of 0.81 and 0.76, suggesting that the risk alleles were somewhat rarer than the non-risk alleles. In the NTR, 36% had ever smoked on a daily basis, 55% had drunk regularly, and 28% had used cannabis. In the F&H, these rates were 49%, 83%, and 41% (for adolescents only), respectively. Using one substance on a moderate level predicted the use of another substance on a moderate level ($\chi 2=111.9$, $p<.01$ for NTR; $\chi 2=16.6$, $p<.01$ for F&H). Likewise, the problematic substance use phenotypes were significantly related ($\chi 2=29.1$, $p<.01$ for NTR; $\chi 2=43.0$, $p<.01$ for F&H), justifying the aggregation in the polysubstance use indices.

Main effects

There was no relationship between the HPRS and moderate or problematic polysubstance use in either sample (see Table 3). In all models, there was a main effect of sex, such that males were more likely than females to show moderate and problematic polysubstance use. Results did not change when analyses were conducted separately for males and females or for young and old cohorts, or when the interactions with these factors were included in the analyses (data not shown). Effects of age were significant in some models, but the coefficients were small and in opposing directions for the samples.

Exploratory analyses

Below, possible explanations for the initial null findings are examined. Because of the post hoc nature of these tests, which increases the multiple testing burden, a stricter α-level of .01 was adopted for the exploratory analyses.

Extended risk score

As a possible explanation, it was investigated whether a more extensive measure of hypodopaminergic risk could predict polysubstance use. The HPRS was extended (eHPRS) using risk alleles in other available polymorphisms that have shown a relation with hypodopaminergic function and substance use (see Table 4). Four additional polymorphisms (in the genes *DRD5, OPRM1, COMT, MAOA*) were selected in the NTR, and one (in *OPRM1* gene) in the F&H sample. The eHPRS was calculated if data for at least five (in the NTR sample) or four polymorphisms (in the F&H sample) were available. Results showed no effect of the eHPRS on either outcome (Supplemental Table A).

Table 3. Results for main analyses using the core hypodopaminergic polygenic risk score.

		moderate poly-substance use		problematic poly-substance use	
	predictor	*b (SD)*	*p*	*b (SD)*	*p*
NTR	HPRS	0.02 (0.13)	.91	-0.02 (0.43)	.96
N= 2384 (moderate)	age	-0.02 (0.00)	<.01**	-0.01 (0.01)	.30
N= 2376 (problematic)	sex	-0.50 (0.09)	<.01**	-0.71 (0.28)	.01*
F&H	HPRS	-0.19 (0.22)	.39	-0.18 (0.48)	.71
N=970 (moderate)	age	0.05 (0.01)	<.01**	-0.04 (0.01)	<.01**
N= 1091 (problematic)	sex	0.57 (0.13)	<.01**	1.49 (0.36)	<.01**

*significant at α=.05 ** significant at α=.01

Gene-environment interaction

Possibly, the effect of the HPRS was obscured by an interaction with environmental influences. If genetic risk would lead to more polysubstance use in one environmental group, but to lower polysubstance use in another, the main effect would not be found in the combined group. One plausible environmental variable is socioeconomic status, of which education level is an important element. For example, it has been found that individuals with a higher education level have a lower risk for alcohol problems, so that they will not develop those unless they have a high genetic liability, whereas environmental risk is more important for individuals with a low education level (Latvala et al., 2011). In both the NTR and F&H sample, information on education level was available. For individuals under age 25, who may not have finished their education, parental education was used as a proxy for socioeconomic status. Education level was dichotomized using cut-offs as described in Table 2. The interaction of education level with the HPRS was explored.

Results of these analyses are summarized in Table 5. In the NTR sample, no significant main or interaction effects for education were observed. In the F&H, there was a trend for an interaction effect (p=.03), such that a higher HPRS predicted less substance use, but only for persons with a low education level.

Relation between HPRS and separate substances

Possibly, the risk score shows no association with an aggregate measure of polysubstance use, but does show an association with separate substance use types. If the association with separate substances would be in opposing directions, they would cancel each other out in the aggregate measure. To test this possibility, six GEE analyses were conducted for the substance use variables separately (moderate and problematic nicotine, alcohol, and cannabis use). No significant relations were found, although there was a trend in the

NTR sample for moderate alcohol use in the direction opposite from what was expected (p=.03, see Supplemental Table B).

Relation between polysubstance use and separate polymorphisms

In the interaction model a negative effect was found of the HPRS on substance use. Therefore, it is possible that the risk alleles were not correctly selected based on the literature. To investigate this possibility, separate GEE analyses for each individual genetic variant were conducted. The results are summarized in Supplemental Table B and C. There were only two associations significant at the α=.01 level, both in the NTR sample, between MAOA and moderate cannabis use and problematic alcohol use. There were as many variants with small positive as with small negative coefficients, so that these cancelled each other out in the sum score. Sample sizes for separate polymorphisms were smaller, so that these analyses may have been underpowered.

Quadratic effects

Both positive and negative coefficients were found for separate polymorphisms and separate substance use outcomes. Possibly, both low and high (rather than normal) dopamine function are predictive of substance use. To test this possibility, the squared centered HPRS was added as a predictor in the model. This quadratic term was zero when a person had an average number of hyperdopaminergic risk alleles, and increased when he/she had a high or low number of alleles. Analyses using this quadratic term revealed no significant associations (see Supplemental Table D).

Table 4. Summary of polymorphisms in more peripherally dopamine-related genes that were included in the explorative hypodopaminergic polygenic risk score(eHPRS).

Gene	Polymorphism	Risk allele	Dopamine-related effect of risk allele	Research findings[b] supportive	opposing
DRD5 dopamine receptor d5 gene	5' di-nucleotide repeat VNTR	148bp	non-functional[a]	*Smoking:* Sullivan et al. (2001) *Substance dependence:* Vanyukov et al. (2001)	-
OPRM1 μ-opioid receptor gene	rs1799971 A118G SNP	G	reduced release	*Smoking:* Kleinjan et al. (2013) *Alcohol:* Miranda et al. (2010) *Drug dependence:* Zhang et al. (2006)[c]	*Alcoholism:* Du and Wan (2009)[c] *Alcoholism and polysubstance abuse:* Schinka et al. (2002)
COMT catechol-O-methyltrans-ferase gene	rs4680 $Val^{158}Met$[b] SNP	G (Val)	increased catabolism	*Smoking:* Munafo et al. (2008) *Alcoholism:* Enoch et al. (2006)[c] *Cannabis:* Isir et al. (2008)[c]	*Smoking:* Beuten et al. (2006) *Alcohol:* Hendershot et al. (2012) *Cannabis:* Verdejo-García et al. (2013)
MAOA monoamine oxidase-A gene	promoter 30bp VNTR[a]	long (>=3.5 repeats)	increased catabolism	*Smoking:* Wiesbeck et al. (2006) *Alcohol:* Nilsson et al. (2011) *Drug abuse:* Gade et al. (1998)	*Smoking:* Jin et al. (2006)[c] *Alcohol:* Samochowiec et al. (2015) *Substance use disorders:* Vanyukov et al. (2007)

[a] The DRD5 polymorphism is likely to be in linkage disequilibrium with variants that decrease dopamine receptor 1 efficiency

[b] A positive research finding indicates that a positive relation was found between the risk allele and substance use; an opposing finding indicates a positive relation between the non-risk allele and substance use.

[c] Non-European ancestry study sample (i.e., Asian, Indian-American)

Table 5. Results for main analyses using the core hypodopaminergic polygenic risk score including the main and interaction effect of family education level.

		moderate polysubstance use		**problematic polysubstance use**	
	predictor	*b(SD)*	*p*	*b(SD)*	*p*
NTR	HPRS	-0.05 (0.40)	.81	-0.19 (1.23)	.85
N= 2315 (moderate)	age	-0.02 (0.00)	<.01**	-0.01 (0.01)	.32
N= 2312 (problematic)	sex	-0.52 (0.09)	<.01**	-0.71 (0.29)	.02*
	education	-0.16 (0.10)	.10	-0.49 (0.30)	.11
	HPRS*education	0.06 (0.25)	.81	0.15 (0.78)	.85
F&H	HPRS	-1.88 (0.80)	.02*	-2.81 (1.49)	.06
N=969 (moderate)	age	0.05 (0.01)	<.01**	-0.04 (0.01)	<.01
N= 1090 (problematic)	sex	0.58 (0.13)	<.01**	1.49 (0.36)	<.01**
	education	-0.16 (0.17)	.33	-0.33 (0.36)	.35
	HPRS*education	1.05 (0.47)	.03*	1.73 (0.91)	.06

*significant at α=.05 ** significant at α=.01

Discussion

This study aimed to test the association between three hypodopaminergic genetic variants and the use of multiple substances over life. In two samples, the hypodopaminergic polygenic risk score did not predict polysubstance use, and this did not change when additional polymorphisms were included.

Possible explanations

Several explanations for the null results can be offered. First, although there was sufficient power to detect an effect of at least R2=0.4-0.8%, it could be that the true effect size was smaller than that. Also, power in the problematic polysubstance use analyses might have been compromised by the low prevalence of this phenotype.

As a second explanation, an interaction between genetic vulnerability and an environmental factor could have muddled the results. It has been suggested that interaction with environmental variables is one of the reasons why molecular genetics studies succeed in explaining only small part of the heritability estimates found in twin studies (Vink, 2016). A plausible candidate for such an environmental variable is socioeconomic status, often indexed by (parental) education level. For example, it has been found that genetic factors are more important for people with a high education level than for those with a low education level in determining the risk for alcohol problems (Latvala, et al., 2011). However, the null-results were not explained by an interaction with family education level, although there were some unexpected trends in the F&H dataset, showing a stronger negative relation between genetic risk and substance use for persons with a low education level.

Third, it might be that the genetic risk scores had opposing effects on the different substance use types, thus obscuring a main effect. This could be driven by one or more polymorphisms that have shown associations in opposing directions for different substances. As we tested this, however, the polygenic risk scores showed hardly any relations with the substance use variables separately, rendering this explanation insufficient.

As a fourth explanation, it was tested whether individual polymorphisms had opposing effects. Selection of hypodopaminergic alleles related to substance use was based on an extensive literature search, but reports were not consistent. Indeed, in both samples, the selected risk alleles showed both positive and negative relations with substance use phenotypes, suggesting that they canceled each other out in the combined scores. However, these individual effects were not significant. This is in line with many studies

that did not find an effect of individual variants in dopamine-related genes on substance use (Creemers et al., 2011; Hiemstra, et al., 2014; Lind et al., 2009; Rasmussen et al., 2009), but in conflict with an even larger number of studies that did find an effect in the direction that was hypothesized or an effect in the opposing direction (see Table 1 and 4).

Finally, it was investigated if there was a quadratic effect of the risk score. This would mean that both hypo- and hyperdopaminergic alleles predict polysubstance use, in contrast to alleles related to normal dopaminergic function. Considering the opposing effects found for the candidate genes (Table 1 and 4) this is a plausible explanation. Also, of the two studies to our knowledge that used a dopaminergic polygenic risk score similar to the one in the current investigation, one found an effect of hypodopaminergic alleles on substance use (Conner, et al., 2010), but the other found a relation between a hyperdopaminergic alleles and addiction (Davis and Loxton, 2013). However, tests of a quadratic term in our study did not suggest that low and high numbers of hypodopaminergic alleles were predictive of polysubstance use as compared to average numbers of alleles.

Concluding, we could not sufficiently explain the null-results with post hoc tests. This suggests that risk alleles in dopamine-related genes do not play a vital role in predicting polysubstance use. Indeed, large GWAS for substance use phenotypes (Stringer et al., 2016; The Tobacco and Genetics Consortium, 2010; Schumann et al., 2016) have rarely identified dopamine-related polymorphisms as their top results, suggesting that these may not be as important as has traditionally been assumed. This might mean that the relation with dopamine function is more indirect. For example, a variation in the CHRNA5 gene (rs16969968) related to smoking addiction reduces nicotine receptor activity, which may hamper the eventual dopamine response to nicotine (Bierut et al., 2008). Alternatively, the genetic etiology of substance use may lie more in other mechanisms than dopamine function, such as the metabolism of the substance. For example, there are indications that variants in the alcohol dehydrogenase (ADH) gene cluster that are related to impaired alcohol metabolism lower the chances of alcohol dependence (Treutlein and Rietschel, 2011).

Strengths and limitations

This study aimed to tackle limitations of previous research. We used a design with polygenic risk scores and aggregate outcome measures in order to counteract power-problems associated with candidate-gene studies. Indeed, power was sufficient to detect reasonably small effect sizes. A second strength was that we replicated the analyses in a separate sample, which is paramount for genetic association studies (Sullivan, 2007). Also, we included VNTRs, that have as of yet not been investigated in GWAS. Furthermore,

the longitudinal nature of the data increased chances of reliably capturing substance use. Finally, we tested several explanations for our results, which gives some indication for the reliability of our findings.

An important limitation of this study lies in the identification of the genetic risk variants based on their proposed relation with hypodopaminergic function, although effects in other directions were explored. We restricted ourselves to genes for which a relation had been found, rather than genes that are in the same pathway but for which a clear effect on dopamine levels has not yet been revealed.

The use of an aggregated outcome measure might be viewed as a strength (as it should increase power to detect effects), but might also have introduced heterogeneity. Preliminary tests of the association among the separate substance use measures however suggested that aggregating them was sensible. Also, relations between the HPRS with separate substance use variables were explored, and testing an aggregate measuring use of no versus 1 or more substance did not change results (not shown). The fact that only adolescent cannabis use was available in the F&H sample is unlikely to have biased the results, as the same results were obtained in the NTR sample, where adults were included in the measure. The measure of problematic cannabis use was based on one question measuring 'regular' use, which may not reliably capture problematic use. Still, it has been found that approximate measures of regular cannabis use (e.g., having used at least ten times) already predict later abuse and dependence (Stenbacka, 2003).

Conclusions and future directions

We found a sum score of hypodopaminergic risk alleles to be unrelated to moderate and problematic polysubstance use. The most likely explanation for these findings seemed to be that these polymorphisms do not play a crucial role in substance use phenotypes. Future research might include (non-SNP) polymorphisms unrelated to dopamine, or might adopt a hypothesis-free approach to circumvent the difficulty with defining risk alleles. Also, studies should include a role for gene-environment interaction, as there were indications that this may alter results. Time may be right for more complex genome-wide models, where interaction, mediation, gene-environment correlation, and opposing effects are included to disentangle the relation between dopamine-related genes and substance use.

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Supplementary Materials

Supplemental materials can also be found online:
Chapter 2 – Candidate-genes for polysubstance use

or copy the following link into the browser:
https://drive.google.com/drive/folders/1qXFblyfbJPRzLEIBGiVTGvoqjzIA5EKv?usp=sharing

Supplementary Table A. Parameter estimates of the GEE models using the explorative hypodopaminergic risk score (eHPRS) as predictor. In other aspects identical to main analyses (using HPRS).

			moderate polysubstance use		**problematic polysubstance use**	
		predictor	*B (SD)*	*p*	*B (SD)*	*p*
NTR	N=1731 (moderate)	eHPRS	-0.22 (0.24)	.36	-0.21 (0.66)	.76
	N=1748	age	-0.02 (0.00)	<.01**	-0.01 (0.01)	.35
	(problematic)	sex	-0.50 (0.11)	<.01**	-0.75 (0.34)	.03*
F&H	N=927 (moderate)	eHPRS	-0.38 (0.29)	.18	-0.28 (0.60)	.65
	N=1045	age	0.05 (0.01)	**<.01****	-0.04 (0.10)	**<.01****
	(problematic)	sex	0.56 (0.13)	**<.01****	1.54 (0.38)	**<.01****

*significant at α=.05 ** significant at α=.01

Supplementary Table B. GEEs for individual genetic variants and separate substance use outcomes. In all models, age and sex were included as covariates, and ethnic outliers were excluded.

		NTR						F&H			
		moderate			problematic			moderate[a]		problematic[a]	
		smk	alc	can	smk	alc	can	smk	alc	smk	alc
HPRS	b	0.15	-0.26	-0.23	0.06	-0.15	0.22	-0.10	-0.32	-0.21	-0.09
	p	.24	**.03***	.10	.84	.41	.44	.57	.20	.58	.71
	N	2406	2440	2356	2325	2443	2291	1245	1177	769	1169
DAT1	b	0.08	-0.07	-0.10	-0.23	-0.09	0.19	-0.01	-0.01	-0.22	-0.02
	p	.35	.37	.23	.17	.43	.32	.93	.97	.23	.89
	N	2360	2393	2310	2279	2396	2246	1203	1136	743	1128
DRD2	b	-0.04	-0.16	-0.11	0.09	-0.02	0.03	-0.02	-0.19	0.12	0.01
	p	.69	.10	.30	.66	.88	.89	.88	.16	.55	.94
	N	1749	1768	1732	1706	1791	1694	1243	1175	768	1167
DRD4	b	0.11	0.01	-0.02	0.15	-0.03	0.04	-0.07	-0.11	0.06	-0.06
	p	.19	.95	.87	.39	.79	.82	.50	.37	.77	.68
	N	2425	2459	2372	2343	2462	2307	1241	1173	769	1165
OPRM1	b	-0.00	-0.04	-0.12	0.45	-0.10	0.46	-0.21	-0.08	-0.12	0.13
	p	.98	.75	.40	.15	.57	.18	.15	.71	.64	.53
	N	1749	1768	1732	1706	1791	1694	1244	1176	768	1168
COMT	b	-0.05	0.19	-0.01	0.01	.09	-0.21				
	p	.45	**.02***	.90	.94	.42	.26				
	N	1749	1768	1732	1706	1791	1694				
DRD5	b	-0.06	0.06	-0.07	-0.29	-0.04	-0.10				
	p	.34	.36	.29	.05	.69	.44				
	N	2426	2460	2373	2344	2463	2309				
MAOA	b	0.20	0.10	0.23	-0.11	0.34	0.38				
	p	**.01***	.17	**<.01****	.52	**<.01****	.10				
	N	2409	2443	2357	2328	2447	2293				

[a] The GEE model for cannabis use could not converge due to low variance (low sample sizes).

*significant at α=.05 **significant at α=.01

Abbreviations: smk=smoking, alc=alcohol, can=cannabis

Supplementary Table C. GEEs for individual genetic variants. In all analyses, age and sex were included as covariates, and ethnic outliers were excluded.

	NTR						F&H					
	moderate			problematic			moderate			problematic		
	N	b	*p*	N	b	*p*	N	b	*p*	N	b	*p*
DAT1	2338	0.01	.93	2329	-0.07	.77	935	0.02	.86	1054	0.02	.94
DRD2	1735	-0.11	.29	1752	0.26	.41	967	-0.07	.56	1089	-0.06	.87
DRD4	2401	0.09	.27	2395	-0.21	.42	967	-0.14	.25	1087	0.00	>.99
OPRM1	1735	-0.18	.16	1752	0.27	.50	969	-0.20	.25	1091	-0.41	.26
COMT	1735	0.07	.37	1752	0.01	.98						
DRD5	2402	-0.02	.73	2396	0.10	.60						
MAOA	2385	0.18	**.02***	2380	0.07	.77						

*significant at α=.05

Supplementary Table D. Parameter estimates of the GEE models using a squared hypodopaminergic risk score (HPRS) as an additional predictor. In other aspects identical to main analyses.

			moderate polysubstance use		problematic polysubstance use	
		predictor	*B (SD)*	*p*	*B (SD)*	*p*
NTR	N=2384 (moderate)	squared HPRS	-0.07 (0.22)	.74	-0.71 (0.58)	.22
	N=2376	HPRS	0.02 (0.13)	.87	0.05 (0.36)	.88
	(problematic)	age	-0.02 (0.00)	**<.01****	-0.01 (0.01)	.29
		sex	-0.50 (0.09)	**<.01****	-0.71 (0.28)	**.01***
F&H	N=970 (moderate)	squared HPRS	-0.37 (0.41)	.38	0.26 (0.93)	.78
	N=1091	HPRS	-0.17 (0.22)	.43	-0.18 (0.46)	.70
	(problematic)	age	0.05 (0.01)	**<.01****	-0.04 (0.10)	**<.01****
		sex	0.57 (0.13)	**<.01****	1.49 (0.36)	**<.01****

*significant at α=.05 ** significant at α=.01

CHAPTER 3

Genetic study of lifetime cannabis use reveals new risk loci, overlap with mental health, and a causal influence of schizophrenia

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Abstract

Cannabis use is a heritable trait that has been associated with adverse mental health outcomes. In the largest genome-wide association study for lifetime cannabis use to date (N=184,765), we identified 8 genome-wide significant independent single nucleotide polymorphisms in 6 regions. All measured genetic variants combined explained 11% of the variance. Gene-based tests revealed 35 significant genes in 16 regions, and S-PrediXcan analyses showed that 21 genes had different expression levels for cannabis users versus non-users. The strongest finding across the different analyses was *CADM2*, which has been associated with substance use and risk-taking. Significant genetic correlations were found with 14 of 25 tested substance use and mental health traits, including smoking, alcohol use, schizophrenia, and risk-taking. Mendelian randomization analysis showed evidence for a causal positive influence of schizophrenia risk on cannabis use. Overall, our study gives new insights about the etiology of cannabis use and its relation with mental health.

Cannabis is a widely used psychoactive substance and its use is associated with various adverse (mental) health outcomes, including psychosis and schizophrenia (Hall & Degenhardt, 2009; Moore et al., 2007; Volkow, Compton, & Weiss, 2014). Successful prevention and intervention efforts aimed at reducing cannabis use, misuse, and related outcomes require a better understanding of why some people use cannabis whereas others do not. Lifetime cannabis use, defined as any use of cannabis during lifetime, is a heritable trait: a meta-analysis of twin studies estimated the heritability to be approximately 45% (Verweij et al., 2010). Twin studies have shown there is substantial overlap in the genetic factors influencing cannabis use and those underlying problematic cannabis use (abuse/dependence) (Agrawal & Lynskey, 2006; Agrawal, Neale, Jacobson, Prescott, & Kendler, 2005).

Several genome-wide association studies (GWASs) have tried to identify genetic variants underlying cannabis use phenotypes (Agrawal et al., 2011; Demontis et al., 2018; Minica et al., 2015; Stringer et al., 2016; Verweij et al., 2013). Recently, Demontis et al. (Demontis et al., 2018) performed the largest GWAS for cannabis use disorder to date with a discovery sample of 2,387 cases and almost 50,000 controls, plus a replication sample of 5,501 cases and ~300,000 controls. They found one genome-wide significant risk locus for cannabis use disorder, a single nucleotide polymorphism (SNP) that is a strong marker for *CHRNA2* expression. Their follow-up analyses showed that cannabis dependent individuals had a decreased expression of this gene in the cerebellum as well as in other brain regions.

The largest GWAS of *lifetime* cannabis use to date is from the International Cannabis Consortium (ICC) and is based on a sample size of 32,330 individuals in the discovery sample along with 5,627 individuals in the replication sample (Stringer et al., 2016). Although no individual SNPs reached genome-wide significance, gene-based tests identified four genes significantly associated with lifetime cannabis use: *NCAM1*, *CADM2*, *SCOC*, and *KCNT2*. Notably, *NCAM1* has previously been linked to other substance use phenotypes (e.g. (Gelernter et al., 2006; Yang et al., 2007)), and following publication of the manuscript, *CADM2* was found to be associated with alcohol consumption (Clarke et al., 2017), personality (Boutwell et al., 2017), reproductive succes and risk-taking behavior (Day et al., 2016) in other GWASs. These results indicate that *CADM2* may play a role in a broader personality profile of sensation seeking and risk taking behaviour in general. In the current paper we present a GWAS of lifetime cannabis use on a substantially larger sample, providing more power to identify genetic variants.

As mentioned, cannabis use has been linked to a variety of mental health outcomes, including substance abuse and dependence and psychiatric disorders (Hall & Degenhardt, 2009). In particular, the relationship between cannabis use and

schizophrenia has been the subject of intensive research and debate. It has long been established that the prevalence of cannabis use is higher in schizophrenia patients (Andreasson, Allebeck, Engstrom, & Rydberg, 1987; Smit, Bolier, & Cuijpers, 2004). A substantial body of evidence supports the hypothesis that cannabis use increases the risk for developing psychoses and schizophrenia (Volkow et al., 2016), but other hypotheses (i.e. schizophrenia increases the use of cannabis, or the association is due to (genetic) pleiotropy) have also been posed. Previous studies have shown that genetic risk factors for cannabis use and schizophrenia are positively correlated (Power et al., 2014; Verweij et al., 2017). However, a genetic correlation does not provide insight in the direction of causation. With Mendelian randomization it is possible to examine the causality of the association between cannabis use and schizophrenia, and recently it has become possible to apply this method using summary statistics from GWASs (Burgess, Scott, Timpson, Davey Smith, & Thompson, 2015). Previous MR studies have investigated the link between lifetime cannabis use and schizophrenia, but findings were inconsistent. Vaucher et al. (Vaucher et al., 2017) tested for causal effects from cannabis use to schizophrenia and found evidence for a causal influence of cannabis use on schizophrenia risk. Gage et al. (Gage et al., 2017) tested bi-directional effects and found weak evidence for a causal effect of cannabis use on schizophrenia and much stronger evidence for a causal effect in the other direction. The results from our GWAS provide more power to examine the causal association between cannabis use and schizophrenia.

In the present study, we perform the largest GWAS for lifetime cannabis use to date. We increase the sample size substantially by meta-analysing GWAS results from the ICC study (N=35,297), along with new data from UK-Biobank (N=126,785) and 23andMe (N=22,683). The combined sample size of this study was N=184,765, a five-fold increase in sample size compared to the previous largest GWAS on lifetime cannabis use. We tested the association of millions of SNPs with lifetime cannabis use, and estimated the heritability of lifetime cannabis use based on all SNPs. Tests of association for individual genetic variants were complemented with gene-based tests of association and S-PrediXcan analysis. The latter was used to identify genes with differential expression levels in cannabis users versus non-users. We further estimated the genetic correlation of lifetime cannabis use with other traits, including use of other substances and mental health traits, such as schizophrenia. Lastly, we performed bi-directional two-sample Mendelian randomization analysis to examine whether there was evidence for a causal relationship from cannabis use to schizophrenia and vice versa.

Results

Genome-wide association meta-analysis

The meta-analysis resulted in 8 independent genome-wide significant SNP associations (linkage disequilibrium [LD] R^2<0.1, window size 250 kb) on chromosomes 3, 7, 8, 11, 16, and 17 (Table 1, Supplementary Table S1, and Figure 1). The top SNP and 2 other independent associations were located in *CADM2* on chromosome 3 (rs2875907, *p*=9.38e-17; rs1448602, *p*=6.55e-11; rs7651996, *p*=2.37e-09). Other hits were located in *ZNF704, SDK1, NCAM1*, *RABEP2/ATP2A1,* and *SMG6* (Figure 2). All SNPs combined explained 11% (h^2_{SNP}=0.11, SE=0.01) of the individual differences in lifetime cannabis use. Supplementary Figure S1-S3 and Table S2 provide information on results of the individual GWASs (ICC, UK-Biobank, and 23andme).

Gene-based test of association and expression

Gene-based tests of associations in MAGMA (De Leeuw, Mooij, Heskes, & Posthuma, 2015) identified 35 genes genome-wide significantly associated with lifetime cannabis use (see Figure 3, Table 2, Supplementary Figure S4, and Supplementary Table S3). These genes were located in 5 regions that were already identified in the SNP-based analysis (including those containing *CADM2* and *NCAM1*) and in 11 different regions (Supplementary Figure S5).

Table 1. Association results (based on linear regression) of 8 independent (R^2<0.01, window size 250 kb) SNPs that are significantly associated with lifetime cannabis use at *p*<5e-08 (conventional genome-wide significant threshold, two-sided).

SNP rs	Chr	Gene	BP	A1	A2	Freq A1	N	β	SE	*p*-value	Direction*
rs2875907	3p12.1	*CADM2*	85,518,580	A	G	0.352	181,675	0.070	0.009	9.38e-17	+++
rs1448602	3p12.1	*CADM2*	85,780,454	A	G	0.756	184,765	-0.062	0.010	6.55e-11	---
rs7651996	3p12.1	*CADM2*	85,057,349	T	G	0.477	184,765	0.049	0.008	2.37e-09	+++
rs10085617	7p22.2	*SDK1*	3,634,711	A	T	0.416	184,765	0.046	0.008	2.93e-08	+++
rs9773390	8q21.13	*ZNF704*	81,565,692	T	C	0.933	44,595	-0.171	0.029	5.66e-09	--?**
rs9919557	11q23.2	*NCAM1*	112,877,408	T	C	0.614	180,428	-0.055	0.009	9.94e-11	---
rs10499	16p11.2	*RABEP2, ATP2A1*	28,915,527	A	G	0.651	179,767	0.053	0.009	1.13e-09	+++
rs17761723	17p13.3	*SMG6*	2,107,090	T	C	0.346	184,765	0.047	0.009	3.24e-08	+++

Chromosomal region (Chr), Gene refers to the gene the SNP is located in or the nearest gene (within 500kb). eQTL target gene (eGene) obtained from the S-PrediXcan analysis, base pairs location SNP on Hg19 (BP), allele 1 (A1), allele 2 (A2), Frequency of allele 1 (Freq A1), number of individuals for which variant was included (N), beta of the effect allele A1 (β), standard error (SE).

* Direction per sample: allele A1 increases (+) or decreases (-) liability for cannabis use, or sample did not contribute to this SNP (?). Order of samples: ICC, 23andMe, UK-Biobank. Independent SNPs were selected as SNPs with linkage disequilibrium R^2<0.1 using a window size of 250 kB.

** SNP was not present in UK-Biobank sample and its effect is rather isolated (see Figure 1b and 2); it might not represent a robust association.

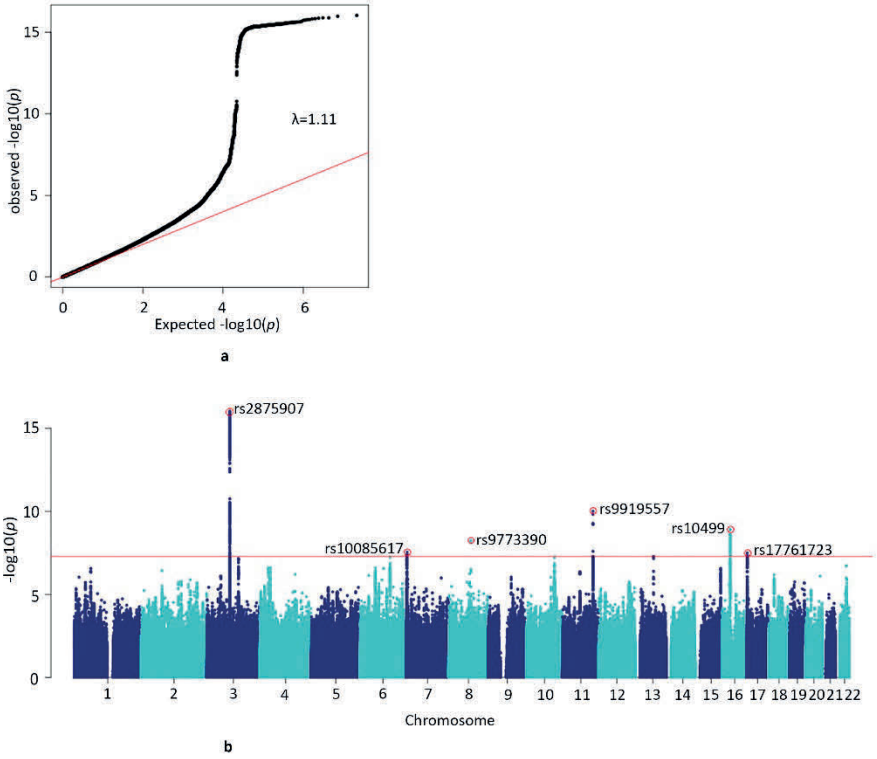


Figure 1. Q–Q and Manhattan plot of the GWAS meta-analysis. **a)** Q–Q plot of the distribution of the –log 10(P) observed for the SNP associations with lifetime cannabis use against those expected under the null hypothesis. Expected –log 10(P) values under the null hypothesis are indicated by the red line. Genomic inflation is indicated by λ in the plot. There was no evidence for population stratification (LD score regression b0 = 1.00, s.e. = 0.007). b, Manhattan plot for the SNP-based GWAS meta-analysis. Results are based on N = 184,765 individuals and NSNPs = 11,733,371 SNPs. The SNP with the lowest P-value for each independent (R2 < 0.1, window size 250 kb) genome-wide significant locus is annotated by a red circle with rsnumber. The red line represents the conventional genome-wide significance threshold of P < 5 × 10–8. The statistical test comprised linear regression; significance was tested two-sided.

S-PrediXcan analysis (Barbeira et al., 2017) revealed 133 Bonferroni-corrected significant associations across tissues targeting 21 unique genes (Supplementary Table S42-S5). Eight genes were also significant in the gene-based test, whereas 13 were novel. For genes identified in multiple tissues, directions of effects were largely consistent across tissues (Supplementary Figure S6). Again, the most significant finding was *CADM2;* genetic variants associated with increased liability to use cannabis are predicted to upregulate expression levels of *CADM2* in 8 (non-brain) tissues, including whole blood (Z=5.88,

p=4.17e-09). Of note, although *CADM2* is expressed more widely in brain compared with other tissues (Supplementary Figure S7), the top SNP rs2875907 regulates the expression of *CADM2* only in non-brain tissues (Supplementary Figure S8). Exploration of S-PrediXcan results in UK-Biobank data (https://imlab.shinyapps.io/ gene2pheno_ukb_neale/) showed that *CADM2* expression is significantly associated with multiple traits, including increased risk-taking, BMI, and reduced feelings of anxiety. Similar to the SNP- and gene-based tests of association, the S-PrediXcan analysis detected a strong signal in a high-LD region at 16p11.2. Supplementary Table S3 provides an overview of all genes that were identified in the gene-based test of association and the S-PrediXcan analyses, along with information about the gene-product and previously identified associations with the gene.

Table 2. Genes significantly associated with lifetime cannabis use, as identified in the MAGMA (p<2.74e-06, which is p<0.05 corrected for 18,293 genes tested) and/or S-PrediXcan analyses (p< 1.92e-07, which is p<0.05 corrected for the 259,825 genes and tissues tested). The MAGMA statistical test is based on multiple regression. Genes that were significant only in the S-PrediXcan analysis are hightlighted in grey.

Locus	Top genes	BP start	BP stop	#SNPs	Z	*p*-value
1p36.31	*KLHL21*	6,640,784	6,672,958	96	4.81	7.65e-07
	PHF13	6,663,756	6,694,093	84	4.61	1.99e-06
2p12	*LRRTM4*	76,969,849	77,754,502	3621	5.19	1.03e-07
3p12.1	*CADM2*	85,003,133	86,128,579	4287	8.96	1.59E-19
4p16.3	*MSANTD1*	3,240,766	3,283,465	231	4.59	2.22e-06
5q12.3	*HTR1A*	63,245,875	63,268,119	64	4.57	2.41e-06
6p12.1	*BEND6*	56,814,773	56,897,450	252	5.22	2.60e-08
	KIAA1586	56,906,343	56,925,023	58	5.09	1.75e-07
	RAB23	57,046,790	57,092,112	86	5.86	2.32e-09
6q21	*REV3L*	111,610,234	111,814,421	539	4.61	1.99e-06
6q25.3	*ARID1B*	157,093,980	157,536,913	1344	5.59	1.15e-08
8q24.3	*ADGRB1*	143,535,377	143,636,369	275	4.71	1.23e-06
10q24.32-33	*NEURL*	103,493,890	103,592,552	17	5.22	1.83e-07
	BORCS7	104,603,967	104,634,718	87	4.72	1.19e-06
	AS3MT	104,624,183	104,666,656	177	5.54	1.53e-08
	CNNM2	104,673,075	104,843,344	549	4.80	8.02e-07
	NT5C2	104,842,774	104,958,063	389	4.81	7.64e-07
11q23.2	*NCAM1*	112,826,969	113,154,158	1263	6.21	2.63e-10
12q24.12	*BRAP*	112,069,950	112,133,790	97	4.87	5.48e-07
	ACAD10	112,118,857	112,199,911	141	5.22	8.96e-08
	ALDH2	112,199,691	112,252,789	112	4.96	3.61e-07

	MAPKAPK5	112,275,032	112,336,228	195	4.87	5.58e-07
	TMEM116	112,364,086	112,456,023	222	4.94	3.96E-07
16p11.2/ 16q12.1	*SBK1*	28,303,840	28335170	23	5.47	4.52e-08
	NPIPB7	28,467,693	28481868	10	5.44	5.46e-08
	CLN3	28,483,600	28,510,897	62	5.84	2.56e-09
	APOBR	28,500,970	28,515,291	49	5.66	7.56e-09
	IL27	28,505,683	28,523,155	57	5.66	7.48e-09
	CCDC101	28,560,249	28,608,111	181	4.90	4.87e-07
	SULT1A2	28,603,264	28,608,391	25	5.40	6.66e-08
	SULT1A1	28,605,196	28,623,625	51	5.30	1.14e-07
	*CDC37P1**	28,700,176	28,701,611	31	5.26	1.42e-07
	EIF3C	28,722,782	28,747,053	14	5.37	8.08-08
	EIF3CL	28,722,785	28,747,053	23	5.47	4.55e-08
	NPIPB9	28,742,728	28,772,850	8	5.41	6.29e-08
	ATXN2L	28,829,369	28,853,558	89	5.85	2.50e-09
	TUFM	28,848,732	28,862,729	55	5.83	2.83e-09
	SH2B1	28,867,939	28,890,534	71	5.72	5.46e-09
	ATP2A1	28,884,192	28,920,830	89	5.97	1.20e-09
	NFATC2IP	28,962,318	28,977,767	8	5.35	8.82e-08
	RABEP2	28,910,742	28,942,339	71	5.43	2.84e-08
17p13.3	*SRR*	2,202,244	2,233,553	121	5.33	5.03e-08
	TSR1	2,220,972	2,245,678	90	5.59	1.12e-08
18q11.2	*C18orf8*	21,078,434	21,118,311	132	5.30	5.65e-08
	NPC1	21,081,148	21,171,581	257	5.30	5.87e-08

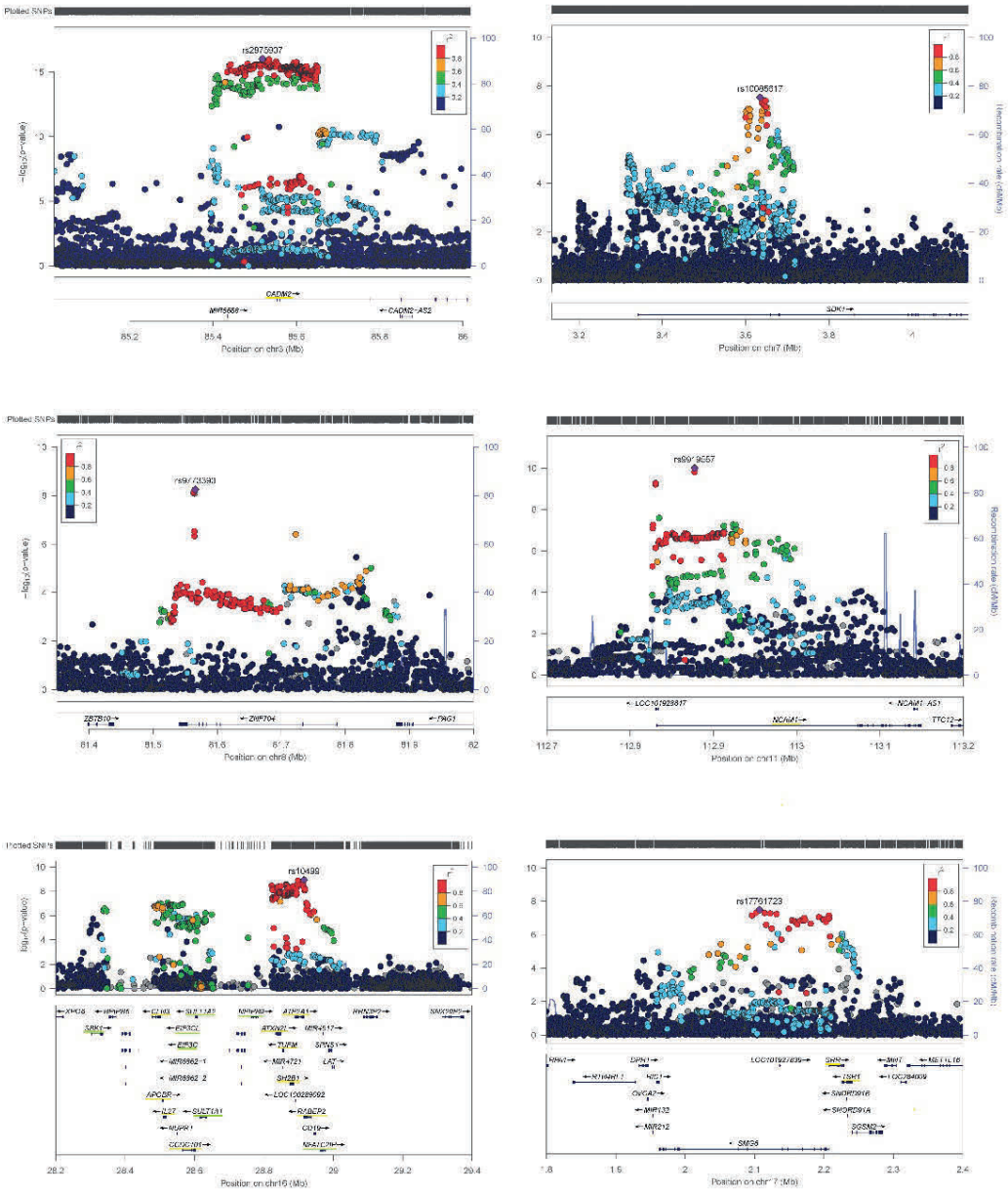
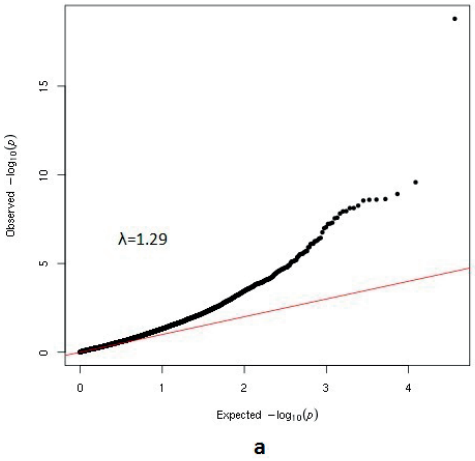
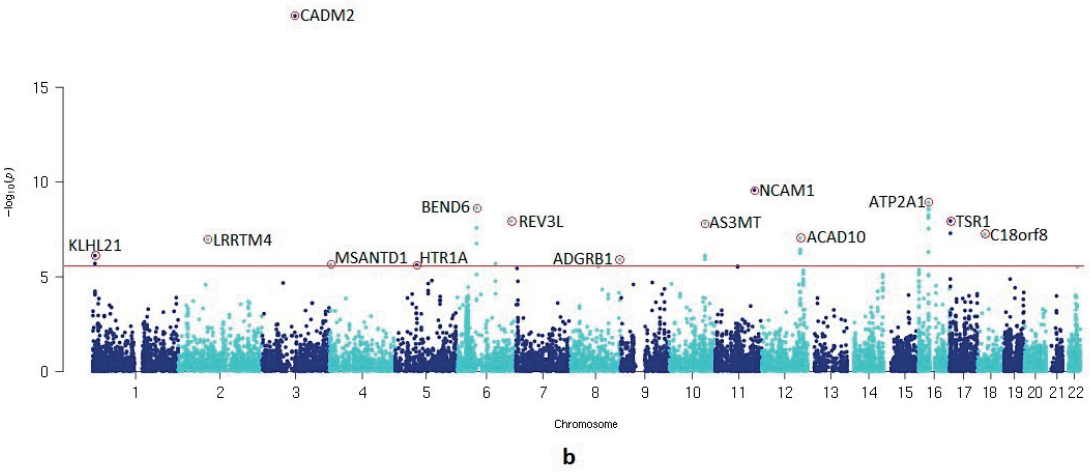


Figure 2. Regional plots of the genome-wide significant SNPs. Underlined in yellow are the genes that were significant in the gene-based test (tested



a



b

Figure 3. Q–Q and Manhattan plot of the gene-based test of association. a, Q–Q plot of the distribution of the –log10(P) for the gene-based association with lifetime cannabis use against those expected under the null hypothesis. Expected –log 10(P) under the null hypothesis are indicated by the red line. Genomic inflation is indicated by λ . The gene-based test was performed in MAGMA, which uses multiple regression (tested two-sided). b, Manhattan plotfor the gene-based test of association. The red line represents the genome-wide significance threshold of P < 2.74 × 10–6 (Bonferroni corrected threshold of P < 0.05 adjusted for 18,293 tests; NSNPs = 5,710,956 were mapped to at least one gene). The top gene (that with the lowest P-value) for each locus is annotated by a red circle and gene symbol.

Genetic correlations with other traits

Using our GWAS results and those of other GWASs, we estimated the genetic correlation of lifetime cannabis use with 25 traits of interest, including substance use, personality, and mental health phenotypes. Fourteen traits were significantly genetically correlated with lifetime cannabis use after correction for multiple testing (Figure 4 and Supplementary Table S6). Positive genetic correlations were found with substance use phenotypes, including smoking and alcohol use and dependence, as well as with mental health phenotypes, including ADHD and schizophrenia. Furthermore, positive genetic correlations were found with risk-taking behaviour, openness to experience, and educational attainment, as well as a negative correlation with conscientiousness.

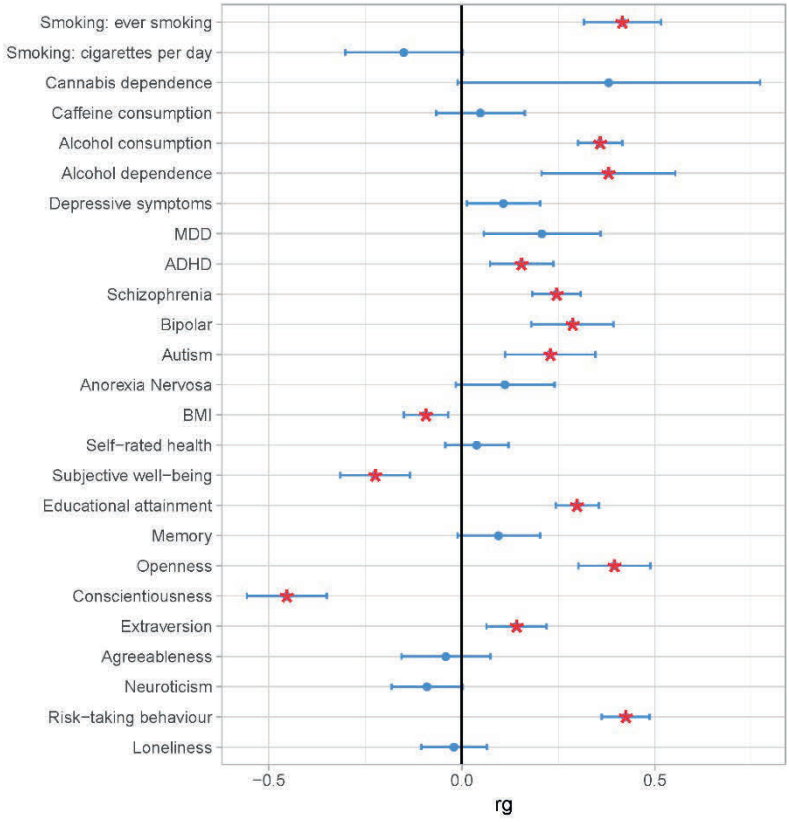


Figure 4. Genetic overlap between lifetime cannabis use and other phenotypes. Blue dots represent point estimates of the genetic correlation, blue error bars represent 95% confidence intervals and red asterisks indicate significant associations after correction for multiple testing (two-sided $P < 0.002$, Bonferroni corrected threshold of $P < 0.05$ adjusted for 25 tests). MDD, major depressive disorder; ADHD, attention deficit hyperactivity disorder; BMI, body mass index.

Causal association between cannabis use and schizophrenia: Two-sample Mendelian randomization

A positive genetic correlation was found between genetic risk factors for cannabis use and schizophrenia (r_g=0.24, SE=0.03, p<0.01). To examine whether there was evidence for a causal effect of cannabis use on schizophrenia risk and vice versa we performed bi-directional two-sample Mendelian randomization (MR) analysis (Burgess et al., 2015). In our main analysis (Inverse Variance Weighted [IVW] regression analysis) we found some weak (non-significant) evidence for a causal influence of lifetime cannabis use on schizophrenia risk, but only for the genetic instrument containing SNPs associated with cannabis use under the *p*-value threshold 1e-05. The IVW regression odds ratio was 1.10 (95% confidence intervals [CIs] 0.99-1.21, p=0.074). We found stronger evidence for a causal positive influence of schizophrenia risk on lifetime cannabis use, the IVW regression odds ratio being 1.16 (95% CIs 1.06-1.27, p=0.001; see Table 3, Supplementary Table S7-S9, and Supplementary Figure S9-S10 for details).

Table 3. Results of the bidirectional two-sample Mendelian randomization analysis between lifetime cannabis use and schizophrenia including results of 4 sensitivity analyses. Significant results (p<0.05; two-sided) are shown in bold.

	Cannabis-Schizophrenia *(p<5e-08, 5 SNPs)*				***Cannabis-Schizophrenia*** *(p<1e-05, 69 SNPs*)*				***Schizophrenia-Cannabis*** *(p<5e-08, 109 SNPs**)*			
	B	**SE**	**OR**	***p***	**B**	**SE**	**OR**	***p***	**B**	**SE**	**OR**	***p***
IVW	0.039	0.158	1.04	0.806	0.091	0.051	1.10	0.074	0.151	0.046	1.16	**0.001**
Weighted Median	-0.048	0.105	0.95	0.649	0.069	0.049	1.07	0.156	0.163	0.049	1.17	**0.001**
MR-Egger SIMEX	-0.044	0.190	0.96	0.827	0.106	0.110	1.11	0.340	0.071	0.293	1.07	0.810
Weighted Mode	-0.084	0.125	0.92	0.536	0.016	0.071	1.02	0.823	0.315	0.178	1.37	0.080
GSMR after HEIDI filtering	-	-	-	-	0.192	0.080	1.21	**0.017**	0.237	0.038	1.27	**5.36e-10**

Inverse Variance Weighted regression analysis (IVW); MR-Egger simulation extrapolation (SIMEX); Generalized Summary-data-based Mendelian Randomization (GSMR; HEIDI outlier analysis detects and eliminates from the analysis instruments that show significant pleiotropic effects on both risk factor and disease); risk coefficient representing the change in outcome for a one-unit increase in the exposure variable (B); standard error of the B (SE (B)); odds ratios represent the odds of schizophrenia for lifetime cannabis users versus non-users (when cannabis is the exposure) or the odds of lifetime cannabis use for those with a schizophrenia diagnosis versus those without (when schizophrenia is the exposure) (OR); p-value (p). *Number of SNPs in instrument was 74 for the GSMR analysis; ** Number of SNPs in instrument was 102 for the GSMR analysis.

We performed 4 sensitivity analyses that rely on distinct assumptions regarding instrument validity, to determine the robustness of these findings. The sensitivity analyses showed a consistent pattern supporting weak evidence for a causal effect of cannabis use on schizophrenia and strong evidence for a causal effect of schizophrenia on cannabis use (Table 3). As an exception, the evidence provided by MR-Egger SIMEX for a causal relation from schizophrenia risk to cannabis use, was very weak. However, since the Egger intercept was not significantly different from 0 (Supplementary Table S10), indicating no pleiotropic effects for the SNPs included in the genetic instruments (Bowden, Davey Smith, & Burgess, 2015), it is likely that this method simply lacked power to be able to reject the null hypothesis of no causal effect (Burgess & Thompson, 2017).

Discussion

SNP- and gene-based tests revealed several SNPs and genes strongly associated with lifetime cannabis use. Overall, 11% of the variation in the phenotype was explained by the combined effect of SNPs, which amounts to approximately 25% of twin-based heritability estimates (Verweij et al., 2010). *CADM2* and *NCAM1,* both identified in the original ICC meta-analysis (Stringer et al., 2016), were among the strongest findings in the SNP-based and gene-based tests. The *CADM2* gene (Cell Adhesion Molecule 2) is a synaptic cell adhesion molecule and is part of the immunoglobulin superfamily. Interestingly, *CADM2* has previously been identified in GWASs of other behavioural phenotypes, including alcohol consumption (Clarke et al., 2017), processing speed (Ibrahim-Verbaas et al., 2016), and number of offspring and risk-taking behavior (Day et al., 2016). A large-scale phenome-wide scan showed that *CADM2* was associated with various personality traits, with the risk variant being associated with reduced anxiety, neuroticism and conscientiousness, and increased risk-taking (Boutwell et al., 2017). Taken together, these findings suggest that risk variants in *CADM2* are associated with a broad profile of a risk-taking, optimistic, and care-free personality (Boutwell et al., 2017). Cannabis use has previously been associated with related personality traits, including high levels of impulsivity and novelty seeking (Martin et al., 2002; Walther, Morgenstern, & Hanewinkel, 2012).

NCAM1 (Neural Cell Adhesion Molecule 1) also encodes a cell adhesion protein and is member of the immunoglobulin superfamily. The encoded protein is involved in cell-matrix interactions and cell differentiation during development (Nielsen, Kulahin, & Walmod, 2010). *NCAM1* is located in the *NCAM1-TTC12-ANKK1-DRD2* gene cluster, which is related to neurogenesis and dopaminergic neurotransmission. This gene cluster has been associated with smoking, alcohol use, and illicit drug use (Bidwell et al., 2015; Ducci et al., 2011; Gelernter et al., 2006; Rubinek et al., 2003) and has been implicated in psychiatric disorders, such as schizophrenia and mood disorders (Atz, Rollins, & Vawter, 2007; Petrovska et al., 2017).

A putatively novel finding comprises the 16p11.2 region (identified in the SNP and gene-based tests of association and in S-PrediXcan analysis). Deletions and duplications in this region have previously been reported to be associated with autism and schizophrenia (Mccarthy et al., 2009; Weiss et al., 2008), while a common 16p11.2 inversion underlies susceptibility to asthma and obesity (Gonzalez et al., 2014). The inversion explains a substantial proportion of variability in expression of multiple genes in this region, including *TUFM* and *SH2B1* (Gonzalez et al., 2014). Given the high LD in this region and

high levels of co-expression of the differentially expressed genes, follow-up studies will be needed to determine which genes are functionally driving the association with cannabis use.

Several of the top genes from the gene-based and/or S-PrediXcan analyses have previously shown an association with other traits, including schizophrenia (e.g., *TUFM*, *NCAM1*), BMI or obesity (e.g. *SH2B1, APOBR, ATXN2L*), alcohol use (e.g. *ALDH2*), intelligence and cognitive performance (*CNNM2, CCDC101*), and externalizing and impulsive phenotypes (e.g. *CADM2*; see Supplementary Table S4). Interesting is also the association with *HTR1A*; this gene has been implicated in alcohol and nicotine co-dependence (Zuo et al., 2013), BMI (Speliotes et al., 2010), psychiatric disorders (Donaldson et al., 2016; Gatt, Burton, Williams, & Schofield, 2015), and antipsychotic pharmacological treatment response (Takekita et al., 2016). At the phenotypic level, associations between cannabis use and psychiatric disorders (Moore et al., 2007) and use of other substances (Walther et al., 2012) are well-established.

There are two previous studies that found significant SNP associations for a cannabis use phenotype. Sherva et al. (2016, (Sherva et al., 2016)) found 3 SNPs significantly associated with cannabis dependence. In our results only one of the SNPs was available (rs77378271) and was not significantly associated with lifetime cannabis use (p=.144). The other 2 SNPs (rs143244591 and rs146091982) or their high LD proxies were not available in our data. The SNPs rs77378271 and rs146091982 were located in genes CSDM1 and SLC35G1 respectively, and neither of those were significant in our gene-based results (p=0.96 and p=0.49, respectively). Demontis et al. (Demontis et al., 2018) found one independent significant signal at chromosome 8 to be associated with cannabis dependence (with top SNP rs56372821, a strong eQTL for *CHRNA2*). Neither the SNP (p=0.55) nor the gene (p=0.52) was significantly associated with lifetime cannabis use in our study. The protein encoded by *CHRNA2* is a subunit of certain nicotinic acetylcholine receptors and Demontis et al. (Demontis et al., 2018) provide three potential biological explanations for the link between cannabis intake and *CHRNA2*. However, it is possible that while *CHRNA2* is associated with cannabis dependence, it does not play a role in the initial stages of cannabis use, which are more related to personality and risk-taking behaviours and less to the actual effects of cannabis intake on the brain.

The genetic correlation analyses revealed genetic overlap of cannabis use with a broad range of traits, including positive associations with substance use and mental health phenotypes. Furthermore, positive genetic correlations were found with risk-taking behaviour, openness to experience, and educational attainment, as well as a negative correlation with conscientiousness. The range of correlations suggests that genetic

liability to lifetime cannabis use should be viewed in a broader context of personality and mental health traits. Specifically, the substantial genetic correlations with risk-taking behaviour and openness to experience may indicate that liability to start using cannabis is an indication of one's personality. The positive genetic correlation between lifetime cannabis use and educational attainment was unexpected and in contrast to a previous study that found a negative genetic correlation between cannabis dependence and educational attainment (Demontis et al., 2018). We therefore investigated phenotypic associations of cannabis use with household income and fluid intelligence using UK-Biobank data. Within Caucasian participants of UK-Biobank (N=438,870), categorically rated household income was higher among lifetime cannabis users compared to non-users ($\chi^2(4)=2243$, $p=2.2e-16$). Cannabis users also scored higher on fluid intelligence ($t(50,856)=25.13$, $p<2e-16$). These findings are in agreement with observations by Patrick et al. (Patrick, Wightman, Schoeni, & Schulenberg, 2012), who showed that cannabis use is associated with higher childhood family social economic status (SES) in a survey of US families. Possibly, environments more often experienced by those with higher SES backgrounds, such as universities, increase accessibility to cannabis, explaining how a positive correlation between lifetime cannabis use and educational attainment in our study could arise.

We also found a significant genetic correlation between cannabis use and schizophrenia ($r_g=0.24$), which is in line with previous findings (Power et al., 2014; Verweij et al., 2017), indicating that genetic risk factors for cannabis use and schizophrenia are positively correlated. As for the causal direction of this correlation, we found weak evidence for a causal link from cannabis use to schizophrenia and much stronger evidence for a causal link from schizophrenia to cannabis use. This suggests that individuals with schizophrenia have a higher risk to start using cannabis. These results are in contrast with results from a MR study by Vaucher et al. (Vaucher et al., 2017), who found strong evidence for a causal effect from cannabis use to schizophrenia (causality in the other direction was not tested). However, our findings are in line with a MR study by Gage et al. (Gage et al., 2017) who used genetic instruments similar to ours and also found weak evidence for a causal effect of cannabis use to schizophrenia and much stronger evidence for a causal effect in the other direction. Our findings may indicate that individuals at risk for developing schizophrenia experience prodromal symptoms or negative affect that make them more likely to start using cannabis to cope or self-medicate (Muller-Vahl & Emrich, 2008). The lack of strong evidence of a causal influence of cannabis use on schizophrenia may be due to the lower power of the instrumental variables. The instrumental variable based on schizophrenia SNPs explained 3.38% of variance in liability to schizophrenia. For cannabis use, the genetic instruments explained 1.12% and 0.15% of the variance in cannabis use for SNPs included with $p<1e-05$ and $p<5e-08$, respectively.

The results of our study should be interpreted in view of its strength and limitations. Important strengths of this study include the analyses of the largest population sample to date which has led to a substantial increase in power to identify genetic variants associated with lifetime cannabis use. The association analyses were complemented with several follow-up analyses to further investigate the genetic basis of cannabis use and the extent to which the genetic aetiology of cannabis use overlaps with that of other complex phenotypes. Strong genetic correlations across a wide spectrum of traits are observed, confirming that lifetime cannabis use is a relevant measure of an individual's vulnerability.

Our study also has several limitations. First, lifetime cannabis use was analyzed as a dichotomous measure combining experimental and regular users in a single group. Additionally, the different samples varied substantially regarding the age of the participants, the prevalence of cannabis use, and the country's policies regarding cannabis use. All these factors may introduce heterogeneity which may reduce the power to detect genetic associations. Secondly, power of some analyses may have been limited. For example, the MR analysis from cannabis to schizophrenia was based on an instrument of only 5 SNPs, and the summary statistics of some traits used for the genetic correlation analyses in LD-score regression (e.g. cannabis dependence) were based on a small sample size. Finally, some regions identified in the SNP-based analyses did not appear in the gene-based analyses. In particular, inspection of the region around rs9773390 (in *ZNF704*) showed that the top SNP in this region was isolated, and that the SNP was only available in two of the three datasets (not in UK-Biobank). SNPs in LD with the top SNP that were included in all three datasets were not genome-wide significant. Thus, this result may not represent a robust association.

In summary, our GWAS of lifetime cannabis use, which is the largest to date, revealed significant SNP and gene associations in 16 regions, 14 of which have not been previously implicated in cannabis use. The most promising candidates for future functional studies are *CADM2*, *NCAM1,* and multiple genes located at 16p11.2. Our findings further indicated a causal influence of schizophrenia on cannabis use and substantial genetic overlap between cannabis use and use of other substances, mental health traits, and personality traits, including smoking and alcohol use, schizophrenia, ADHD, and risk-taking.

Online methods

Samples

Data from three sources were obtained: ICC, 23andMe, and UK-Biobank (total N=184,765). We used existing GWAS summary statistics from the **ICC**, based on data from 35,297 individuals of European ancestry from 16 cohorts from Northern America, Europe, and Australia (Stringer et al., 2016). Details regarding ethical approval and informed consents of the ICC cohorts can be found in the supplementary material of the original ICC paper (Stringer et al., 2016). The overall sample included 55.5% females and the age ranged between 16 and 87 years with a mean of 35.7 years. An average of 42.8% of the individuals had used cannabis during their lifetime. The second set of results was derived from the personal genetics company **23andMe** Inc.. Data were available for 22,683 individuals of European Ancestry who provided informed consent and answered surveys online according to a human subjects protocol approved by Ethical & Independent Review Services, a private institutional review board. The sample included 55.3% females and the age ranged between 18 and 94 years with a mean of 54.0 years. Within the sample, 43.2% had used cannabis during their lifetime. The third sample was obtained from **UK-Biobank**. Data were available for 126,785 individuals of European ancestry. The sample included 56.3% females and the age ranged between 39 and 72 years with a mean of 55.0 years. Within the sample, 22.3% had used cannabis during their lifetime. Ethical approval for UK-Biobank data collection procedures has been provided by the North West Multi-centre Research Ethics Committee (MREC), the National Information Governance Board for Health & Social Care (NIGB), and the Community Health Index Advisory Group (CHIAG).

Phenotype and covariates

For all participants, self-report data were available on whether the participant had ever used cannabis during their lifetime: yes (1) versus no (0). Measurement instruments and phrasing of the questions about lifetime cannabis use differed across the samples. For the ICC study this has been described for each cohort in the original paper (Stringer et al., 2016). As part of their online questionnaire, 23andMe used the following phrase to examine lifetime cannabis use: 'Have you ever in your life used the following: Marijuana?'. The UK-Biobank – as part of an online follow-up questionnaire - asked: 'Have you taken CANNABIS (marijuana, grass, hash, ganja, blow, draw, skunk, weed, spliff, dope), even if it was a long time ago?'.

Genotyping and imputation

Genotyping was performed on various genotyping platforms and standard quality control checks were performed prior to imputation. Genotype data were imputed using the 1000

Genomes phase 1 release reference set (Abecasis et al., 2012) for ICC and 23andMe, and the Haplotype Reference Consortium reference set (Mccarthy & Das, 2016) for the UK-Biobank sample. Information about samples, genotyping, imputation, and quality control is summarized in Supplementary Table S8. After quality control, the ICC sample comprised 35,297 individuals and 6,643,927SNPs, the 23andMe sample 22,683 individuals and 7,837,888 SNPs, and the UK-Biobank sample 126,785 individuals and 10,827,718 SNPs.

Statistics

All statistical tests were two-sided and –unless stated otherwise- we used the conventional *p*-value of 0.05 for significance testing. When necessary, Bonferroni correction for multiple testing was applied. Randomization and blinding procedures do not apply to our study design.

Genome-wide association analyses and meta-analysis

We conducted the GWASs in 23andMe and UK-Biobank samples separately. Associations between the binary phenotype and SNPs were tested using a logistic regression model accounting for the effects of sex, age, ancestry, and genotype batch (and age^2 in the UK-Biobank sample). The GWAS for UK-Biobank was performed in PLINK 1.9 (Chang et al., 2015) and for 23andMe using an internally developed pipeline. We then meta-analyzed the GWAS results from ICC, 23andMe, and UK-Biobank. Prior to conducting the meta-analysis, additional quality control of the summary statistics of each study was conducted in EasyQC (Winkler & Day, 2014). Because of varying GWAS methods and sample characteristics, slightly different quality control criteria were used for the 3 samples (see Supplementary Table S8). All 3 samples were aligned with the Haplotype Reference Consortium panel using the EasyQC R-package (Winkler & Day, 2014), to ensure that rs-numbers and chromosome-basepair positions referred to the exact same variants and to correct for strand effects. Variants were deleted if they had a minor allele frequency (MAF) diverging more than 0.15 from that in the reference panel.

We applied genomic control to the three GWAS files prior to meta-analysis. Inflation due to stratification was estimated using LD-score regression, which can differentiate inflation due to population stratification from that due to real signal. The intercept was used to correct the standard errors (SEs) of the estimated effect sizes as follows: $SE_{GC} = \sqrt{LDSC\ intercept * SE^2}$. The intercepts were b_0= 1.005 (SE=0.007) for ICC, b_0=1.004 (SE=0.007) for 23andMe, and b_0=1.022 (SE=0.008) for UK-Biobank. We then performed a fixed effects meta-analysis based on effect sizes (log odds ratios [OR]) and standard errors in METAL (Willer, Li, & Abecasis, 2010). We applied the conventional *p*-value threshold of 5e-08 as indication of genome-wide significance. The meta-analysis was performed on

11,733,371 SNPs that passed quality control. The combined sample size of the meta-analysis was 184,765 individuals, although the sample size varied per SNP due to differential missingness across samples.

Manhattan and QQ-plots for the GWAS, meta-analysis, and gene-based test results were created using the R-package qqman (Turner, 2014). Regional plots were created using LocusZoom (Pruim et al., 2010), with varying window size for optimal visualization.

Gene-based test of association

Testing associations on the level of protein-coding genes can be more biologically meaningful and is more powerful (lower multiple testing burden) than testing solely on the level of SNPs. Gene-based analysis was used to test associations for the combined effect of SNPs in protein-coding genes taking into account LD between the SNPs and the size of the gene. The analysis was conducted in MAGMA (v 1.6) (De Leeuw et al., 2015), which uses the 1000 Genomes reference-panel (phase 3, 2012) to control for LD. SNPs were mapped to genes if they were located in or within 10 kb from the gene, such that 5,710,956 SNPs (49%) could be mapped to at least one of 18,293 protein-coding genes in the reference panel. The significance threshold was set at p<2.74e-06 (Bonferroni correction 0.05/18,293).

Identification of genes with differential expression levels between cannabis users and non-users

We used S-PrediXcan to integrate eQTL (expression quantitative trait loci) information with our GWAS summary statistics to identify genes of which genetically predicted expression levels are associated with cannabis use (Barbeira et al., 2017). Briefly, S-PrediXcan estimates gene expression weights by training a linear prediction model in samples with both gene expression and SNP genotype data. The weights are then used to predict gene expression from GWAS summary statistics, while incorporating the variance and co-variance of SNPs from an LD reference panel. We used expression weights for 48 tissues from the GTEx Project (V7) and the DGN whole blood cohort generated by Gamazon et al. (Gamazon & Wheeler, 2015), and LD information from the 1000 Genomes Project Phase 3 (Delaneau & Marchini, 2014). These data were processed with beta values and standard errors from the lifetime cannabis use GWAS meta-analysis to estimate the expression-GWAS association statistic. We used a transcriptome-wide significance threshold of p<1.92e-07, which is the Bonferroni corrected threshold when adjusting for all tissues and genes (i.e. N=259,825 gene-based tests in the GTEx and DGN reference sets).

We used the GTExPortal (https://www.gtexportal.org/home/; GTEx Analysis Release V7)(Carithers et al., 2015) to obtain gene expression levels of *CADM2* across tissues. We

used the same portal to plot a multi-tissue eQTL comparison of the top SNP rs2875907. The multi-tissue eQTL plot shows both the single-tissue eQTL *p*-value and the multi-tissue posterior probability from METASOFT (Sul, Han, Ye, Choi, & Eskin, 2013).

SNP-based heritability analysis

The proportion of variance in liability to cannabis use that could be explained by the aggregated effect of the SNPs (h^2_{SNPs}) was estimated using LD-Score regression analysis (Bulik-Sullivan, Loh, Finucane, Ripke, & Yang, 2015). The method is based on the premise that an estimated SNP effect-size includes effects of all SNPs in linkage disequilibrium (LD) with that SNP. A SNP that tags many other SNPs will have a higher probability of tagging a causal genetic variant compared to a SNP that tags few other SNPs. The LD score estimates the amount of genetic variation tagged by a SNP within a specific population. Accordingly, assuming a trait with a polygenic architecture, SNPs with a higher LD-score have on average stronger effect sizes than SNPs with lower LD-scores. When regressing the effect size from the association analysis against the LD score for each SNP, the slope of the regression line provides an estimate of the proportion of variance accounted for by all analysed SNPs (Bulik-Sullivan, Loh, et al., 2015). For this analysis, we included 1,179,898 SNPs that were present in all cohorts and the HapMap 3 reference panel. Standard LD scores were used as provided by Bulik-Sullivan et al. (2015) based on the Hapmap 3 reference panel, restricted to European populations (Altshuler et al., 2010).

Genetic correlations with other substances and mental health phenotypes

We used cross-trait LD-Score regression (Bulik-Sullivan, Finucane, Anttila, Gusev, & Day, 2015) to estimate the genetic correlation between lifetime cannabis use and 25 other traits using GWAS summary statistics. The genetic covariance is estimated using the slope from the regression of the product of z-scores from 2 GWASs on the LD score. The estimate represents the genetic covariation between the 2 traits based on all polygenic effects captured by SNPs. Summary statistics from well-powered GWASs were available for 25 relevant substance use and mental health traits, including nicotine, alcohol and caffeine use, schizophrenia, depression, bipolar disorder, and loneliness (Supplementary Table S6). To correct for multiple testing we adopted a Bonferroni corrected *p*-value threshold of significance of 0.002 (0.05/25). LD scores were based on the HapMap 3 reference panel, restricted to European populations.

Causal association between cannabis use and schizophrenia: Two-sample Mendelian randomization

We performed two-sample Mendelian randomization analyses (MR) (Burgess et al., 2015) to examine whether there was evidence for a causal relationship from cannabis use to schizophrenia and vice versa. Analyses were performed with the R package of database

and analytical platform *MR-Base* (Hemani et al., 2017) and with the gsmr R package which implements the GSMR (Generalized Summary-data based Mendelian Randomization) method (Zhu et al., 2018).

MR utilizes genetic variants strongly associated with an exposure variable as an 'instrument' to test for causal effects of the exposure on an outcome variable. This approach minimizes the risk of spurious findings due to confounding or reverse causation present in observational studies, provided that the following assumptions are met: 1) the genetic instrument is predictive of the exposure variable, 2) the genetic instrument is independent of confounders, and 3) the genetic instrument is not directly associated with the outcome variable, other than by its potential causal effect through the exposure (i.e. there is no directional pleiotropy) (Davey Smith & Hemani, 2014). Two-sample MR refers to the application of MR methods to well-powered summary association results estimated in non-overlapping sets of individuals (Burgess et al., 2015) in order to reduce instrument bias towards the exposure-outcome estimate.
Bi-directional causal effects were tested between lifetime cannabis use and schizophrenia. We used genetic variants from our cannabis GWAS as well as those from the largest schizophrenia GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) to serve as instruments (*gene-exposure association*). For lifetime cannabis use we used 2 genetic instruments; 1) an instrument including all independent genetic variants that were genome-wide significantly associated with lifetime cannabis use (p<5e-08; 5 SNPs), and 2) an instrument including independent variants with a more lenient significance threshold (p<1e-05; 69 SNPs). For schizophrenia we used one genetic instrument, including independent genetic variants that were genome-wide significantly associated with schizophrenia (instrument p<5e-08; 109 SNPs). Information on the included SNPs in the genetic instruments is provided in Supplementary Table S6.

Genetic variants were pruned (R^2<0.001) and the remaining genetic variants (or proxies [R^2≥0.8] when an instrumental SNP was not available in the other GWAS) were then identified in GWAS summary-level data of the outcome variable (*gene-outcome association*). Note that not all independent SNPs identified in the exposure dataset have been included in the analyses, because not all exposure SNPs or their proxies were also available in the outcome dataset and because some SNPs were palindromic (see Supplementary Table S6).

Evidence for both a gene-exposure and a gene-outcome association suggests a causal effect, provided that the MR assumptions are met. To combine estimates from individual genetic variants we applied Inverse-Variance Weighted (IVW) linear regression (Ehret et

al., 2011). In addition, 4 sensitivity analyses more robust to horizontal pleiotropy were applied, each relying on distinct assumptions regarding instrument validity: Weighted Median (Bowden, Davey Smith, Haycock, & Burgess, 2016), MR-Egger SIMEX (Bowden et al., 2015), Weighted Mode (Hartwig, Davey Smith, & Bowden, 2017), and Generalized Summary-data based Mendelian Randomization (GSMR) (Zhu et al., 2018). These sensitivity analyses rely on orthogonal assumptions, making their inclusion important for triangulation. The Weighted Median approach provides a consistent estimate of the causal effect even when up to 50% of the weight comes from invalid instruments (Bowden, Davey Smith, et al., 2016). MR-Egger regression applies Egger's test to MR instruments that consist of multiple genetic variants (Bowden et al., 2015; Burgess & Thompson, 2017). MR-Egger provides a consistent estimate of the causal effect, provided that the strength of the genetic instrument (the association between SNPs and exposure) does not correlate with the effect the instrument has on the outcome (i.e. the InSIDE assumption: Instrument Strength Independent of Direct Effect). This is a weaker assumption than the assumption of no pleiotropy. MR-Egger may, however, be biased when the NOME (NO Measurement Error) assumption is violated – i.e. the assumption that the SNP-exposure associations are *known* rather than *estimated.* Violation of NOME can be quantified with the I^2 statistic, which ranges between 0 and 1. A value below 0.9 indicates a considerable risk of bias. This bias can be corrected for with MR-Egger simulation extrapolation (SIMEX, Bowden, Del Greco, et al., 2016). Since I^2 ranged between 0.7-0.9 for our analyses, we report results from MR-Egger SIMEX in Table 3. The Weighted Mode methods can produce an unbiased result, as long as the most common causal effect estimate is a consistent estimate of the true causal effect: (the Zero Modal Pleiotropy Assumption (ZEMPA), Hartwig et al., 2017). Finally, we performed GSMR, a method which leverages power from multiple genetic variants while accounting for LD between these variants (Zhu et al., 2018). Because GSMR accounts for LD, genetic variants that were included in GSMR instruments were pruned at a higher threshold of R^2<0.05 (instead of R^2<0.001 for the other MR analyses). Zhu et al. (2018) showed that the gain of power from including SNPs in higher LD than 0.05 is limited. GSMR also allows extra filtering for SNPs that are suspected to have pleiotropic effects on both the exposure and the outcome (HEIDI filtering).

To calculate variance explained (R^2) by the instrument, first we selected a single SNP to obtain an estimate of the phenotypic variance, var(y). Assuming effect sizes are normally distributed, we used the quantile function of the student t-distribution to transform the *p*-value of the SNP association into an estimate of t, t^. The number of degrees of freedom and N were based on the effective sample size (4/(1/cases+1/controls)). The effective sample sizes were estimated at N=130,072 for schizophrenia and N=180,934 for cannabis

use. The corresponding value of r was calculated using the formula $t = \frac{r}{\sqrt{(1-R^2)/(N-2)}}$ and obtained the R^2 that corresponds to t with the online tool http://vassarstats.net/rsig.html. Subsequently, we approximated the variance of the phenotype y using $var(y) = \frac{2*MAF*(1-MAF)*\beta^2}{R^2}$ in which MAF denotes the Minor Allele Frequency and β the effect size of the specific SNP. Finally, we used the estimated value of *var(y)* to calculate the R^2 for the remaining SNPs of interest using $R^2 = (2 * MAF * (1 - MAF) * \beta^2)/var(y)$; and summed the R^2 of all SNPs of interest included in the instrumental variable to obtain an estimate of the total R^2 explained by the instrument.

Data availability

General information on study design and data availability are included in the Life Sciences Reporting Summary. Summary statistics (based on the UK-Biobank and ICC samples) are available via LDhub (http://ldsc.broadinstitute.org/gwashare/). The result from the top 10,000 SNPs based on all three subsamples (i.e. including the 23andMe sample), codes, scripts are available upon reasonable request. Full summary statistics can only be provided after permission by 23andMe.

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Supplementary Materials

Supplemental to this paper is a large number of figures and tables, of which an overview can be found below. They can be view online at:
Chapter 3 – Genome-wide association study for cannabis use

or copy this link in the browser:
https://drive.google.com/drive/folders/1232cFoZuc1nf-Evj0gLbKMjHOaqGuWKu

Supplementary item	Short title
Supplementary Figure 1	GWAS results for the ICC cohort
Supplementary Figure 2	GWAS results for the 23andMe cohor
Supplementary Figure 3	GWAS results for the UK-Biobank cohort
Supplementary Figure 4	QQ-plot for the gene-based test of association
Supplementary Figure 5	Regional plots for the significant genes in the gene-based test
Supplementary Figure 6	Summary of the effect direction for significant genes identified by S-PrediXcan across human tissues and cell types
Supplementary Figure 7	Plot of gene expression levels in transcripts per million (TPM; on y-axis) of CADM2 across GTEx tissues (on x-axis)
Supplementary Figure 8	eQTL plot of rs2875907 depicting single-tissue eQTL effect sizes
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Supplementary Figure 10	Scatter plots depicting MR results for bidirectional two-sample Mendelian randomization analyses between lifetime cannabis use and schizophrenia diagnosis
Supplementary Table 1	All genome-wide significant SNP-associations in the meta-analysis
Supplementary Table 2	Independent genome-wide significant associations with lifetime cannabis use in the UK-Biobank sample
Supplementary Table 3	Description of the genome-wide significant associations in the gene-based test of association and the S-PrediXcan analysis, with a short (non-comprehensive) overview of relevant literature findings on gene–phenotype associations
Supplementary Table 4	Significant S-prediXcan associations after correction for multiple testing
Supplementary Table 5	Summary of S-PrediXcan associatons by target gene
Supplementary Table 6	Results from LD score regression analysis: genetic correlations between lifetime cannabis use and various traits of interest
Supplementary Table 7	SNPs included in the genetic instruments used for bidirectional two-sample Mendelian randomization analyses between lifetime cannabis use and schizophrenia diagnosis

Supplementary Table 8	Cochran's heterogeneity statistic (Q) for Inverse Variance Weighted (IVW) bidirectional two-sample Mendelian randomization analyses between lifetime cannabis use and schizophrenia diagnosis
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CHAPTER 4

A molecular genetics study mapping the common genetic architecture of substance use traits

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Abstract

Genetic vulnerability is an important predictor of substance use. Previous research has shown that different substance use traits correlate both on the observed as well as on the genetic level.

We used summary level data from genome-wide association studies (GWASs) and Genomic SEM to map the common underlying genetic architecture of 12 substance use traits related to use of nicotine, alcohol, cannabis, and cocaine. Subsequently, we conducted GWASs on the common latent factors to identify genetic markers associated with these factors. We then estimated the genetic correlations between the common factors and a broad range of mental health, personality, physical and cognitive traits. Finally, zooming in on the psychiatrically most relevant trait, we tested the causal relationships between the latent substance dependence factor and five common psychiatric disorders using Mendelian Randomization and SEM pathway analysis.

We identified five latent genetic substance use factors which we refer to as dependence, cannabis, smoking, alcohol, and smoking initiation. The GWASs for these factors confirmed previously discovered variants and identified novel loci. The genetic correlations showed substantial overlap with other traits. Dependence and the smoking factors correlated mostly with unfavorable outcomes. The strongest evidence for causal effects was found for causal effects of liability to ADHD and schizophrenia on dependence.

We found evidence for common genetic factors underlying substance use, which showed diverging patterns of (causal) relationships with other traits. Mapping these relationships provides new insight in the etiology of substance use.

Introduction

The use of legal and illegal psychoactive substances is prevalent worldwide [1-3]. Substance abuse and dependence are among the most common mental health disorders [4]. Both legal and illegal substance use can have deleterious consequences for mental and physical health [5], making the etiology of these behaviors an important topic for study.

Genetic vulnerability is an important risk factor for substance use. Twin-based heritability estimates lie around 40-50% for many substance use outcomes, such as smoking initiation and quantity [6-8], alcohol consumption [9], coffee consumption [10], and cannabis initiation [11]. For substance use disorders heritability estimates are often higher, with estimates around 50-75% for alcohol, nicotine, and caffeine, and around 70% for cocaine and opioids [12]. In the last decade, genome-wide association studies (GWASs) have aimed to identify genetic variants (SNPs) associated with substance use. Most substance use GWASs have reached sufficient sample sizes to detect genetic signal, even for substance dependence, which is rarer and thus difficult to investigate in large samples. Although the power has not always been sufficient to identify genome-wide significant SNPs, these GWAS result in significant SNP-based heritability estimates and strong genetic correlations across the (mental) health domain [13-15]. Variance explained by all SNPs tested in the GWAS (SNP-based heritability) is estimated to lie around 10% for many substance use outcomes [14-18].

Given the high co-morbidity and overlapping risk factors (such as sensation seeking and religion) for different substance use traits e.g., [19], it is not surprising that there is also overlap in genetic vulnerability. Twin studies have shown that a common genetic liability factor might underlie different kinds of substance use behavior [20, 21]. Possibly, within this common factor, there are components that overlap for the same stage of use (e.g., initiation, quantity, abuse/dependence) across different substances. For example, there are indications that the genetic factors underlying problematic substance use are distinguishable from those underlying normative use [22], as are factors underlying licit versus illicit substance dependence [23] .

Genetic overlap has also been found on the level of the measured DNA. Substantial genetic correlations are found between different substance use phenotypes e.g., between cannabis, alcohol, and tobacco use; [17, 23, 24], especially for the same kinds of measures for different substances e.g., smoking initiation and cannabis use initiation, or cigarettes use quantity and alcohol use quantity; [25]. Furthermore, it seems that there is also genetic overlap between substance use and other psychiatric and physical health traits. To name

a few examples, smoking and alcohol use traits show genetic overlap with mood disorders, ADHD, BMI and cardiovascular disease [18]. Information on shared genetic etiology can explain why different substance use behaviors often occur together, and why they seem to be associated with phenomena such as psychiatric disorders.

Taking this a step further, genetic information can be used to disentangle shared and unique factors in the etiology of related traits and to test causal relationships. In Mendelian Randomization (MR) GWAS summary statistics are used to create SNP-instruments to measure an 'exposure' trait. The assumption is that SNPs cannot be influenced by confounders and are randomly distributed across the population. If there is an effect of a substance use SNP on some outcome, the idea is that this effect can only occur through the SNP effect on substance use. In other words, the relationship should be causal. Using the MR framework, several studies have shown causal relationships between different substance use traits and psychiatric disorders. For example, schizophrenia liability seems to cause cannabis initiation [17], smoking risk could cause schizophrenia [26], ADHD liability was found to cause several substance use behaviors [27], and depression liability could lead to alcohol dependence [28].

In the current paper, we used data from 12 powerful GWASs (related to use of nicotine, alcohol, cannabis, and cocaine) to map the common underlying genetic architecture of these traits. By conducting GWASs on the common factors, we aimed to identify variants and genes that have general effects on substance use. By testing genetic correlations between the common substance use factors and 37 other complex trait we provided a broad overview into general and distinctive features of the genetic architecture of substance use. Subsequently, we zoom in on a potential reason for the high phenotypic and genetic overlap between substance use *dependence* and 5 common psychiatric disorders by testing pathway models and causal relationships.

Results

We investigated the common genetic architecture of substance use by co-analyzing available GWAS summary statistics for five smoking traits (initiation, age at initiation, pack years, dependence, and cessation), three alcohol traits (consumption in drinks per week, drinking frequency, and dependence), three cannabis traits (initiation, use frequency, and dependence), and one cocaine trait (dependence; see Table S1). For pack years of smoking and cannabis use frequency no published GWAS summary statistics were available; we ran these GWASs ourselves in the UK Biobank cohort [29]. The GWAS for pack years of smoking resulted in 18 independent genome-wide significant hits and a significant SNP-heritability of 11%. For cannabis frequency, there were no significant associations at the SNP-level, but there was a significant gene-based association with *SLC45A3* and SNP-heritability was significant at 5%. Full methods and results for these GWASs can be found in Supplementary Information 1.

As an initial exploration of the overlap between the 12 substance use traits, we conducted Linkage Disequilibrium Score regression[30]. The overlap was substantial (see Figure 1 and Table S2). Smoking initiation, age at smoking initiation, and alcohol consumption were important nodes with genetic correlations to all other traits. Alcohol dependence showed quite strong correlations with (a smaller subset of) other traits. Alcohol frequency and cannabis use frequency showed the least (strong) overlap with other traits.

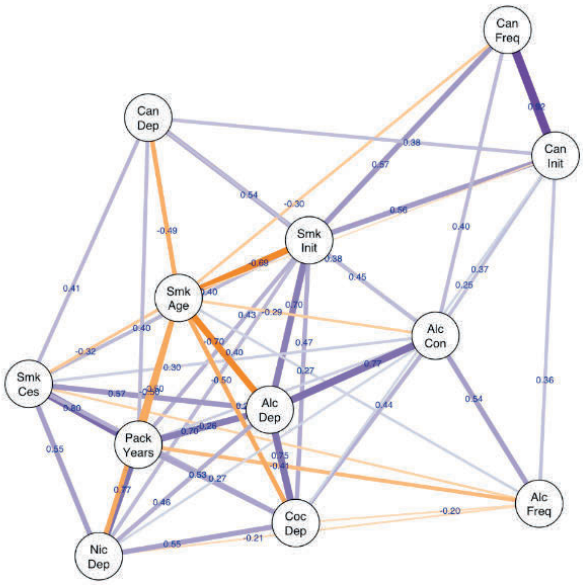

Figure 1. Genetic correlation structure between the substance use traits, with in purple positive relations and in orange negative relations. Thicker and darker colored lines indicate stronger correlations. Only significant correlations are included in the figure (Bonferroni corrected for the number of tested traits, p= .05/12=0.004). Note. alccon=alcohol consumption, alcdep=alcohol dependence, alcfreq=alcohol use frequency, candep= cannabis dependence, canfreq= cannabis use frequency, caniniti= cannabis initiation, cocdep= cocaine dependence, nicdep= nicotine dependence, pack years=pack years of smoking, smkage= smoking age at initiation, smkces= smoking cessation, and smkinit= smoking initiation.dependence, nicdep= nicotine dependence, pack years=pack years of smoking, smkage= smoking age at initiation, smkces= smoking cessation, and smkinit= smoking initiation.

Factor structure underlying the genetic architecture of substance use

We explored in how many common latent factors the substance use traits clustered by conducting exploratory factor analysis in Genomic Structural Equation Modelling (Genomic SEM, [31]), using a cut-off value of ≥0.30 for the factor loadings. The factor solutions with 1 to 4 factors did not fit the data well. Model fit increased until leveling off at a 5-factor solution (Table S3). This model was fit in a confirmatory factor analysis, resulting in good fit with AIC=294.17, CFI=.981, SRMR=.084, and $\chi^2(41)=220.17$, p=2.27E-26; see Table S4). Adding more factors did not increase model fit and led to a solution that was less parsimonious (e.g., factors with only one indicator). Thus, the best solution was the model containing 5 latent genetic factors. The CFA results for indicators of the factors with their factor loadings are depicted in Figure 2. Models using 'varimax' instead of 'promax' rotation produced similar results (Table S5).

The first factor comprised cannabis dependence, alcohol dependence, cocaine dependence, alcohol use quantity, and smoking cessation. We dub this factor

'dependence'. The second factor consisted of smoking initiation, lifetime cannabis use, and cannabis use frequency; we call this factor *'cannabis'*. The third factor was made up by the traits pack years of smoking, smoking dependence, and smoking cessation; we call this factor '*smoking'*. The fourth '*alcohol*' factor has two indicators, alcohol use frequency and alcohol use quantity. The final factor is defined by smoking initiation and age at smoking initiation; we call it '*smoking initiation*'. Note that age at smoking loads positively and smoking initiation negatively, so that a higher factor score indicates a smaller chance at initiation and a higher age at initiation. Smoking initiation, smoking cessation, and alcohol use quantity had cross loadings. The factor loading of smoking cessation on the *dependence* factor had become smaller than 0.30 in the CFA (p=.177). All other factor loadings were larger than 0.5 and significant at p<.001.

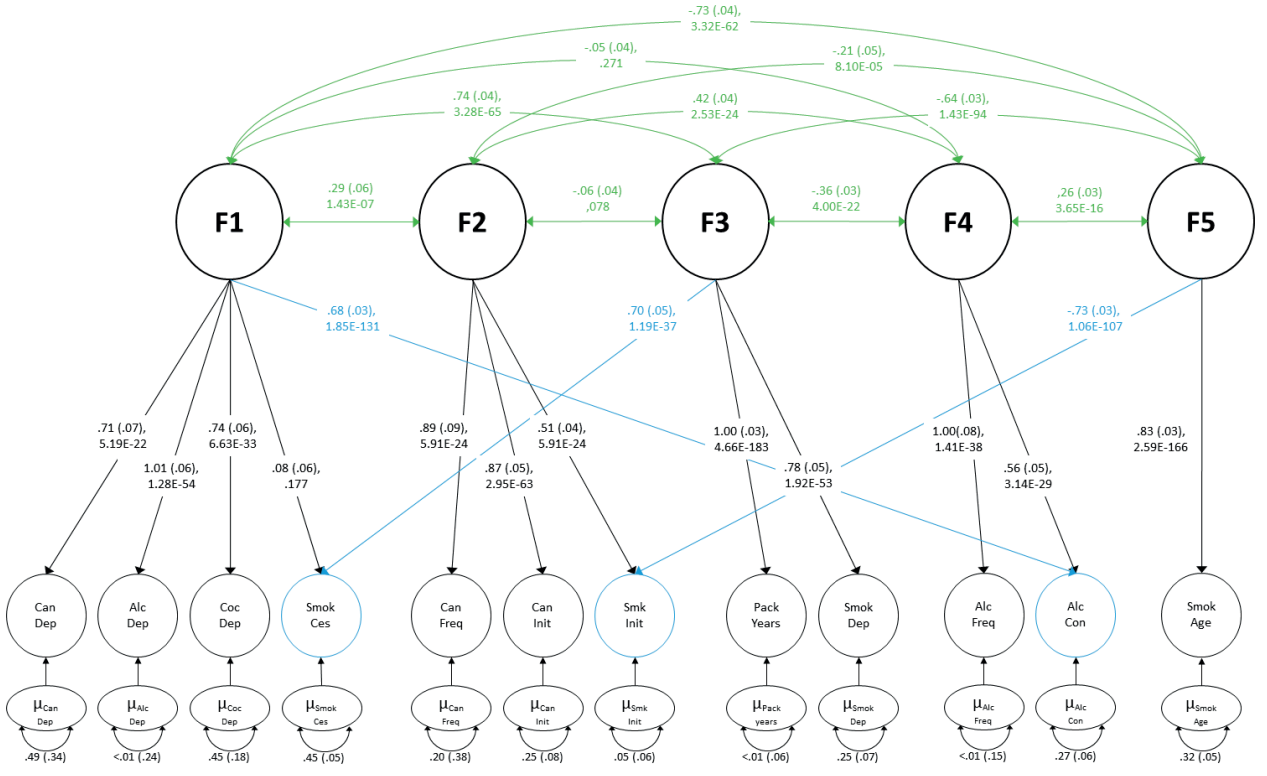

Figure 2. Factor structure underlying substance use traits with standardized factor loadings (standard deviation). F1=dependence; F2=cannabis; F3=smoking; F4=alcohol, F5= smoking initiation. In blue are cross-loadings from indicators that contribute to more than 1 factor. Correlations between the latent factors are shown in double headed green arrows

GWAS on the latent genetic substance use factors

Subsequently, GWAS analyses were conducted for the identified factors. Full results are presented in Tables S6-S30 and Figures S1-S5. Figure 3 shows a condensed circular Manhattan plot with the GWAS results of all five factors, with the outer ring depicting the results of factor 1, the second of factor 2, etc. Note that the y-scales vary per factor due to variations in statistical power. Sixteen genomic regions contained associations with two factors, and one region showed signal for three factors (Table S31). This region on chromosome 2 around the *LINC01833* gene contained associations with *dependence*, *smoking*, and *smoking initiation*. The highest number of overlapping regions was observed between the *smoking* and *smoking initiation* factor.

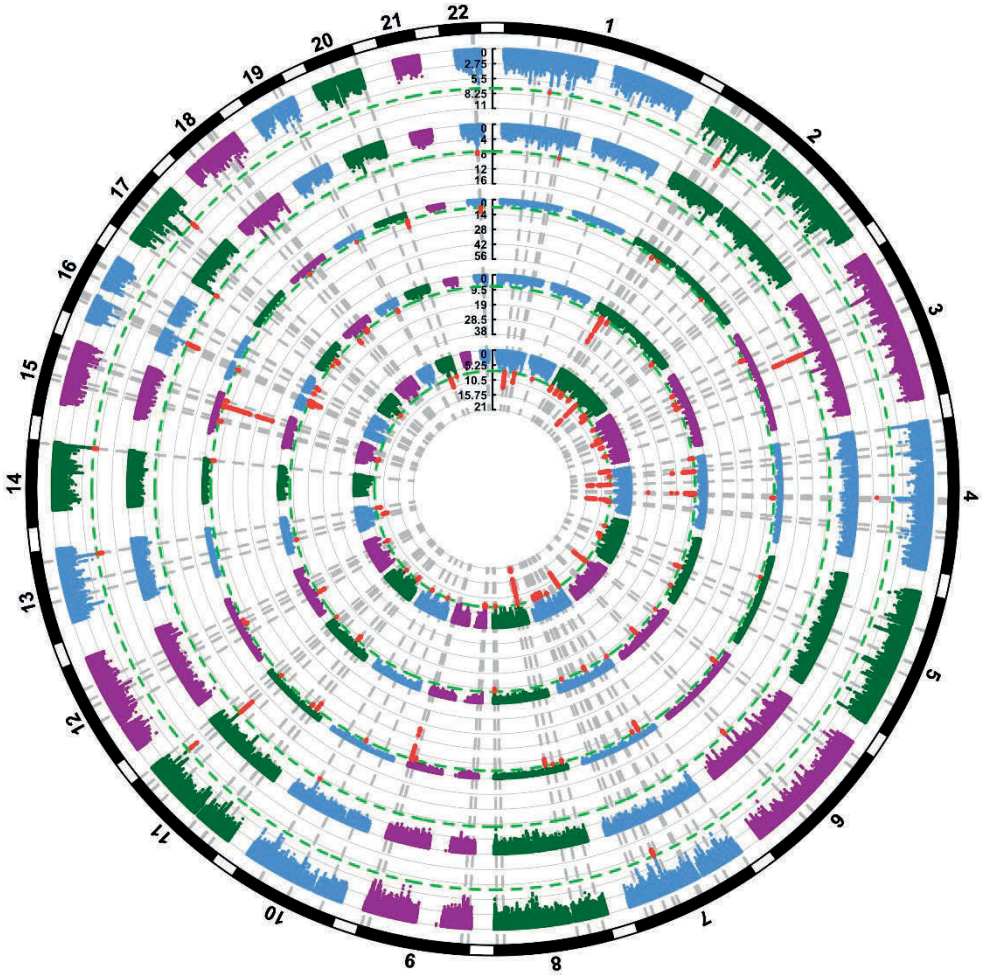

Figure 3. Circular Manhattan plot of the GWAS on the common factor structure of substance use. From the outside in: the profiles of association for F1-*dependence*, F2-*cannabis*, F3-*smoking*, F4-*alcohol*, and F5-*smoking initiation*. The red dots represent SNPs exceeding the genome-wide significance threshold (p<5E-08). The y-axis was scaled to maximize visibility of the SNP results and differs per factor.

Of the eleven genome-wide significant SNP that were identified for the latent *dependence* factor eight were unique for this factor. The strongest association was found for rs1229984 in the *ADH1B* gene on chromosome 4 (Table S6), which was also associated with the *alcohol* factor. Look-up in the GWAS catalog [32] showed that this SNP has been implied in alcohol (dependence) traits and risk-taking behavior (Table S7). The gene-based test of association revealed 40 genes significantly associated with *dependence* (Table S8). Hits included *PCCA* and *ADH7* which have previously been implicated in alcohol use, smoking, cognitive phenotypes, BMI and mental health disorders (Table S9). Most identified genes had previously been implicated in some substance use trait, although *PCCA*, *ZFYVE21*, and *NSF* had not been identified for any of the specific traits included in the *dependence* factor. Putatively novel findings that had not been reported for any substance use trait before included the *XRCC3* and *AL049840.1* genes. The genes associated with the *dependence* factor showed no significant enrichment for tissue expression, although *p*-values were the smallest for expression in brain regions (Table S10).

The *cannabis* factor was uniquely associated with 28 independent genome-wide significant hits (Table S11) and 42 genes (Table S13). Identified SNPs and genes had previously been implicated in a wide range of substance use behaviors, psychiatric disorders, and cognitive traits (Table S12, S14). For the *smoking* factor 129 SNPs were independently genome-wide significantly associated, with rs146009840 in the *HYKK* gene on chromosome 15 as the strongest hit (Table S16). This SNP has previously been implicated in respiratory traits (Table S17). There were 161 significant genes (Table S18), many of which had been implicated in smoking behavior before (Table S19). There were 129 SNPs independently significantly associated with the *alcohol* factor (Table S21). The strongest association was the same as the top-SNP for the *dependence* factor (rs1229984 in the *ADH1B* gene, Table S22). Most of the significant genes (Table S23) had been previously identified in alcohol traits (Table S24). One hundred thirty independent SNPs (Table S26) and 169 genes (Table S28) were significantly associated with the *smoking initiation* factor. The top-SNP was rs35290231 in *PTPRF*, which has previously been associated with smoking and cognitive ability traits (Table S27). The most strongly associated genes were previously implicated in cognitive traits, mental health, and smoking (Table S29). For the *cannabis, smoking, alcohol,* and *smoking initiation* factors gene expression analysis showed enrichment in all brain tissues, although enrichment levels did not always survive correction for multiple testing (Table S15, S20, S25, and S30). In addition, the pituitary showed enrichment for the *smoking initiation* factor. None of the other tested tissues showed enrichment for substance use.

Heterogeneity analyses

For the *dependence* factor, there were 4 independent loci across the genome with significant heterogeneity (Q), indicating that their association with the substance use traits was not well accounted for by the pathway through the latent *dependence* factor (Table S32). This means that the observed SNP effects on the indicators were not proportional to their loadings on the latent factor [33]. The rs1229984 SNP in *ADH1B,* that was a top-hit both for *dependence* and *alcohol,* had heterogeneous effects on the first factor, such that it was strongly associated with alcohol dependence, but not or reversely with the other dependence traits. There were two loci containing heterogeneous SNP-effects for *smoking*. These same two regions contained SNPs with heterogeneous effects on the *alcohol* factor. Both loci (around the *CYP2A6* gene on chromosome 15 and in the nicotine receptor gene cluster on chromosome 19) have been implicated in nicotine metabolism. They seem to have rather specific effects on traits that do not generalize well to other traits. For factor 2 (*cannabis*) and 5 (*smoking initiation)* no significant heterogeneity was observed. Figure S6 visualizes to what extent the genetic signal for the factors overlaps with heterogeneity signal.

Genetic correlation patterns of the latent substance use factors with 37 other complex traits

In order to investigate the overlap between the substance use factors and 37 socio-cognitive, mental health, and physical traits genetic correlations were calculated in LD Score Regression (Table S35). Results are presented in Figure 5, with full results given in Table S36. The factors showed different patterns of association across traits. For example, the *dependence*, *smoking*, and *cannabis* factors showed positive genetic overlap with many mental health disorders, whereas the alcohol factor showed negative correlations. The negative correlations for the *smoking initiation* factor indicates that a younger age at smoking initiation is related to less favorable mental health outcomes. The *dependence* and *smoking* factor show remarkably similar associations, with negative correlations with cognitive ability and social outcomes, whereas the *cannabis*, *alcohol*, and *smoking initiation* factors show positive correlations. For most physical traits, the *dependence*, *smoking*, and *smoking initiation* factors clustered together and showed genetic overlap with less favorable outcomes, such as heart disease, lower self-rated health, and high BMI, whereas the *alcohol* and *cannabis* factors showed opposite patterns.

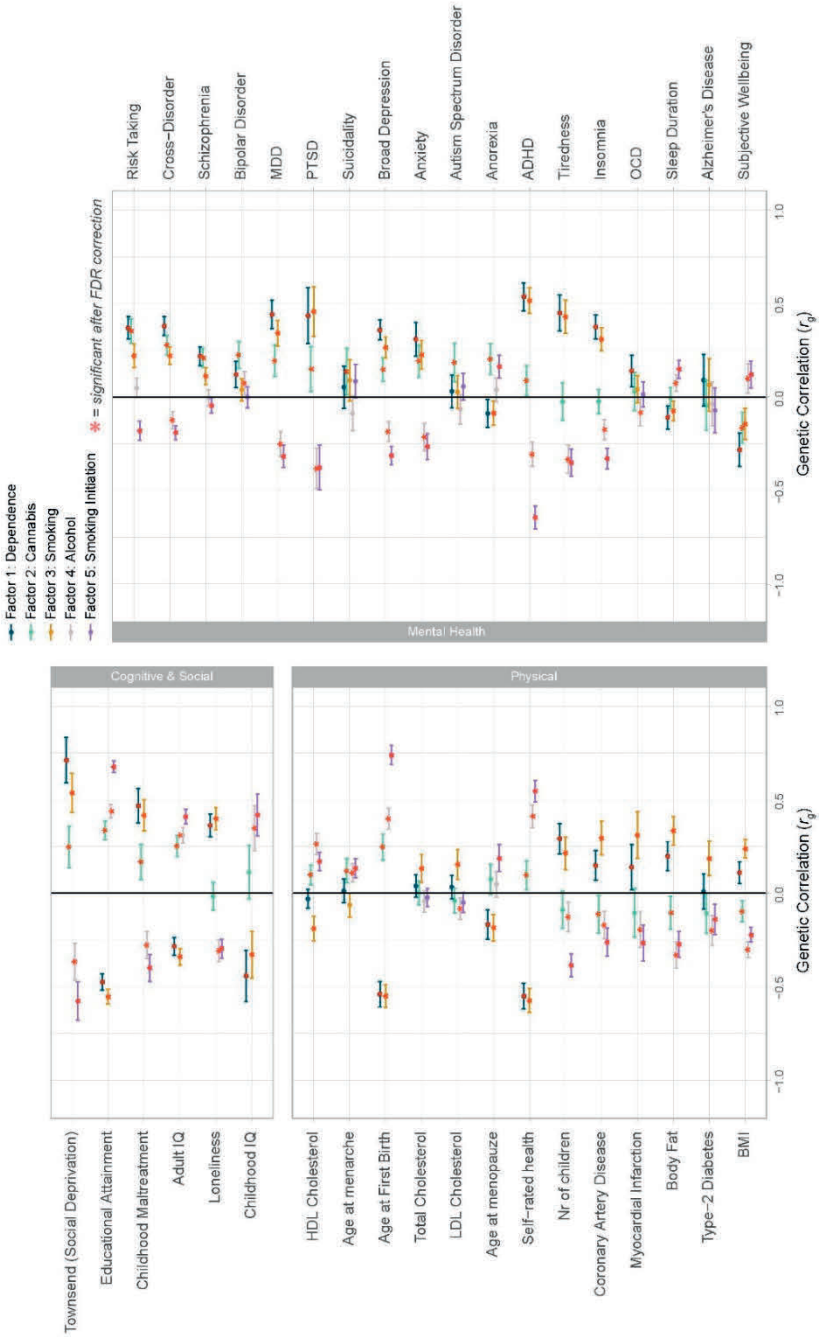

Figure 5. Genetic overlap between the substance use factors and other traits (see Table S33-34).

Testing causal relationships between the substance use factors and psychiatric disorders
Using Mendelian Randomization (MR) we tested the causal direction(s) between the substance use factors and psychiatric disorders. We focused on the most recent and largest available summary statistics from the Psychiatric Genomics Consortium, with information on Attention Deficit/ Hyperactivity Disorder (ADHD), bipolar disorder, major depressive disorder, and schizophrenia. Furthermore, we looked at the relationship with a more general psychopathology factor, captured by the 'cross disorder' GWAS on general liability to at least 4 different psychiatric disorders (Table S37). The results are summarized in Figure 6, with details given in Tables S38 and Figures S7-16. For the MR analysis from the *dependence* factor to ADHD there were strong indications for outlier effects; one outlier SNP was identified and excluded from the *dependence* instrument before conducting MR. We found evidence for positive causal effects of *dependence* liability on ADHD risk and on the cross disorder trait across the main IVW and sensitivity analyses. However, there was significant heterogeneity in these analyses, suggesting that the effects were not uniform across instrument SNPs and should be interpreted with caution. Furthermore, in the GSMR analysis there appeared an effect of liability to *dependence* on schizophrenia risk. GSMR is better powered than the other methods, which may explain why this effect did not reach significance in the other tests (although they were in the same direction). The effect of *dependence* liability on schizophrenia risk appeared to be driven by a single SNP without pleiotropic effects on schizophrenia (rs1229984 in the *ADHB1B* gene).

In the other direction, there was some evidence for a positive effect of liability to ADHD and schizophrenia on *dependence* risk. For ADHD, the effect did not reach significance in the weighted mode analysis, suggesting that the effect may have been driven in part by one or a few SNPs with strong effects, that may be due to pleiotropy. For the effect of liability to schizophrenia on *dependence* risk, the leave-one-out analysis showed some indications for strong outliers. We identified 6 significant (at p<.001) outliers with radial MR and excluded these before MR analysis. The MR Egger analysis for this relationship showed significant pleiotropy, and the pleiotropy corrected estimate showed no significant effects, suggesting that the effect could have been driven by pleiotropy. For the cross disorder trait, the GSMR analysis showed a significant positive effect, but this effect did not reach significance in the other analyses. All analyses with *dependence* as outcome, except the one with schizophrenia, showed low I^2, indicating substantial NOME violation. Also, there was heterogeneity of SNP effects in the cross disorder, depression, and schizophrenia analyses.

In sum, there was some evidence for causal effects of liability to ADHD and the cross disorder trait on *dependence* risk, and in the other direction of liability to *dependence* on ADHD and schizophrenia risk (Figure 5). However, all effects showed some violation of MR assumptions, indicating that they should be interpreted with caution.

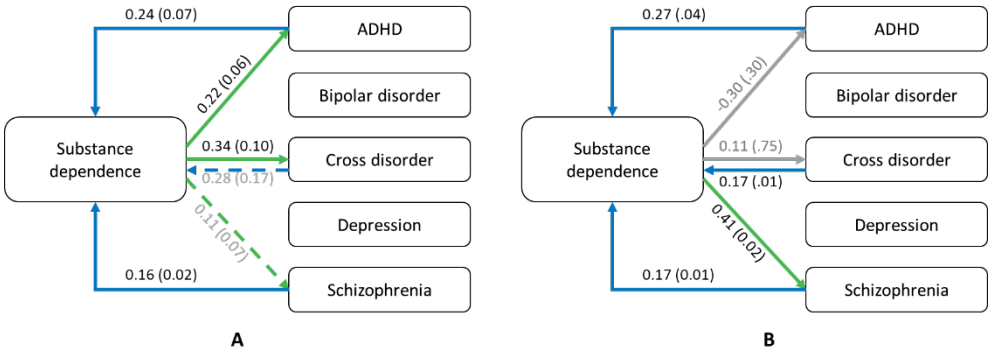

Figure 6. Evidence for causal relationships between the substance *dependence* factor and psychiatric traits. **Panel A.** Schematic summary of MR results, with solid lines indicating considerable evidence across sensitivity analyses for a positive causal effect (green: *dependence* as exposure, blue: *dependence* as outcome) and dashed lines indicating weak evidence for an effect. Per relationship the IVW beta with standard error is given. The results per tested relationship are depicted in Figures S7-16 and summarized in Table S38. **Panel B.** The same relationships, but now with the parameters from the Genomic SEM pathway models, that controlled for mediation through the other substance use factors. Per pathway the standardized estimate (β) and the *p*-value is given (in grey *p*-values above .05). The full models per tested relationship can be found in Figures S17-21.

To corroborate these findings, we conduct follow-up analysis on the causal relationships for which we found any evidence. Using Genomic SEM pathway analysis, we compare two competing models for the effect of *dependence* on psychiatric traits and vice versa (see Figures S17-21 and Tables S39-44). We test if the effects of psychiatric traits on *dependence* are mediated through the other substance use factors, or alternatively, if the effects of the substance use factors on psychiatric traits are mediated through *dependence.* Results are shown in Table 1 and Figure 6B. None of the effects of psychiatric traits on *dependence* were (fully) mediated through the other substance use factors, providing converging evidence for direct (causal) effects of psychiatric traits on *dependence*. In the other direction, the effect of *dependence* on ADHD and cross disorder was largely accounted for by the other substance use factors (particularly F2 and F3), which was not in line with the causal relationships found in the MR analyses. For the effect of *dependence* on schizophrenia we do find evidence corroborating MR analyses.

Table 1. Model fit and path estimates for Genomic SEM pathway analyses assessing effects of *dependence* on psychiatric traits and vice versa. In all models, we included the full correlation matrix between all factors (F1-dependence, F2-cannabis, F3-smoking, F4-alcohol, F5-smoking initiation), factor indicators (the separate substance use traits), and psychiatric traits (ADHD, bipolar disorder, cross disorder, depression, and schizophrenia). Parameters for all the effects in the model can be found in Supplementary Tables S39-S44. Models with lower AIC as compared to the alternative model fit the data better. CFI>.90 and SRMR<.08 are taken as indications of good model fit. *P*-values below .05 are boldfaced.

Predictor	**Outcome**	**AIC**	**CFI**	**SRMR**	**β**	***p***
Dependence	ADHD	1519	.979	.071	-.30	.296
ADHD	Dependence	1405	.981	.078	.27	**.042**
Dependence	Cross disorder	1058	.987	.067	.11	.751
Cross disorder	Dependence	2299	.967	.086	.17	**.012**
Dependence	Schizophrenia	977	.988	.066	.41	**.021**
Schizophrenia	Dependence	5637	.914	.111	.17	**.005**

Discussion

In this study, we used a data driven approach to investigate common liability for substance use by conducting an exploratory factor analyses on 12 sets of summary statistics from substance use GWAS. There were 5 common factors underlying substance use traits that we named substance dependence, cannabis use, smoking, alcohol use, and smoking initiation. Combining summary statistics resulted in high power for detecting genetic signal. GWAS analysis on these substance use factors resulted both in confirmation of previous GWAS findings as well as in putatively novel findings of variants that had not before been implicated in the source GWAS. Top SNP and genes for the factors follow previously identified associations, with an important role for ADH1B in dependence (which has often been implied in alcohol traits), CADM2 in cannabis, the nicotine receptor genes in smoking, ADH1B again for alcohol, and PTPRF for smoking initiation. Novel findings include XRCC3 for dependence, AC027228.1 for smoking, a cluster around SNX17 for alcohol, and AC110781.3 for smoking initiation. The XRCC3 gene (associated with dependence) plays an important role in repairing DNA damage, providing an interesting avenue of research given the previously reported association between substance abuse and DNA damage[34]. Novel associations for alcohol (ZNF513, EIF2B4, and MPV17) cluster on chromosome 2 around SNX17, a gene that was found in a GWAS on interaction effects between lipid levels and alcohol use[35]. Proteins coded by the SNX17 gene are involved in intracellular trafficking. Less is known about AC027228.1 (identified for smoking) and AC110781.3 (for smoking initiation); future research will have to shed more light on the function and implications of these genes.

Most SNP effects were consistent with the common factor models, such that only a handful had heterogeneous effects that could not be explained by the factor. Strong heterogeneous effects were detected for SNPs in ADH1B, CYP2A6, and the nicotine receptor gene cluster. These genes are closely involved in the metabolism of alcohol and nicotine, respectively. Presumably, these roles explain why these loci have substance-specific and even trait-specific associations that cannot be explained by a general substance use factor. Loci with more general effects might be interesting targets for follow-up research. For example, PPP1R13B or BPTF had common effects on the dependence factor. PPP1R13B is involved in programmed cell death and has been implicated in schizophrenia, whereas BPTF might play a role in transcription and has been associated with Alzheimer's disease.

Genetic signal for most substance use factors was over-expressed in brain regions. For the dependence factor enrichment levels in the brain did not reach significance, but this is

likely due to the lower power (i.e., lower sample size) of this factor. Outside of the brain tissues, only the pituitary showed enrichment for expression of substance use-related variants (for the smoking initiation factor). Overall, the (frontal) cortex, nucleus accumbens, and cerebellum tissues showed the strongest enrichment, with no distinct enrichment profiles for the different factors. The role of the nucleus accumbens in substance use and addiction is well established [36]. The frontal cortex is likely involved in substance use and the development of addiction due to its role in motivation and inhibitory control [37]. The role of the cerebellum in addiction is less well-established. It has been proposed that it mediates the links between motor and reward systems [38].

The genetic correlation analyses showed diverging patterns for the different substance use factors, but also showed clustering between some factors. The dependence, smoking, and smoking initiation factors showed similar patterns. In general, higher dependence, higher smoking, and younger age at smoking initiation (i.e., lower scores on the initiation factor) were associated with less favorable outcomes, including adverse physical and mental health outcomes, lower scores on cognitive outcomes, and poorer social outcomes. The alcohol factor, on the other hand, showed overlap with more favorable outcomes across all domains, including lower chances for psychiatric disorders and physical disease. This could be driven by the correlations with socioeconomic and cognitive traits like educational attainment, of which it is well-established that it has important advantages for health [39]. The cannabis factor was similar to the alcohol factor in some respects, but more akin to the other factors in other respects. It was associated with higher educational attainment, IQ, and somewhat more favorable physical outcomes, but also with more psychiatric disorders. These patterns may be due to the higher rates of cannabis use in higher education on the one hand (at least in some populations, [17]) and causal effects of cannabis use on psychiatric traits on the other [40-42]. Overall, these results seem to show a split between ‘adaptive’ and ‘maladaptive’ substance use phenotypes, and seem to argue that smoking is rather more akin to substance use disorders than to moderate alcohol and cannabis use. Also, as the correlations will in part have been driven by causal effects, these results emphasize the importance of adopting a comprehensive approach to substance use treatment, taking into account social, cognitive, mental health, personality, and physical health factors.

Mendelian randomization analyses yielded some evidence for causal effects of ADHD and schizophrenia on substance dependence, and weak evidence for an effect of a cross-disorder trait (general vulnerability for psychiatric disorders) on dependence. Our findings of causal effects of substance dependence line up with previous results from observational studies [43-45]. Previous MR studies have shown similar evidence for causal effects of substance use on psychiatric disorders, such as cannabis initiation on

schizophrenia [17], substance use on ADHD [46], and smoking initiation on externalizing behavior [47]. In the other direction, we found evidence for causal effects of ADHD and schizophrenia, and weak evidence for an effect of the cross disorder trait on dependence. Causal effects from psychiatric traits on substance use could be explained through 'self-medication' mechanisms, for example when individuals use alcohol or drugs to alleviate anxiety symptoms [48, 49].

However, all findings from the MR analysis should be interpreted with caution, given the violation of some of MR's assumptions. These violations were probably due to the use of a genetic common factor in these analyses, which is inherently heterogeneous. Also, the power of the dependence factor and most of the psychiatric traits summary statistics was limited given low sample sizes and low SNP-based heritability (Table S37). In order to corroborate the MR findings, we conducted pathway analyses in Genomic SEM, testing to what extent the relationship between psychiatric traits and substance dependence was accounted for by the other substance use factors. The effects of ADHD, cross disorder, and schizophrenia on substance dependence were not (fully) mediated through the other substance use factors, supporting the interpretation that these effects were at least in part causal. Likewise, there was evidence for a direct, causal effect of schizophrenia on substance dependence. However, the effect of dependence on ADHD and the cross disorder trait was fully mediated by the other substance use factors, suggesting that these effects may not be causal but driven by pleiotropy. These findings warrant future research attention. The results suggest that at least in some cases substance dependence and psychiatric disorders are causally linked in both directions, which is crucial information considering that the advice is often to treat them consecutively rather than simultaneously in clinical practice [50].

This study adds to a growing body of studies that try to uncover common factors in the genetic etiology of psychiatric traits. Recent studies focusing on psychiatric traits identified different common factor structures, such as mood disturbances versus rare serious mental illness [51], a four-factor structure of externalizing, internalizing, thought problems, and neurodevelopmental disorders [52], three factors of mood/psychotic disorders, compulsive disorders, and early neurodevelopmental disorders [53], or even a single 'p' factor underlying all psychiatric disorders [54]. Few studies have included substance use disorders in these investigations[55]. One study that did found that adding substance use disorders changed the underlying vulnerability factors for psychiatric disorders, with most of them loading on a heterogeneous factor that also included ADHD, depression, autism, and Tourette's syndrome [56]. No studies as of yet have focused solely on substance use, thereby not only including disorders but also moderate use traits. Our

study showed that substance use traits do not fall in a single category, and that different types of substance use show diverging genetic relationships to psychiatric disorders.

The findings discussed above also show that similar study designs can lead to diverging conclusions. Factor structures can shift depending on the included traits and model specification. This is an important caveat to bear in mind when interpreting findings from (genomic) factor analyses. A strength of the current study's design is that it was fully data-driven: we included all available substance use traits that met our criteria and conducted our follow-up analyses based on the results without making any adaptations to the model.

Several important conclusions can be drawn from this study. First, we identify genetic variants and genes that have rather general effects and are implied in different substance use traits. These may form promising starting points for follow-up research into underlying biological mechanisms of substance use. Second, we show that different substance use factors show diverging and substantial overlap with other traits. Interestingly, substance dependence and the smoking factors show rather similar patterns such that they correlate genetically with unfavorable outcomes. Third, there was some evidence for causal relationships between substance use dependence and psychiatric disorders. The strongest evidence was found for bidirectional causal effects between ADHD and substance dependence, and for a causal effect of schizophrenia on substance dependence. Our results provide new insights into common and distinct genetic features of substance use, and show how these traits are related with each other and with other psychiatric traits. This new step in mapping the genetics of substance use brings us another step closer to unravelling the etiology of these costly behaviors.

Online methods

Data sources for the substance use GWAS summary statistics

Publicly available summary statistics from GWAS on substance use traits (published before October 2020) were assessed. Due to limited availability of GWASs in other ethnicities, we focused solely on GWAS in European ancestry individuals. In order to ensure the quality of the data we selected phenotypes with a significant SNP-based heritability. If multiple GWAS for the same phenotype were available, we used the summary statistics from the one with the largest (effective) sample size. We selected 10 traits for which we were able to obtain summary statistics (see Table S1). When applicable, we requested permission from 23andMe to use summary statistics including this cohort. In addition, for 2 phenotypes we ran GWAS analyses in UK Biobank cohort [29], namely smoking pack years and cannabis use frequency (for GWAS methods, refer to Supplementary Information 1). Pack years is a measure of tobacco exposure, defined as (the equivalent of) the number of years a person has smoked one pack of cigarettes per day. Eventually, we included summary statistics for five smoking traits (initiation, age at initiation, pack years, dependence, and cessation), three alcohol traits (consumption in drinks per week, drinking frequency, and dependence), three cannabis traits (initiation, use frequency, and dependence), and one cocaine trait (dependence). The summary statistics were cleaned for further processing, filtering out rare variants (MAF<.01) and aligning with reference data (1000 Genomes, phase 3). If the source GWAS did not report SNP-based heritability (h^2_{SNP}) or estimated it in an alternative manner, this was computed with linkage disequilibrium (LD) score regression [30], so that it could be compared across phenotypes.

Investigating the common genetic architecture of substance use

As a first step, we mapped the genetic correlations between the 12 substance use traits using LD score regression. This tool assesses the overlap in GWAS summary statistics for different traits using the relationship between the test statistics and linkage disequilibrium scores in the population to tease apart true genetic effects from bias. To visualize the results we used the igraph R package to create a network plot of the genetic correlations [57].

Next, we identified the genetic architecture underlying the different substance use traits using the R-package Genomic Structural Equation Modeling Genomic SEM, [31]. Genomic SEM can deal with an substantial extent of overlap between samples. We conducted a series of exploratory factor analysis (EFA) with 'promax' rotations (as well as 'varimax' for comparison). Subsequently, the factor structure suggested by EFA (with standardized

loadings of ≥0.3) was tested using confirmatory factor analysis (CFA). The fit of the factor solution was evaluated using commonly used thresholds for good fit of Comparative Fit Index (CFI)>.90 and Standardized Root Mean Square Residual (SRMR) <.08 [58]. We compare the resulting best model with models with one factor more or a factor less, and prioritize the one with the lower AIC. As χ^2 is susceptible to large sample sizes and will be inevitably significant in all factor solutions using these large sets of summary statistics, it, too, was only used to compare models.

GWAS analyses on the identified substance use factors

Subsequently, we ran GWASs within Genomic SEM on the defined factors. These multivariate GWASs estimate the SNP effects from the Genomic SEM model on the common factor. Genome-wide significant SNPs (p<5E-08) were clumped using PLINK1.9 [59] with independency defined as R^2<.01 and SNPs within 250kb viewed as a single locus. To determine effective sample size of the factors we used the formula from Mallard et al. (2020) [51]. We created a circular Manhattan plot to visualize the SNP profiles for the factors using the Rpackage CMplot [60]. We also ran genome-wide heterogeneity tests in Genomic SEM to test to what extent the SNP-effects were mediated through the latent factors or were more consistent with an independent pathway model of specific effects on indicators. Miami plots were created to show how much of the genetic signal for the factors was significantly heterogeneous using the Hudson R package. Gene-based tests and functional gene mapping analyses for the per-factor GWAS results were run in FUMA [61]. As p-value cut-off for the gene-based tests we used p<.05/19,080=2.62E-6 to correct for the number of genes tested.

Genetic correlations between factors and other traits

To assess how the genetic architecture of the latent genetic substance use factors overlapped with that of other traits, we used the summary statistics to compute genetic correlations using LD score regression [30]. We selected a set of 37 physical and mental health traits and cognitive/ social traits for which GWAS summary statistics were publicly available (see Table S34). To correct for multiple testing, we computed false discovery rate p-values. Results were visualized using the R package ggplot [62].

Mendelian Randomization analysis on the dependence factor and psychiatric traits

Finally, we conducted Mendelian Randomization (MR) analyses to examine causal relationships between the first factor we identified (*dependence*) and 5 common psychiatric disorders from the Psychiatric and Genomics Consortium (PGC; attention deficit and hyperactivity disorder, bipolar disorder, cross disorder, depression, and schizophrenia). MR uses SNPs with a robust association to an 'exposure' trait as an instrument to test causal associations with 'outcome' traits. Assuming that a SNP cannot

be influenced by confounders and are randomly distributed across the population, an association between the SNP and an outcome can only occur through the exposure trait. This is evidence that a causal effect exists of the exposure trait on the outcome trait.

We selected psychiatric disorders for which at least 10 genome-wide significant SNPs were reported and that had a significant genetic correlation with the *dependence* factor. Traits meeting these criteria were attention deficit hyperactivity disorder (ADHD), bipolar disorder, major depressive disorder, schizophrenia, and a 'cross-disorder' factor for psychopathology [53; see Table S36]. As instruments we selected SNPs that were reported as genome-wide significant independent hits in the source GWAS. For the cross-disorder trait, we selected SNPs that were significantly associated with at least four different psychiatric disorders as our instrument.

Analyses were conducted in the MR-base R-package [63] and GSMR package. In addition to the standard Inverse Variance Weighted (IVW) analyses we conducted several sensitivity tests. We used radial MR to exclude outliers [64], the F-statistic to assess instrument strength [65], Generalised Summary-data-based MR (GSMR) to account for LD and increase power to detect effects [66], weighted mode and weighted median to offer estimates robust to effect size outliers that could be due to pleiotropy [67], and the Q-statistic to assess SNP effect heterogeneity [68]. MR-Egger was used to test and correct for pleiotropy [69]. We used the I^2-statistic to check for no measurement error assumption violation [70]. If NOME violation was too strong (I^2<0.6) MR Egger was not reported; if there was some violation (I^2=0.6-0.9) MR Egger was corrected with simulation extrapolation SIMEX[70].

Genomic SEM pathway analysis on the dependence factor and psychiatric traits

We aimed to corroborate the findings from the MR analysis on causal relationships between *dependence* and psychiatric traits by testing the direction of association while controlling for the effects of the other substance use factors. If the relationships between psychiatric traits and *dependence* are not completely accounted by the other factors this supports the interpretation that the relationships are (partly) causal. Within the Genomic SEM model used to test the factor structure, we added the same five psychiatric traits as in the MR (ADHD, bipolar disorder, cross disorder, depression, and schizophrenia) as well as all factors (*dependence, cannabis, smoking, alcohol,* and *smoking initiation)* and their indicators (Table S1). First, we tested if the effects of *dependence* on ADHD, cross disorder, and schizophrenia that we identified in the MR analysis were accounted for by the other substance use factors, by regressing the psychiatric trait as well as the *dependence* factor on the other substance use factors. In other words, we test if the effects of the substance use factors on the psychiatric trait are mediated through *dependence.* Within the models, we allowed for cross loadings and correlations among factors and indicators. Second, in

the other direction, we tested if the effect of ADHD, cross disorder, and schizophrenia on *dependence* were mediated through the other substance use factors. If the effect of the predictor on the outcome was no longer significant when allowing pathways through the other factors, this is interpreted as contra-evidence for direct causal effects. If this effect is only attenuated when allowing for mediation effects, it is said to be partially mediated. We compare models using the fit indices, with a lower AIC, CFI>.90 and SRMR<.08 taken as indications of good model fit.

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Supplementary materials

There is a large number of supplementary materials that accompany this paper. They can be viewed at:
Chapter 4 – Genetic architecture of substance use

or copy this link into the browser:
https://drive.google.com/drive/folders/1E5dSPGwXlq-8Rgk9rUEhYMUwdp2svkUx

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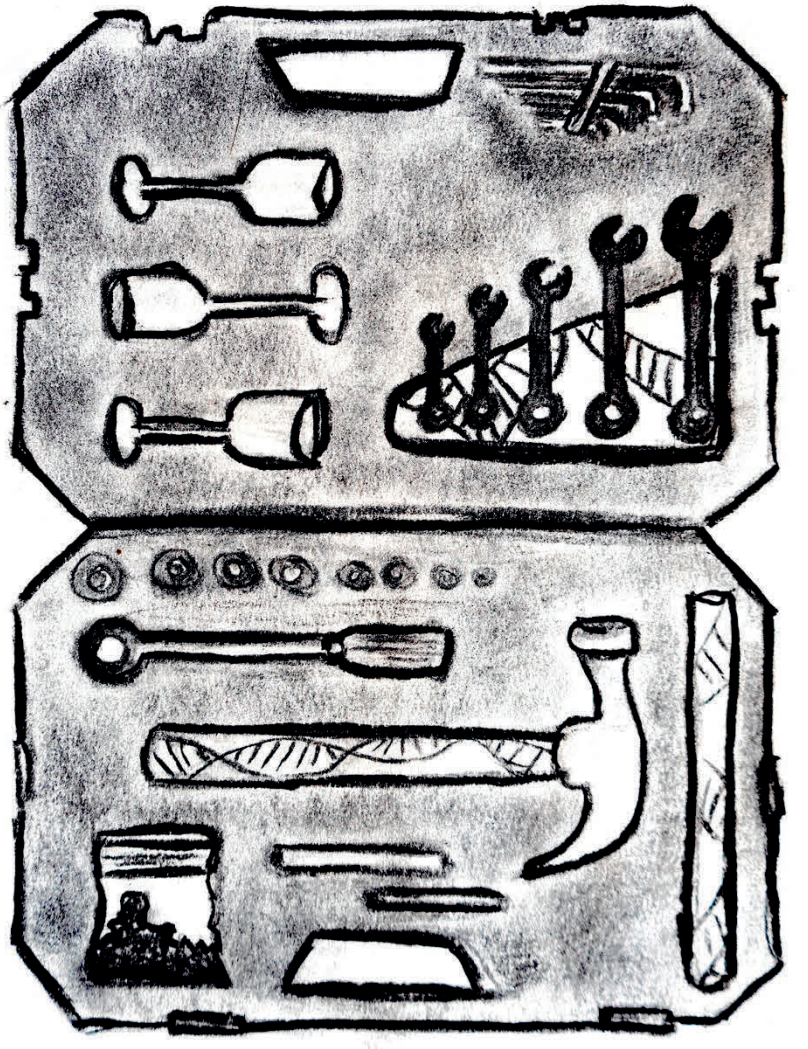
PART 2

LEVERAGING GENE FINDINGS



CHAPTER 5

Associations between the CADM2 gene, substance use, risky sexual behavior, and impulsivity-related traits

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Abstract

Risky behaviors, such as substance use and unprotected sex, are associated with various physical and mental health problems. Recent genome-wide association studies indicated that variation in the cell-adhesion molecule 2 (*CADM2*) gene plays a role in risky behaviors and self-control. In this phenome-wide scan for risky behavior, it was tested if underlying common vulnerability could be (partly) explained by pleiotropic effects of this gene, and how large the effects were. SNP-level and gene-level association tests within 4 samples (25 and Up, Spit for Science, Netherlands Twin Register, and UK Biobank and meta-analyses over all samples (combined sample of 362,018 participants) were conducted to test associations between *CADM2,* substance- and sex-related risk behaviors and various measures related to self-control. We found significant associations between the *CADM2* gene, various risky behaviors, and different measures of self-control. The largest effect sizes were found for cannabis use, sensation seeking, and disinhibition. Effect sizes ranged from 0.01% to 0.26% for single top SNPs and from 0.07 to 3.02% for independent top SNPs together, with sufficient power observed only in the larger samples and meta-analyses. In the largest cohort, we found indications that risk-taking proneness mediated the association between *CADM2* and latent factors for lifetime smoking and regular alcohol use. This study extends earlier findings that *CADM2* plays a role in risky behaviors and self-control. It also provides insight into gene-level effect sizes and demonstrates the feasibility of testing mediation. These findings present a good starting point for investigating biological etiological pathways underlying risky behaviors.

KEYWORDS
CADM2, self-control, multi-cohort, phenome-wide, risky behavior, substance use

1 | INTRODUCTION

Risky behaviors, such as substance use (e.g. nicotine, alcohol, cannabis) and unprotected sexual contact, are important factors contributing to physical and mental health problems.[1] As a result, these risk factors for morbidity and mortality[2] are included in the global Sustainable Development Goals, set up and agreed on by all member states of the United Nations in 2015 to ensure more healthy lives and promote quality of life worldwide.[3] For instance, substance use contributes to approximately 12% of deaths worldwide,[4] due to factors such as an increased risk of respiratory and vascular diseases, various forms of cancer, stroke, suicide or overdose.[5] Approximately 4% of the global burden of disease, as measured in disability-adjusted life years (DALYs),[6] is attributable to alcohol and tobacco use, and 0.8% to illicit drugs.[4] Furthermore, risky sexual behavior (e.g., unprotected sexual intercourse with multiple partners) contributes another 6.3% of the total global burden of disease, as it is associated with the risk of sexually transmitted infections (STIs), HIV or cervical cancer.[5,7]

Various studies indicate that risky behavior has a substantial genetic component. For instance, a substantial part of the variation in the initiation of substance use can be explained by genetic factors: alcohol (37%),[8] nicotine (44%)[9] and cannabis (40-48%)[10]. Even higher heritability estimates are shown for substance use disorders, e.g. alcohol: 45-73%,[8,11] nicotine: 44-75%,[8,9,11] and cannabis: 37-59%.[10,11] Furthermore, the heritability of risky sexual behavior was estimated by previous research to be around 33%.[12] It is assumed that different risky behaviors might merely reflect different phenotypic manifestations of (partly) shared underlying genetic vulnerabilities.[13,14] However, it is largely unknown which genetic and biological mechanisms underpin the heritability of risky behaviors.[15]

Recent large genome-wide association studies (GWASs) have independently implicated a gene located on chromosome 3 encoding cell adhesion molecule 2 (*CADM2*) in various risky behaviors including alcohol (ab)use,[16] lifetime cannabis use,[17] number of sexual partners,[16] and age at first sexual intercourse.[18] Proteins encoded by *CADM2* are involved in glutamate signaling, GABA transport and neuron cell-cell adhesion, especially in the prefrontal and anterior cingulate cortices.[19] These brain regions are well known for their role in cognitive control and motivational salience, which are in turn involved in impulse regulation and self-control.[20,21]

Low self-control, as indexed by high impulsivity, sensation seeking, and disinhibition, has been associated with engaging in risky behavior, including unprotected sexual intercourse[12] and substance use (initiation) or abuse.[22,23] A review by Bezdjian et al. showed heritability for different indices of self-control of around 50% across 41 studies including around 27,000 infants, children, adolescents and adults[24]. These findings suggest that genetic factors, at least in part, modulate various aspects of self-control.

Specifically, *CADM2* has been associated with sensation seeking,[23] hyperactivity, and impulsivity.[25] This suggests potential shared heritability between reduced self-control and risky behavior, most likely due to overlapping underlying biological processes.[12,22,23] As such, reduced self-control might act as intermediate phenotype, linking *CADM2* and various risky behaviors.

Candidate-gene studies have traditionally selected plausible candidate-genes based on a theory on the underlying biological mechanisms, e.g. relating the dopamine cascade to ADHD[26] or substance use.[27] This approach is limited by current knowledge of the biology of investigated behaviors.[27] In addition, candidate-gene studies are often restricted by a lack of available data resulting in underpowered or small-scale designs[28] and examination of only a few (or a single) phenotype(s).[29] Consequently, these limitations have rendered the candidate-approach largely unsuccessful.[30,31]

We propose to apply GWAS techniques on a single gene, whose candidate-gene status is anchored in a body of (hypothesis-free) GWASs. In this first phenome-wide association study (PHeWAS) [32] for *CADM2* and risky behavior, the multiple testing burden is much lower than in GWASs, which should increase power. This study aims to establish if power increases substantially enough to detect associations in smaller samples, thereby also providing insight into gene-level effect sizes. By looking at several risky behavior phenotypes concurrently, we furthermore investigate the link between genetic variation in *CADM2* and substance- and sex-related risk behaviors more comprehensively than single phenotype studies. Doing so, we aim to examine if the involvement of *CADM2* in various risky behaviors and self-control related constructs (i.e. pleiotropy, when a single gene influences the expression of multiple phenotypic traits) can explain the potential genetic overlap between various aspects of reduced self-control and multiple risky behaviors. By combining data from four different cohorts, and analyzing a range of risky behaviors and indices of self-control, we aim to increase reliability and robustness of findings.[29] Finally, we explore if reduced self-control might mediate the relationship between *CADM2* and various risky behaviors.

In data across four European ancestry population-based samples from different countries, we tested here whether single nucleotide polymorphisms (SNPs) in *CADM2* are associated with risk behavior, including 1) substance use and abuse (alcohol, tobacco, cannabis, and other drugs), 2) sexual risk behavior (number of sex partners, sexual risk-taking, and age at first sexual intercourse), and 3) indices of reduced self-control (disinhibition, sensation seeking, risk-taking proneness, and ADHD symptoms). We conduct factor analyses to explore common underlying vulnerability factors. Furthermore, we explore whether relationships between *CADM2* and risk behaviors are mediated by a self-control trait.

2 | MATERIALS AND METHODS

2.1 | Subjects and procedures

Data from 443,693 participants from four different data sources were used, including the Queensland Twin Registry's '25 and Up' (25Up: *N* = 2,133) study in Australia,[33] 'Spit for Science' (S4S: *N* = 2,994) study in the U.S.,[34] the 'Netherlands Twin Register' (NTR: *N* = 12,120) repository in The Netherlands,[35] and the 'UK Biobank' (UKB: *N* = 426,446) in the United Kingdom.[36] Although 25UP and S4S are considerably smaller than the others, they have not been included in previous risk behavior GWAS and have data on phenotypes that were not available in NTR and UKB, making them valuable additions. All studies were performed in accordance with the Declaration of Helsinki and were approved by local ethical committees. Study details are described in articles referenced in the Supplementary Methods section.

2.2 | Measures

Genotyping and quality control

We used available genotyped or imputed SNP information in and around *CADM2* (chr 3 (3p12.1), bp 83,951,945 - 86,126,470, GRCh37/hg19). Per sample genotyping, imputation and quality control (QC) procedures can be found in Supplementary Table S1. Variants with a minor allele frequency (MAF) below 1%, a genotype missingness rate above 5%, or deviations from Hardy-Weinberg Equilibrium (HWE) of p<1e-10 were excluded from further analysis. SNPs were aligned with the 1000 Genomes reference panel (phase 3)[37], removing ambiguous SNPs and SNPs that had a MAF that diverged more than 0.15 from that in the reference panel. Following these procedures, n_{25Up}=297, n_{S4S}=2,972 , n_{NTR}=6,166, n_{UKB}=4,638 SNPs were available and retained for analysis. Genetic data and data on at least one phenotype were available for N_{25Up}=2,133, N_{S4S}=2,994, N_{NTR}=12,120, and N_{UKB}=426,446 individuals (total *N* = 443,693). The per-phenotype sample size range was N_{25Up}=419-2,071, N_{S4S}=503-2,384, N_{NTR}=581-9,432, and N_{UKB}=23,423-362,018 individuals.

Outcome measures

In this study, we adopted a PHeWAS approach, meaning that we tested the association between CADM2 and all risk behavior and self-control measures that were available in the datasets. In order to provide an overview of all measures, we grouped them into six categories: lifetime experience with substance use (regarding tobacco, cannabis, and other substances), age at initiation of substance use (regarding alcohol, tobacco, cannabis, and other substances), average substance use level (regarding alcohol and

tobacco), regular substance (ab)use (including regular alcohol, tobacco, and cannabis use, and any behavioral/substance addiction), sexual risk behavior (including the number of sexual partners, sexual risk-taking, and age at first sexual intercourse) and self-control (including disinhibition, sensation seeking, risk-taking proneness, and symptoms of ADHD). Variables with a total *N* of < 1,000 were excluded as they could not be analyzed due to a lack of statistical power. Preprocessing of the data included combining measures (e.g., across different waves), removing outliers, and excluding inconsistent or invalid response patterns. An overview of all 23 outcome measures included can be found in Table 1. More detailed information about the (cleaning and combining of the) measures is given in Supplementary Table S2.

2.3 | Data-analysis

Primary analyses were performed separately within each cohort and combined in meta-analyses. Identical analysis procedures were used in all individual datasets. Phenotype data cleaning, preparation, and descriptive analyses were conducted using the Statistical Package for the Social Sciences (SPSS; version 25).[38]

To test whether *CADM2* SNPs were associated with separate risk behavior outcomes, association analyses were firstly conducted in PLINK (version 1.9).[39] For dichotomous phenotypes, logistic regression was used; for continuous variables, we used linear regression. Covariates included sex, age, and highest level of education, as we aimed to capture the influence of *CADM2* on risk behavior and self-control, that was independent of these factors (e.g. education has shown to be associated both with *CADM2* and risk behavior).[40] Furthermore, principal components for ancestry (PCs) were included. PCs are used to control for possible stratification effects that arise when a genetic factor and a trait show a spurious correlation due to systematic differences in allele frequencies between groups of different genetic ancestry. We used the PCs as calculated by the institute we received the data from, following their recommendations on how many PCs were appropriate to control for ancestry stratification effects within their specific sample. Because S4S participants were recruited at university, parental rather than own education level was included as a covariate in this sample. In 25Up, S4S, and NTR we used ten PCs to control for population stratification, while in UKB we included 40 PCs. We controlled for clustering due to genetic relatedness in the twin datasets (25Up and NTR) by using the --family option in PLINK and excluded individuals that showed high genetic relatedness in the other datasets (see Supplementary Table S1).

Second, to assess the overall effect of the variants at the gene-level, the association results were analyzed using Multi-marker Analysis of GenoMic Annotation gene-based tests (MAGMA, version 2).[41] Because not all phenotypes were present in all cohorts, we conducted these analyses separately per cohort. SNPs were mapped to

CADM2 using 1000Genomes phase 3 data. We used the *snp-wise = top* procedure, which is more sensitive when only a small proportion of SNPs in the gene shows an association. To control for the number of phenotypes tested, we computed the Benjamini-Hochberg False Discovery Rate (FDR)[42] *p*-values within each variable category, using R (version 3.6.2).[43] When reporting the results, we present uncorrected *p*-values with an asterisk indicating if the FDR-corrected *p*-value was below *p*=.05.

Thirdly, we conducted two meta-analyses for those phenotypes that were present in multiple datasets in order to maximize power to detect associations. The first meta-analysis was performed on the results from the per-cohort gene-based tests using the meta-analysis procedure in MAGMA. This method aggregates the Z-values for the gene-based associations within the individual cohorts while taking sample size into account, in a procedure similar to 'normal' meta-analysis. The results give an indication of the strength of the association with *CADM2* across cohorts. The second meta-analysis was used to get per-SNP effects, that can be used to estimate the variance in the phenotype explained by SNPs in the gene (R^2). To conduct these meta-analyses, odds ratios for binary outcome variables were converted to betas with corresponding standard errors in the input files and all continuous variables were standardized. The meta-analysis was conducted in METAL[44] based on standard errors and effect estimates (rather than on sample size) so that β and se(β) could be obtained.

Using the results from the SNP-based meta-analysis we computed R^2 (the procedure is described in Supplementary Methods II). To give an indication of how the resulting effect size estimates impacted power, we conducted post-hoc power analyses for the meta-analysis. The analysis was conducted based on the observed effect sizes as a function of the minimum and maximum sample size. We used the compromise power analysis option from the G*power package for the *F* test family with a single predictor.[45]

Mediation analysis with latent factors

A secondary aim of this study was to test whether the association between *CADM2* and risky behavior would be mediated by one or more indices of self-control. Assuming that latent factors would be stronger measures of underlying risky behavior propensity than the separate phenotypes (and to limit the number of analyses), we used factor scores in the mediation analyses. Assuming that *CADM2* is associated with risky behavior and reduced self-control in general rather than specific behaviors or constructs per se, such latent factors might show stronger relationships with *CADM2.* We used a data-driven approach without a priori specifying the nature of the factors or the number of factors to extract. We expect clustering due to the overlap in the measures, but the actual clustering could differ per sample. We used principal component analysis with principal axis factoring (PAF/PFA) including oblique (oblimin) rotation; missing values were replaced

with the mean.[46] The analyses were conducted separately for each cohort and factors with an Eigenvalue >1 that explained >10% of the variance were extracted from the dataset (see Supplementary Table S3). Subsequently, individual factor scores were computed using regression.

To test if a self-control trait can explain the association between *CADM2* and risky behavior we tested mediation following Baron & Kenny's procedure (see Figure 1, including *p*-values rather than regression weights as MAGMA does not provide such estimates). [47] We first tested the relationship between *CADM2* and the risk behavior factor (path *c*) in MAGMA, and if that was significant, we tested the association between the self-control trait (mediator) and the risk behavior factor in SPSS (path *b*). If path *b* and *c* were significant, and there was an association between a self-control trait and *CADM2* in the gene-based test (path *a*) we tested in a final step the relationship between *CADM2* and the risk behavior factor outcome, while controlling for the self-control mediator, in MAGMA (path *c'*). When in path *c'* the relationship between the risk behavior and *CADM2* was attenuated while controlling for self-control, mediation was assumed.[48] In all paths, we controlled for the effects of age, sex, and education, and in the analyses involving genetic data, we controlled for the PCs.

As an addition to see if common propensity would indeed show a stronger association with *CADM2* we also meta-analyzed factors that were made up of similar indicators in different cohorts. We used similar procedures for these analyses as for the separate phenotypes in MAGMA.

3 | RESULTS

3.1 | Demographics and descriptives

The sample size of people included in at least one analysis consisted of 443,693 individuals (maximum sample size per analysis *N*=362,018). Slightly more than half of the participants (54%) were female (25Up: 61%, S4S: 58%, NTR: 62%, UKB: 54%) and age ranged from 18 to 94 with a weighted mean age of 38 years (25Up: *M*=30.1, *SD*=4.3; S4S: *M*=20.7, *SD*=1.5; NTR: *M*=44.8, *SD*=16.9; UKB: *M*=54.7, *SD*=8.0). Furthermore, most participants had a moderate (49%) or high (33%) level of education (largest group 25Up: 41.7% moderately high, S4S: 77.5% high, NTR: 45.7% high, UKB: 32.4% high education). Cohort descriptions are provided in Table 1, including a description of the mean (continuous variables) and prevalence rates (dichotomous variables) for all outcome measures. Due to different operationalizations and sample compositions in the four cohorts, most descriptives cannot be directly compared. In the association analyses we controlled for age, sex, and education level, and we conducted meta-analysis either on per-sample Z-scores for the association (in MAGMA) or on standardized regression weights (in METAL) to control for sample differences.

3.2 | Associations for *CADM2* with risk behavior and self-control

The associations between *CADM2* and risk behavior and indices of self-control are shown in Table 2. Associations that were significant after FDR-correction for multiple testing (at $p<.05$) are indicated with an asterisk. Both lifetime tobacco use and lifetime cannabis use were associated with *CADM2* in the meta-analyses. In the individual samples, these associations were significant in NTR and UKB, but not in 25Up and S4S. No significant associations were found for lifetime use of other substances (i.e., recreational drugs), although it must be noted that this variable was not present in the largest sample (UKB). None of the age at initiation of substance use variables were associated with *CADM2*. The smallest *p*-value was .049 in the NTR sample for age at alcohol initiation. After correction for multiple testing, this finding was no longer significant. The meta-analyses revealed associations between both average alcohol consumption and average number of cigarettes per day and *CADM2* that seem to be largely driven by significant associations in the UKB sample. Regular alcohol use, problematic alcohol use, regular tobacco use, and nicotine dependence were all associated with *CADM2* in the meta-analyses. In the individual study analyses, only regular alcohol use was after correction significantly associated with *CADM2* in a sample (S4S) other than the UKB. The number of sexual partners was associated with *CADM2* in 25Up, UKB and the meta-analysis, age at first

sexual intercourse in UKB and the meta-analysis, but not in the individual 25Up, S4S or NTR samples.

As for the analyses of indices of self-control, a significant association between *CADM2* and disinhibition (significant in the NTR and meta-analysis), sensation seeking (in NTR) and risk-taking personality (in UKB) was observed. As the constructs of sensation seeking and risk-taking personality were only measured in one study, no meta-analyses could be performed.

SNP-based meta-analyses were conducted in order to get per-SNP estimates that could be used to compute explained variances. Results show little overlap between the top-SNPs for different phenotypes (see Supplementary Table S4). Only 31 SNPs showed a significant association with multiple independent phenotypes.

Effect sizes of the associations and power analyses

The variance explained by all independently associated SNPs in *CADM2* taken together ranged from 0.07% for regular alcohol use to 3.02% for regular cannabis use (*M*=1.05%, *SD*=1.09%, *Mdn*=0.45%). The sample sizes included in the analyses ranged from 2,094 to 362,018 individuals (see Table 2). It does not seem to be the case that phenotypes from a particular sample or specific category have higher R^2 than the others. Also, there does not seem to be an effect of the number of SNPs in the analysis on the size of R^2 (r= -.27, p>.05). As most effect sizes were below 1% we set the power analysis parameters at R^2=0.001% to 1% as a range for the effect size and 2,000-400,000 as a range for the sample size. For an effect size of 0.001% even a sample size of 400,000 results in a power level of only 50%, whereas for an effect size of 1% a sample size of 8,000 suffices to achieve 80% power. In our study, the average observed effect size of the top SNP was R^2=0.11%, resulting in sufficient (>80%) power levels at sample sizes of at least *N*=7,100. A visualization of power as a function of effect size and the SNP sample size is provided in Supplementary Figure S1a and S1b.

Mediation analysis with latent factors

Factor analysis of the 14-20 outcomes per sample overall identified five factors with Eigenvalues above 1 and explained variance >10%, of which two appeared to be made up by similar variables in multiple cohorts (see Supplementary Table S3). The latent factor lifetime substance use was present in 25Up and S4S and was not significantly associated with *CADM2*. A tobacco (ab)use factor could be discerned in all datasets, but was only significantly associated with *CADM2* in UKB with *p*=8.45e-06. In UKB there were two other factors, one for lifetime smoking and one for regular alcohol use, which were both associated with *CADM2* (*p*=1.01e-22 and *p*=5.84e-13, respectively). Finally, in NTR there was a self-control factor that was associated with *CADM2* (*p*=2.28e-08).

Thus, there were three risk behavior factors that could be used for the mediation analyses, all extracted from the UKB. There was only one measure of self-control included in the UKB, namely risk-taking proneness (yes/no). Results of the analysis using this measure as a mediator between *CADM2* and the three risk-taking behavior factors, are presented in Figure 1 (with *p*-values rather than regression weights as MAGMA does not provide such estimates). Path *a* for the association between *CADM2* and risk-taking proneness controlling for sex, age, and PCs was tested earlier and found to be significant (see Table 2). Paths *c1-c3* for the associations between *CADM2* and the outcomes (risk behavior factors) were reported in Table 3. Paths *b1-b3* between risk-taking proneness and the risk behavior factors were all significant (tobacco [ab]use factor OR=1.27, *p*<.001; lifetime smoking factor, OR=1.27, *p*<.001; and alcohol abuse factor OR=1.21, *p*<.001). In step c', the associations between CADM2 and lifetime smoking and risky alcohol use factors were attenuated when including the mediator (p=1.01e-22 to 1.51e-18 and 5.84e-13 to 5.05e-09, respectively), suggesting partial mediation by risk-taking proneness. The association between tobacco (ab)use and CADM2 was enhanced (p=4.34e-05 to 9.14e-07) when controlling for risk-taking proneness, which suggests that there was no mediation effect.

4 | DISCUSSION

In this multi-cohort study, it was shown that *CADM2* is associated with multiple substance use and abuse traits, sex-related risky behavior, and different indices of self-control. Meta-analyses showed significant associations between *CADM2* and lifetime experience with tobacco and cannabis use, average alcohol and cigarette consumption, regular/problematic alcohol and tobacco use, number of sexual partners, age at first sexual intercourse, and disinhibition. Furthermore, in the per-sample analyses there were significant associations with sensation seeking, behavioral or substance addiction, and risk-taking proneness. The variance explained by a single *CADM2* SNP ranged from 0.01% (for average alcohol consumption, cigarettes per day, nicotine dependence, and the number of sexual partners) to 0.26% (sensation seeking). Independent top SNPs together explained between 0.07% (regular alcohol use) and 3.02% (regular cannabis use) of the variance. Finally, the self-control trait 'risk-taking proneness' was found to be a significant partial mediator of the associations between *CADM2* and latent factors for lifetime smoking and regular alcohol use.

The results of this study are in line with results from recent GWAS, indicating associations of *CADM2* with substance use and abuse (including alcohol consumption, lifetime cannabis use, and general drug experimentation),[16,17,23,49] sexual risk behavior (such as age at first sexual intercourse and number of sexual partners),[16,28] and different aspects of self-control (sensation seeking, hyperactivity, and risk-taking propensity).[17,18,23,25] Our study finds support for these findings in a large, hypothesis-driven, multi-cohort and phenome-wide study for risk behavior, indicating that the role of *CADM2* in risky behaviors and reduced self-control is robust. This is also in line with some earlier reported genetic correlations for various forms of risky behaviors,[40] suggesting overlapping genes directly or indirectly influence these behaviors. The observed mediation effect of risk-taking proneness is in line with previous suggestions that the association between substance use and *CADM2* might be (partially) mediated by reduced self-control.[49] Our results suggest that variability in *CADM2* may give rise to various aspects of reduced self-control underlying multiple expressions of risky behavior. This corresponds with proposed shared genetic and neurobiological mechanisms underlying various risky behaviors.[13,14]

CADM2 is mainly expressed in the brain (predominantly prefrontal and anterior cingulate cortices (PFC and ACC)), the central nervous system and its peripheral nerve fibers.[23,50] The PFC and ACC are generally involved in cognitive functions concerned with motivation and controlling behavior.[51] The ACC has been associated with error detection and response inhibition, whereas several regions within the PFC are involved in reward learning and decision-making processes, which can all be linked to self-control and risky behavior.[52-54] By affecting brain functions in these regions, variation in *CADM2* may result

in different manifestations of reduced self-control and risky behavior. Future research could further delineate which neurobiological mechanisms are involved in the link between *CADM2,* reduced self-control and risky behaviors.

Looking at the individual SNPs (see Supplementary Table S4), we observe that most top SNPs cluster in the region roughly around 85,500,000 (see Supplementary Figure S2). This is a region containing large numbers of expression quantitative trait loci (eQTLs; panel C). eQTLs are places in the genome that influence to what extent a gene comes to expression, that is, how much is transcribed to messenger RNA. Only a few SNPs are among the top ten independent SNPs for more than one phenotype. This suggests that the effects of *CADM2* were not driven by one strong causal SNP. Six SNPs were associated with three different (but overlapping) primary phenotypes (sensation seeking, any behavioral/substance addiction, and risk-taking proneness). Another SNP that was a top SNP more than twice was rs1271459, associated with ever tobacco use, regular tobacco use, and age at first sexual intercourse. SNPs associated with multiple distinct phenotypes might be more central to the functioning of the gene. As an illustration, we looked up this rs1271459. No information was available for this SNP itself, but Its proxy rs9820373 is a significant eQTL for *CADM2* expression in the subcutaneous adipose tissue (p_{fdr}=5.4E-4).[55] This is interesting as *CADM2* has been associated with BMI [56], potentially through impulsive over-eating.

4.1 | Strengths and limitations

This study has to be viewed in light of its strengths and limitations. Data from separate cohorts with different characteristics were used, which results in a large sample size and high generalizability. It also induces measure heterogeneity, which on the one hand may have limited the power to detect effects in the meta-analyses, and on the other hand further substantiates the robustness of findings. This study included a range of risky behavior and self-control phenotypes, potentially expanding the findings. Furthermore, previous research also indicates that *CADM2* may play a role in phenotypically heterogeneous risk-taking behaviors and personality.[17,23] Future studies might further explore the role of *CADM2* in other potentially related phenotypes, such as (a lack of) physical activity, eating patterns or overweight, gambling, reckless driving etc.[1] and should investigate if these results generalize to populations with different age ranges or different genetic ancestry.

In this study, we observed explained variances between 0.01 and 3.02%. The 25UP and S4S samples were too small to detect significant effects in the individual samples. Virtually all phenotypes reached significance only after adding data from the larger samples (NTR and UKB). The comparison of 4 cohorts with different sample sizes

has shown that in general samples of over 7,000 individuals are needed to find significant effects with these effect sizes (see Supplementary Figure S1).[45,49] This means that for the phenotypes that were available in UK Biobank, the addition of the other samples has not led to a substantial increase in information over and above what we already learned from previous studies. This is the first study to our knowledge, using this method to give a concrete indication of what sample sizes are needed to detect the effect of a single gene. We may conclude that we must be cautious to draw conclusions from individual small samples, but that these smaller samples can be combined in meta-analyses, especially for (possibly more detailed) phenotypes that are not available in large-scale data sets.

This is the first study aiming to shed light on effect sizes that can be expected on the level of genes. Although small, these effects are substantially larger than those of single variants, as have traditionally been investigated in candidate-gene research. Also, given that behavior arises as a result of a complex interplay between environment and a large number of genes with small effects, the effect sizes of *CADM2* that we find could actually be considered substantial. Looking at the level of genes rather than SNPs is biologically more meaningful and could provide clues on underlying biological mechanisms, which in turn will contribute to a better understanding of transgenerational transmission of risky behaviors and provide clues for designing treatment and prevention programs.

This study shows the feasibility and added value of novel variations of the more common analyses in the field of behavior genetics, including genetic association analyses on factor analyzed traits and mediation analyses. New questions might be answered using such techniques, providing more insight into underlying common vulnerability patterns and etiological mechanisms. However, there were some limitations to the mediation analyses, including the lack of control for family relatedness and covariates in the PCA, and the impossibility of calculating regression weights for the associations with *CADM2*. Also, we used Baron & Kenny's procedure to test for mediation only for outcomes that showed a significant relationship with CADM2.[47] Technically, mediation could arise in the absence of such a relationship. Bootstrapping is a more recently developed non-parametric method that can increase power to detect mediation. However, this approach has not yet been implemented in the area of genetic association analysis. Future research might develop techniques to tackle these limitations. In conclusion, the mediation results in this study suggest mediation testing may be feasible, but improved statistical tools applicable to behavioral genetics need to be developed.

Next to the genetic etiology of risk behaviors, we recognize the generally known influence of environmental factors.[12] For example cultural, parenting or peer norms can influence substance- and sex-related risky behaviors. What remains largely unknown is to what extent the impact of genetic and environmental risks are additive or interactive. The variants in *CADM2* identified here lend themselves well to future gene-environment

interaction testing, provided a multi-cohort study and a combined SNP measure are used to ensure sufficient power.

4.2 | Conclusions

This comprehensive multi-cohort study has shown the feasibility of a phenome-wide association study for risky behavior to confirm previous findings on associations between *CADM2* and manifestations of risky behavior and reduced self-control from GWASs on individual phenotypes. It was shown that single SNPs in *CADM2* could explain 0.01 to 0.26% of the variance and a combination of independent top SNPs together 0.07 to 3.02%. This study provides more insight into the relatively small effect sizes that can be expected from association studies. Furthermore, results revealed that a self-control trait might partially mediate the associations between *CADM2* and substance-related risky behavior (lifetime smoking and regular alcohol use). Future studies should further explore the biological underpinnings of the observed relationships between *CADM2*, reduced self-control, and various risky behaviors.

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TABLE 1 Descriptive statistics of participant characteristics and study variables for the four cohort studies.

	25Up (*N* = 2,133)			S4S (*N* = 2,994)			NTR (*N* = 12,120)			UKB (*N* = 426,446)		
Variable	***N***	**Range / *n***	***M* (*SD*) / %**	***N***	**Range / *n***	***M* (*SD*) / %**	***N***	**Range / *n***	***M* (*SD*) / %**	***N***	**Range / *n***	***M* (*SD*) / %**
DEMOGRAPHICS												
Age	2,131	21.8-44.2	30.1 (4.3)	2,452	18.1-32.1	20.7 (1.5)	12,013	18.0-94.0	44.8 (16.9)	426,446	36.0-72.0	54.7 (8.0)
% Male	2,133	832	39.0%	2,994	1,255	41.9%	12,120	4,564	37.7%	426,446	196,462	46.1%
% No/primary education [a]	2,132	250	11.7%	2,963	5	0.2%	9,471	295	3.1%	426,446	75,577	17.7%
% Secondary education		572	26.8%		238	8.0%		1,954	20.6%		114,042	26.7%
% Certificate or diploma		890	41.7%		425	14.3%		2,892	30.5%		98,540	23.1%
% Masters/postgraduate/PhD		420	19.7%		2,295	77.5%		4,330	45.7%		138,287	32.4%
LIFETIME SUBSTANCE USE												
% Ever used tobacco	2,128	1,496	70.3%	2,876	1,949	67.8%	11,870	5,745	48.4%	425,114	259,676	61.1%
% Ever used cannabis	2,118	1,331	62.8%	2,953	1,966	66.6%	10,012	2,412	24.1%	138,524	30,627	22.1%
% Ever used other substance(s) [b]	2,062	1,104	53.5%	2,968	1,067	36.0%	10,055	1,153	11.5%	NA	NA	NA
AGE AT INITIATION OF SUBSTANCE USE												
M age alcohol initiation	1,995	11-33	16.1 (1.9)	2,469	11-22	16.5 (1.8)	9,762	11-30	15.8 (2.1)	NA	NA	NA
M age tobacco initiation	471	11-34	17.7 (3.5)	1,554	11-21	15.9 (2.0)	6,862	11-30	15.4 (2.3)	32,381	11-69	17.9 (5.7)
M age cannabis initiation	1,275	12-36	18.4 (3.5)	1,393	11-19	14.4 (2.0)	9,729	11-40	17.9 (3.3)	NA	NA	NA
M age other substance initiation	1,037	11-37	20.2 (3.9)	617	11-24	15.1 (2.1)	11,381	11-40	21.4 (5.2)	NA	NA	NA
AVERAGE SUBSTANCE USE												
M alcohol units per month	1,929	1-395	25.2 (37.7)	2,594	0-199	23.1 (30.4)	8,670	0-192	34.9 (27.3)	304,654	0-200	8.8 (7.8)
M cigarettes per day	430	0-30	5.3 (7.4)	1,422	1-4	1.3 (0.6)	4,819	0-60	13.8 (9.4)	132,310	1-140	18.3 (10.1)
M tobacco using days	430	1-30	14.8 (13.4)	1,817	1-28	6.4 (8.5)	2,016	4-30	23.7 (9.9)	NA	NA	NA
REGULAR SUBSTANCE (AB)USE												
% Regular alcohol use	2,062	450	21.8%	2,662	220	8.3%	11,081	2,722	24.6%	138,693	42,564	30.7%
M problematic alcohol use (AUDIT)	NA	NA	NA	2,603	0-11	3.5 (2.7)	6,248	0-34	4.1 (3.6)	138,682	0-39	5.0 (4.2)
% Regular tobacco use	1,490	489	32.8%	2,979	427	14.3%	8,051	2,386	29.6%	299,866	133,640	44.6%
M nicotine dependence (FTND)	NA	NA	NA	1,553	0-9	1.1 (1.9)	4,475	0-10	2.8 (2.4)	132,541	0-9	1.8 (1.9)
% Regular cannabis use	1,317	303	23.0%	2,953	397	13.4%	1,493	232	15.5%	29,704	10,465	35.2%
% Any behavioral/substance addiction	NA	NA	NA	NA	NA	NA	NA	NA	NA	137,122	8,278	6.0%
SEXUAL RISK BEHAVIOR												
M number of sexual partners	1,719	0-300	13.3 (24.4)	1,023	0-25	1.3 (1.8)	NA	NA	NA	351,099	1-300	6.6 (13.0)
M/% sexual risk behavior	1,925	0-10	2.1 (2.4)	1,014	375	37.0%	NA	NA	NA	NA	NA	NA
M age at first sexual intercourse	NA	NA	NA	919	11-28	16.3 (3.1)	1,071	12-18	17.1 (1.2)	375,311	11-69	19.1 (3.8)
SELF-CONTROL												
M disinhibition	NA	NA	NA	2,757	1-18	11.0 (2.1)	9,785	5-25	14.2 (3.7)	NA	NA	NA
M sensation seeking	NA	NA	NA	NA	NA	NA	9,229	4-20	11.3 (2.5)	NA	NA	NA
% risk-taking proneness	NA	NA	NA	NA	NA	NA	NA	NA	NA	412,571	111,571	27.0%
M/% ADHD	2,051	7-88	39.0 (10.4)	657	106	16.1%	8,046	0-12	2.3 (1.9)	NA	NA	NA

Note. The variations in sample sizes are due to question branching and the pooling of data of several measurement waves. *N* = total number of participants with phenotypic and genetic data available per variable and subsample; Range = minimum and maximum score/answer; *n* = number of participants scoring positive on this variable within the subsample; *M* = mean within this cohort; *SD* = standard deviation within this cohort; % = percentage of the total number of participants in a subsample. [a] Education levels of participants or their parents in the S4S study. [b] Includes the use of other substances than alcohol, tobacco, and cannabis.

TABLE 2 Results from the MAGMA gene-based tests per sample, with meta-analysis results for variables that were present in two or more datasets. Explained variance is given for the top SNP and the independent SNPs together, for significant associations in the meta-analysis (or cohort analysis for phenotypes present in only one cohort).

	25Up		S4S		NTR		UKB		Meta-analysis				
Variable	***p***	***N***	***p***	***N***	***p***	***N***	***p***	***N***	***p***	***N***	**% R^2 top**	**#SNPs**	**% R^2**
LIFETIME SUBSTANCE USE													
Ever used tobacco	.919	2,071	.601	2,279	**.002***	9,432	**1.12e-20***	348,237	**2.23e-21***	362,018	0.18%	63	0.78%
Ever used cannabis	.424	2,061	.502	2,374	**.008***	8,022	**2.30e-17***	128,132	**3.51e-18***	140,588	0.06%	83	2.28%
Ever used other substance(s)	.858	2,008	.184	2,380	.179	8,073	-	-	.241	12,460			
AGE AT INITIATION OF SUBSTANCE USE													
Age alcohol initiation	.818	1,940	.887	2,048	**.049**	7,784	-	-	.318	11,772			
Age tobacco initiation	.929	458	.737	1,343	.370	5,664	.444	23,423	.519	30,888			
Age cannabis initiation	.587	1,247	.139	1,130	.820	1,877	-	-	.568	4,254			
Age other substance initiation	.172	1,010	.922	503	.443	581	-	-	.485	2,094			
AVERAGE SUBSTANCE USE													
Average alcohol units	.678	1,876	.274	2,169	.202	7,211	**1.71e-07***	257,221	**1.35e-07***	268,477	0.01%	69	0.17%
Average cigarettes per day	**.021**	419	.177	1,242	.429	4,016	**.002***	100,604	**.001***	106,281	0.01%	53	0.27%
Average tobacco using days	.178	419	.632	1,505	.216	1,633	-	-	.264	3,557			
REGULAR SUBSTANCE (AB)USE													
Regular alcohol use	.605	2,007	**.010***	2,275	.076	8,927	**4.07e-04***	128,294	**6.95e-05***	141,503	0.04%	52	0.07%
Problematic alcohol use (AUDIT)	-	-	.351	2,151	.536	5,369	**8.52e-12***	128,286	**2.50e-11***	135,806	0.02%	67	0.31%
Regular tobacco use	.787	1,455	.829	2,384	**.021**	6,634	**5.10e-20***	240,850	**5.84e-20***	251,323	0.02%	90	1.22%
Nicotine dependence (FTND)	-	-	**.047**	1,330	.546	3,831	**7.37e-05***	100,730	**5.50e-05***	105,891	0.01%	59	0.31%
Regular cannabis use	.710	1,288	.155	2,373	.999	1,216	**.033***	28,800	.110	33,677	0.04%	50	3.02%
Any behavioral/substance addiction	-	-	-	-	-	-	**.001***	126,817	-	-	0.04%	37	0.66%
SEXUAL RISK BEHAVIOR													
Number of sexual partners	**.023***	1,677	.468	997	-	-	**2.31e-06***	295,706	**1.21e-06***	298,380	0.01%	54	0.12%
Sexual risk behavior	.673	1,873	.529	990	-	-	-	-	.657	2,863			
Age at first sexual intercourse	-	-	.173	896	.416	941	**2.86e-18***	315,749	**2.08e-18***	317,586	0.02%	91	0.30%
SELF-CONTROL													
Disinhibition	-	-	.612	2,316	**4.36e-04***	8,169	-	-	**.003***	10,485	0.16%	45	2.47%
Sensation seeking	-	-	-	-	**8.00e-07***	7,667	-	-	-	-	0.26%	34	2.66%
Risk-taking proneness	-	-	-	-	-	-	**1.89e-26***	338,031	-	-	0.03%	68	0.21%
ADHD	.136	1,995	.533	647	.088	6,525	-	-	.052	9,167			

p = MAGMA p-value (when bold: $p < .05$, when bold and underscored: $p < .01$.); * = p-value is also significant when corrected for false discovery rate (FDR); *N* = sample size per variable and subsample; %R^2 top = percentage of variance explained by the top SNP; #SNPs = number of independent SNPs in *CADM2* (LD R^2=0.1%) included in the meta-analysis; % R^2 based on those independent SNPs (SNP IDs are shown in Supplementary Table S4); AUDIT = Alcohol Use Disorders Identification Test; FTND = Fagerström Test for Nicotine Dependence.

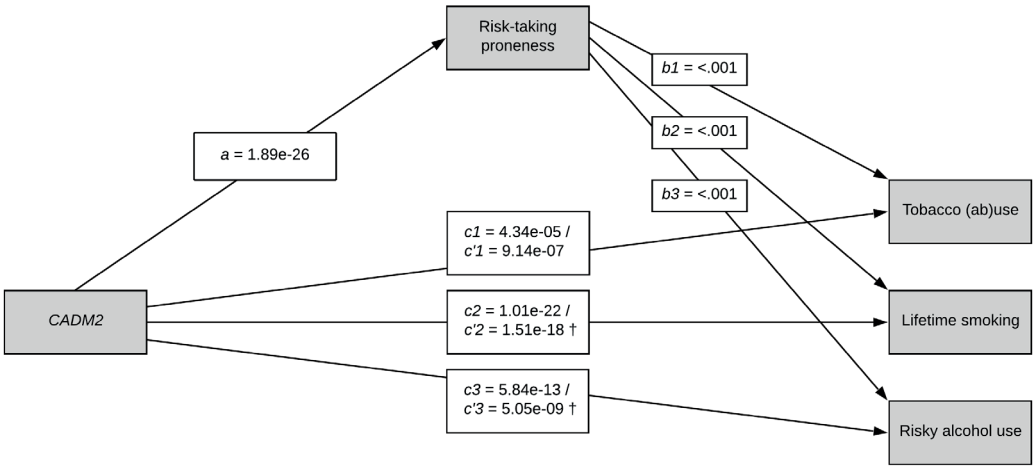
TABLE 3 Results for gene-based analyses between *CADM2* and factors from the PCA. Factors were extracted if they had an Eigenvalue above 1 and explained ≥ 10% of the variance in the data, resulting in 5 factors in total, of which 2 could be detected in multiple cohorts. Below the table, the factor indicators (variables with factor loading > 0.4 on the factor) per sample are given.

	25Up		S4S		NTR		UKB		Meta-analysis				
Factor	***p***	***N***	***p***	***N***	***p***	***N***	***p***	***N***	***p***	***N***	**% R^2 top**	**#SNPs**	**% R^2**
Lifetime substance use[a]	.581	2076	.733	2389					.723	4465	0.09%	12	0.58%
Tobacco (ab)use[b]	.057	2076	.085	2389	.084	9471	**4e-05**	348950	**8e-06**	362886	0.13%	44	2.47%
Lifetime smoking[c]							**1e-22**	348950			0.02%	80	0.24%
Risky alcohol use							**6e-13**	348950			0.01%	63	0.10%
Self-control					**2e-08**	9471					0.24%	44	2.76%

p = MAGMA p-value (when bold: $p < .05$, when bold and underscored: $p < .01$.); *N* = sample size per factor and cohort; %R^2 top = percentage of variance explained by the top SNP; #SNPs = number of independent SNPs in *CADM2* (LD R^2=0.1%) included in the meta-analysis; % R^2 based on those independent SNPs (SNP IDs are shown in Supplementary Table S4).

[a] Lifetime substance use was defined by ever used cannabis, ever used tobacco and ever used other substances in 25UP and by ever used cannabis and ever used other substances in S4S.
[b] Tobacco (ab)use was defined by nicotine dependence and average cigarettes per day in 25UP, by regular tobacco use, average tobacco using days and ever used tobacco in S4S, by the same variables in NTR, and by nicotine dependence, average cigarettes per day, and age at smoking initiation in UKB.
[c] Lifetime smoking was defined by regular tobacco use and ever used tobacco in UKB.
[d] Risky alcohol use was defined by problematic alcohol use (AUDIT), regular alcohol use, and average alcohol units per month in UKB.
[e] Self-control was defined by disinhibition, sensation seeking, and ADHD in NTR.

Figure 1 Significance of associations between *CADM2* and risk behavior factors, with and without a mediating effect of risk-taking proneness. Path *a*: the effect of the predictor (*CADM2*) on the mediator (risk-taking proneness); path *b*: the effect of the mediator on the outcome factors (tobacco (ab)use, lifetime smoking, and risky alcohol use); path *c*: the effect of the predictor on the outcome variables; path *c'*: the effect of the predictor on the outcome variables controlling for the mediator



† C' paths with attenuated p-values, indicating a partial mediation effect.

Supplementary Materials

Supplemental materials can also be found online:
Chapter 5 – CADM2 and risk behavior

or copy this link into the browser:
https://drive.google.com/drive/folders/1Y1Jk4y2JjjQI4MNtLGmqVv-ZH5CzJ6Lm

Supplementary methods I – Study details

25Up study
This study is an extension of previous Brisbane Longitudinal Twin Studies. The data was collected between 2016 and 2018 from twins and their non-twin siblings. The main aim of these studies was to longitudinally assess genetic, psychological, behavioral, and demographic risk factors for various mental disorders in a large cohort of Australian twins and their non-twin siblings, by using questionnaire, genome-wide genetic and pedigree information. Content and methods of the 25Up study are further described in a recent article.[1]

S4S study
The Spit for Science (S4S) study is a prospective, longitudinal study of the genetic and environmental influences on behavioral and emotional health among undergraduate students at a large urban university in the mid-Atlantic region.[2] The study examined questions concerning for instance demographic topics, mental health, life experiences, and various (risk) behaviors. In total, there were 7 waves of data collection used in this study. We selected adult Caucasian students that had survey and genetic data available.

NTR study
This sample comprised participants registered at the Netherlands Twin Register,[3] an ongoing longitudinal study of Dutch twins and their family members. Survey questions encompass topics as physical and mental health, lifestyle, and personality. NTR participants were included in this study for whom genotype data were available and who completed questions related to risk behavior (substance use and sexual risk behavior) and indices of self-control in one or more waves of the longitudinal survey project. We used data from the adult sample (ANTR) wave 1 to 8 (1991-2010) and 10 (2013-2014) to 12 (2013-2016) and the young (YNTR) sample at age 18. DNA collection procedures have been described elsewhere.[3]

UKB study

The UK-Biobank constitutes a large nationwide effort to follow the (psychological) health of half a million UK individuals.[4] Genetic, behavioral, medical, and imaging data were collected from 2006 onwards, and more data is still being collected. Data from participants was included in this study if genetic information was available and someone filled in at least one questionnaire on one wave (regarding substance use, sexual risk factors or self-control).

Supplementary methods II – Procedure for estimating effect size

Using the results from the SNP-based meta-analysis we computed explained variance based on previously reported procedures.[5] For continuous phenotypes, we used the formula $R^2=\frac{2\beta^2 MAF(1-MAF)}{2\beta^2 MAF(1-MAF)+(se(\beta))^2 2N\ MAF(1-MAF)}$ to estimate the explained variance of the top SNP, with *MAF* being the minor allele frequency from the 1000 Genomes reference panel[6], β the effect estimate from the meta-analysis, and *se* the corresponding standard error.[7] For the binary phenotypes, we first estimated the *t*-value corresponding to the *p*-value using the quantile function of the student *t*-distribution, with the degrees of freedom based on the effective sample size $N=4/(\frac{1}{N_{cases}}+\frac{1}{N_{controls}})$, and calculated $R^2=(\frac{t}{\sqrt{N+t^2-2}})^2$. To check if considering multiple SNPs in the gene would increase explained variance we calculated and summed R^2 for independent SNPs (r^2≤0.10%) that showed an association with two-sided *p*<.100. This lenient threshold was used in order to integrate effect sizes that did not reach significance in the smaller cohorts, in an approach equivalent to what is often done for polygenic risk scores.[8] For the binary phenotypes, the summed R^2 was approached using $\frac{2MAF(1-MAF)\beta^2}{var(y)}$ with var(y)= $\frac{2MAF(1-MAF)\beta^2}{R^2}$ from the top SNP.

SUPPLEMENTARY TABLE S1 Genotyping and imputation procedures used

	25Up	S4S	NTR	UKB
bp start (GRCh37/hg19)	83,901.945	83,951,945	84,068,424	83,951,956
bp stop (GRCh37/hg19)	85,568,580	86,126,470	86,031,960	86,126,239
genotyping platform	Illumina 610k SNP	Affymetrix BioBank	Affymetrix 6.0[a] Illumina 660 Illumina 1M Perlegen-Affymetrix Typed using GONL sequence data	UK-Biobank Axiom UKBiLEVE
imputation platform	PLINK HapMap	SHAPEIT2/ IMPUTE2 1000 genomes	Minimac3/ Eagle HRC	SHAPEIT3/ IMPUTE 4 HRC/ 1000 genomes / UK10K
sample QC procedures	Described by Gillespie et al.[9]	Described by Peterson et al.[10]	Described by Willemsen et al.[3]	Described by Bycroft et al.[11]
relatedness threshold	NA[b]	$\hat{\pi}$ = 0.35	NA[b]	KING = .0625
$N_{ethnic\_outliers}$	74	3,307	1,591	63,144
$N_{individuals}$ **after QC**	2,133	2,994	12,120	426,446
N_{SNPs} **before QC**	321	3,312	7,021	7,290
N_{SNPs} **after QC**	297	2,972	6,166	4,638

NA = not applicable; QC = quality control; PMID = PubMed identifier.
[a] Genotyping platform was included as a covariate in the NTR association analyses.
[b] In the family-based samples from 25Up and NTR related individuals were not excluded (the analyses controlled for family structure in the data).

SUPPLEMENTARY TABLE S2 Overview of included variables and used measures in the 25Up, S4S, NTR, and UKB studies. For each construct the composite measure, the number of items it was based on, and the number of times these items were measured. If an item was measured on multiple waves, we checked the consistency of reporting where possible (e.g. age at initiation variables) and took the highest or average of the reported instances (indicated with 'maximum' or 'average' in the table) depending on the type of measure. Outliers and unreliable response patterns were removed and data were standardized before analysis

Variable	25Up	S4S	NTR	UKB
DEMOGRAPHICS				
Age	Maximum score: 1 item age at last survey completion	Maximum score: 1 item (7 waves) age at last survey completion	Maximum score: 1 item (12 waves) age at last survey completion	Raw score: 1 item (1 wave) year of birth
Sex	Biologically determined sex	Biologically determined sex	Biologically determined sex	
Education	Raw score: 1 item highest (partially) completed education level	Maximum score: 2 items (1 wave) education level mother education level father	Maximum score: 1 item (12 waves) completed education level	Maximum score: 1 item (6 waves) educational qualifications
LIFETIME SUBSTANCE USE				
Ever used tobacco	Dichotomous ever/never: 1 item ever tried tobacco product	Dichotomous ever/never: 4 items (7 waves) lifetime smoking smoking frequency nicotine dependence age at smoking initiation	Dichotomous ever/never: 1-5 items (12 waves) lifetime smoking smoking frequency (also in ex-smokers) tobacco products per day (also in ex-smokers) nicotine dependence	Dichotomous ever/never: 4 items (3 waves) smoking status ever smoking pack years of smoking (number of packs p.d*number of years smoking) proportional pack years of smoking
Ever used cannabis	Dichotomous ever/never: 1 item ever tried cannabis	Dichotomous yes/no: 1 item (7 waves) past year cannabis use	Dichotomous ever/never: 1-3 items (5 waves) lifetime use age at first (regular) use past year use	Dichotomous ever/never: 2 items (1 waves) ever taking cannabis maximum use frequency
Ever used other substance(s)	Dichotomous ever/never: 13 items ever tried a specific drug	Dichotomous ever/never: 3 items (3 waves) past year stimulant use past year cocaine use past year opioid use	Dichotomous ever/never: 1-16 items (6 waves) lifetime (experimental) use of specific drug or drug category regular use age at first (regular) use	NA
AGE AT INITIATION OF SUBSTANCE USE				
Age alcohol initiation	Raw score: 1 item age first full-serve alcohol	Average score: 1 item (7 waves) age first time drunk	Average score: 1 item (7 waves) age at first alcohol use	NA
Age tobacco initiation	Raw score: 1 item age at daily smoking initiation	Average score: 1 item (6 waves) age first cigarette	Average score: 1 item (7 waves) age first cigarette	Average score: 1 item (2 waves) age at (regular) smoking initiation
Age cannabis initiation	Raw score: 1 item age at first use cannabis	Average score: 1 item (1 wave) age at first use cannabis	Average score: 1 item (5 waves) age first use cannabis/ soft drug	NA
Age other substance initiation	Raw score: 13 items age at first use specific drugs	Average score: 3 items (1 wave) age at first use stimulant age at first use cocaine age at first use opioid	Average score: 2-8 items (5 waves) age first (experimental) use of specific drug or drug category	NA
AVERAGE SUBSTANCE USE				
Average alcohol units per month	Total score: 2 items alcohol use frequency glasses consumed per drinking occasion	Average score: 1 item (7 waves) glasses consumed per month	Average score: 1-2 items (10 waves) glasses of alcohol on weekdays glasses of alcohol on weekend days	Average total score: 6 items (3 waves) weekly red wine weekly champagne/ white wine weekly beer/ cider weekly spirits weekly fortified wine weekly other alcohol

Average cigarettes per day	Raw score: 1 item tobacco products used per day	Maximum score: 1 item (7 waves) cigarettes smoked per day	Maximum score: 1-4 items (11 waves) cigarettes per day for current smokers cigarettes per day at the period of heaviest smoking for current and ex-smokers tobacco products per day	Average score: 2 items (3 waves) cigarettes per day for current smokers cigarettes per day for ex-smokers
Average tobacco using days	Total score: 1 item tobacco using days per year	Average score: 1 item (7 waves) tobacco use frequency	Average score: 1 item (6 waves) smoking frequency	NA
REGULAR SUBSTANCE (AB)USE				
Regular alcohol use	Dichotomous yes/no: 1 item drinking ≥ 4 times per week	Dichotomous ever/never: 1 item (7 waves) drinking ≥4 times per week	Dichotomous ever/never: 1-2 items (10 waves) drinking ≥ 4 times per week	Dichotomous yes/no: 1 item (1 wave) drinking ≥ 4 times per week
Problematic alcohol use (AUDIT)	NA	Maximum score: 1 item (7 waves) total score AUDIT	Total score: 1 item (1 wave) total score AUDIT	Total score: 1 item (1 wave) total score AUDIT
Regular tobacco use	Dichotomous ever/never: 1 item daily smoking	Dichotomous ever/never: 1 item (7 waves) daily smoking cigarettes per day	Dichotomous ever/never: 1 item (6 waves) daily smoking cigarettes per day	Dichotomous ever/never: 2 items (3 waves) daily smoking cigarettes per day
Nicotine dependence (FTND)	NA	Maximum score: 1 item (7 waves) total score FTND	Maximum score: 1-2 items (5 waves) total score FTND in ever smokers total score FTND in ex-smokers total score FTND in current smokers	Maximum sum score: 3 items (3 waves) total score FTND based on 3 items (time to first cigarette, difficulty To refrain, cigarettes per day)
Regular cannabis use	Dichotomous ever/never: 1 item used ≥ weekly	Dichotomous reported at least twice/ less: 1 item (7 waves) used ≥ 6 times a year	Dichotomous ever/never: 1 item (3 waves) regular use age at regular use	Dichotomous yes/no: 1 item (1 wave) at least monthly cannabis use
Any behavioral/substance addiction	NA	NA	NA	Dichotomous yes/no: 1 item (1 wave) ever addicted to substance or behavior
SEXUAL RISK BEHAVIOR				
Number of sexual partners	Raw score: 1 item number of lifetime sexual partners	Maximum score: 1 item (2 waves) number of sex partners past 3 months	NA	Average score: 1 item (3 waves) number of lifetime sexual partners
Sexual risk behavior	Total score: 2 items no need for affection in sex prefer several sexual relationships at once	Dichotomous ever/never: 4 items (2 waves) unprotected sex while not in relationship unprotected sex with multiple people sex under the influence of drugs sex under the influence of alcohol	NA	NA
Age at first sexual intercourse	NA	Average score: 1 item (2 waves) age at first time sex	Average score: 1 item (2 waves) age at first time sex	Average score: 1 item (3 waves) age at first time sex
SELF-CONTROL				
Disinhibition	NA	Maximum score: 1 item (1 wave) impulsivity total score (UPPS-P)	Maximum score: 1 item (6 waves) disinhibition (subscale from SSS)	NA
Sensation seeking	NA	NA	Maximum score: 1 item (6 waves) sensation seeking total score	NA

			(SSS)	
Risk-taking proneness	NA	NA	NA	Maximum score: 1 item (3 waves) inclined to take risks
ADHD	Total score: 1 item ADHD symptom score (ASRS)	Dichotomous ever/never: 2 items (3 waves) ever diagnosed with ADHD taking medication for ADHD	Maximum score: 1 item (3 waves) ADHD symptom score (ASRS)	NA

Note. ADHD/ADD= attention deficit (hyperactivity) disorder; ASRS= ADHD Self-Report Scale[12]; AUDIT= Alcohol Use Disorders Identification Test[13]; FTND= Fagerström Test for Nicotine Dependence[14]; UPPS-P= Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency, Impulsive Behavior Scale,[15] SSS= Sensation Seeking Scale.[16]

SUPPLEMENTARY TABLE S3 Results of factor-analyses including explained variances and pattern matrices of **(A)** 25Up, **(B)** S4S, **(C)** NTR and **(D)** UKB study data. Pattern matrices show results for factors with eigenvalues ≥ 1.00 indicating more explained variance than a single observed variable, and variable loadings < .30 are not considered significant [17] and therefore not reported.

A) 25Up data.

Factor	Eigenvalue	% of Variance	Cumulative %
1	2.84	17.75	17.75
2	1.98	12.34	30.09
3	1.47	9.17	39.26
4	1.35	8.43	47.70
5	1.08	6.76	54.45
6	1.05	6.57	61.02

	Factor						
Variable	**1**	**2**	**3**	**4**	**5**	**6**	**Factor label**
Ever used cannabis	.90						Lifetime substance use
Ever used tobacco	.69						
Ever used other substance(s)	.40						
Age alcohol initiation	-.37						
Average cigarettes per day		.90					Tobacco (ab)use
Average tobacco using days		.84					
Age cannabis initiation			.60				Age at initiation of substance use
Age other substance initiation			.34				
Age tobacco initiation			.33				
Regular alcohol use				.79			Alcohol (ab)use
Average alcohol units per month				.77			
Regular tobacco use					.76		NA
Regular cannabis use					.35		
ADHD					*		
Number of sexual partners						.55	Sexual risk behavior
Sexual risk behavior						.51	

* Variable loading < .30

C) NTR data.

B) S4S data

Factor	Eigenvalue	% of Variance	Cumulative %
1	4.10	20.52	20.52
2	2.10	10.52	31.04
3	1.30	6.49	37.52
4	1.24	6.19	43.72
5	1.20	98	49.70
6	1.04	5.20	54.90
7	1.02	5.08	59.97

	Factor							
Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Factor label**
Ever used cannabis	.73							Lifetime substance use
Ever used tobacco	.72							
Ever use other substance(s)	.45							
Disinhibition	*							
Nicotine dependence (FTND)		.78						Tobacco (ab)use
Average cigarettes per day		.71						
Risk score ADHD		*						
Average alcohol units per month			.82					Alcohol (ab)use
Regular alcohol use			.60					
Problematic alcohol use (AUDIT)			.39					
Number of sexual partners				.67				Sexual risk behavior
Sexual risk behavior				.45				
Age first sexual intercourse				*				
Age tobacco initiation					.59			Age at initiation of substance use
Age alcohol initiation					.51			
Age cannabis initiation					*			
Age other substance initiation					*			
Regular tobacco use						.80		Tobacco (ab)use
Average tobacco using days						.73		
Regular cannabis use							-.64	-

* Variable loading < .30

Factor	Eigenvalue	% of Variance	Cumulative %
1	2.72	14.33	14.33
2	2.06	10.87	25.20
3	1.65	8.66	33.85
4	1.42	7.47	41.32
5	1.22	6.40	47.72
6	1.12	5.87	53.59
7	1.02	5.36	58.95

Variable	1	2	3	4	5	6	7	Factor label
Sensation seeking	.92							Self-control
Impulsivity/disinhibition	.81							
ADHD	*							
Regular tobacco use		1.00						Tobacco (ab)use
Average tobacco using days		.43						
Ever used tobacco		.33						
Average alcohol units per month			.79					Alcohol (ab)use
Regular alcohol use			.60					
Problematic alcohol use (AUDIT)			.55					
Average cigarettes per day				.76				Tobacco (ab)use
Nicotine dependence (FTND)				.74				
Age alcohol initiation					.60			Age at initiation
Age tobacco initiation					.43			
Age at first sexual intercourse					*			
Ever other substance(s)						.70		Lifetime substance use
Ever used cannabis						.56		
Age cannabis initiation							-.57	-
Regular cannabis use							*	
Age other substance initiation							*	

* Variable loading < .30

D) UKB data.

Factor	Eigenvalue	% of Variance	Cumulative %

1	2.35	16.80	16.80
2	1.53	10.93	27.72
3	1.47	10.47	38.20
4	1.18	8.42	46.61
5	1.04	7.46	54.07

	Factor					
Variable	**1**	**2**	**3**	**4**	**5**	**Factor label**
Regular tobacco use	.96					Tobacco (ab)use
Ever used tobacco	.91					
Problematic alcohol use (AUDIT)		.77				Alcohol (ab)use
Regular alcohol use		.71				
Average alcohol units per month		*				
Nicotine dependence (FTND)			.70			Tobacco (ab)use
Average cigarettes per day			.56			
Age tobacco initiation			*			
Number of sexual partners				.57		(Sexual) risk behavior
Age at first sexual intercourse				-.34		
Risk-taking proneness				*		
Any behavioral/substance addiction					.52	-
Ever used cannabis					*	
Regular cannabis use					*	

* Variable loading < .30

SUPPLEMENTARY TABLE S4 The number of SNPs that was present in at least one sample in the analysis (#SNPs), the number of independent SNPs (with R^2=0.1, LD merging distance 250kb, and a two-sided p<.1), and the name of the 10 independent SNP(s) with the smallest p-values and the corresponding bp position. SNPs that occur among the top-ten for multiple phenotypes are denoted with a superscript number. SNPs with a red superscript appear more than two times in primary phenotypes, green appear twice in primary phenotypes, and blue appear multiple times, but only once in a primary phenotype (the other time(s) in a factor)

Variable	#SNPs	#independent SNPs	SNPs	bp
LIFETIME SUBSTANCE USE				
Ever used tobacco	1,756	63	rs1433708	85481979
			rs11127908	85869285
			rs12714592 [1]	84387950
			rs1248857 [2]	85018612
			rs1448602	85780454
			rs1485582	84688653
			rs113364248 [3]	85741923
			rs13319945	84025580
			rs1248809	84934915
			rs12637371	84219156
Ever used cannabis	6,383	83	rs7636243	85472033
			rs4473564 [4]	85893048
			rs1248857 [2]	85018612
			rs9868293	84808756
			rs9856889	84424761
			rs9821126	85487124
			rs6804996	85468913
			rs9832634	85135013
			rs114226996 [5]	85143307
			rs9869787	84048375
Ever used other substance(s)	6,214	58	rs74928832	85086455
			rs79337314	85841743
			rs74569441	85409260
			rs60859036	84668629
			rs116240880	84210507
			rs4635723	85153235
			rs9846520 [6]	84299389
			rs60407397	85381838
			rs73136796	85465561
			rs34584686	85345197
AGE AT INITIATION OF SUBSTANCE USE				
Age alcohol initiation	6,215	41	rs60538752	84043066
			rs78355395	84906860
			rs9816329	86115541
			rs74332784	84313596

			rs57547677	84981296
			rs111993139	85266708
			rs9861858	83998003
			rs3887138	85648957
			rs17501983	84833794
			rs2123163	85243797
Age tobacco initiation	6,383	35	rs10514735	85798950
			rs12489914	84987790
			rs7625608	84964399
			rs9826386	84265870
			rs79077228	84093466
			rs7625199	85721301
			rs13076735	84160147
			rs55829275	85804632
			rs74337284 [7]	84564209
			rs76446023	85997149
Age cannabis initiation	6,215	38	rs76578522	85392436
			rs114612207	86063849
			rs114382596	85683952
			rs114226996 [5]	85143307
			rs980333	84838485
			rs7622685	84692835
			rs77687507	84171360
			rs78495499	84586445
			rs115454880	85628672
			rs116593166	84356123
Age other substance initiation	6,215	37	rs114228638	84028252
			rs79526794	85006615
			rs11713922	84294532
			rs73136106	86070879
			rs79664787	84498183
			rs11127830	84024847
			rs73147245	85759865
			rs74355494	85258972
			rs4261889	84741624
			rs116230250	84991998
AVERAGE SUBSTANCE USE				
Average alcohol units	6,383	69	rs9839708 [8]	85058885
			rs9990096 [9]	85411193
			rs76363701	85436059
			rs7611991 [10]	85759558
			rs7616936	85803785
			rs80134033	85816461
			rs78223691	84976059

			rs111594685 [11]	86070777
			rs76517098	84303077
			rs115217146	84906889
Average cigarettes per day	6,383	53	rs116772105 [12]	84202337
			rs7652808	85603643
			rs9814165	84223116
			rs73125330	84288773
			rs9836564	83997097
			rs55765801	85685077
			rs115888131	84498841
			rs77025486	85830581
			rs62250660	85446954
			rs17735321 [13]	85113612
Average tobacco using days	6,215	25	rs77114692	85076445
			rs28366554	84185799
			rs76813943	83961148
			rs73141532	85652614
			rs9876301	85991634
			rs73130743 [14]	85288268
			rs12633811	83962883
			rs111468253	84297075
			rs12637767	85206681
			rs114807292	85599240
REGULAR SUBSTANCE (AB)USE				
Regular alcohol use	6,281	52	rs12495758 [15]	85554262
			rs4301023	85057281
			rs116559208 [16]	84462556
			rs114242255	84201642
			rs79187939	84191354
			rs13085678	85358591
			rs75504236	84030818
			rs375750	84987517
			rs115730277	84173238
			rs9872971	85344951
Problematic alcohol use (AUDIT)	6,379	67	rs12495758 [15]	85554262
			rs1248860	85015779
			rs4473564 [4]	85893048
			rs114375956	85193798
			rs76873181	84208185
			rs75902824	84343551
			rs72919209	85318295
			rs13060392	84880419
			rs2326267	85394420
			rs117898875	84272987

Regular tobacco use	6,380	90	rs7650284	85472227
			rs62261746 [17]	85958954
			rs7611991 [10]	85759558
			rs9834688	85035279
			rs12714592 [1]	84387950
			rs515207	84926866
			rs9835484	84742570
			rs113364248 [3]	85741923
			rs6773147	83988022
			rs9864886	84133542
Nicotine dependence (FTND)	6,379	59	rs73131909 [18]	83960956
			rs116383974	84576787
			rs9990096 [9]	85411193
			rs114011108	85693334
			rs116772105 [12]	84202337
			rs116240935	85285448
			rs74745315 [19]	85012608
			rs79749903	85249455
			rs9832119	84972676
			rs78780329 [20]	85100973
Regular cannabis use	5,676	50	rs2044723	85646002
			rs113817396	83973343
			rs74337284 [7]	84564209
			rs114185016	84313475
			rs1988552	85849851
			rs2172846	85004637
			rs2875889	85394742
			rs1454089	84852101
			rs4513464	83966741
			rs6549016	85180270
Any behavioral/substance addiction	4,557	37	rs1003985 [21]	85870303
			rs10049108 [22]	85702021
			rs10084664 [23]	85964737
			rs1013839 [24]	85243777
			rs1014796 [25]	85544470
			rs10154865 [26]	85409299
			rs1017638 [27]	84751572
			rs10212311 [28]	84297635
			rs10212377 [29]	84299345
			rs10212504 [30]	84299387
SEXUAL RISK BEHAVIOR				
Number of sexual partners	5,677	54	rs9824301	85682888
			rs4856269	85406735

			rs66680800	85985324
			rs73132094 [31]	84893889
			rs114398534	84219421
			rs62250579	85024652
			rs62250575	84987256
			rs73130743 [14]	85288268
			rs7646381	85705615
			rs17735321 [13]	85113612
Sexual risk behavior [a]	NA	NA	NA	NA
Age at first sexual intercourse	6,379	91	rs2044725	85645873
			rs12714592 [1]	84387950
			rs62261746 [17]	85958954
			rs12714603	84656669
			rs13316157	84115210
			rs74843558	85655439
			rs9839708 [g]	85058885
			rs79314616	84801526
			rs62255523	84804511
			rs67028245	85394772
SELF-CONTROL				
Disinhibition	6,211	45	rs10212294	85668496
			rs9816652	85067018
			rs79361329	84594046
			rs76062229	84211693
			rs76508707	85397049
			rs79874755	84116343
			rs17437928	85406521
			rs13088475	85019056
			rs74446005	85733229
			rs114307462	84764593
Sensation seeking	6,109	35	rs1003984 [32]	85870199
			rs1003985 [21]	85870303
			rs1003986 [33]	85870363
			rs10049108 [22]	85702021
			rs10049397 [34]	84857250
			rs1005690 [35]	85358806
			rs10084664 [23]	85964737
			rs10084716 [36]	85964639
			rs1013839 [24]	85243777
			rs1014796 [25]	85544470
Risk-taking proneness	4,557	69	rs1003985 [21]	85870303
			rs10049108 [22]	85702021
			rs10084664 [23]	85964737
			rs1013839 [24]	85243777

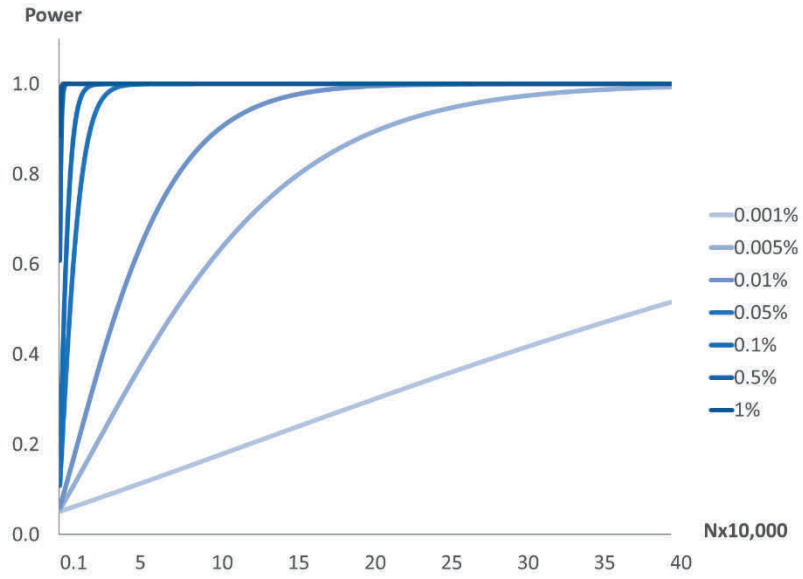
			rs1014796 [25]	85544470
			rs10154865 [26]	85409299
			rs1017638 [27]	84751572
			rs10212311 [28]	84297635
			rs10212377 [29]	84299345
			rs10212504 [30]	84299387
ADHD	3,436		rs17023190	85752400
			rs111594685 [11]	86070777
			rs114459574	84931784
			rs73132094 [31]	84893889
			rs7615964	85660567
			rs116559208 [16]	84462556
			rs77423946	85644855
			rs3860559	84765953
			rs112117757	84525528
			rs17021771	84796650
FACTORS				
Lifetime substance use (meta-analysis)	2,464	12	rs6419760	84870987
			rs60750563	84489985
			rs116351045	85507460
			rs77430012	85473111
			rs2171140	85126955
			rs75892230	85506121
			rs6764254	84613365
			rs61555026	84685842
			rs73843277	85122712
			rs9864651	85514116
Tobacco (ab)use (meta-analysis)	6,352	44	rs73131909 [18]	83960956
			rs9863620	85448493
			rs13059122	84181443
			rs9846520 [6]	84299389
			rs78780329 [20]	85100973
			rs12629798	85905591
			rs62250718	85523783
			rs1874866	84988633
			rs17735321[13]	85113612
			rs74745315 [19]	85012608
Lifetime smoking (UKB)	4,557	81	rs1003985 [21]	85870303
			rs10049108 [22]	85702021
			rs10084664 [23]	85964737
			rs1013839 [24]	85243777
			rs1014796 [25]	85544470
			rs10154865 [26]	85409299
			rs1017638 [27]	84751572

			rs10212311 [28]	84297635
			rs10212377 [29]	84299345
			rs10212504 [30]	84299387
Regular alcohol use (UKB)	4,557	64	rs1003985 [21]	85870303
			rs10049108 [22]	85702021
			rs10084664 [23]	85964737
			rs1013839 [24]	85243777
			rs1014796 [25]	85544470
			rs10154865 [26]	85409299
			rs1017638 [27]	84751572
			rs10212311 [28]	84297635
			rs10212377 [29]	84299345
			rs10212504 [30]	84299387
Self-control (NTR)	6,109	45	rs1003984 [32]	85870199
			rs1003985 [21]	85870303
			rs1003986 [33]	85870363
			rs10049108 [22]	85702021
			rs10049397 [34]	84857250
			rs1005690 [35]	85358806
			rs10084664 [23]	85964737
			rs10084716 [36]	85964639
			rs1013839 [24]	85243777
			rs1014796 [25]	85544470

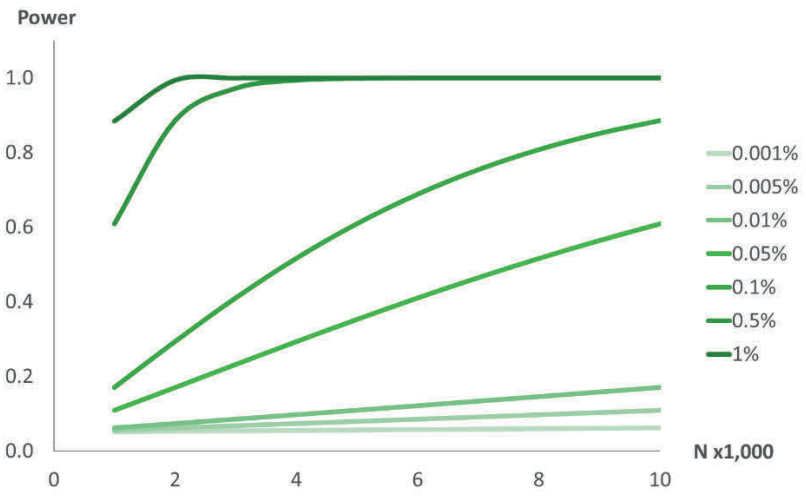
[a] Not available in the SNP-based meta-analysis

SUPPLEMENTARY FIGURE S1 **(A)** Power as a function of sample size (*N*) and effect size (%R^2) **(B)** as well as reported in more detail for sample sizes below 10,000.

A)

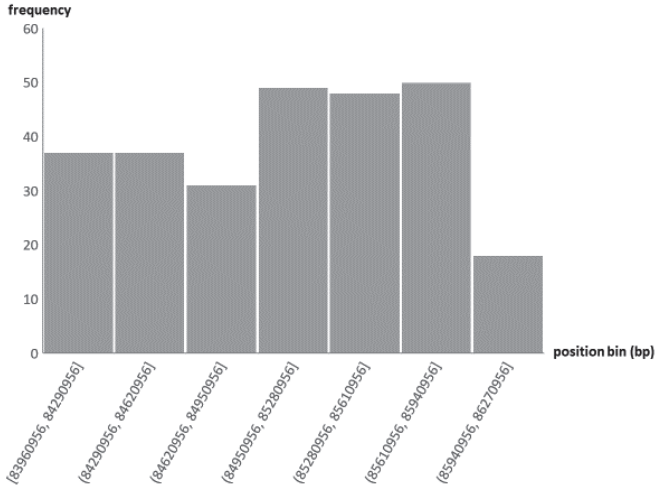


B)

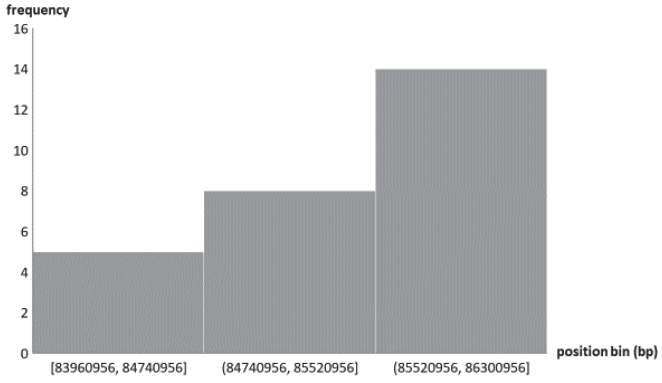


SUPPLEMENTARY FIGURE S2 Frequency distribution of the basepair positions of the independent SNPs that were most strongly associated with the included risk behavior phenotypes with **(panel A)** showing the distribution in 10 equal-sized bins (330,000 bp), **(panel B)** showing the distribution in 3 bins (780,000). Most of the associated top SNPs fell in the bins between 84,950,956 and 85,940,956, which is a region containing many cis eQTLs **(panel C)**

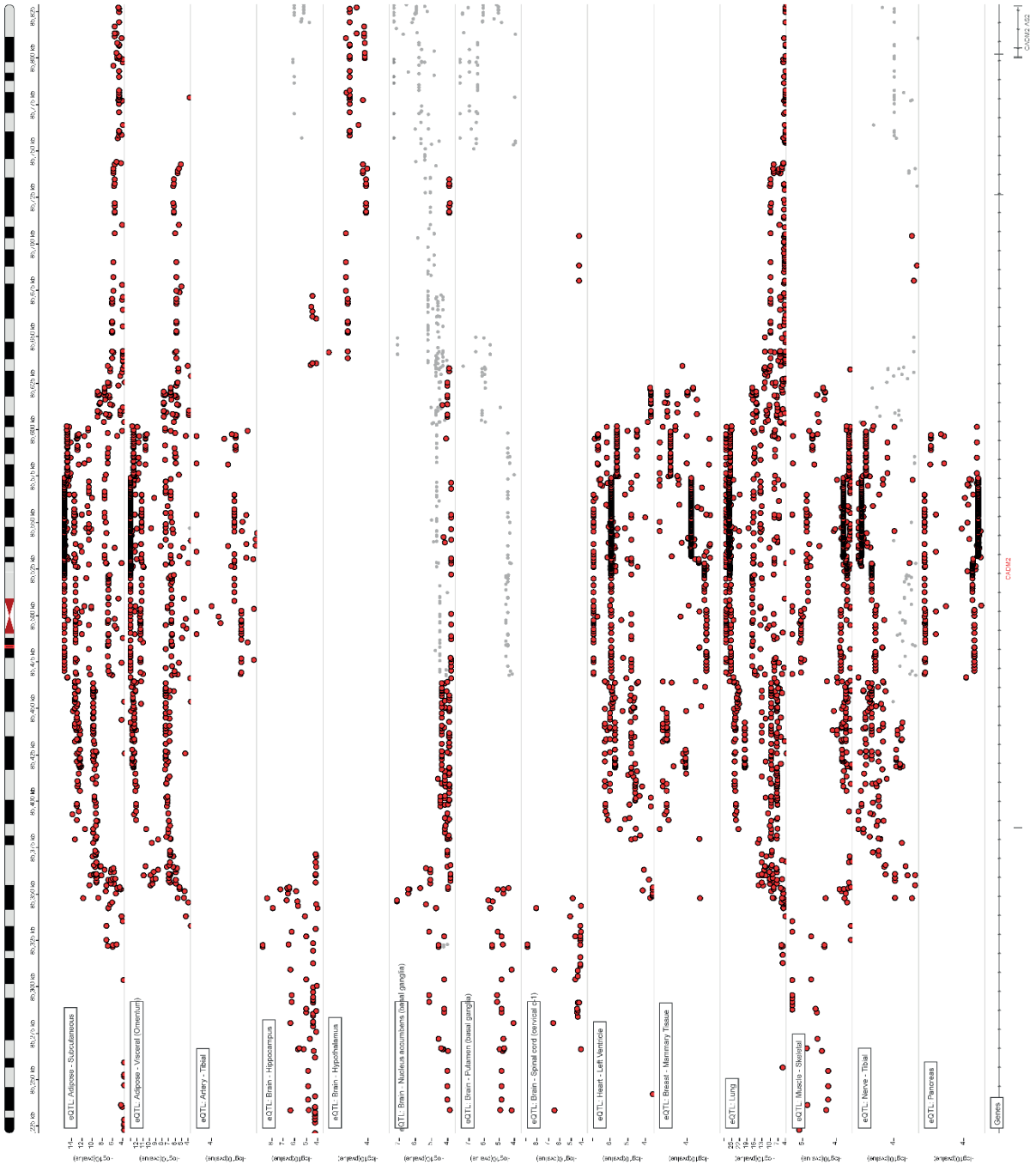
Panel A)



Panel B)



Panel C)


eQTL: Adipose - Subcutaneous
eQTL: Adipose - Visceral (Omentum)
eQTL: Artery - Tibial
eQTL: Brain - Hippocampus
eQTL: Brain - Hypothalamus
eQTL: Brain - Nucleus accumbens (basal ganglia)
eQTL: Brain - Putamen (basal ganglia)
eQTL: Brain - Spinal cord (cervical c-1)
eQTL: Heart - Left Ventricle
eQTL: Breast - Mammary Tissue
eQTL: Lung
eQTL: Muscle - Skeletal
eQTL: Nerve - Tibial
eQTL: Pancreas
Genes
CADM2
CADM2 ASO

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CHAPTER 6

The CADM2 gene and behavior: A phenome-wide scan in UK Biobank

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Abstract

This phenome-wide association study examined SNP and gene–based associations of the *CADM2* gene with 241 psycho-behavioral traits in the UK Biobank (N=12,160 – 453,496). We found significant associations for 56 traits, replicating previously reported associations with substance use, risk-taking and health behaviors, and uncovering novel associations with sleep and dietary traits. We conclude that *CADM2* is involved in many psycho-behavioral traits, suggesting a common denominator in the biology of these traits.

In the last 15 years, genome-wide association studies (GWASs) have identified tens of thousands of associations between genetic variants and a range of human behavioral and physical traits. One gene that has popped up surprisingly often in behavioral GWASs is the Cell Adhesion Molecule 2 gene (*CADM2*). Common variations (single nucleotide polymorphisms, SNPs) in the *CADM2* gene have been implicated in various traits, including personality[1], cognition and educational attainment[2,3], risk-taking behavior[4], reproductive success[5], autism spectrum disorders[6], substance use[7,8], physical activity[9], and BMI/ obesity[10].

CADM2 encodes a member of the synaptic cell adhesion molecules (SynCAMs) involved in synaptic organization and signaling, suggesting that alterations in *CADM2* expression affect neuronal connectivity. *CADM2* is abundant in brain regions important for reward processing and addiction, including the frontal anterior cingulate cortex[3], substantia nigra, and insula[11]. Given its common appearance in GWASs and its central role in brain functioning, *CADM2* is a gene that warrants further exploration.

In this study we perform a phenome-wide association analysis (PheWAS), in which we test for associations of *CADM2* (on SNP and gene level) with a comprehensive selection of psycho-behavioral phenotypes as measured in the UK Biobank cohort. Results will provide insights about whether the role of *CADM2* is confined to a specific set of traits or is involved in a wider range of phenotypes. This will inform future studies on the function of *CADM2* and the neurobiological underpinnings of different psycho-behavioral traits. An additional advantage is that the multiple testing burden is reduced as compared to genome-wide studies, resulting in higher power levels.

UK Biobank is a nationwide study in the United Kingdom containing phenotypic and genetic information for up to 500,000 individuals[12]. We analyzed 12,218 to 453,496 UK Biobank participants with European ancestry for whom genetic and phenotypic data were available. About half (54.3%) of the sample was female, and mean age was M=56.8 (range 39-73, SD=8.0). We extracted the *CADM2* region 250 kb up- and downstream (bp 84,758,133 to 86,373,579 on 3p12.1, GRCh37/hg19) and selected 4,265 SNPs with call rate>95%, minor allele frequency >1%, and *p*-value for violation of Hardy-Weinberg equilibrium of $p_{HWE}>10^{-6}$ (QC details are described in Abdellaoui, 2020[13]).

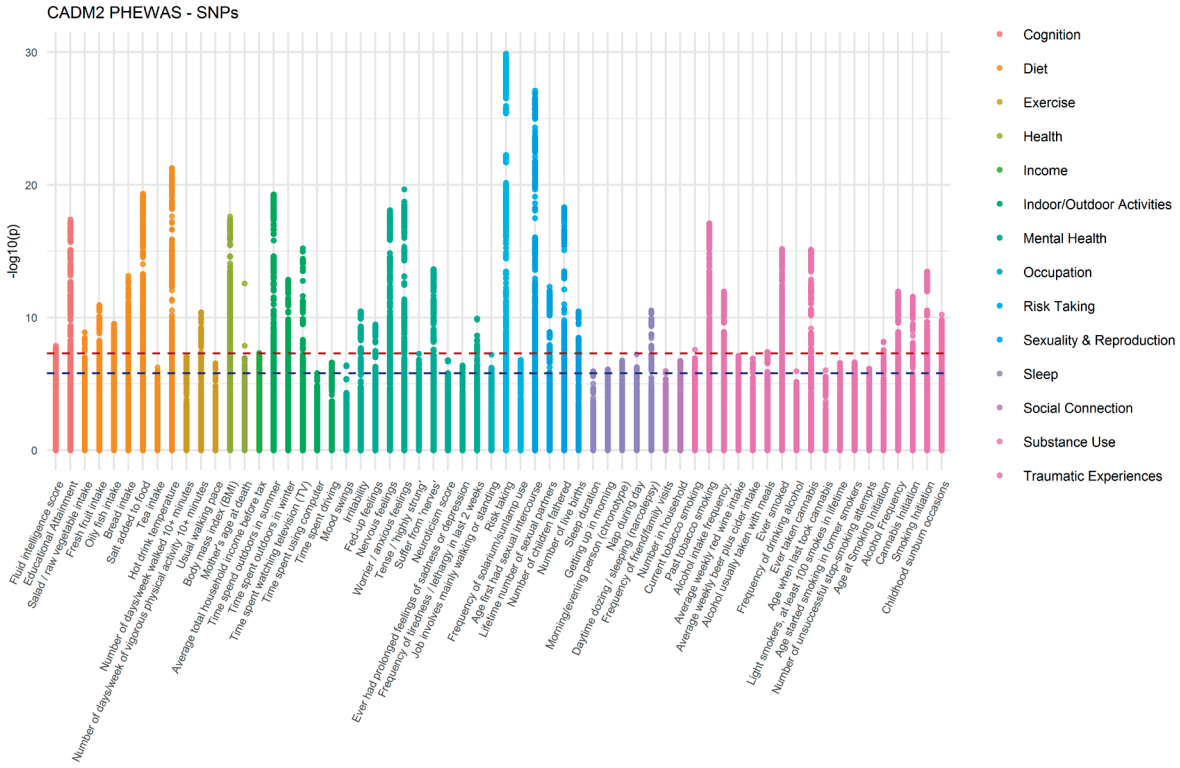
We selected 241 psychological and behavioral phenotypes from the UK Biobank with an effective sample size above N=10,000 ($N_{eff}=4/\frac{1/N_{cases}}{1/N_{controls}}$). In order to maximize sample size, we used the first available measurement for each individual; if the first instance was not available, we took the second, otherwise the third, etc. In addition, we included eight

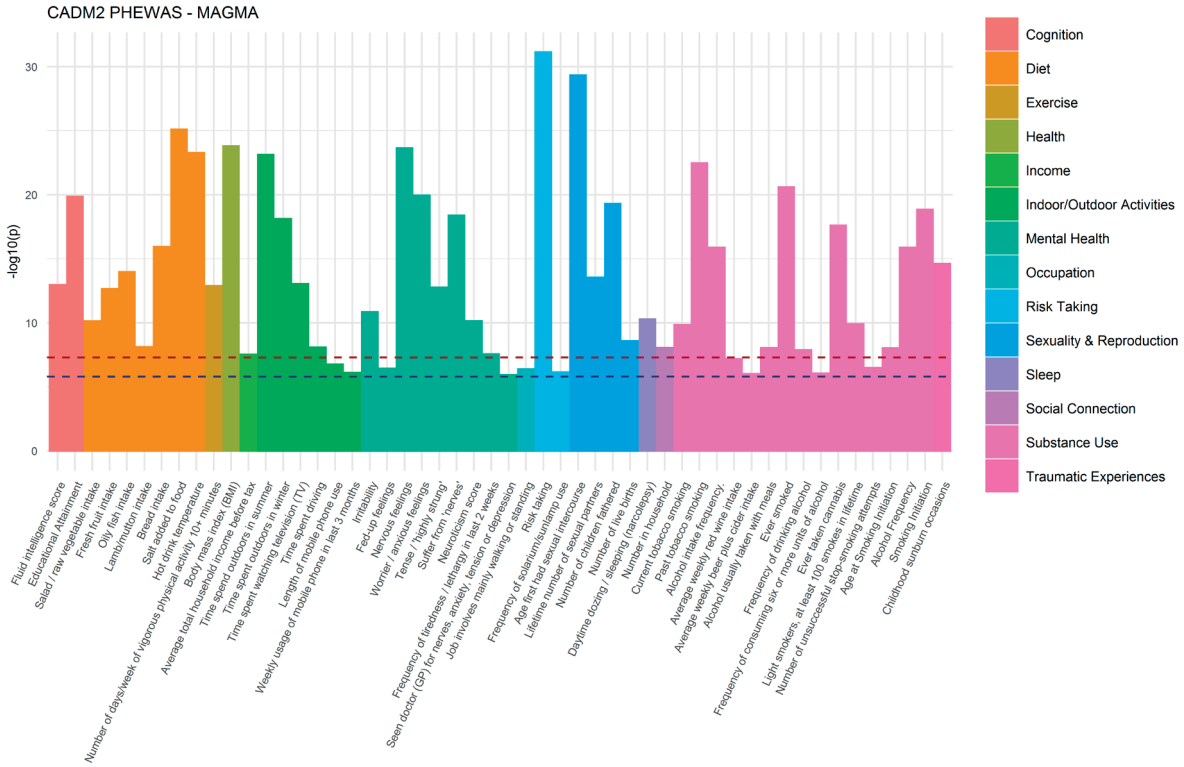
traits that were derived for recent genetic studies, including seven substance use traits and educational attainment (for an overview of all included traits, see Supplemental Table S1). Continuous phenotypes were cleaned such that theoretical implausible were set on missing and extreme values at 4 SDs from the mean were put on missing. Binary and ordinal variables were left unchanged. Ordinal were analyzed as continuous

The SNP-based association analyses were performed in GCTA fastGWA[14], taking into account genetic relatedness. Analyses were controlled for effects of age, sex, and 25 principal components (PCs, to control for genetic ancestry[15]). We used linear mixed modeling for all traits and Haseman-Elston regression to estimate the genetic variance component. To test the significance of association on the level of the gene we conducted a MAGMA gene-based test[16], which aggregates the SNP effects (regardless of direction) in a single test of association. We used the default SNP-wise mean procedure (averaging SNP effects across the gene) and check the results of the SNP-wise top procedure for comparison (more sensitive when only a small proportion of SNPs has an effect). As significance threshold for the SNP-based test we adopt a genome-wide significance threshold of p<5E-08. As this is rather stringent given that we test within a single gene, we also use a significance threshold of .05 corrected for the number of independent SNPs (n=133, at R^2=0.10 and 250kb) and the number of traits, resulting in .05/(133*241)=1.56E-06. For the gene-based test we used a threshold of 2.62E-05, corresponding to .05 divided by the total number of genes (19,082). To provide an estimation of the effect size, we used $R^{2=}\frac{2\beta^2 MAF(1-MAF)}{2\beta^2 MAF(1-MAF)+(se(\beta))^2 2N\ MAF(1-MAF)}$, as described in 17, with adaptations for binary traits as described in[8].

On the SNP-level, 38 traits reached significant associations at a genome-wide corrected p-value (5E-08), and 62 traits at the lenient threshold of p<1.56E-06 (Figure 1a, Table 1). In the gene-based test, 56 out of 241 traits showed significant associations under a p-value of 2.62E-06 (Figure 1b, Table 1). Of those, there were 48 traits whose top-SNP reached significance under p<1.56E-06. The strongest associations were found for cognitive ability, risk taking, diet, BMI, daytime sleeping, sedentary behaviors, nervousness-like traits, reproductive traits, and substance use. There were virtually no associations with occupational, traumatic experiences, social connection and non-worry related depression traits. Full SNP- and gene-based results are provided in Table S2 and S3a. Table S3b shows the gene-based results for the SNP-wise top procedure, showing some differences with the SNP-wise mean results (correlation between the p-values from the respective tests was r=.69).

Figure 1. PheWAS results. Panel **a)** shows the subset of significant associations of the SNP-based test per trait . The x-axis shows the traits (colored by trait category) and the y-axis the *p*-values of the association. Each dot represents a SNP association. SNPs exceeding the red horizontal line have a *p*-value significant at a genome-wide threshold of *p*=5E-08. The blue horizontal line represents the suggestive threshold of *p*=1.56E-06. Full SNP-based results are given in Supplementary Figure 1. Panel **b)** shows the subset of significant results of the MAGMA gene-based test, with *p*-values on the y-axis. The red dotted line represents a threshold of *p*=2.62E-06. The full gene-based results are depicted in Supplementary Figure S2.





The SNPs that showed the highest number of significant trait-associations (with a maximum of 27 traits at p<1.56E-6, Table S4) clustered around loci at 85.53 and 85.62 Mb. As can be seen in Figure 2, most SNPs that were independently (LD R^2<0.01, distance >250kb) significantly associated with at least one trait cluster in the middle of the gene, a region rich in eQTLs.

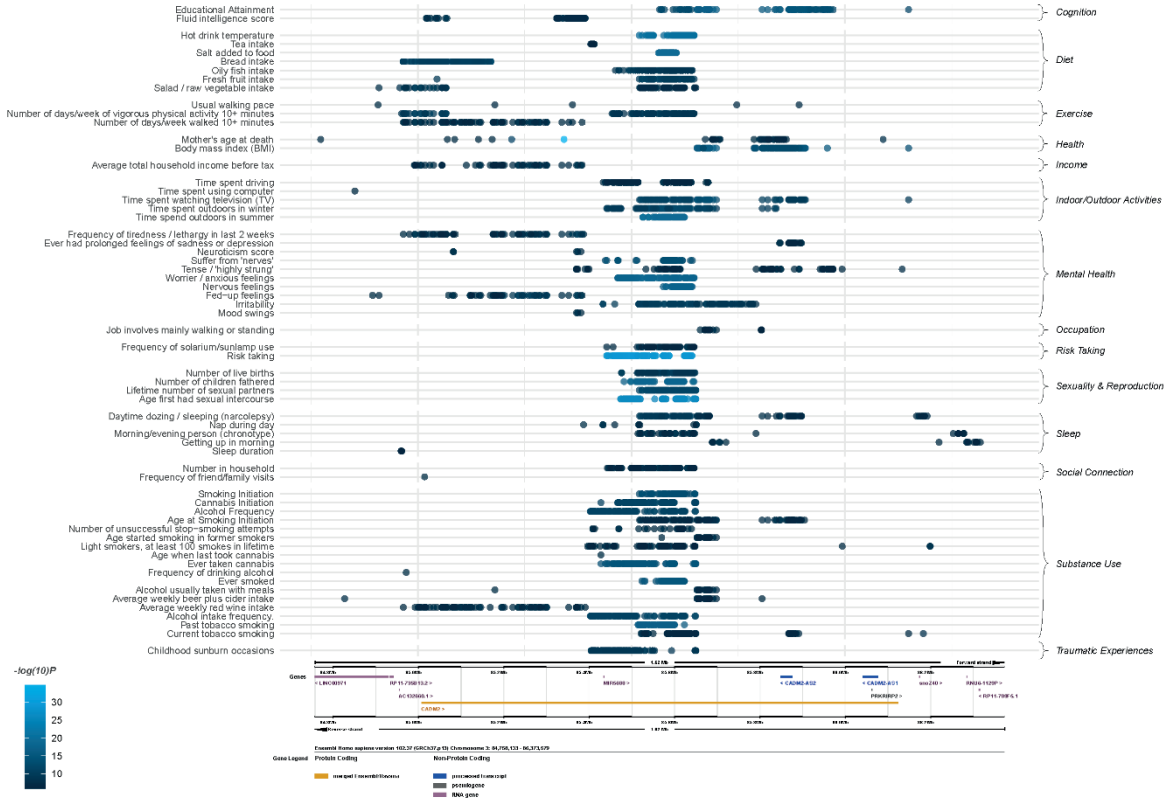


Figure 2. The top 100 most significant SNPs for each trait with at least 1 significant SNP. The x-axis represents the base pair position, and the panel below shows information on the CADM2 transcripts as derived from https://www.ensembl.org/.

Table 1. Phenotypes with a significant association with *CADM2* according to the MAGMA gene-based test (SNP-wise mean) at $p<2.62E-06$. The top-SNP for the phenotype is given with the minor allele (A1), beta (β), *p*-value (*p*), and percentage of explained variance in the respective trait (R^2 (%)). Most top-SNPs were significant at $p<1.56E-6$ (bold-faced).

Category	Variable label	N	*p*(Gene)	Top SNP	A1	β	*p*	R^2 (%)
Diet	Bread intake	446,539	1.13E-16	rs2326128	A	0.016	6.78E-14	0.013
Diet	Fresh fruit intake	450,404	2.29E-13	rs2326319	G	0.015	1.15E-11	0.010
Diet	Hot drink temperature	448,846	5.49E-24	rs17023019	A	-0.012	5.21E-22	0.021
Diet	Lamb/mutton intake	450,954	7.76E-09	rs10865611	G	0.007	3.24E-06	0.005
Diet	Oily fish intake	451,386	1.08E-14	rs11712915	C	0.014	2.95E-10	0.009
Diet	Salad / raw vegetable intake	446,150	7.76E-11	rs1248825	A	-0.014	1.26E-09	0.008
Diet	Salt added to food	453,496	8.03E-26	rs17516256	G	0.017	4.61E-20	0.018
Exercise and sedentary behavior	Length of mobile phone use	447,996	1.81E-07	rs13092059	A	0.023	2.41E-06	0.005
Exercise and sedentary behavior	Number of days/week of vigorous physical activity 10+ minutes	431,856	1.31E-13	rs2326123	T	-0.030	3.96E-11	0.010
Exercise and sedentary behavior	Time spend outdoors in summer	427,599	7.72E-24	rs62250754	G	0.020	5.04E-20	0.020
Exercise and sedentary behavior	Time spent driving	440,626	8.47E-09	rs11922956	A	0.011	2.36E-07	0.007
Exercise and sedentary behavior	Time spent outdoors in winter	425,396	7.82E-19	rs960986	T	-0.016	1.41E-13	0.013
Exercise and sedentary behavior	Time spent watching television (TV)	448,565	9.29E-14	rs9867437	C	-0.017	5.96E-16	0.015
Exercise and sedentary behavior	Weekly usage of mobile phone in last 3 months	382,548	8.01E-07	rs6785555	G	-0.020	1.68E-06	0.005
Mental health	Ever thought that life not worth living	146,036	2.75E-06	rs10511068	G	0.011	3.47E-04	0.008
Mental health	Fed-up feelings	444,267	3.91E-07	rs1452117	A	0.007	3.24E-10	0.009

Mental health	Frequency of tiredness / lethargy in last 2 weeks	440,245	2.87E-08	rs818215	C	-0.011	1.18E-10	0.010
Mental health	Irritability	352,855*	1.47E-11	rs6800177	T	0.007	3.33E-11	0.010
Mental health	Nervous feelings	320,799*	2.35E-24	rs1449386	T	-0.008	7.93E-19	0.018
Mental health	Neuroticism score	367,393	7.56E-11	rs818219	C	-0.012	1.59E-07	0.010
Mental health	Seen doctor (GP) for nerves, anxiety, tension or depression	450,553	1.25E-06	rs12631564	A	0.005	2.77E-06	0.005
Mental health	Suffer from 'nerves'	294,167*	4.22E-19	rs7652808	T	-0.007	2.21E-14	0.013
Mental health	Tense / 'highly strung'	253,874*	1.77E-13	rs9811546	A	-0.005	5.28E-08	0.006
Mental health	Worrier / anxious feelings	436,089*	1.17E-20	rs62250713	A	-0.010	2.18E-20	0.019
Occupation	Job involves mainly walking or standing	258,887	4.25E-07	rs11928368	T	-0.018	6.07E-08	0.014
Physical health	Body mass index (BMI)	450,828	1.65E-24	rs11915747	G	-0.019	2.41E-18	0.017
Physical health	Childhood sunburn occasions	337,064	2.41E-15	rs9880919	A	0.018	5.85E-11	0.013
Reproductive	Age first had sexual intercourse	396,343	4.89E-30	rs62263912	G	-0.025	7.88E-28	0.031
Reproductive	Lifetime number of sexual partners	370,052	3.06E-14	rs4856598	A	0.017	4.74E-13	0.015
Reproductive	Number of children fathered	205,419	5.41E-20	rs1368750	T	0.028	4.87E-19	0.039
Reproductive	Number of live births	245,522	2.67E-09	rs6782190	G	0.020	3.27E-11	0.018
Risk taking	Frequency of solarium/sunlamp use	446,769	7.52E-07	rs62250713	A	0.011	1.50E-07	0.007
Risk taking	Risk taking	339,917*	7.58E-32	rs6762267	C	0.011	1.27E-30	0.030
Sleep	Daytime dozing / sleeping (narcolepsy)	451,906	5.28E-11	rs960986	T	-0.007	2.86E-11	0.010
Social interaction	Frequency of friend/family visits	451,221	2.56E-06	rs146388815	A	0.017	.004	0.002
Socioeconomic status and intelligence	Average total household income before tax	390,639	3.09E-08	rs426444	T	-0.015	4.59E-08	0.008
Socioeconomic status and intelligence	Educational attainment transformed to ISCED categories, derived from PMID 27225129	449,653	1.44E-20	rs11915747	G	0.095	3.86E-18	0.017

Socioeconomic status and intelligence	Fluid intelligence score	176,234	1.32E-08	rs73845427	A	-0.054	1.32E-08	0.018
Socioeconomic status and intelligence	Number in household	450,321	8.87E-09	rs10865611	G	0.011	1.82E-07	0.007
Substance use	Age at smoking initiation, derived from PMID 30643251	146,485	9.66E-09	rs4513466	T	-0.021	6.43E-09	0.023
Substance use	Age started smoking in former smokers	113,014	2.76E-06	rs67219198	A	0.023	2.27E-07	0.023
Substance use	Alcohol intake frequency	453,215	1.34E-16	rs9814516	T	-0.026	1.09E-12	0.011
Substance use	Alcohol usually taken with meals	208,057*	9.28E-09	rs12493621	C	0.008	3.85E-08	0.013
Substance use	Average weekly beer plus cider intake	320,900	1.01E-06	rs79948904	G	-0.035	6.61E-05	0.036
Substance use	Average weekly red wine intake	320,895	6.88E-08	rs1452121	T	-0.014	7.36E-08	0.009
Substance use	Current tobacco smoking	453,302	1.45E-10	rs56262138	A	-0.005	2.60E-08	0.007
Substance use	Ever smoked	432,971*	2.58E-21	rs6790699	A	0.009	6.39E-16	0.014
Substance use	Ever taken cannabis	146807	2.51E-18	rs62263912	G	0.029	7.44E-16	0.046
Substance use	Frequency of alcohol use, derived from PMID 30874500	453,216	1.34E-16	rs9814516	T	-0.026	1.09E-12	0.011
Substance use	Frequency of consuming six or more units of alcohol	134,928	9.03E-07	rs1691471	T	0.020	1.37E-05	0.013
Substance use	Frequency of drinking alcohol	146,834	1.38E-08	rs9832119	T	-0.025	1.06E-06	0.017
Substance use	Lifetime cannabis use, derived from PMID 30150663	100,951*	2.32E-16	rs62263912	G	0.011	2.63E-12	0.033
Substance use	Light smokers, at least 100 smokes in lifetime	124,065*	1.23E-10	rs62253088	T	0.011	2.49E-07	0.020
Substance use	Number of unsuccessful stop-smoking attempts	103,486	3.26E-07	rs13077660	C	0.026	7.05E-07	0.026
Substance use	Past tobacco smoking	417,712	3.40E-23	rs6780346	C	-0.024	7.54E-18	0.018
Substance use	Smoking initiation, derived from PMID 30643251	167,714*	1.49E-19	rs62263910	G	0.008	3.26E-14	0.019

*For binary traits, the effective sample size is given (determined using $N_{eff}=4/\frac{1/N_{cases}}{1/N_{controls}}$).

This PheWAS showed that *CADM2* was involved in a wide spectrum of traits, thereby replicating and extending on previous findings. For most of the associations we find, similar associations have been reported in previous literature (Table S4). However, some specific associations, such as those with the specific dietary traits, daytime sleeping, and occupation, have not been reported before. Table S5 summarizes (functional implications of) putatively novel SNPs for which no phenotypic associations have been reported before. This study is the first that provides a comprehensive overview across a spectrum of psycho-behavioral traits.

On the gene-level, 56 of 241 tested traits showed a significant association. All categories except the one for traumatic experiences contained traits that were associated with *CADM2*, with diet (7 out of 20 traits), substance use (17/60), risk taking (2/5), socioeconomic status and intelligence (4/8), and physical health (2/6) containing the most significantly associated traits. The variance explained by *CADM2* was highest for lifetime cannabis use, followed by number of children fathered, age at first sexual intercourse, and risk taking. Overall, effect sizes were small (less than 0.05% for cannabis initiation). Few associations were found in the social interaction, sleep, and occupational categories. Also, there were not many mental health traits that showed an association (10 out 53 traits). It is interesting to note the significant associations with worry and nervousness-like traits in the absence of association with (other) depression- and anxiety-related traits. There may be something specific to these seemingly overlapping traits, translating to distinct biological pathways.

Future (animal) research is needed to explain these links between *CADM2* and this spectrum of psycho-behavioral traits in terms of biological processes. For example, it could be that *CADM2* is important for the learning aspects of behavior, given its role in synaptic connectivity. Speculatively, *CADM2* could be mainly involved in reward-learning and conditioning, giving rise to associations with risk behavior and substance use.

It needs to be noted that sample sizes for the phenotypes differed substantially (from N=12,218 to 453,496), so that it is possible that the pattern of associations was driven in part by differences in power. The correlation between sample size and *p*-value of the gene-based test was moderate, r=-.38 (p=7E-10) showing that well-powered traits were more likely to result in a significant association. It is clear that high power was a requirement: the effect sizes of CADM2 were diminutive. Also, our tests were limited to the psycho-behavioral traits measured in the UK-Biobank; inclusion of more measures, such as longitudinal or non-self-report measures could contribute to a more complete picture. Still, the range of tested traits was quite broad and enabled us to discern interesting patterns.

This study presents the first comprehensive and rigorous test of associations between *CADM2* and psycho-behavioral traits, showing strong associations for a wide range of traits (many akin to health behavior). Results could be used as starting point for future (animal) research into the function of *CADM2.* Research on the trait-associations and function of *CADM2* will further our understanding of the biology of behavior.

Supplementary materials

Supplementary materials accompanying this paper can be viewed at:
Chapter 6 – Phenome-wide association study of CADM2

or past this link into the browser:
https://drive.google.com/drive/folders/1mPGHTuNYcX4u7PCxqLrXcf7uqHEL1vqu

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CHAPTER 7

Causal relationships between substance use and insomnia

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Abstract

Background: Poor sleep quality and insomnia have been associated with the use of tobacco, alcohol, and cannabis, but it is unclear if there is a causal link. In this Mendelian Randomization (MR) study we examine if insomnia causes substance use and/or if substance use causes insomnia.

Methods: MR uses summary effect estimates from a genome-wide association study (GWAS) to create a genetic instrumental variable for a proposed 'exposure' variable and then identifies that same genetic instrument in an 'outcome' GWAS. Using GWASs of insomnia, smoking (initiation, heaviness, cessation), alcohol use (drinks per week, dependence), and cannabis initiation, bi-directional causal effects were tested. Multiple sensitivity analyses were applied to assess the robustness of the findings.

Results: There was strong evidence for positive causal effects of liability to insomnia on all substance use phenotypes (smoking traits, alcohol dependence, cannabis initiation), except alcohol per week. In the other direction, there was strong evidence that smoking initiation increased insomnia risk (smoking heaviness and cessation could not be tested as exposures). We found no evidence that alcohol use per week, alcohol dependence, or cannabis initiation causally affect insomnia risk.

Conclusions: There were unidirectional effects of liability to insomnia on alcohol dependence and cannabis initiation, and bidirectional effects between liability to insomnia and smoking measures. Bidirectional effects between smoking and insomnia might give rise to a vicious circle. Future research should investigate if interventions aimed at insomnia are beneficial for substance use treatment.

1. Introduction

Insomnia (trouble falling and/or staying asleep) is associated with substance use, including alcohol, nicotine , and cannabis use. Worldwide, individuals drink on average a glass of alcohol per day. A fifth of US and European adults smoke (WHO, 2016a), and a quarter to half of them have tried cannabis (EMCDDA, 2011). Both insomnia (Bin et al., 2012) and substance use (WHO 2016b, 2017, 2018) have serious consequences for health and well-being. Insight into the etiological processes underlying these associations might provide clues for prevention and intervention.

Alcohol, nicotine, and/or cannabis use have been associated with increased prevalence of insomnia (Angarita et al., 2016; Sabanayagam and Shankar, 2011). These comorbidities may reflect overlapping genetic etiology and/or causal relationships. For smoking, previous studies showed a genetic correlation with insomnia (Gibson et al., 2018; Jansen et al., 2019). As for causal relationships, experimental studies have investigated the acute effects of substance use on insomnia. Alcohol use shortened sleep onset latency, but led to sleep disruptions in the second half of sleep (Ebrahim et al., 2013). Cannabis intake likewise resulted in reduced sleep onset latency, but the effects of cannabis on sleep quality were less clear (Babson et al., 2017). Although smokers often cite its relaxing effects, nicotine intake was found to actually disturb sleep (Irish et al., 2015). Reversed causation -from insomnia to substance use- may also play a role. For example, adolescents with low sleep quality have shown a stronger inclination for later substance use (Hasler et al., 2016), although strong causal inferences cannot be made based on observational designs.

Mendelian Randomization (MR) can be used for causal inference in complex relationships (Lawlor et al., 2008). A previous MR study found that insomnia increased smoking heaviness and decreased chances of cessation and found no effects in the other direction (Gibson et al., 2018). We extend this work by using genetic data from the largest GWASs to date to examine genetic correlations and causal associations between insomnia and substance use, including alcohol and cannabis use.

2. Materials and methods

First, we estimated genetic correlations between insomnia and substance use with LDscore regression (Bulik-Sullivan et al., 2015). Second, we used MR to test for causal effects of insomnia on substance use and vice versa. We used the Two-Sample MR R-package (Hemani et al., 2018) with GWAS summary statistics from non-overlapping samples (Table 1). The rationale behind MR is that genetic variants are randomly distributed in the population and therefore not affected by confounders. This makes them suited as instrumental variables to test causal effects of an 'exposure' on an 'outcome'. Assumptions underlying MR are that the genetic instruments predict the exposure robustly (1) and are not independently associated with confounders (2) or the outcome (3). The latter two assumptions could be violated in case of horizontal pleiotropy (where one genetic variant directly affects multiple traits).

Insomnia cases were people that reported they 'usually' had trouble falling asleep at night or often woke up in the middle of the night; controls 'never/rarely' or 'sometimes' experienced this. Smoking initiation was defined as ever having smoked regularly (yes/no), smoking heaviness as cigarettes smoked per day, smoking cessation as having quit smoking (yes/no), and alcohol per week as the number of standard drinks consumed per week. Alcohol dependence (yes/no) was based on clinician's diagnosis or on a semi-structured interview based on DSM-IV criteria. Cannabis initiation was defined as ever having used cannabis (yes/no). For the genetic instruments we selected independent SNPs that were genome-wide significantly associated with the exposure variable in the source GWAS (p<5E-8; Table 1). For cannabis initiation there were only 2 genome-wide significant variants after excluding the UK Biobank sample, so for this phenotype we included SNPs that reached a 'suggestive' threshold of p<1E-5. Smoking heaviness and cessation could not be used as exposures, as the insomnia summary statistics could not be stratified on smoking status. Instrument strength was estimated by summing the variance explained (R^2) by each independent instrument SNP in the exposure (Table 1).

The main analysis was an inverse-variance weighted (IVW) meta-analysis of the SNP-outcome association divided by the SNP-exposure association for each SNP. Sensitivity analyses were used to assess the robustness of the IVW findings against violation of the MR assumptions. Weighted median and weighted mode regression correct for effect size outliers that could represent pleiotropic effects (Hartwig et al., 2017). MR-Egger regression provides an intercept that indicates the presence of pleiotropy, and adjusts the regression coefficient for such effects (Bowden et al., 2015). MR-Egger relies on the NO Measurement Error (NOME) assumption, violation of which can be tested with the I^2-

statistic. When I^2 was between 0.6 and 0.9, simulation extrapolation (SIMEX) was used to correct the MR-Egger for NOME violation; if I^2 was below 0.6 MR-Egger was not reported (Bowden et al., 2016). We also applied Generalised summary-data-based MR (GSMR), which gains statistical power by taking low levels of linkage disequilibrium between the included SNPs into account, and deletes effect size outliers. Using Steiger filtering, MR analyses were repeated excluding SNPs that explained more variance in the outcome than the exposure, and again retaining only SNPs that explained significantly (p<.05) more variance in the exposure (to rule out reverse causation; Hemani et al., 2017). Cochran's Q-statistic was used to assess SNP effect heterogeneity (Bowden et al., 2018) and the F-statistic for weak instrument bias (Burgess et al., 2011). Finally, leave-one-out IVW analyses were used to give an indication of disproportional effects of single SNPs (Hemani et al., 2018). Rather than assessing the strength of the statistical evidence by p-values only, we also consider the effect sizes across the IVW and sensitivity analyses to inform our interpretation.

Table 1. Sources of the genome-wide association summary statistics used for the two-sample MR, the number of SNPs in the IVW exposure instrument (being the independent lead SNPs as reported in the source GWAS that were also present in the outcome SNP set, #exposure SNPs), the variance explained in the respective phenotype by these instrument SNPs (Instrument R^2), and the genetic correlation of each substance use trait with insomnia (r_g) with its associated p-value. For the computation of r_g we used the full GWAS summary statistics except for insomnia, where 23andMe participants were excluded.

Phenotype	Source	Sample	#exposure SNPs[a]	Instrument R^2	r_g, SE (p)
Insomnia	Jansen et al. (2019)	excl. 23andMe N=386,533	248	0.89%	NA
Smoking initiation	Liu et al. (2019)	excl. UKB* N=848,460	360	1.16%	.23, .02 (2.09E-23)
Smoking heaviness	Liu et al. (2019)	excl. UKB N=143,210	NA[a]	NA[a]	.27, .03 (5.42E-17)
Smoking cessation	Liu et al. (2019)	excl. UKB N=216,590	NA[a]	NA[a]	.28, .04 (5.56E-12)
Alcohol per week	Liu et al. (2019)	excl. UKB N=630,154	91	0.59%	.03, .02 (.029)
Alcohol dependence	Walters et al. (2018)	N=46,568	8	0.36%	.29, .07 (1.42E-5)
Cannabis initiation	Pasman et al. (2018)	excl. UKB N=57,980	32[b]	1.33%	.04, .03 (.205)

*UKB=UK Biobank

[a] The effect of smoking heaviness and cessation on insomnia could not be tested because the insomnia GWAS could not be stratified on smoking status

[b] p<1e05

3. Results

There were moderate genetic correlations between the insomnia GWAS and all substance use GWASs except alcohol per week (small overlap) and cannabis initiation (no significant overlap; Table 1).

Insomnia to substance use

The IVW analyses showed strong evidence for causal effects of liability to insomnia on all substance use traits except alcohol use per week (Table 2). For all analyses except insomnia-on-cannabis initiation there was evidence for SNP-effect heterogeneity, although leave-one-out analyses did not show the effects were driven by a single SNP (Figures S1-S6). The insomnia instrument had low explained variance, but did not suffer from weak instrument bias (F>10). I^2, F, and Q statistics are presented in Table S1. The effect of insomnia on substance use retained similar effect sizes across the weighted mode, median, and GSMR analyses (although effect estimates became less precise). MR-Egger results were not reported because the I^2 statistic was below 0.6. For smoking and alcohol use per week outcomes the proportion of SNPs that explained more variance in the outcome than in insomnia varied from 5.4 to 20.6% (Table S2). The Steiger-filtered IVW with those SNPs excluded showed only slightly attenuated effect sizes. However, when retaining only SNPs that significantly explained more variance in insomnia than in the outcome, strong evidence remained only for an effect on smoking initiation. For alcohol dependence (36.0%) and cannabis initiation (25.4%) large proportions of SNPs explained more variance in the outcome than in the exposure. Filtering those out led to substantial attenuation of effects (Table 2).

Substance use to insomnia

The IVW analyses showed a causal effect of smoking initiation on insomnia risk, and no effects of other traits. In the weighted median, mode, and GSMR sensitivity analyses the effect size of smoking initiation was roughly equal, although statistical evidence was slightly weaker (substantially weaker in the weighted mode). Smoking initiation-on-insomnia was the only analysis with sufficiently high I^2 to allow for MR-Egger intercept interpretation, showing no evidence for pleiotropy (p=.347), although the MR-Egger estimate was substantially attenuated. Less than 4% of the instrument SNPs explained more variance in insomnia outcome than in smoking initiation (Table S2). Filtering those out hardly changed results, although retaining only SNPs that explained significantly more variance in the exposure did attenuate the effects (Table 2). There was no evidence for heterogeneity or weak instrument bias (Table S1, Figures S7-S10).

4. Discussion

There were moderate genetic correlations between insomnia and smoking traits and alcohol dependence, such that insomnia was genetically associated with higher levels of substance use. The genetic correlation with alcohol per week was small but significant, and there was no significant correlation with cannabis initiation.

Overall, we found more evidence for causal effects from liability to insomnia to substance use than vice versa. MR results suggest that insomnia leads to heavier smoking, increased chances of smoking initiation, alcohol dependence, and cannabis initiation, and decreased chances of smoking cessation. The finding that insomnia caused heavier smoking and lowers chances of smoking cessation confirms results from Jansen et al. (2019) and Gibson et al. (2018) on smoking. As a possible interpretation, a desire to smoke may be induced by sleep deprivation (Hamidovic and de Wit, 2009). The causal effects of insomnia on alcohol use may be interpreted in light of a self-medication framework, as alcohol has somnolent properties (Goodhines et al., 2019). For cannabis the same reasoning might apply, although this interpretation seems more likely for a measure of cannabis use frequency rather than lifetime use. While we found an effect of insomnia on alcohol dependence, we found no effect on alcohol use. This might be due to the measure of alcohol use in quantity per week, which does not distinguish drinking large quantities in one evening from drinking one glass with dinner daily; the first would impair sleep quality more than the latter. The genetic architecture of drinking frequency seems to differ from that of drinking quantity (Marees et al., 2019).

In the other direction, we found an effect of smoking initiation on insomnia. A previous study testing this relationship did not find this effect, possibly due to lower power (Gibson et al., 2018). The effect of smoking on insomnia might be due to nicotine's stimulant properties (Greenland et al., 1998), although we could not test the effect of smoking heaviness. The absence of an effect of alcohol use and dependence on insomnia is in contrast with experimental literature that suggested a negative effect of alcohol on sleep quality (Ebrahim et al., 2013). Our results might be due to low instrument strength for the alcohol phenotypes. Also, the genetic instruments capture lifetime vulnerability to alcohol use and dependence, which is not directly comparable to the immediate effects of alcohol tested in experiments.

Results were reasonably robust against MR assumption violation. However, the effects of insomnia on alcohol dependence and cannabis initiation were in part driven by pleiotropic SNPs, suggesting caution in interpreting these findings. Although the analysis

of smoking initiation on insomnia did not show strong evidence for it, pleiotropy might also play a role in this association. For example, smoking initiation was found to be positively associated with ADHD liability in children that have not started smoking yet, indicating that it might represent something different than only the inclination to smoke (Treur et al., 2019). A limitation might be the use of instruments that explained limited amounts of variance in their respective phenotype (0.36-1.33%). Sensitivity analyses correcting for this showed attenuation in effect sizes. Another limitation is that we investigated a simplistic measure of cannabis use (ever vs, never); however, to date no suitable, sufficiently powered GWAS are available on more in-depth cannabis use phenotypes, such as use frequency or quantity. For cannabis initiation we used a more inclusive *p*-value threshold, which might have increased chances of pleiotropy. However, filtering out instruments that explained more variance in the exposure than the outcome did not have strong effects on the results.

To summarize, we find genetic overlap between insomnia and substance use, evidence for causal effects from insomnia to most substance use traits, and a causal effect of smoking initiation on insomnia. Future research should focus on underlying mechanisms and potential implications for clinical practice. As has been found previously (Patterson et al., 2017), our results suggest that treatment for substance use and insomnia could be optimized when attention is devoted to both issues.

Table 2. Results for the MR analyses with the IVW representing the main analysis and the remaining representing the results for the sensitivity analyses.

		insom> smok init	insom> smok heav	insom > smok ces	insom > alc week	insom > alc dep	insom > can init	smok init> insom	alc week> insom	alc dep > insom	can init > insom
IVW	**N_{SNPs}**	205	204	205	205	203	201	316	78	5	28
	beta (SE)	0.13 (0.02)	0.11 (0.02)	-0.08 (0.02)	-0.01 (4E-3)	0.16 (0.04)	0.18 (0.06)	0.11 (0.03)	0.12 (0.13)	0.05 (0.05)	7E-4 (0.01)
	OR (SE)	NA	NA	NA	NA	1.18 (0.09)	1.20 (0.14)	1.11 (0.06)	1.13 (0.25)	1.05 (0.09)	1.00 (0.02)
	***p*-value**	**1.58E-12**	**1.67E-12**	**6.37E-8**	.115	**5.43E-5**	**.003**	**3.68E-5**	.349	.292	.937
Weighted median	**N_{SNPs}**	205	204	205	205	203	201	316	78	5	28
	beta (SE)	0.08 (0.02)	0.08 (0.02)	-0.07 (0.02)	-0.01 (5E-3)	0.19 (0.06)	0.16 (0.09)	0.11 (0.04)	0.09 (0.19)	0.04 (0.06)	-2E-3 (0.01)
	OR (SE)	NA	NA	NA	NA	1.21 (0.14)	1.17 (0.19)	1.11 (0.08)	1.10 (0.34)	1.05 (0.11)	1.00 (0.03)
	***p*-value**	**3.70E-7**	**1.41E-5**	**6.42E-4**	.263	**1.64E-3**	.086	**3.79E-3**	.624	.456	.866
Weighted mode	**N_{SNPs}**	204	204	205	205	203	201	316	78	5	28
	beta (SE)	0.10 (0.03)	0.07 (0.04)	-0.08 (0.04)	-0.01 (10E-3)	0.28 (0.13)	0.20 (0.18)	0.10 (0.11)	0.09 (0.46)	0.04 (0.08)	3E-3 (0.01)
	OR (SE)	NA	NA	NA	NA	1.32 (0.29)	1.22 (0.35)	1.11 (0.21)	1.10 (0.65)	1.04 (0.15)	1.00 (0.03)
	***p*-value**	**4.15E-4**	.060	**.059**	.470	**.031**	.246	.354	.843	.634	.816
MR-Egger	**N_{SNPs}**	[a]	[a]	[a]	[a]	[a]	[a]	316	78	5[b]	28[b]
	beta (SE)							0.03 (0.09)	0.11 (0.21)	0.02 (0.04)	-4E-3 (9E-3)
	OR (SE)							1.03 (0.16)	1.12 (0.96)	1.02 (4E-3)	1.00 (0.01)
	***p*-value**							.737	.579	.703	.671
GSMR	**N_{SNPs}**	194	200	201	200	202	200	306	71	[c]	28
	beta (SE)	0.11 (0.01)	0.09 (0.01)	-0.07 (0.01)	-0.01 (3E-3)	0.13 (0.04)	0.16 (0.06)	0.11 (0.02)	0.10 (0.13)		7E-4 (0.01)
	OR (SE)	NA	NA	NA	NA	1.14 (0.04)	1.17 (0.07)	1.12 (0.03)	1.11 (0.13)		1.00 (0.01)
	***p*-value**	**5.00E-26**	**4.08E-14**	**2.92E-7**	.076	**8.85E-4**	**.012**	**3.29E-5**	.424		.939

IVW After Steiger filtering[d]	**N_{SNPs}**	181	169	194	191	130	150	305	69	5	28
	beta (SE)	0.11 (0.02)	0.09 (0.01)	-0.08	-0.01 (4E-3)	0.08 (0.04)	0.13 (0.06)	0.10 (0.03)	0.11 (0.13)	0.05 (0.05)	7E-4 (0.01)
	OR (SE)	NA	NA	NA	NA	1.09 (0.04)	1.14 (0.07)	1.10 (0.06)	1.12 (0.25)	1.05 (0.09)	1.00 (0.02)
	***p*-value**	**9.02E-12**	**1.09E-11**	**4.49E-8**	.091	.055	**.046**	**2.92E-4**	.388	.292	.937
IVW After Steiger filtering with p<.05 [e]	**N_{SNPs}**	74	28	60	90	0	1	143	30	5	28
	beta (SE)	0.07 (0.01)	0.03 (0.02)	-0.03 (0.02)	-3E-3 (4E-3)			0.02 (0.03)	0.11 (0.13)	0.05 (0.05)	7E-4
	OR (SE)	NA	NA	NA	NA			1.02 (0.07)	1.12 (0.25)	1.05 (0.09)	1.00 (0.02)
	***p*-value**	**5.65E-07**	0.134	0.052	0.398			0.641	0.388	0.292	0.937

Odds ratios are given for binary outcomes (note that smoking initiation and cessation were not binary because in the summary statistics they were rescaled to SD units)

[a] MR-Egger was not reported because I2 was below 0.6;

[b] SIMEX-corrected MR-Egger is reported because I2 was >.06 and <.09

[c] GSMR was not performed for the alcohol dependence to insomnia analysis as there were too few SNPs

[d] The IVW after Steiger filtering, i.e. after filtering out all SNPs that explained more variance in the outcome than in the exposure; see also Table S2.

[e] The IVW after keeping only those SNPs that explained more variance in the exposure than in the outcome; see also Table S2.

Abbreviations: insom=insomnia; smok init=smoking initiation; smok heav=smoking heaviness; smok ces= smoking cessation; alc week=alcohol use per week; alc dep=alcohol dependence; can init=cannabis initiation; IVW=inverse variance weighted meta-analysis; OR=odds ratio; GSMR=generalised summary-data-based Mendelian randomization; NSNPs =number of SNPs that was retained in the analyses after filtering for high LD, palindromic, and ambiguous SNPs, with additional HEIDI filtering in the GSMR and filtering for pleiotropic SNPs in the Steiger analyses.

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Supplementary Materials

Below is an overview of included Supplementary Materials accompanying this paper; they can be found on the following pages.

The full Supplementary Materials can be viewed online at:
Chapter 7 – Causal relationship between insomnia and substance use

or past this link in the browser:
https://drive.google.com/drive/folders/1eYAi1mD9mZ1WPDSFSkUG3IjzAf0znFUx

Supplementary item	Short title
Supplementary Table S1	Robustness checks for the two-sample MR analyses
Supplementary Table S2a	Number of SNPs filtered out in Steiger analyses
Supplementary Table S2b	IVW results after Steiger filtering with p<.05
Supplementary Figure S1a	Scatter plot for insomnia to smoking initiation
Supplementary Figure S1b*	Leave-one-out plot for insomnia to smoking initiation
Supplementary Figure S2a	Scatter plot for insomnia to cigarettes per day
Supplementary Figure S2b*	Leave-one-out plot for insomnia to cigarettes per day
Supplementary Figure S3a	Scatter plot for insomnia to smoking cessation
Supplementary Figure S3b*	Leave-one-out plot for insomnia to smoking cessation
Supplementary Figure S4a	Scatter plot for insomnia to alcohol per week
Supplementary Figure S4b*	Leave-one-out plot for insomnia to alcohol per week
Supplementary Figure S5a	Scatter plot for insomnia to alcohol dependence
Supplementary Figure S5b*	Leave-one-out plot for insomnia to alcohol dependence
Supplementary Figure S6a	Scatter plot for insomnia to cannabis initiation
Supplementary Figure S6b*	Leave-one-out plot for insomnia to cannabis initiation
Supplementary Figure S7a	Scatter plot for smoking initiation to insomnia
Supplementary Figure S7b*	Leave-one-out plot for smoking initiation to insomnia
Supplementary Figure S8a	Scatter plot for alcohol per week to insomnia
Supplementary Figure S8b*	Leave-one-out plot for alcohol per week to insomnia
Supplementary Figure S9a	Scatter plot for alcohol dependence to insomnia
Supplementary Figure S9b*	Leave-one-out plot for alcohol dependence to insomnia
Supplementary Figure S10a	Scatter plot for cannabis initiation to insomnia
Supplementary Figure S10b*	Leave-one-out plot for cannabis initiation to insomnia

*

Supplementary Table S1. Robustness checks for the two-sample MR analyses.

	I-squared	Cochran's Q (*p*)	F
insom > smok init	0.49	1025.40 (1.43E-109)	11.54
insom > cig day	0.51	369.63 (8.07E-12)	11.58
insom > smok ces	0.49	316.84 (6.83E-07)	11.54
insom > alc week	0.49	465.90 (1.57E-22)	11.54
insom > alc dep	0.49	236.45 (.049)	11.35
insom > can init	0.48	219.86 (.160)	11.59
smok init > insom	0.9	127.66 (>.999)	27.74
alc week > insom	0.97	33.62 (>.999)	38.23
alc dep > insom	0.79	20.87 (.995)	29.17
can init > insom	0.77	6.11 (>.999)	21.57

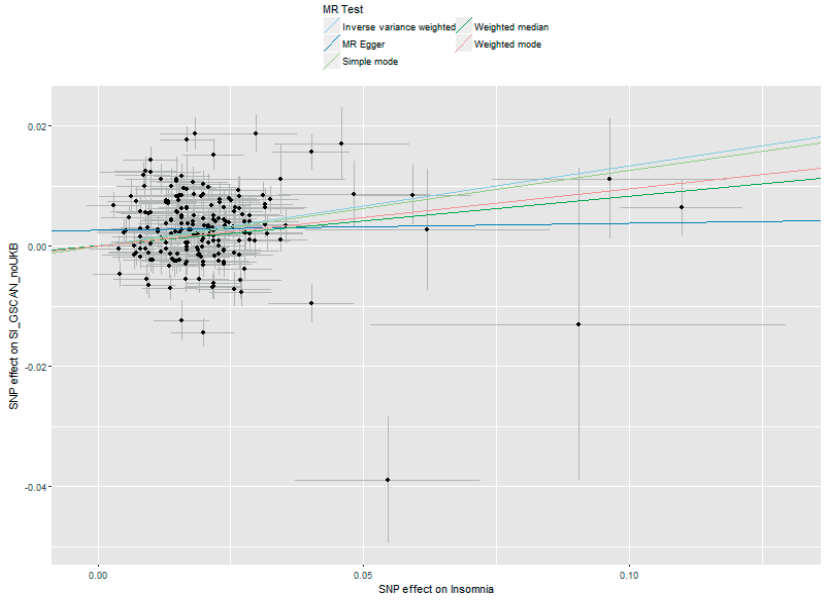
Shaded columns indicate values that meet criteria for NOME assumption violation, heterogeneity, or weak instrument bias, respectively (I2<0.6, significant Q, F<10)

Table S2a.Number of SNPs that explained more variance in the outcome than in the exposure (Steiger filtered) with percentage of the original number of SNPs (original IVW). In addition, the number of SNPs that explained more variance in the outcome with p<.05

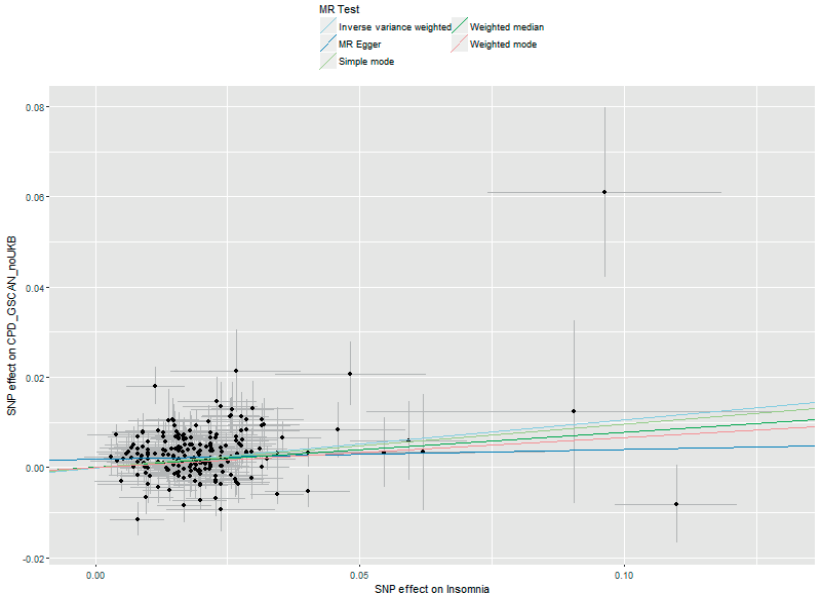
	#SNPs	original IVW	Steiger filtered (%)
insom > smok init	205	24 (11.71%)	131 (63.90%)
insom > cig day	204	42 (20.59%)	176 (86.27%)
insom > smok ces	205	11 (5.37%)	145 (70.73%)
insom > alc week	205	14 (6.83%)	115 (56.10%)
insom > alc dep	203	73 (35.69%)	203 (100%)
insom > can init	201	51 (25.37%)	200 (99.50%)
smok init > insom	316	11 (3.48%)	173 (54.75%)
alc week > insom	78	9 (11.54%)	48 (61.54%)
alc dep > insom	5	0 (0%)	0 (0%)
can init > insom	28	0 (0%)	0 (0%)

*Odds ratios are given for binary outcomes (note that smoking initiation and cessation were not binary because in the summary statistics they were rescaled to SD units)

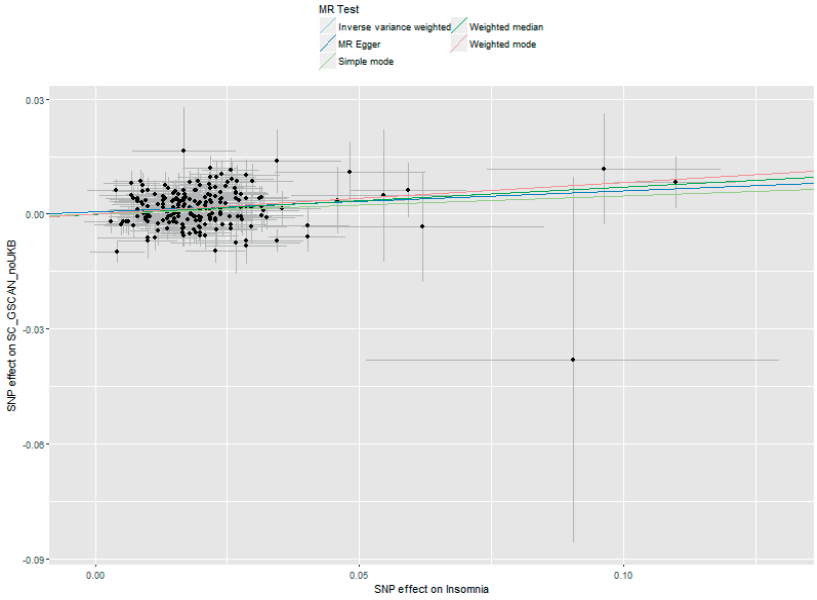
Supplementary Figure S1a. Scatter plot for insomnia to smoking initiation



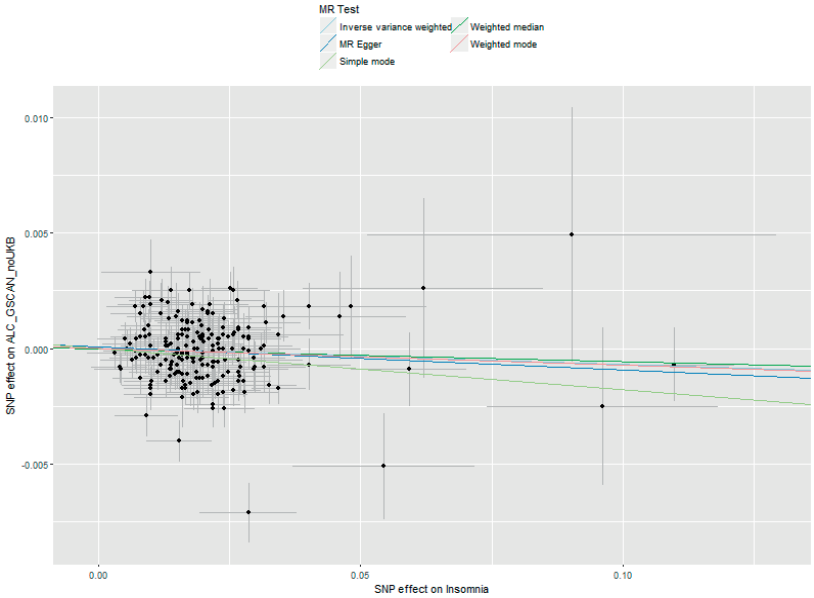
Supplementary Figure S2a. Scatter plot for insomnia to cigarettes per day



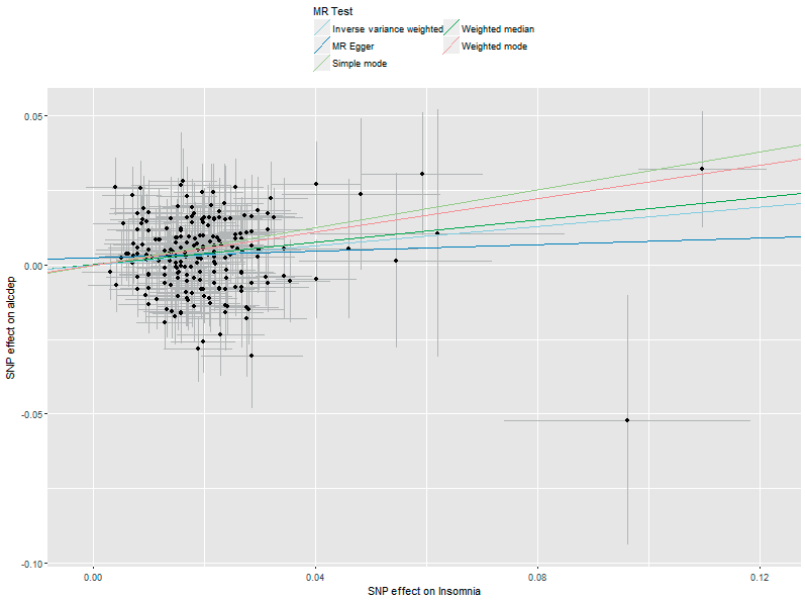
Supplementary Figure S3a. Scatter plot for insomnia to smoking cessation



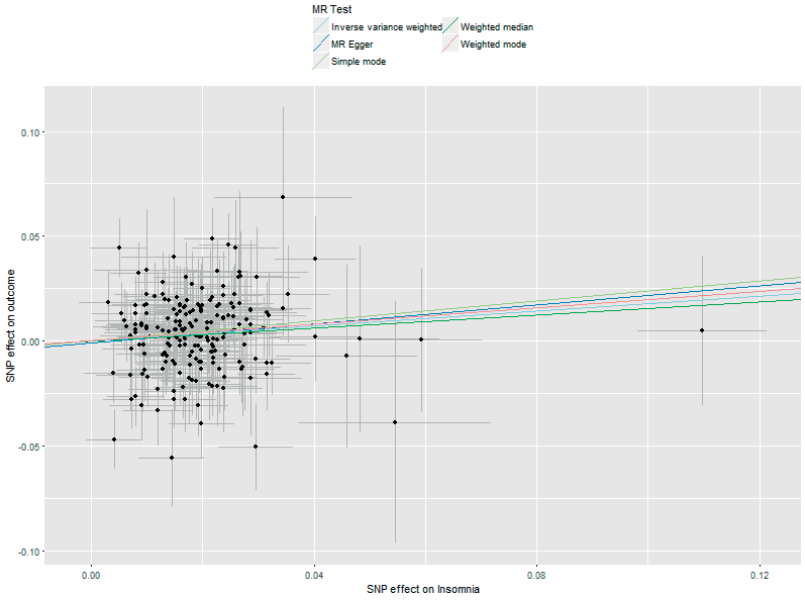
Supplementary Figure S4a. Scatter plot for insomnia to alcohol per week



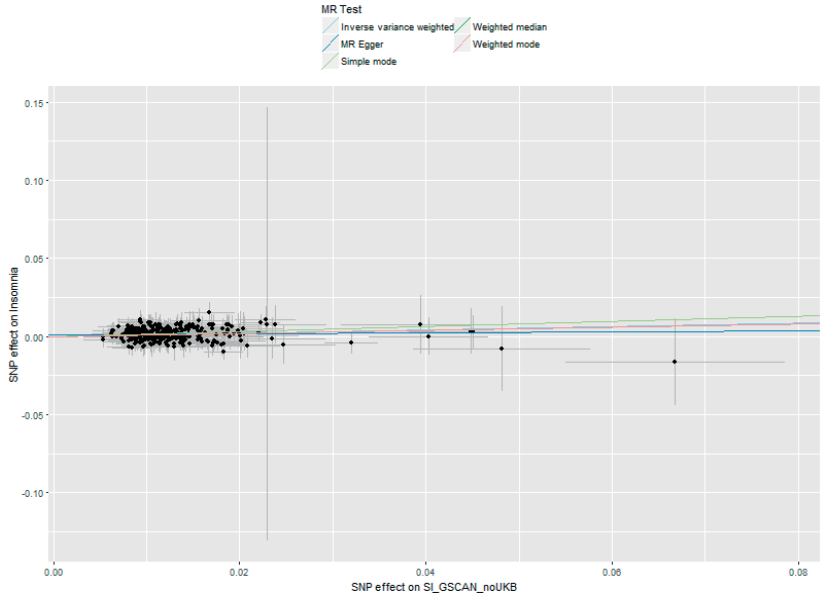
Supplementary Figure S5a. Scatter plot for insomnia to alcohol dependence



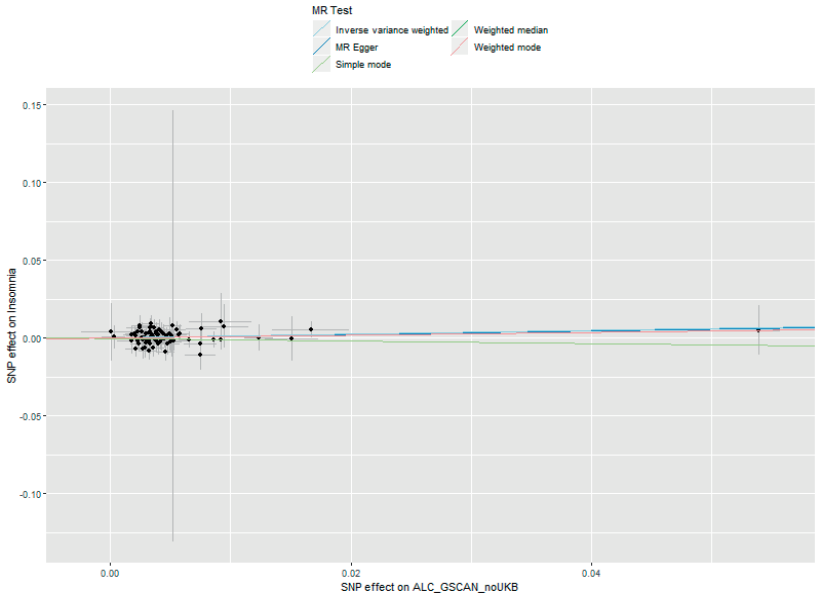
Supplementary Figure S6a. Scatter plot for insomnia to cannabis initiation



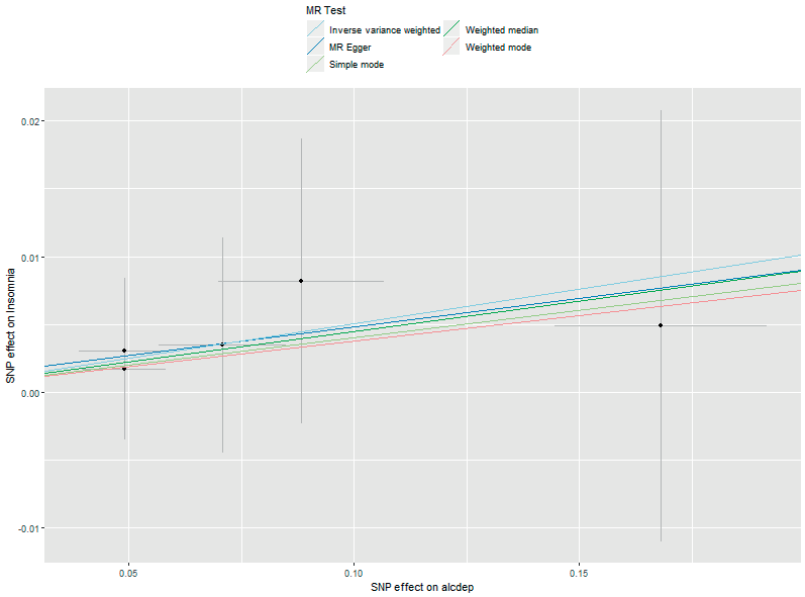
Supplementary Figure S7a. Scatter plot for smoking initiation to insomnia



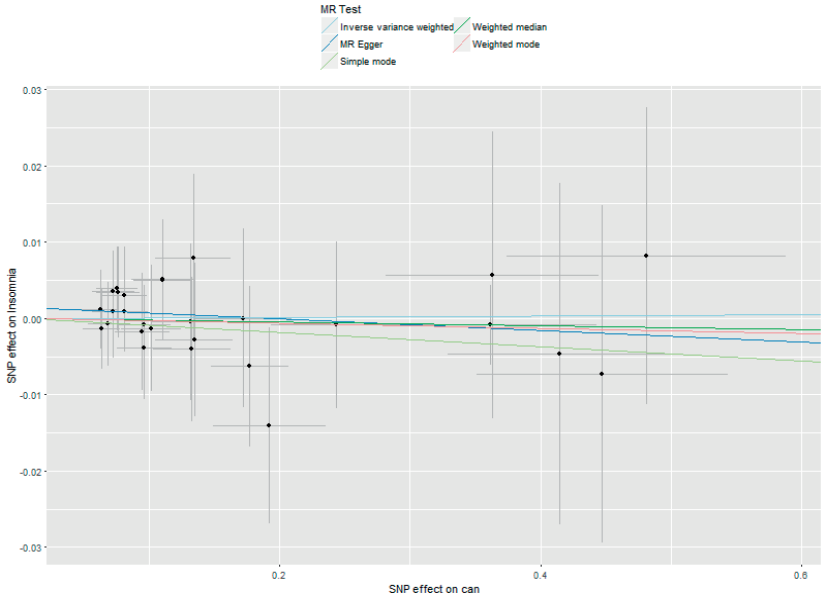
Supplementary Figure S8a. Scatter plot for alcohol per week to insomnia



Supplementary Figure S9a. Scatter plot for alcohol dependence to insomnia



Supplementary Figure S10a. Scatter plot for cannabis initiation to insomnia



Corrigendum

It has come to the attention of the authors that a correction is needed to our paper "Causal relationships between substance use and insomnia". For our alcohol and smoking genetic instruments and outcomes we used summary statistics from the GSCAN meta-analyses (Liu et al., 2019). As the full summary statistics including data from 23andMe could not be provided by the GSCAN authors, we performed our own meta-analyses of 23andMe data (which we obtained from 23andMe through application) with summary statistics from all other GSCAN samples, excluding UK-Biobank. We could not replicate the exact method that GSCAN used for their meta-analyses (rareGWAMA), as that would have required information from the individual contributing cohorts. We rather used METAL to conduct the meta-analyses (Willer et al., 2010), and weighted the SNP-effects by the inverse of the standard errors. However, as the phenotype measurements in the original samples were heterogeneous, effect sizes and standard errors were not on the same scale, so SNP effects should have been weighted by their sample sizes.

We re-conducted the meta-analyses, now based on sample size. As N-weighted meta-analysis results in z-scores rather than betas and standard errors, we had to derive these using procedures described before (Taylor et al., 2016). For beta, we used $\beta = \frac{z-score}{\sqrt{N} * \frac{1}{\sqrt{EAF(1-EAF)}}}$, with EAF representing the frequency of the effect allele in a European ancestry reference panel (The 1000 Genomes Project Consortium, 2015). The corresponding standard error was computed using $se = \frac{\beta}{z-score}$. We observed genomic inflation in the summary statistics for some of the traits, in which case the standard error was corrected using the LD-score regression intercept with $se_{corrected} = \sqrt{se^2 * intercept}$ and corresponding *p*-values were computed. The genetic correlations between the old and new summary statistics were high (for smoking initiation r_g=.98, cigarettes per day r_g=.82, smoking cessation r_g=.96, alcohol per week r_g>.99). Using the new summary statistics, we recreated the genetic instruments and reran the MR analyses following identical procedures as before.

The new instruments had slightly higher instrument R^2 and showed similar genetic correlations with insomnia (see Table 1). Results from the MR analyses are presented in Table 2. Comparing the results with the original ones, there are some fluctuations in betas and *p*-values in both directions, but the overall patterns were the same; i.e., for all analyses the direction and significance of effects and remained the same, such that the new results do not lead to alternative interpretations. Additional statistics for robustness checks and sensitivity analyses can be found in the updated Supplementary Material. The

statistics show only minor differences from the previous results. One notable exception was the lower I^2 for the analysis from smoking initiation on insomnia (indicating substantial NOME violation), so that MR Egger analysis for this relationship could no longer be interpreted. Figures S1a-S10a display the scatter plots for the MR analyses.

Overall, we conclude that changes in results were negligible and did not merit different interpretations. Thus, the conclusions of our original publication stand: we found evidence for positive causal effects of liability to insomnia on all substance use phenotypes (smoking traits, alcohol dependence, cannabis initiation), except alcohol per week. Also, we found strong evidence that smoking initiation increased insomnia risk.

Table 1. Sources of the genome-wide association summary statistics used for the two-sample MR, the number of SNPs in the IVW exposure instrument (being the independent lead SNPs as reported in the source GWAS that were also present in the outcome SNP set, #exposure SNPs), the variance explained in the respective phenotype by these instrument SNPs (Instrument R^2), and the genetic correlation of each substance use trait with insomnia (r_g) with its associated *p*-value. For the computation of r_g we used the full GWAS summary statistics except for insomnia, where 23andMe participants were excluded.
[a] The effect of smoking heaviness and cessation on insomnia could not be tested because the insomnia GWAS could not be stratified on smoking status

	#exposure SNPs[a]		**Instrument R^2**		**r_g, SE (*p*)**	
Phenotype	Old	New	Old	New	Old	New
Smoking initiation	360	366	1.16%	1.49%	.23, .02 (2.09E-23)	.22, .02 (2.88E-23)
Smoking heaviness	NA[a]	NA[a]	NA[a]	NA[a]	.27, .03 (5.42E-17)	.30, .04 (7.07E-12)
Smoking cessation	NA[a]	NA[a]	NA[a]	NA[a]	.28, .04 (5.56E-12)	.25, .04 (5.92E-12)
Alcohol per week	91	99	0.59%	0.68%	.03, .02 (.029)	.02, .03 (.058)

Table 2. Comparison of MR results using summary statistics based on a standard error-weighted meta-analysis and based on N-weighted meta-analysis (shaded). Odds ratios are given for binary outcomes (note that smoking initiation and cessation were not binary because in the summary statistics they were rescaled to SD units)

		insom> smok init	**insom> smok heav**	**insom > smok ces**	**insom > alc week**	**smok init> insom**	**alc week> insom**
IVW	**N_{SNPs}**	205	204	205	205	316	78
	beta (SE)	0.13 (0.02)	0.11 (0.02)	-0.08 (0.02)	-0.01 (4.29E-3)	0.11 (0.03)	0.12 (0.13)
	OR (SE)	NA	NA	NA	NA	1.11 (0.06)	1.13 (0.25)
	***p*-value**	**1.58E-12**	**1.67E-12**	**6.37E-8**	.115	**3.68E-5**	.349
IVW new	**N_{SNPs}**	202	203	203	204	311	78
	beta (SE)	0.14 (0.03)	0.11 (0.01)	-0.12 (.02)	-0.01 (0.01)	0.10 (0.02)	0.02 (0.05)
	OR (SE)	NA	NA	NA	NA	1.10 (0.05)	1.02 (0.09)
	***p*-value**	**9.81E-9**	**1.51E-14**	**8.66E-11**	.239	**7.37E-6**	.606
Weighted median	**N_{SNPs}**	205	204	205	205	316	78
	beta (SE)	0.08 (0.02)	0.08 (0.02)	-0.07 (0.02)	-0.01 (4.95E-3)	0.11 (0.04)	0.09 (0.19)
	OR (SE)	NA	NA	NA	NA	1.11 (0.08)	1.10 (0.34)
	***p*-value**	**3.70E-7**	**1.41E-5**	**6.42E-4**	.263	**3.79E-3**	.624
Weighted median new	**N_{SNPs}**	202	203	203	204	311	78
	beta (SE)	0.11 (0.03)	0.09 (0.02)	-0.05 (0.05)	-0.01 (0.01)	0.09 (0.03)	0.01 0.08)
	OR (SE)	NA	NA	NA	NA	1.03 (0.07)	1.01 (0.15)
	***p*-value**	**1.22E-5**	**1.99e-5**	**2.20E-4**	.642	**3.03E-3**	.889
Weighted mode	**N_{SNPs}**	204	204	205	205	316	78

	beta (SE)	0.10 (0.03)	0.07 (0.04)	-0.08 (0.04)	-0.01 (9.80E-3)	0.10 (0.11)	0.09 (0.46)
	OR (SE)	NA	NA	NA	NA	1.11 (0.21)	1.10 (0.65)
	***p*-value**	**4.15E-4**	.060	.059	.470	.354	.843
Weighted mode new	**N_{SNPs}**	202	203	203	204	311	78
	beta (SE)	0.13 (0.05)	0.07 (0.02)	-0.07 (0.05)	-2.93E-3 (9.1E-3)	0.07 (0.08)	0.02 (0.07)
	OR (SE)	NA	NA	NA	NA	1.07 (0.17)	1.02 (0.14)
	***p*-value**	**6.32E-3**	**1.60E-3**	.183	.747	.382	.830
MR-Egger	**N_{SNPs}**	[a]	[a]	[a]	[a]	316	78
	beta (SE)					0.03 (0.09)	0.11 (0.21)
	OR (SE)					1.03 (0.16)	1.12 (0.96)
	***p*-value**					.737	.579
MR-Egger new	**N_{SNPs}**	[a]	[a]	[a]	[a]	[a]	78
	beta (SE)						-4.83E-3 (0.07)
	OR (SE)						1.00 (0.64)
	***p*-value**						.945
IVW After Steiger filtering[d]	**N_{SNPs}**	181	169	194	191	305	69
	beta (SE)	0.11 (0.02)	0.09 (0.01)	-0.08	-0.01 (3.84E-3)	0.10 (0.03)	0.11 (0.13)
	OR (SE)	NA	NA	NA	NA	1.10 (0.06)	1.12 (0.25)
	***p*-value**	**9.02E-12**	**1.09E-11**	**4.49E-8**	.091	**2.92E-4**	.388
IVW After Steiger filtering new	**N_{SNPs}**	186	177	189	189	256	71
	beta (SE)	0.11 (0.02)	0.10 (0.01)	-0.11 (0.02)	-0.01 (0.01)	0.07 (0.02)	0.02 (0.05)

	OR (SE)	NA	NA	NA	NA	1.07 (0.56)	1.02 (0.56)
	***p*-value**	**4.83E-7**	**8.05E-13**	**4.02E-10**	.321	**.003**	.631
IVW After Steiger filtering with p<.05 [e]	**NSNPs**	74	28	60	90	143	30
	beta (SE)	0.07 (0.01)	0.03 (0.02)	-0.03 (0.02)	-3.02E-3 (3.58E-3)	0.02 (0.03)	0.11 (0.13)
	OR (SE)	NA	NA	NA	NA	1.02 (0.07)	1.12 (0.25)
	p-value	**5.65E-07**	.134	.052	.398	.641	.388
IVW After Steiger filtering with p<.05 new	**NSNPs**	89	30	53	86	63	36
	beta (SE)	0.08 (0.02)	2.45E-3 (0.02)	-0.03 (0.02)	1.10E-3 (0.01)	0.05 (0.04)	0.02 (0.05)
	OR (SE)	NA	NA	NA	NA	1.05 (0.57)	1.02 (0.56)
	p-value	**4.53E-5**	.918	.082	.870	.256	.754
GSMR	**N_{SNPs}**	194	200	201	200	306	71
	beta (SE)	0.11 (0.01)	0.09 (0.01)	-0.07 (0.01)	-0.01 (3.15E-3)	0.11 (0.02)	0.10 (0.13)
	OR (SE)	NA	NA	NA	NA	1.12 (0.03)	1.11 (0.13)
	***p*-value**	**5.00E-26**	**4.08E-14**	**2.92E-7**	.076	**3.29E-5**	.424
GSMR new	**N_{SNPs}**	191	201	201	201	304	71
	beta (SE)	0.14 (0.02)	0.10 (0.01)	-0.09 (0.01)	-3.62E-3 (6.05E-3)	0.10 (0.02)	0.02 (0.05)
	OR (SE)	NA	NA	NA	NA	1.10 (0.03)	1.02 (0.05)
	***p*-value**	**6.70E-19**	**4.09E-14**	**1.04E-10**	.549	**1.64E-5**	.753

[a] MR-Egger was not reported because I^2 was below 0.6;
[b] SIMEX-corrected MR-Egger is reported because I^2 was >.6 and <.9
[c] GSMR was not performed for the alcohol dependence to insomnia analysis as there were too few SNPs
[d] The IVW after Steiger filtering, i.e. after filtering out all SNPs that explained more variance in the outcome than in the exposure; see also Table S2.
[e] The IVW after keeping only those SNPs that explained more variance in the exposure than in the outcome; see also Table S2.

Abbreviations: insom=insomnia; smok init=smoking initiation; smok heav=smoking heaviness; smok ces= smoking cessation; alc week=alcohol use per week; alc dep=alcohol dependence; can init=cannabis initiation; IVW=inverse variance weighted meta-analysis; OR=odds ratio; GSMR=generalised summary-data-based Mendelian randomization; N_{SNPs} =number of SNPs that was retained in the analyses after filtering for high LD, palindromic, and ambiguous SNPs, with additional HEIDI filtering in the GSMR and filtering for pleiotropic SNPs in the Steiger analyses.

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CHAPTER 8

Investigating the causal nature of the relationship between subcortical brain volume and substance use

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Abstract

Background: Structural variation in subcortical brain regions has been linked to substance use, including the most prevalent substances nicotine and alcohol. It may be that pre-existing differences in subcortical brain volume affect smoking and alcohol use, but there is also evidence that smoking and alcohol use can lead to structural changes. We assess the causal nature of this complex relationship with bi-directional Mendelian randomization (MR). Methods: MR uses genetic variants predictive of a certain trait ('exposure') as instrumental variables to test causal effects on a certain outcome. Due to random assortment at meiosis, genetic variants shouldn't be associated with confounders, allowing less biased causal inference. We employed summary-level data of the largest available genome-wide association studies of subcortical brain region volumes (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen, and thalamus; n=50,290) and smoking and alcohol use (smoking initiation, n=848,460; cigarettes per day, n=216,590; smoking cessation, n=378,249; alcohol drinks per week, n=630,154; alcohol dependence, n=46,568). The main analysis, inverse-variance weighted regression, was verified by a wide range of sensitivity methods. Results: There was strong evidence that alcohol dependence decreased amygdala and hippocampal volume and that smoking more cigarettes per day decreased hippocampal volume. From subcortical brain volumes to substance use, there was no or weak evidence for causal effects. Conclusions: Our findings suggest that alcohol dependence and smoking can causally reduce subcortical brain volume. This adds to accumulating evidence that alcohol and smoking can be detrimental to the brain, and subsequently mental health, warranting more recognition in public health efforts.

Introduction

Subcortical brain regions have consistently been implicated in substance use, playing a crucial role in the brain's reward system1. It is thought that addiction reflects a vicious cycle of intoxication, withdrawal and craving, with (subcortical) brain circuits mediating these three stages2. However, the causal nature of the relationship between structural variation in subcortical brain regions and substance use is largely unclear. Subcortical brain volume and substance use are both substantially genetically influenced, and there is evidence that they share (part of) their genetic aetiology3. Alternatively, the relationship may be causal, such that pre-existing differences in subcortical brain volume assert a direct effect on substance use. Causal effects in the other direction are also plausible, i.e., substance use affecting brain structure. Most likely, the complex relationship between subcortical brain volume and substance use is due to a combination of these mechanisms, making it challenging to identify causal relationships.

The two addictive substances which are responsible for the majority of substance use related morbidity and mortality worldwide, are nicotine and alcohol4,5. Most literature on the relationship of subcortical brain volumes with smoking and alcohol use is based on relatively small cross-sectional studies, reporting mixed findings. Smoking has been linked to smaller nucleus accumbens6,7, amygdala7–9, hippocampus10, pallidum9 and thalamus7,9,11 volumes, to smaller8 and larger12 caudate volume, and to larger putamen volume6. Alcohol (ab)use has been associated with smaller nucleus accumbens13,14, amygdala15, hippocampus13,14,16, pallidum14,17 and with smaller18 and larger19 caudate, smaller13,14,16 and larger20 thalamus and smaller14 and larger20 putamen volumes. Recently, the ENIGMA addiction working group attempted to resolve these inconsistent findings with a mega-analysis of subcortical thickness and surface area (volume being its product), among 1,628 controls and 2,277 individuals with dependence on alcohol, nicotine, cocaine, methamphetamine, and/or cannabis21. Smoking was associated with greater thickness and surface area for all subcortical regions, with the strongest associations found for the nucleus accumbens and the hippocampus, while alcohol dependence was associated with lower thickness and surface area, with the strongest associations found for the hippocampus, amygdala, thalamus, and putamen.

Few longitudinal imaging studies have investigated relationships between subcortical brain volume and substance use. Recently, a study was published that obtained structural brain measures and extensive survey-data for 714 individuals at ages 14 and 19. Using a machine learning method, the authors found that alcohol and cannabis use were associated with accelerated cortical thinning and a (mild) increase in subcortical volumes22. While these were longitudinal analyses, the study's observational nature

means there is potential for bias due to (unmeasured) confounding and reverse causality. Whereas a randomized controlled trial (RCT) to assess causal relationships between subcortical brain volume and substance use would be unfeasible, a promising alternative is Mendelian randomization (MR)23. Instead of experimental manipulation, MR uses genetic variants as proxies for the proposed independent variable. Because genes are randomly transmitted from parents to offspring at conception, genetic variants should not be associated with confounders (e.g. socio-economic status). Reverse causation is also not possible, as the genetic independent variable is fixed at birth.

MR has already been applied to assess the relationship of smoking and alcohol use with psychiatric disorders. For instance, there was evidence that ADHD increases the odds of smoking and alcohol dependence and also that smoking causally increases ADHD risk24. Bi-directional causal relationships were also reported between smoking and depression25, while depression was found to causally increase the odds of alcohol dependence with no evidence for the reverse26. Individuals with psychiatric disorders, including ADHD27 and depression28, are known to have smaller subcortical volumes compared to health controls. It may be that subcortical brain volume mediates causal relationships of smoking and alcohol with psychiatric disorders. A recent MR study found no clear evidence for a causal effect of smoking on hippocampal volume29, but the analyses were based on much smaller genetic samples than currently available and other subcortical regions were not included. We conduct the first comprehensive MR study using the largest genetic data-sets available on the volume of seven subcortical brain regions (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen, thalamus) and substance use (smoking initiation, cigarettes smoked per day, smoking cessation, alcohol drinks per week, and alcohol dependence), to probe bi-directional, causal relationships.

Methods and materials

Mendelian randomization

Mendelian randomization (MR) is based on the premise that genetic markers can be used as proxies for a variable that is hypothesized to be a risk factor, or 'exposure', for another 'outcome' variable. Single Nucleotide Polymorphisms (SNPs) are the most commonly used genetic markers. The validity of MR relies on three core assumptions: 1) the association of the genetic instrument with the exposure is robust (ensured by selecting SNPs that reached genome-wide significance, i.e. p<5e-08); 2) the instrument is not associated with confounding variables; 3) the instrument does not influence the outcome through any other path than the exposure. Horizontal pleiotropy, where a SNP directly affects multiple traits, could lead to the second and third assumptions being violated. In order to assess whether the assumptions were met, we applied a range of sensitivity methods, described below.

Data

We took summary-level data from a published GWAS on subcortical brain volumes (n=13,17130) and meta-analyzed these with summary-level data from a GWAS we ran using data from 37,119 UK Biobank participants (Supplementary Methods). This resulted in a total sample of 50,290 for the volume of subcortical brain structures nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen, and thalamus. For substance use, we used summary-level data from the single largest available GWAS on smoking and alcohol use31 (smoking initiation n=848,460, cigarettes per day n=216,590, smoking cessation n=378,249, drinks per week n=630,154; note that UK Biobank was excluded to prevent sample overlap and we meta-analyzed data from the remaining cohorts with data from 23andme, see Supplementary Methods) and a separate GWAS on alcohol dependence32 (n=46,568). Our meta-analyses were n-weighted, due to measurement variance in the original samples, resulting in z-scores. To allow MR analysis, we constructed beta coefficients and standard errors using these z-scores, the effect allele frequencies and sample size33. While the unit of MR estimates based on such constructed betas and standard errors cannot be reliably interpreted, the direction of effect and statistical strength of the evidence can.

Because we obtained the exposure estimates and the outcome estimates from separate samples, it's impossible to verify if individuals in the outcome sample were affected by said exposure. Therefore, when we refer to an exposure causally affecting an outcome, this should be interpreted as an effect of the 'liability to' that exposure.

Main analysis

For clarifying purposes, we describe the exact analysis approach for one specific relationship, where hippocampal volume is the exposure (i.e., the independent variable) and smoking initiation is the outcome (i.e., the dependent variable). First, SNPs that robustly predict hippocampal volume (p<5E-08) were identified in the hippocampus GWAS, and their effect size estimates and standard errors extracted. These same SNPs – i.e., the genetic instrument – were then identified in the smoking initiation GWAS, and their effect size estimates and standard errors extracted. To estimate the causal effect, the SNP-smoking initiation association was divided by the SNP-hippocampal volume association for each individual SNP, and the estimates of multiple SNPs combined with Inverse-Variance Weighted (IVW) regression. IVW provides the first indication of a causal effect by indicating the degree to which SNPs that predict the exposure (hippocampal volume), also predict the outcome (smoking initiation). We tested causal relationships with subcortical brain volumes as the exposures and smoking initiation, cigarettes per day, smoking cessation, alcohol per week and alcohol dependence as the outcomes – and in the other direction, with smoking initiation, alcohol per week and alcohol dependence as the exposures and subcortical volumes as the outcomes. If less than 10 SNPs p<5e-08 were available, we additionally constructed a genetic instrument containing SNPs under a more lenient p-value threshold of p<1e-05. We clumped SNPs for independence at r2<0.01 and 10,000 kb34.

Because the GWAS for cigarettes per day consisted of smokers only31, genome-wide significant SNPs from that study aren't appropriate to use as proxies for cigarettes per day in never smokers. Therefore, the complete subcortical brain volume dataset (n=50,290), consisting of both smokers and never smokers, could not be used. For UK Biobank participants (n=37,119), we had information on smoking behaviour available and could perform GWASs of subcortical brain volumes in never smokers (n=22,555) and in ever smokers (n=14,564). We then applied summary-level MR with cigarettes per day as the exposure, in never and ever smokers separately. This approach provides an additional test of horizontal pleiotropy, as it allowed us to check MR assumptions 2 and 3 – the genetic instrument should not be associated with the outcome through other routes than the exposure. If the genetic instrument for cigarettes per day predicts subcortical brain volume in never smokers, this indicates horizontal pleiotropy because there can't be a true causal effect35.

Sensitivity analyses

The F-statistic was computed to assess instrument strength for all exposures, with F>10 reflecting a sufficiently strong instrument36. In order to test the robustness of a potential causal finding with IVW, we performed six sensitivity methods with different and partly

contrasting assumptions. First, we applied weighted median regression, which produces a reliable causal estimate as long as 50% or more of the total weight of the genetic instrument comes from SNPs that are valid (not biased)37. Second, we applied weighted mode regression, which clusters the SNPs in the genetic instrument based on their causal estimates, and selects the estimate of the SNP-cluster with the largest weight as the final causal estimate. This results in an unbiased value if the SNPs in that cluster are valid and the most common causal effect estimate is indeed the true causal effect38. Third, we conducted MR-Egger, which permits the intercept to deviate from zero, allowing a formal test of horizontal pleiotropy (when there is no horizontal pleiotropy, the intercept should be zero)39. MR-Egger is reliable as long as the InSIDE (Instrument Strength Independent of Direct Effect) assumption is met, meaning that the strength of the instrument (SNP-exposure association) should not correlate with the direct effect of the SNPs on the outcome. MR-Egger also requires sufficiently strong genetic instruments, indicated as the NOME (No Measurement Error) assumption. This can be assessed with the IGX2 (regression dilution) statistic, which ranges between 0 and 1. A lower value represents a higher chance that the NOME assumption is violated40. If IGX2 is ≥0.9 NOME is unlikely to be violated and the results can be reliably interpreted. If IGX2 is 0.6 – 0.9, NOME may have been violated but this can be corrected for with MR-Egger simulation extrapolation (SIMEX). If IGX2 is <0.6, MR-Egger results are likely biased and can't be reliably interpreted. Fourth, we conducted GSMR (Generalised Summary-data-based Mendelian Randomization), which accounts for very low levels of linkage disequilibrium (LD) between SNPs and sampling variance in the estimated SNP effects, to attain higher statistical power. GSMR identifies and removes SNPs that are likely outliers based on their effect size (HEIDI-filtering)41. Fifth, we applied MR-PRESSO (Pleiotropy Residual Sum and Outlier), which compares the observed residual sum of squares to the expected residual sum of squares for each SNP, and re-runs outlier-corrected IVW analyses42. Sixth, we performed Steiger filtering, a method that is used to identify potential bias from reverse causation. It calculates the amount of variance that each SNP explains in both the exposure and the outcome and tests whether the explained variance is, as would be expected, higher for the exposure than the outcome. SNPs that explain more variance in the outcome than the exposure are excluded after which MR is repeated43.

We computed Cochran's Q statistic to assess heterogeneity across the causal estimates of the SNPs included in each instrument36 – high heterogeneity points to horizontal pleiotropy. It should be noted that it is also possible for a true causal effect to run through multiple, very separate biological pathways, resulting in heterogeneity. To assess variability in the power of the genetic instruments, we computed the amount of variance that each instrument explained in the proposed exposure variable44.

Analyses were conducted using the TwoSampleMR package for R34, the GSMR package for R41 and the MR-PRESSO package for R42.

Appraisal of the evidence

We didn't correct for multiple testing explicitly because we analyse phenotypes for which, a priori, there are plausible hypotheses why they are (causally) associated and we want to avoid appraising the evidence based on an arbitrary threshold. We ascribe a finding as showing strong evidence, evidence, weak evidence, or no clear evidence for a causal effect, based on both the IVW regression – adhering to the interpretation of p-values suggested by Sterne and Davey Smith (2001)45 – and the sensitivity methods. Because sensitivity methods rely on stricter assumptions than IVW their statistical power is lower. It is to be expected that the statistical evidence, but not the effect size, decreases with stricter sensitivity methods, even for a true causal effect.

Results

All genetic instruments showed sufficient strength as indicated by the mean F-statistic, ranging between 15.23 and 68.94 (Table S1). Based on the IGX2 statistic, MR-Egger could reliably be performed for all relationships, except when smoking initiation was the exposure (Tables S2 and S3). The amount of variance that the genetic instruments for substance use explained in the corresponding substance use variables ranged between 0.56% and 1.43%. For subcortical brain volumes it ranged between 0.17% and 3.97% (Table S4). A graphical display of all relationships with (weak or strong) evidence for causality is provided in Figure 1.

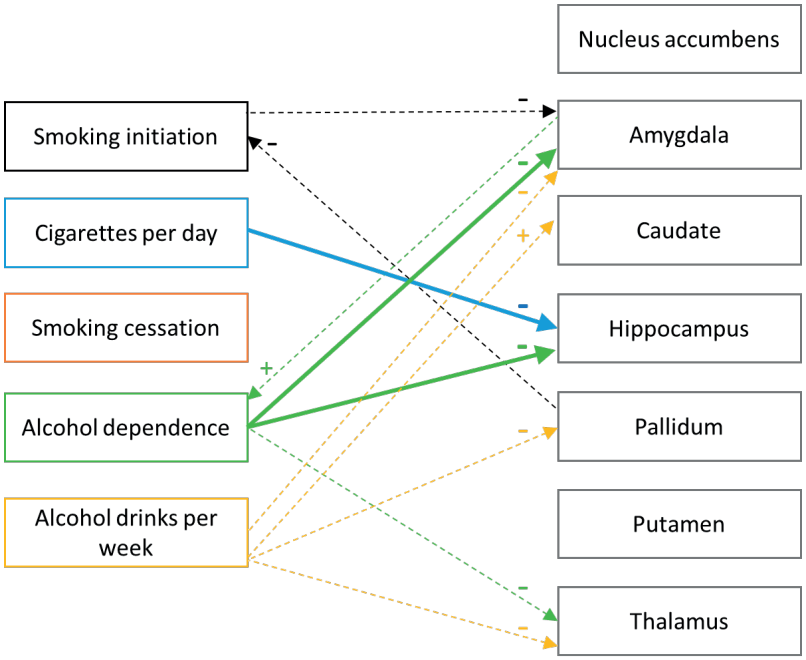

Figure 1. Graphical display of the relationships for which there was evidence for causality. Minus (–) signifies a negative, decreasing effect, while plus (+) signifies a positive, increasing effect. The thicker lines reflect evidence or strong evidence while the dotted, thinner lines signify weak evidence for causality. Note that for all relationships causal effects were tested in both directions, except for smoking cessation which was only tested as an outcome variable.

Causal relationships from subcortical volumes to substance use

There was weak evidence that a larger pallidum volume decreased the odds of initiating smoking (betaIVW=-0.04, p=0.053). Weighted median, weighted mode, and GSMR corroborated this finding, showing similar effect sizes and stronger statistical evidence (Table 1). While there was no clear evidence for horizontal pleiotropy (Egger-intercept=-0.003, p=0.332; Table S5), the regression coefficient of MR-Egger did not indicate a causal effect (Table 1). There was strong evidence for heterogeneity among the SNP-effects (Cochran's Q p=2.4E-05; Table S6). MR-PRESSO identified 2 SNP-outliers but there was no distortion of the causal estimate after outlier removal (Table S7). Steiger filtering did not identify SNPs that explained more variance in the outcome than the exposure (Table S8). There was weak evidence that a larger amygdala volume increased alcohol dependence risk (p<1E-05 betaIVW=0.08, p=0.046), corroborated by weighted median, weighted mode and GSMR sensitivity methods, but not MR-Egger. There was no clear evidence for horizontal pleiotropy (Egger-intercept=0.003, p=0.400), nor for heterogeneity (p=0.621). MR-PRESSO did not identify any SNP-outliers nor did Steiger filtering identify SNPs that explained more variance in the outcome than the exposure. With a 2-SNP instrument (p<5E-08), there was a similar sized, positive effect, but no clear statistical evidence (betaIVW=0.09, p=0.522).

There was very weak evidence that a larger amygdala volume increased the number of alcohol drinks per week (betaIVW=0.06, p=0.098), but sensitivity analyses were not possible due to the p<5E-08 instrument only containing 2 SNPs, and with 40 SNPs under p<1E-05 there was no clear evidence for an effect (betaIVW=0.01, p=0.289).

None of the other analyses showed clear evidence for causal effects of subcortical volumes on substance use.

Causal relationships from substance use to subcortical volumes

There was weak evidence that smoking initiation decreased amygdala volume (betaIVW=-0.05, p=0.046), but the effect was only consistent with the GSMR method (Table 2). There was no clear evidence for horizontal pleiotropy (Egger-Intercept=-0.001 p=0.457), but strong evidence for heterogeneity (Cochran's Q p=2.4E-07). MR-PRESSO identified 1 SNP-outlier, which did not impact the results. Steiger filtering excluded 44 SNPs but after running the analyses with the 302 remaining SNPs evidence for a causal effect remained (betaIVW=-0.06, p=0.013).

In the analyses stratified for smoking status, there was strong evidence that smoking more cigarettes per day decreased hippocampal volume in smokers (betaIVW=-94.73, p=1.8E-06; Table 3). Results were consistent with weighted median, weighted mode, MR-

Egger and GSMR methods, albeit with a smaller effect size for the latter. There was no clear evidence for horizontal pleiotropy (Egger-intercept=0.633, p=0.568) nor heterogeneity (p=0.357). No SNP-outliers were identified with MR-PRESSO. Steiger filtering identified 9 SNPs that were more predictive of the outcome than the exposure, but after excluding these (leaving 40 SNPs) strong evidence for causality remained, consistent across sensitivity methods. There was also weak evidence for a negative effect of cigarettes smoked per day on hippocampal volume in never smokers – indicating horizontal pleiotropy – with a much smaller, less significant effect size (betaIVW=-30.40, p=0.050) and less consistency across sensitivity methods. Taken together, some horizontal pleiotropy exists, but on top of that, there is likely a decreasing effect of cigarettes smoked per day on hippocampal volume.

There was evidence for a negative effect of cigarettes smoked per day on putamen volume, but this relationship seems pleiotropic, given that the effect size and statistical evidence in ever and never smokers are nearly indiscernible (betaIVW=-68.86, p=0.018 and betaIVW=-71.41, p=0.003, respectively). Similarly, there was weak evidence for an effect of cigarettes per day on amygdala and thalamus volume in never smokers, pointing to horizontal pleiotropy.

There was strong evidence for a decreasing effect of liability to alcohol dependence on amygdala volume (betaIVW=-0.15, p=0.007) and evidence for a decreasing effect on hippocampal volume (betaIVW=-0.11, p=0.037). These results were consistent across weighted median, weighted mode, MR-Egger, and GSMR methods (Table 2). There was no clear evidence for horizontal pleiotropy for alcohol dependence-to-amygdala (Egger-intercept=0.007, p=0.468) and weak evidence for alcohol dependence-to-hippocampus (Egger-intercept=0.023, p=0.080). There was no clear evidence for heterogeneity in the SNP-effects (p=0.950 and p=0.691, respectively). MR-PRESSO did not identify SNP-outliers nor did Steiger filtering exclude SNPs that explain more variance in the outcome than the exposure.

There was weak evidence that alcohol dependence decreased thalamus volume (betaIVW=-0.09, p=0.097), which was corroborated by MR-Egger, but not the other sensitivity methods. There was no clear evidence for horizontal pleiotropy (Egger-intercept=0.021, p=0.150), nor was there heterogeneity (p=0.493). No SNP-outliers were identified with MR-PRESSO and Steiger filtering did not exclude any SNPs.

There was evidence that more alcoholic drinks per week increased caudate volume (betaIVW=0.20, p=0.032). The effect was consistent in size, albeit with lower statistical evidence, with weighted median, but attenuated with weighted mode, MR-Egger and

GSMR. There was no clear evidence for horizontal pleiotropy (Egger-intercept=0.001, p=0.629), but strong evidence for heterogeneity (p=6.4E-10). MR-PRESSO identified 5 SNP-outliers, which did not distort the causal estimate. Steiger filtering identified 26 SNPs that explained more variance in the outcome than in the exposure and after removing these (leaving 66 SNPs) there was no clear evidence for a causal effect. Taken together, the evidence that alcoholic drinks per week has a positive effect on caudate volume was weak.

There was weak evidence that more drinks per week decreased pallidum volume (betaIVW=-0.15, p=0.096), an effect which was consistent and even stronger in size and statistical evidence across weighted median, weighted mode, MR-Egger and GSMR. There was evidence for horizontal pleiotropy (Egger-intercept=0.003, p=0.049) and strong evidence for heterogeneity (p=2.2E-08). MR-PRESSO identified 2 SNP-outliers, but there was no distortion in the causal estimate before and after outlier removal. With Steiger filtering 35 SNPs were excluded (leaving 58 SNPs), but after running the analyses again weak evidence for a causal effect remained.

Finally, from drinks per week to both amygdala and thalamus volume there were sizable negative effects which, while there was no clear evidence for the main IVW method, appeared much stronger with the different sensitivity methods (Table 2). There was no indication of horizontal pleiotropy (Egger-intercept=0.001, p=0.537 and 0.001, p=0.459, respectively) but there was evidence for heterogeneity (p=0.005 and p=0.001, respectively). There were no SNP-outliers with MR-PRESSO and while Steiger filtering excluded 27 and 25 SNPs, respectively, weak evidence for causality remained.

Discussion

This is the first study that applied Mendelian randomization to assess bi-directional, causal relationships between volume of subcortical brain regions and a range of substance use behaviours. Our most robust findings were that (liability to) alcohol dependence causally decreased amygdala and hippocampal volume, and smoking more cigarettes per day causally decreased hippocampal volume.

The evidence that alcohol dependence decreased amygdala and hippocampal volume was particularly strong. This is in line with work showing that in individuals with alcohol dependence subcortical brain regions are smaller and have a lower thickness and surface area than in healthy controls – with the largest differences reported for the amygdala and hippocampus13–18,21,46. Given MR's powerful premise and the consistency of our findings across many sensitivity analyses, we are able to make stronger conclusions that this is due to causal effects of alcohol. It had been previously hypothesised that alcohol can cause cell death or reduced cell density, subsequently resulting in volume loss46. For instance, chronic alcohol consumption is known to induce the release of tumour necrosis factor alpha (TNF-α), a cytokine involved in potentiating neuro-inflammation which in turn can cause neuronal death47. When number of alcoholic drinks per week was the exposure, we found weak evidence that more drinks per week decreases amygdala, pallidum, and thalamus volume. This discrepancy in strength of evidence is likely due to the fact that alcohol dependence is the more severe phenotype, reflecting prolonged and heavy exposure of the brain to alcohol.

We found strong evidence that smoking more cigarettes per day (in smokers) decreases hippocampal volume and weak evidence that being a smoker versus being a non-smoker decreases amygdala volume – implying that exposure to cigarette smoking can induce structural subcortical brain changes. While the literature on potential biological mechanisms responsible for such effects is scarce, animal work has shown that exposure to nicotine can induce apoptosis in hippocampal cells48,49. In contrary to our findings, and to those of other observational studies6–11, a large ENIGMA study found smoking to be associated with greater thickness and surface area of all subcortical regions21. This discrepancy may be due to the fact that the ENIGMA-study was observational and its findings influenced by confounding factors.

There is an ongoing discussion as to whether differences in brain structure between substance (ab)users and controls reflect pre-existing differences, or whether they are the result of alterations caused by substance use. Our results mostly point to the latter, with

robust evidence for negative effects of alcohol dependence and smoking on some subcortical volumes, without (similarly robust) evidence for causal effects from subcortical volumes to substance use. This is important knowledge with potentially far reaching consequences. Volume loss might lead to cognitive deficits and a higher chance of developing mental illness, given that smaller volume of the amygdala and hippocampus has been implicated in the most common psychiatric disorders50,51. For instance, it is thought plausible that smoking-related structural brain changes in regions that connect fear response areas (e.g. amygdala) impact trait anxiety states, subsequently leading to an anxiety disorder52. More research is needed to explicitly test pathways from smoking and alcohol use to subcortical brain volume, and subsequently to psychiatric symptoms.

The current study has some important strengths. We used the largest available genetic datasets, which allowed us to test causal effects with sufficiently powered genetic instruments in both directions. We used a diverse and extensive set of sensitivity methods in order to assess the robustness of our findings and whether or not the assumptions underlying MR were met, allowing us to make claims about causality with considerable certainty. There are also limitations to note. While MR can provide less biased causal inference, there may be bias stemming from sources that have so far been less emphasised. One important source is 'genetic nurturing', which occurs when the genotype of parents directly affects offspring phenotypes even if the responsible genetic variants weren't transmitted53. Second, assortative mating, i.e., spouses showing higher phenotypic similarity than expected by chance, may impact MR estimates if this similarity arises because individuals with a particular genetic predisposition choose their mate based on a genetically influenced phenotype53. The effect of both phenomena is that bias from confounding is reintroduced. Finally, while the GWASs we employed corrected for population structure, some geographic clustering may remain54. When these become available, large-scale within-family GWAS would be able to correct for more fine-grained (geographical/family) clusters, providing superior genetic estimates to use in MR53.

In sum, we report robust evidence that heavy alcohol use causally affects the brain, decreasing subcortical brain volume (at least as it pertains to the amygdala and the hippocampus). There was also considerable, but more tentative, evidence that smoking causally decreases amygdala and hippocampus volume. These findings provide additional proof that smoking and alcohol use can have a detrimental effect on the brain and it may implicate structural changes as a pathway connecting substance use to the development of (other) psychiatric disorders. We feel that, combined with accumulating evidence from other types of research, this justifies more recognition in public health efforts and clinical practice.

Table 1. Mendelian randomization analysis with subcortical brain volumes as the exposures and smoking and alcohol use as the outcomes

Exposure	Outcome	SNP	IVW			Weighted median			Weighted mode				MR-Egger#		SNP	GSMR		
		n	b	95% CIs	p	b	95% CIs	p	b	95% CIs	p	b	95% CIs	p	n	b	95% CIs	p
NAc	SI	13	0.02	-0.05 to 0.09	0.542	-0.01	-0.08 to 0.06	0.789	-4.3E-04	-0.12 to 0.12	0.994	-0.24	-0.37 to -0.11	0.003	13	0.02	-0.03 to 0.06	0.519
Amyg *p*<5E-08	SI	2	0.10	-0.12 to 0.31	0.388	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyg *p*<1E-05	SI	40	-0.01	-0.04 to 0.03	0.770	3.6E-03	-0.04 to 0.04	0.864	1.0E-03	-0.04 to 0.04	0.957	0.04	-0.01 to 0.10	0.134	40	5.6E-04	-0.03 to 0.03	0.966
Caudate	SI	53	-0.02	-0.05 to 0.02	0.298	-5.8E-03	-0.04 to 0.03	0.740	-2.1E-03	-0.05 to 0.04	0.931	0.05	-0.06 to 0.17	0.352	49	4.3E-03	-0.02 to 0.03	0.702
Hippo	SI	19	0.02	-0.07 to 0.01	0.644	0.02	-0.05 to 0.09	0.545	0.04	-0.10 to 0.17	0.599	-0.30	-0.81 to 0.21	0.271	19	5.4E-04	-0.04 to 0.04	0.978
Pallidum	SI	25	-0.04	-0.08 to 0.07E-04	0.053	-0.05	-0.09 to -0.01	0.024	-0.07	-0.12 to -0.03	0.006	1.8E-03	-0.09 to 0.09	0.970	23	-0.05	-0.07 to -0.02	0.001
Putamen	SI	35	-3.8E-03	-0.05 to 0.04	0.859	-0.02	-0.05 to 0.01	0.200	-0.02	-0.05 to 0.01	0.266	0.02	-0.08 to 0.11	0.745	29	1.8E-03	-0.02 to 0.02	0.880
Thalamus	SI	12	-0.05	-0.16 to 0.05	0.291	-0.06	-0.14 to 0.03	0.177	-0.06	-0.20 to 0.08	0.395	-0.16	-0.36 to 0.04	0.139	10	3.5E-03	-0.06 to 0.05	0.903
NAc	SC	13	0.02	-0.02 to 0.06	0.307	0.02	-0.03 to 0.07	0.477	0.04	-0.03 to 0.11	0.304	0.06	-0.04 to 0.17	0.265	13	-0.02	-0.06 to 0.02	0.303
Amyg *p*<5E-08	SC	2	-0.14	-0.32 to 0.04	0.131	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyg *p*<1E-05	SC	39	-0.01	-0.04 to 0.01	0.406	-0.03	-0.07 to 0.01	0.150	-0.02	-0.08 to 0.04	0.501	-0.07	-0.19 to 0.05	2.309	39	0.01	-0.01 to 0.04	0.348
Caudate	SC	53	0.01	-0.02 to 0.04	0.706	-2.4E-03	-0.04 to 0.03	0.886	-0.01	-0.07 to 0.05	0.848	-8.5E-03	-0.09 to 0.07	0.831	50	-0.01	-0.03 to 0.01	0.389
Hippo	SC	19	0.01	-0.04 to 0.06	0.586	-0.01	-0.06 to 0.04	0.742	-0.03	-0.12 to 0.06	0.523	-0.03	-0.27 to 0.21	0.800	19	-0.01	-0.05 to 0.02	0.528
Pallidum	SC	25	0.02	-0.01 to 0.06	0.110	0.01	-0.03 to 0.05	0.560	0.01	-0.04 to 0.05	0.781	-0.02	-0.08 to 0.05	0.635	25	-0.02	-0.05 to 0.00	0.061
Putamen	SC	35	0.01	-0.02 to 0.05	0.513	8.5E-03	-0.03 to 0.05	0.660	0.01	-0.05 to 0.06	0.797	-0.03	-0.12 to 0.06	0.532	35	-0.01	-0.03 to 0.01	0.355
Thalamus	SC	13	3.7E-03	-0.06 to 0.07	0.907	4.3E-03	-0.06 to 0.06	0.889	0.01	-0.07 to 0.08	0.864	-0.10	-0.31 to 0.11	0.361	13	2.9E-03	-0.04 to 0.04	0.893
NAc	CPD	13	0.04	-0.01 to 0.09	0.111	0.04	-0.02 to 0.11	0.198	0.06	-0.05 to 0.16	0.312	0.07	-0.05 to 0.19	0.254	13	0.04	-0.01 to 0.09	0.129
Amyg *p*<5E-08	CPD	2	0.02	-0.11 to 0.16	0.741	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyg *p*<1E-05	CPD	40	-0.01	-0.05 to 0.03	0.582	0.01	-0.04 to 0.06	0.770	0.01	-0.05 to 0.08	0.723	0.02	-0.04 to 0.09	0.480	40	-0.01	-0.04 to 0.03	0.732
Caudate	CPD	53	-6.1E-04	-0.04 to 0.04	0.974	-0.02	-0.06 to 0.02	0.250	-0.04	-0.13 to 0.04	0.322	1.9E-03	-0.10 to 0.10	0.969	50	-0.02	-0.04 to 0.01	0.171
Hippo	CPD	19	0.02	-0.03 to 0.07	0.339	0.03	-0.02 to 0.09	0.246	0.04	-0.03 to 0.11	0.302	-0.05	-0.24 to 0.13	0.575	19	0.03	-0.01 to 0.06	0.177

Pallidum	CPD	25	0.01	-0.02 to 0.05	0.488	-0.01	-0.06 to 0.03	0.553	-0.03	-0.08 to 0.03	0.403	0.05	-0.02 to 0.13	0.155	25	0.01	-0.02 to 0.04	0.485
Putamen	CPD	35	-6.9E-03	-0.04 to 0.02	0.664	2.8E-04	-0.04 to 0.04	0.989	0.01	-0.04 to 0.06	0.636	0.02	-0.05 to 0.10	0.544	34	1.4E-03	-0.03 to 0.03	0.917
Thalamus	CPD	12	0.03	-0.07 to 0.03	0.541	0.04	-0.05 to 0.12	0.390	0.02	-0.13 to 0.17	0.802	0.08	-0.19 to 0.35	0.569	11	0.06	0.00 to 0.12	0.037
NAc	AD	13	-0.01	-0.12 to 0.01	0.922	-0.06	-0.22 to 0.10	0.446	-0.09	-0.34 to 0.17	0.514	0.26	-0.06 to 0.57	0.134	13	-0.01	-0.12 to 0.10	0.884
Amyg *p*<5E-08	AD	2	0.09	-0.18 to 0.36	0.522	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyg *p*<1E-05	AD	39	0.08	1.2E-03 to 0.05	0.046	0.10	-0.01 to 0.22	0.081	0.09	-0.04 to 0.21	0.193	0.01	-0.15 to 0.16	0.916	39	0.08	0.00 to 0.15	0.059
Caudate	AD	53	0.02	-0.04 to 0.08	0.521	3.2E-03	-0.08 to 0.09	0.942	-6.5E-03	-0.19 to 0.07	0.921	-0.07	-0.22 to 0.09	0.455	53	0.02	-0.04 to 0.07	0.513
Hippo	AD	19	0.07	-0.03 to 0.07	0.156	0.09	-0.03 to 0.21	0.158	0.11	-0.24 to 0.47	0.280	0.08	-0.30 to 0.46	0.667	19	0.07	-0.03 to 0.17	0.167
Pallidum	AD	25	0.01	-0.06 to 0.08	0.796	-0.05	-0.15 to 0.05	0.326	-0.04	-0.16 to 0.09	0.560	-0.05	-0.21 to 0.10	0.508	25	0.01	-0.06 to 0.08	0.796
Putamen	AD	36	-0.03	-0.11 to 0.06	0.563	0.02	-0.09 to 0.13	0.700	0.04	-0.07 to 0.16	0.465	0.02	-0.20 to 0.23	0.870	35	-4.2E-03	-0.07 to 0.06	0.900
Thalamus	AD	13	0.03	-0.13 to 0.08	0.748	0.05	-0.11 to 0.21	0.538	0.08	-0.14 to 0.30	0.506	0.40	-0.16 to 0.96	0.180	12	0.04	-0.07 to 0.16	0.458
NAc	ALC	13	0.01	-0.04 to 0.06	0.626	-3.6E-03	-0.04 to 0.03	0.856	-0.01	-0.07 to 0.04	0.616	-0.15	-0.28 to 0.02	0.039	11	0.01	-0.02 to 0.04	0.456
Amyg *p*<5E-08	ALC	2	0.06	-0.01 to 0.12	0.098	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyg *p*<1E-05	ALC	40	0.01	-0.01 to 0.03	0.289	0.01	-0.01 to 0.04	0.388	0.01	-0.03 to 0.05	0.520	-0.04	-0.08 to 0.7E-03	0.048	40	0.01	-0.01 to 0.03	0.239
Caudate	ALC	52	2.8E-03	-0.02 to 0.02	0.784	-4.6E-03	-0.02 to 0.02	0.648	9.8E-03	-0.02 to 0.04	0.481	0.02	-0.04 to 0.07	0.535	44	-0.01	-0.02 to 0.01	0.285
Hippo	ALC	19	0.01	-0.02 to 0.04	0.472	0.02	-0.01 to 0.04	0.271	0.02	-0.02 to 0.05	0.448	-0.19	-0.29 to 0.09	0.002	18	6.7E-04	-0.02 to 0.02	0.945
Pallidum	ALC	25	-0.01	-0.04 to 0.01	0.304	-0.02	-0.04 to 4.3E-03	0.116	-0.02	-0.05 to 5.0E-03	0.128	-0.03	-0.08 E 0.03	0.326	23	-0.02	-0.03 to 0.00	0.049
Putamen	ALC	34	-5.5E-03	-0.02 to 0.01	0.576	-4.9E-03	-0.02 to 0.01	0.628	-2.3E-03	-0.02 to 0.02	0.839	-0.01	-0.07 to 0.05	0.742	33	-0.01	-0.02 to 0.01	0.200
Thalamus	ALC	13	1.0E-03	-0.04 to 0.04	0.957	-0.01	-0.04 to 0.03	0.701	-0.01	-0.07 to 0.05	0.740	0.18	0.09 to 0.28	0.002	13	-1.4E-03	-0.03 to 0.02	0.915

MR-Egger reports SIMEX-corrected values if the IGX2 statistic (regression dilution) was below 0.9. If the number of SNPs included in the *p*<5E-08 instrument was <10, a lowered SNP-inclusion threshold (*p*<1E-05) was also reported. SNP = Single Nucleotide Polymorphism, IVW = Inverse-Variance Weighted regression analysis, GSMR = Generalized Summary Level Mendelian randomization, NAc = nucleus accumbens, Amyg = Amygdala, Hippo = Hippocampus, SI = smoking initiation, SC = smoking cessation, CPD = cigarettes smoked per day, DPW = alcohol drinks per week, AD = alcohol dependence.

Table 2. Mendelian randomization analysis with smoking and alcohol use as the exposures and subcortical brain volumes as the outcomes

Exposure	Outcome	SNP	IVW			Weighted median			Weighted mode			MR-Egger#			SNP	GSMR		
		n	b	95% CIs	p	b	95% CIs	p	b	95% CI	p	b	95% CIs	p	n	b	95% CIs	p
SI	NAc	346	-0.04	-0.10 - 0.01	0.114	-0.03	-0.10 - 0.04	0.381	-0.02	-0.13 - 0.09	0.743	-	-	-	339	-0.05	-0.09 - 0.00	0.050
SI	Amygdala	346	-0.05	-0.07 - -9E4	0.046	4E-4	-0.07 - 0.07	0.991	0.01	-0.11 - 0.09	0.856	-	-	-	340	-0.05	-0.09 - 0.00	0.058
SI	Caudate	346	3E-3	-0.10 - 0.07	0.919	-0.02	-0.10 - 0.06	0.660	-6E-3	-0.14 - 0.13	0.929	-	-	-	335	-0.01	-0.06 - 0.04	0.671
SI	Hippo	346	-2E-3	-0.09 - 0.05	0.939	-0.02	-0.09 - 0.06	0.680	7E-4	-0.13 - 0.13	0.992	-	-	-	340	-0.02	-0.07 - 0.03	0.449
SI	Pallidum	346	-0.04	-0.09 - 0.02	0.166	-0.02	-0.09 - 0.05	0.502	-0.01	-0.14 - 0.13	0.934	-	-	-	338	-0.03	-0.08 - 0.02	0.206
SI	Putamen	346	0.02	-0.09 - 0.08	0.614	-0.01	-0.09 - 0.06	0.728	-0.03	-0.15 - 0.09	0.618	-	-	-	336	0.01	-0.04 - 0.06	0.614
SI	Thalamus	345	-0.03	-0.09 - 0.03	0.304	-0.02	-0.09 - 0.06	0.640	-0.01	-0.13 - 0.12	0.900	-	-	-	340	-0.01	-0.06 - 0.04	0.666
AD	NAc	10	-0.06	-0.21 - 0.05	0.301	-0.06	-0.21 - 0.08	0.388	-0.06	-0.26 - 0.13	0.545	-0.32	-0.77 - 0.12	0.189	10	-0.07	-0.20 - 0.06	0.309
AD	Amygdala	10	-0.15	-0.31 - -0.04	0.007	-0.17	-0.31 - -0.04	0.013	-0.17	-0.34 - -0.01	0.074	-0.28	-0.60 - 0.05	0.276	10	-0.15	-0.28 - -0.02	0.023
AD	Caudate	10	0.04	-0.14 - 0.16	0.552	2E-3	-0.14 - 0.14	0.977	0.01	-0.17 - 0.18	0.934	0.49	-0.02 - 1.01	0.099	10	0.02	-0.11 - 0.15	0.734
AD	Hippo	10	-0.11	-0.26 - -0.01	0.037	-0.11	-0.26 - 0.04	0.156	-0.08	-0.28 - 0.11	0.417	-0.50	-0.87 - -0.13	0.031	10	-0.12	-0.25 - 0.01	0.077
AD	Pallidum	10	-0.02	-0.13 - 0.11	0.756	0.03	-0.13 - 0.18	0.737	0.12	-0.07 - 0.32	0.252	-0.50	-1.08 - 0.08	0.130	10	0.01	-0.12 - 0.14	0.883
AD	Putamen	10	-0.03	-0.16 - 0.08	0.624	-0.01	-0.16 - 0.13	0.874	-0.02	-0.21 - 0.16	0.806	0.13	-0.37 - 0.62	0.630	10	-0.01	-0.14 - 0.12	0.891
AD	Thalamus	10	-0.09	-0.21 - 0.02	0.097	-0.06	-0.21 - 0.09	0.443	-0.03	-0.21 - 0.15	0.774	-0.46	-0.91 - -4E-3	0.084	10	-0.01	-0.14 - 0.12	0.891
ALC	NAc	92	-0.13	-0.39 - 0.04	0.122	-0.18	-0.39 - 0.04	0.111	-0.17	-0.38 - 0.05	0.137	-0.18	-0.46 - 0.10	0.200	86	-0.13	-0.26 - 0.01	0.070
ALC	Amygdala	92	-0.11	-0.46 - 0.04	0.147	-0.25	-0.46 - -0.03	0.025	-0.24	-0.48 - -2E4	0.053	-0.17	-0.42 - 0.07	0.174	87	-0.13	-0.27 - 0.00	0.057
ALC	Caudate	92	0.20	-0.06 - 0.39	0.032	0.18	-0.06 - 0.41	0.138	0.09	-0.12 - 0.31	0.401	0.14	-0.16 - 0.45	0.361	85	0.14	0.00 - 0.28	0.054

ALC	Hippo	92	-5E-3	-0.34 - 0.14	0.949	-0.14	-0.34 - 0.06	0.170	-0.18	-0.40 - 0.03	0.100	-0.10	-0.33 - 0.14	0.411	87	-0.03	-0.17 - 0.10	0.635
ALC	Pallidum	92	-0.15	-0.52 - 0.03	0.096	-0.29	-0.52 - -0.05	0.017	-0.31	-0.53 - -0.09	0.007	-0.39	-0.68 - -0.10	0.010	87	-0.17	-0.31 - -0.04	0.013
ALC	Putamen	92	-0.01	-0.29 - 0.19	0.896	-0.06	-0.29 - 0.17	0.605	-0.13	-0.36 - 0.10	0.258	-0.12	-0.45 - 0.21	0.482	83	-0.01	-0.15 - 0.13	0.839
ALC	Thalamus	92	-0.13	-0.48 - 0.03	0.104	-0.26	-0.48 - -0.04	0.022	-0.27	-0.50 - -0.04	0.025	-0.21	-0.46 - 0.05	0.118	86	-0.13	-0.27 - 0.00	0.058

MR-Egger reports SIMEX-corrected values if the IGX2 statistic (regression dilution) was below 0.9, or was not reported if the IGX2 statistic was below 0.6. SNP = Single Nucleotide Polymorphism, IVW = Inverse-Variance Weighted regression analysis, GSMR = Generalized Summary Level Mendelian randomization, NAc = nucleus accumbens, Hippo = Hippocampus, SI=smoking initiation, DPW=alcohol drinks per week, AD=alcohol dependence.

Table 3. Mendelian randomization analysis with cigarettes per day as the exposure and subcortical brain volumes as the outcomes – stratified on smoking status (ever versus never smokers)

Exposure	Outcome	Smok	SNP	IVW			Weighted median			Weighted mode			MR-Egger#			SNP	GMSR		
		stat	n	b	95% CIs	p	b	95% CIs	p	b	95% CIs	p	beta	95% CIs	p	n	b	95% CIs	p
CPD	Nac	Ever	49	2.28	-8.14 to 12.71	0.668	-1.41	-14.32 to 11.51	0.831	2.75	-7.42 to 12.92	0.598	9.95	-5.16 to 25.07	0.203	45	6.77	-0.07 to 19.43	0.294
CPD	Amygdala	Ever	49	8.41	-14.10 to 30.91	0.464	12.86	-14.93 to 40.65	0.365	7.27	-17.02 to 31.56	0.560	5.02	-28.22 to 38.25	0.769	45	5.62	-10.38 to 35.2	0.710
CPD	Caudate	Ever	49	-18.59	-60.66 to 23.48	0.386	-23.17	-71.06 to 24.72	0.343	-30.68	-74.45 to 13.09	0.176	-59.16	-119.27 to 0.95	0.060	44	-13.30	-41.1 to 38.09	0.612
CPD	Hippo	Ever	49	-94.73	-133.57 to -55.89	1.8E-06	-121.36	-170.28 to -72.43	1.2E-06	-108.97	-153.96 to -63.99	1.9E-05	-106.98	-164.19 to -49.77	0.001	45	-46.57	-75.96 to 7.78	0.093
CPD	Pallidum	Ever	49	-13.43	-44.75 to 17.89	0.401	-21.67	-51.32 to 7.98	0.152	-23.51	-50.45 to 3.44	0.094	-1.89	-47.95 to 44.18	0.936	45	7.19	-8.43 to 36.07	0.626
CPD	Putamen	Ever	49	-68.86	-125.68 to -12.05	0.018	-56.36	-119.91 to 7.18	0.082	-41.29	-90.27 to 7.69	0.105	-7.59	-88.06 to 72.87	0.854	45	-30.52	-64.02 to 31.43	0.334
CPD	Thalamus	Ever	49	-1.95	-55.21 to 51.30	0.943	27.72	-40.07 to 95.51	0.423	0.60	-54.88 to 56.07	0.983	-5.23	-83.93 to 73.47	0.897	44	22.31	-13.84 to 89.18	0.513
CPD	NAc	Never	49	-9.50	-17.86 to -1.14	0.026	-11.83	-21.82 to -1.83	0.020	-12.70	-21.59 to -3.82	0.007	-16.20	-28.26 to -4.14	0.011	45	-5.72	-15.86 to 4.43	0.269
CPD	Amygdala	Never	49	-15.66	-34.05 to 2.73	0.095	-20.19	-42.69 to 2.30	0.079	-19.79	-39.25 to -0.33	0.052	-20.93	-48.02 to 6.15	0.136	44	4.83	-18.75 to 28.41	0.688
CPD	Caudate	Never	49	-12.17	-50.53 to 26.19	0.534	-8.08	-52.37 to 36.21	0.721	-18.29	-53.10 to 16.52	0.308	-36.42	-92.27 to 19.42	0.207	43	0.29	-41.27 to 41.86	0.989
CPD	Hippo	Never	49	-30.40	-60.75 to -0.04	0.050	-26.96	-66.70 to 12.79	0.184	-24.92	-62.12 to 12.28	0.195	-9.42	-53.78 to 34.94	0.679	45	-21.44	-65.2 to 22.32	0.337
CPD	Pallidum	Never	49	-16.57	-44.82 to 11.69	0.250	-35.33	-61.56 to -9.10	0.008	-23.44	-44.09 to -2.80	0.031	-16.97	-58.70 to 24.76	0.429	43	11.91	-14.08 to 37.9	0.369
CPD	Putamen	Never	49	-71.41	-119.23 to -23.58	0.003	-69.84	-116.59 to -23.08	0.003	-66.83	-108.38 to -25.28	0.003	-76.98	-147.58 to -6.37	0.038	44	-53.17	-101.94 to -4.39	0.033
CPD	Thalamus	Never	49	-45.30	-92.05 to 1.46	0.058	-67.14	-117.07 to -17.20	0.008	-52.49	-92.41 to -12.57	0.013	-65.05	-133.66 to 3.57	0.069	44	-17.06	-70.36 to 36.24	0.530

MR-Egger reports SIMEX-corrected values if the IGX2 statistic (regression dilution) was below 0.9. SNP = Single Nucleotide Polymorphism, IVW = Inverse-Variance Weighted regression analysis, GSMR = Generalized Summary Level Mendelian randomization, NAc=nucleus accumbens, Hippo = Hippocampus, CPD=cigarettes smoked per day. Note that for the analyses presented in table 3, SNP effects for subcortical brain volume were in units of mm^3, as opposed to the constructed betas and SEs that were used for the analyses presented in table 1 and table 2 – this explains the difference in the size of the causal estimates in table 3 compared to table 1 and table 2.

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Supplementary Materials

The full Supplementary Materials can also be viewed online at:
Chapter 8 – Causal relationship between brain region volumes and substance use

or past this link in the browser:
https://drive.google.com/drive/folders/1FQ7MwF67QqE7XV1zi0tocNUiCKDWBVi1

GWAS and meta-analysis procedures

GWAS subcortical brain volumes

To perform the GWAS on subcortical brain volumes in UK Biobank participants, we first extracted UKB participants of European ancestry. Individuals of non-European ancestry, as determined by Principal Component Analysis in GCTA[1], were excluded. We performed Single Nucleotide Polymorphism (SNP) quality control on unrelated Europeans (N=312,927) – using the Hapmap 3 reference panel – filtering out SNPs with MAF < 0.01, missingess > 0.05 and HWE p < 10^{-10}, leaving 1,246,531 SNPs. After also adding related participants, we created a dataset of 1,246,531 common, QC-ed SNPs for 456,064 UK Biobank participants of European ancestry. For a more detailed description of our QC and data processing pipeline, see Abdellaoui et al., 2019[2].

Of the total, QC-ed UK Biobank dataset, 37,119 participants had MRI-imaging data on subcortical brain volumes available. We performed GWAS for seven subcortical brain regions; nucleus accumbens (UKB data fields 25023 (left) and 25024 (right)), amygdala (25021 (left) and 25022 (right)), caudate (25013 (left) and 25014 (right)), hippocampus (25019 (left) and 25020 (right)), pallidum (25017 (left) and 25018 (right)), putamen (25015 (left) and 25016 (right)), and thalamus (25011 (left) and 25012 (right)). For each brain region, we first summed the left and right volume measures (in mm^3). For the GWAS, we ran a linear mixed model (LMM) GWAS using fastGWA[3]. As covariates, we added a sparse genetic relatedness matrix (GRM) which controls for cryptic relatedness as well as population stratification[4] and the first 25 principle components (PCs), and we applied LDSC-intercept based genomic control (GC)[2]. Other covariates that were added were sex, age and total brain volume (UKB data field 25010).

After performing GWAS for subcortical brain structures in all participants, we also ran GWAS for subcortical brain structures stratified for smoking status (to allow Mendelian randomization analyses with cigarettes per day as the exposure variable, see main paper). All 37,119 UK Biobank participants described above had provided information on their lifetime smoking behaviour (UKB data field 20160), falling into the category of either ever

smoker (n=14,564) or never smoker (n=22,555). We performed GWAS using the exact same approach described above, in these two groups separately.

Meta-analysis GWAS samples subcortical brain volumes

We performed a meta-analysis of the summary statistics from our GWAS analyses on UK Biobank participants (the complete sample described above) and the summary statistics from a previous ENIGMA GWAS[5] for all brain regions. We performed this meta-analysis in METAL[6], and it was based on sample size (instead of standard error) because there was measurement variance in the original samples. SNPs with a sample size below 1,000 were not included in the meta-analysis. Subsequently, the LD score regression intercept was calculated using LDSC[7]. Z-scores resulting from the meta-analysis were converted to betas using the formula $\beta = \frac{z-score}{\sqrt{N} * \frac{1}{\sqrt{EAF(1-EAF)}}}$ (following procedures from Taylor et al., 2016[8]). The corresponding standard error was computed using $se = \frac{\beta}{z-score}$. We corrected the standard error for genomic inflation with the LD score regression intercept with $se_{corrected} = \sqrt{se^2 * intercept}$ and computed corresponding p-values in R. To create genetic instruments for exposures, we clumped significant (5e-08 / 1e-05) hits using PLINK, using R^2 <0.01 and 10,000 kb as our independence threshold.

Meta-analysis GWAS samples substance use

We performed a meta-analysis of the summary statistics from the single largest available GWAS on smoking and alcohol use[9] (excl. UK-Biobank) and the summary statistics from 23andMe (requested from 23andMe, Inc) for smoking initiation, cigarettes per day, smoking cessation, and alcohol per week in METAL[6], based on sample size. Subsequently, the LD score regression intercept was calculated using LDSC[7]. Z-scores resulting from the meta-analysis were converted to betas with similar procedures as described above. To create genetic instruments for exposures, we selected the SNPs (rs-numbers) that were reported to be significantly related with the traits in the original GSCAN paper[9] and extracted their estimates (betas, standard errors and p-values) from our own meta-analysis.

SNPs included in the various genetic instruments

Below is an overview of the SNPs that were included in the various genetic instruments used for Mendelian randomization analyses. Note that we selected SNPs which had initially, that is, in the original, complete GWAS study, been reported as genome-wide associated (p<5E-08), to include in the instruments. In the case of smoking initiation, smoking cessation, cigarettes smoked per day and alcohol drinks per week, we excluded UK Biobank from the original GWAS study to prevent sample overlap (which causes bias in MR), and then took the effect estimates from a meta-analyses of the remaining samples. This means that for some of the included SNPs for those exposures the significance level may be higher than 5E-08.

SNPs included for subcortical brain volumes as exposures:

	Nucleus accumbens	**Amygdala 5E-08**	**Amygdala 1E-05**	**Caudate**	**Hippocampus**	**Pallidum**	**Putamen**	**Thalamus**
1	rs1178320	rs2347701	rs10130307	rs10439608	rs10494303	rs11196876	rs10439608	rs11827239
2	rs11916858	rs79340609	rs10504722	rs10800064	rs11068205	rs11639504	rs10765500	rs12146713
3	rs2153960		rs10850141	rs10887759	rs11245365	rs11976703	rs11196979	rs16956241
4	rs2608301		rs10868746	rs112510871	rs11737577	rs12445022	rs11197861	rs1789167
5	rs321414		rs111592563	rs115053453	rs12189145	rs12703184	rs11204421	rs321348
6	rs34255419		rs11230889	rs11669745	rs12523793	rs1291602	rs11576262	rs385301
7	rs34312154		rs116991338	rs11676147	rs13089287	rs1328360	rs11765218	rs429358
8	rs3789364		rs11790097	rs117407140	rs2279681	rs196798	rs1187155	rs45499402
9	rs4148557		rs118133428	rs1187155	rs2287509	rs2231142	rs12197456	rs62288104
10	rs62288106		rs118182651	rs12256016	rs3111623	rs28439696	rs12457893	rs7026018
11	rs6658111		rs12251016	rs12749024	rs429358	rs2844510	rs12806934	rs75179968
12	rs66717543		rs12363897	rs12952581	rs58499557	rs2974367	rs13403450	rs76928645
13	rs7940646		rs12586189	rs13172721	rs61921502	rs34658078	rs1346914	rs806794
14			rs12605433	rs1347008	rs62063437	rs34736685	rs1430332	
15			rs13100173	rs1422191	rs62374671	rs4887023	rs1544528	
16			rs13124981	rs1743179	rs6664992	rs6037025	rs2139559	
17			rs132867	rs17764251	rs6876370	rs6058317	rs2244479	
18			rs17150071	rs17773700	rs7040792	rs62079082	rs2710880	
19			rs202923	rs208829	rs77956314	rs6723226	rs3131633	
20			rs2336943	rs2196448		rs73186053	rs314688	
21			rs2347701	rs2817145		rs76891681	rs3815071	
22			rs28399637	rs28366595		rs7950543	rs398652	
23			rs4586727	rs28535536		rs8014725	rs4714854	
24			rs4797344	rs2910056		rs9426738	rs4748999	
25			rs4818252	rs2926043		rs9922520	rs4916907	
26			rs61353762	rs34259020			rs556151	
27			rs61921502	rs34811474			rs6138937	
28			rs62282701	rs3820870			rs62136802	
29			rs62359209	rs4888010			rs634368	
30			rs6658111	rs4984975			rs6428671	
31			rs6979446	rs4985152			rs7101609	
32			rs7014578	rs55830072			rs7734654	

33			rs707097	rs56000151			rs79787807	
34			rs7539819	rs6135525			rs8017172	
35			rs7604451	rs62037364			rs806794	
36			rs76436457	rs62082217			rs9838026	
37			rs79340609	rs62365541				
38			rs80223973	rs6483195				
39			rs895330	rs6703416				
40			rs9853475	rs7040561				
41				rs7084454				
42				rs7198936				
43				rs7245004				
44				rs72631228				
45				rs73144681				
46				rs75268943				
47				rs7747401				
48				rs7949956				
49				rs8017172				
50				rs888234				
51				rs9369275				
52				rs9903088				
53				rs994539				

Note that if the number of SNPs differed slightly for different exposure-outcome combinations, we report the instrument with the largest amount of SNPs here.

SNPs included for substance use behaviours as exposures:

	Smoking initiation	Cigarettes per day	Alcohol dependence	Alcohol drinks per week
1	rs10042827	rs1024323	rs113659074	rs10004020
2	rs1004787	rs10454798	rs1154445	rs10028756
3	rs10060196	rs10519203	rs1229863	rs1004787
4	rs1008078	rs10742683	rs1229984	rs10085696
5	rs1022376	rs1115019	rs34929220	rs10236149
6	rs1022528	rs11264100	rs3811802	rs10438820
7	rs10233018	rs113001570	rs4388946	rs10506274
8	rs10272990	rs117824460	rs6827898	rs10750025
9	rs10279261	rs11846838	rs79171978	rs10753661
10	rs1030015	rs11940255	rs894368	rs10876188
11	rs10446419	rs12438181		rs10978550
12	rs10490159	rs12924872		rs11030084
13	rs1050847	rs13253502		rs1123285
14	rs1059490	rs143200968		rs113443718
15	rs10698713	rs146009840		rs1154414
16	rs10789369	rs1592485		rs11625650
17	rs10805858	rs182317		rs11692435
18	rs10853981	rs2084533		rs11739827
19	rs10858334	rs215600		rs11940694
20	rs10873871	rs2236951		rs12088813
21	rs10885480	rs2273500		rs1217091
22	rs10905461	rs258321		rs1229984
23	rs10914684	rs2741351		rs12499107
24	rs10935779	rs28681284		rs1260326
25	rs10945141	rs28813180		rs12655091
26	rs10953957	rs3025383		rs12795042
27	rs10966092	rs34973462		rs12907323
28	rs11057005	rs4144686		rs13024996
29	rs1106363	rs4236926		rs13032049
30	rs11076962	rs4485470		rs13066454
31	rs11078713	rs56113850		rs13094887
32	rs1108130	rs59208569		rs13107325
33	rs1109480	rs6078373		rs13250583
34	rs11162019	rs62447179		rs13383034
35	rs1116690	rs632811		rs17029090
36	rs11191269	rs699165		rs1713676
37	rs11192347	rs7125588		rs17177078
38	rs11258417	rs72740955		rs17665139
39	rs1126757	rs7281463		rs2011092
40	rs112725451	rs73229090		rs2165670
41	rs113230003	rs7431710		rs2178197
42	rs1139897	rs75596189		rs2180870
43	rs114976176	rs7599488		rs2472297
44	rs1150668	rs7766641		rs2764771
45	rs11587399	rs78408772		rs281379
46	rs11594623	rs790564		rs2854334
47	rs11611651	rs7951365		rs28601761
48	rs11642231	rs8040868		rs28680958
49	rs11651955	rs8192726		rs28929474
50	rs11678980			rs35034355
51	rs11692435			rs35538052

52	rs11713899			rs36052336
53	rs1173461			rs3748034
54	rs117657830			rs378421
55	rs11766326			rs3803800
56	rs11768481			rs3809162
57	rs117734003			rs4092465
58	rs11780471			rs4501255
59	rs11783093			rs4548913
60	rs11791671			rs4690727
61	rs118202			rs4699791
62	rs11872397			rs4815364
63	rs1187820			rs4842786
64	rs11889814			rs4916723
65	rs11956866			rs4938230
66	rs12022778			rs500321
67	rs12027999			rs5024204
68	rs12053870			rs55872084
69	rs12130857			rs55932213
70	rs12195240			rs56030824
71	rs12244388			rs56337305
72	rs12474587			rs58107686
73	rs12517438			rs60654199
74	rs12530388			rs62044525
75	rs12563365			rs62250685
76	rs12633090			rs6460047
77	rs12642744			rs6787172
78	rs12714017			rs682011
79	rs12739243			rs6951574
80	rs12740789			rs705687
81	rs12755632			rs7074871
82	rs12855717			rs7185555
83	rs12878369			rs72859280
84	rs12918191			rs77165542
85	rs1291821			rs79139602
86	rs13007361			rs7950166
87	rs13066050			rs79616692
88	rs13109980			rs823114
89	rs13110073			rs828867
90	rs13261666			rs9607814
91	rs13319205			rs9838144
92	rs13392222			rs9950000
93	rs13437771			
94	rs1373178			
95	rs1381287			
96	rs1381775			
97	rs1385108			
98	rs1389171			
99	rs13906			
100	rs139896			
101	rs1413119			
102	rs1435479			
103	rs1435672			
104	rs1435741			

105	rs1445649			
106	rs1449012			
107	rs147052174			
108	rs1514176			
109	rs1518393			
110	rs1549979			
111	rs1555445			
112	rs1561112			
113	rs1565735			
114	rs160631			
115	rs1632941			
116	rs16826827			
117	rs16828799			
118	rs1713676			
119	rs1714521			
120	rs17165769			
121	rs17197663			
122	rs1722666			
123	rs17229285			
124	rs1733760			
125	rs1737329			
126	rs1759433			
127	rs17616642			
128	rs17692129			
129	rs1772572			
130	rs1799068			
131	rs1811739			
132	rs181508347			
133	rs1834306			
134	rs1863161			
135	rs1889571			
136	rs1901477			
137	rs1910236			
138	rs1927901			
139	rs1930371			
140	rs1935571			
141	rs1944689			
142	rs2010921			
143	rs2028269			
144	rs2063976			
145	rs2155646			
146	rs2173019			
147	rs2196356			
148	rs221988			
149	rs2276825			
150	rs2279829			
151	rs2289791			
152	rs2306866			
153	rs2319545			
154	rs2344976			
155	rs2378662			
156	rs238896			
157	rs2526390			

158	rs2539706			
159	rs2587507			
160	rs2637869			
161	rs2710634			
162	rs2734390			
163	rs2796793			
164	rs281296			
165	rs28408682			
166	rs28441558			
167	rs28717373			
168	rs2901785			
169	rs290601			
170	rs2925128			
171	rs2938134			
172	rs2939756			
173	rs2952251			
174	rs2959084			
175	rs301807			
176	rs3098272			
177	rs3115418			
178	rs3172494			
179	rs3218116			
180	rs329124			
181	rs34342129			
182	rs34399632			
183	rs34553878			
184	rs34940743			
185	rs34970111			
186	rs35375873			
187	rs35656245			
188	rs357304			
189	rs359247			
190	rs359431			
191	rs3740977			
192	rs3764351			
193	rs3800227			
194	rs3810496			
195	rs3811038			
196	rs3820277			
197	rs3843905			
198	rs3847244			
199	rs3909281			
200	rs3934797			
201	rs4044321			
202	rs42417			
203	rs4264267			
204	rs4275621			
205	rs4310804			
206	rs4476253			
207	rs4543050			
208	rs45444697			
209	rs4674916			
210	rs4674993			

211	rs4727189			
212	rs4752018			
213	rs4759229			
214	rs4785187			
215	rs4788676			
216	rs4790874			
217	rs4818005			
218	rs4822102			
219	rs4837631			
220	rs4877285			
221	rs4886207			
222	rs4912332			
223	rs540860			
224	rs55786907			
225	rs55913542			
226	rs55944129			
227	rs56208390			
228	rs56367474			
229	rs56902655			
230	rs57153235			
231	rs58400863			
232	rs586699			
233	rs59537158			
234	rs6011779			
235	rs6050446			
236	rs6058782			
237	rs6073075			
238	rs60833441			
239	rs61533748			
240	rs61884449			
241	rs61886926			
242	rs619087			
243	rs61959481			
244	rs62007780			
245	rs62052916			
246	rs62098013			
247	rs62106258			
248	rs62137126			
249	rs62180324			
250	rs62193862			
251	rs62246017			
252	rs62340589			
253	rs62618693			
254	rs6265			
255	rs6437769			
256	rs6438436			
257	rs644740			
258	rs6452785			
259	rs6497840			
260	rs6568832			
261	rs67050670			
262	rs6730325			
263	rs6731872			

264	rs6750107			
265	rs6750529			
266	rs6756212			
267	rs67777803			
268	rs6782116			
269	rs6874731			
270	rs6890961			
271	rs6936160			
272	rs6948707			
273	rs6968380			
274	rs6986430			
275	rs6993429			
276	rs7024924			
277	rs7026534			
278	rs7072776			
279	rs7134009			
280	rs71367544			
281	rs71592686			
282	rs71602617			
283	rs7188873			
284	rs7192140			
285	rs72780746			
286	rs72789626			
287	rs72790288			
288	rs72898831			
289	rs72938304			
290	rs73008357			
291	rs7333559			
292	rs73831818			
293	rs74697736			
294	rs748832			
295	rs7505855			
296	rs75210106			
297	rs75674569			
298	rs75919030			
299	rs7600835			
300	rs7631379			
301	rs7640107			
302	rs76460663			
303	rs7657022			
304	rs76608582			
305	rs76841737			
306	rs7696257			
307	rs77215829			
308	rs77283305			
309	rs7743165			
310	rs7802996			
311	rs7809303			
312	rs7836565			
313	rs7867822			
314	rs7901883			
315	rs7929518			
316	rs7943721			

317	rs79476395			
318	rs7969559			
319	rs8005334			
320	rs80054503			
321	rs8027457			
322	rs8050598			
323	rs8083764			
324	rs8096225			
325	rs8103660			
326	rs876793			
327	rs910912			
328	rs925524			
329	rs9288999			
330	rs9302604			
331	rs9323328			
332	rs9331343			
333	rs951740			
334	rs9538162			
335	rs9540731			
336	rs9545155			
337	rs9627272			
338	rs963354			
339	rs9787523			
340	rs9826984			
341	rs9841807			
342	rs9850597			
343	rs9922607			
344	rs9936784			
345	rs9941217			
346	rs9987376			

Note that if the number of SNPs differed slightly for different exposure-outcome combinations, we report the instrument with the largest amount of SNPs here.

Supplementary table 1. Mean F statistic for all SNPs included in the genetic instruments used for Mendelian randomization analyses

Exposure	Threshold	SNPs	F statistic
Accumbens	5.00E-08	13	39.05
Amygdala	5.00E-08	2	36.77
Amygdala*	1.00E-05	40	23.43
		39	23.47
Caudate	5.00E-08	53	43.17
		52	42.60
Hippocampus	5.00E-08	19	49.02
Pallidum	5.00E-08	25	48.43
Putamen*	5.00E-08	36	53.84
		35	54.44
		34	53.04
Thalamus	5.00E-08	13	36.62
		12	36.62
Smoking initiation*	5.00E-08	346	15.23
		345	15.25
Drinks per week	5.00E-08	92	39.48
Alcohol dependence	5.00E-08	10	29.15
Cigarettes per day	5.00E-08	49	68.94

Supplementary table 2. IGX2 (regression dilution) statistic which assesses the reliability of MR-Egger results - with subcortical brain volumes as the exposures

		Outcome				
Exposure	**Threshold**	*Smoking initiation*	*Smoking cessation*	*Cigarettes per day*	*Alcohol dependency*	*Drinks per week*
Accumbens	5E-08	0.75 (u)	0.75 (u)	0.75 (u)	0.75 (u)	0.75 (u)
Amygdala	5E-08	0.80 (u)	0.80 (u)	0.80 (u)	0.80 (u)	0.80 (u)
Amygdala	1E-05	0.91 (w)	0.88 (w)	0.79 (u)	0.89 (w)	0.79 (u)
Caudate	5E-08	0.86 (w)	0.81 (w)	0.81 (u)	0.81 (w)	0.81 (u)
Hippocampus	5E-08	0.74 (w)	0.63 (w)	0.62 (u)	0.63 (w)	0.63 (u)
Pallidum	5E-08	0.91 (w)	0.91 (u)	0.91 (u)	0.91 (u)	0.91 (u)
Putamen	5E-08	0.94 (w)	0.93 (w)	0.89 (u)	0.93 (w)	0.89 (u)
Thalamus	5E-08	0.71 (u)	0.84 (w)	0.71 (u)	0.84 (w)	0.71 (u)

The IGX2 statistic is a measure for violation of the NOME ('No Measurement Error') assumption. For all genetic instruments the highest IGX2 statistic was reported (either unweighted, or weighted for standard error). If both were ≥0.9 then weighted was reported as default. If IGX2 was 0.9 or higher, bias as a result of NOME violation is not likely, and MR-Egger results were reported in the manuscript. If IGX2 was lower than 0.9, SIMEX ('Simulation Extrapolation') was performed on MR-Egger to correct for possible bias resulting from NOME violation. If IGX2 was below 0.6, MR-Egger (SIMEX) was not reported since bias as result of NOME violation renders the results unreliable (u) and (w) represent the weighted and unweighted IGX2 respectively.

Supplementary table 3. IGX2 (regression dilution) statistic which assesses the reliability of MR-Egger results - with substance use variables as the exposures

	Outcome						
Exposure	**Accumbens**	**Amygdala**	**Caudate**	**Hippocampus**	**Pallidum**	**Putamen**	**Thalamus**
Smoking initiation	0.54 (w)	0.54 (w)	0.54 (w)	0.54 (w)	0.54 (w)	0.54 (w)	0.54 (w)
Cigarettes per day (ever)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)
Cigarettes per day (never)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)	0.97 (w)
Drinks per week	0.91 (w)	0.91 (w)	0.91 (w)	0.91 (w)	0.91 (w)	0.91 (w)	0.91 (w)
Alcohol dependency	0.77 (w)	0.77 (w)	0.77 (w)	0.77 (w)	0.77 (w)	0.77 (w)	0.77 (w)

The IGX2 statistic is a measure for violation of the NOME ('No Measurement Error') assumption. For all genetic instruments the highest IGX2 statistic was reported (either unweighted, or weighted for standard error). If both were ≥0.9 then weighted was reported as default. If IGX2 was 0.9 or higher, bias as a result of NOME violation is not likely, and MR-Egger results were reported in the manuscript. If IGX2 was lower than 0.9, SIMEX ('Simulation Extrapolation') was performed on MR-Egger to correct for possible bias resulting from NOME violation. If IGX2 was below 0.6, MR-Egger (SIMEX) was not reported since bias as result of NOME violation renders the results unreliable (u) and (w) represent the weighted and unweighted IGX2 respectively.

Supplementary table 4. Overview of the amount of variance that the genetic instruments explained

Exposure	Threshold	SNPs	Explained variance (%)
Accumbens	5E-08	13	0.70
Amygdala	5E-08	2	0.17
Amygdala	1E-05	40	0.97
Caudate	5E-08	53	3.97
Hippocampus	5E-08	19	1.60
Pallidum	5E-08	25	1.82
Putamen	5E-08	35	2.18
Thalamus	5E-08	12	0.70
Smoking initiation	5E-08	346	1.43
Drinks per week	5E-08	92	0.56
Alcohol dependence	5E-08	10	0.59
Cigarettes per day (ever smokers)	5E-08	49	1.27

Explained variance was computed as described by Pasman et al. Nature Neuroscience,2019, 21:1161-1170

Supplementary table 5. MR-egger intercepts, providing an estimation of horizontal pleiotropy.

exposure	Threshold	outcome	intercept	SE	p
Nucleus accumbens	5E-08	Smoking initiation	0.012	0.006 to 0.019	0.004
Nucleus accumbens	5E-08	Smoking cessation	-3E-03	-0.008 to 0.003	0.375
Nucleus accumbens	5E-08	Cigarettes per day	-0.002	-0.009 to 0.004	-0.002
Nucleus accumbens	5E-08	Drinks per week	0.008	0.001 to 0.015	0.048
Nucleus accumbens	5E-08	Alcohol dependency	-0.013	-0.031 to 0.004	0.154
Amygdala	5E-08	Smoking initiation	-	-	-
Amygdala	5E-08	Smoking cessation	-	-	-
Amygdala	5E-08	Cigarettes per day	-	-	-
Amygdala	5E-08	Drinks per week	-	-	-
Amygdala	5E-08	Alcohol dependency	-	-	-
Amygdala	1E-05	Smoking initiation	-0.003	-0.006 to -3.0E-04	0.036
Amygdala	1E-05	Smoking cessation	0.003	-0.001 to 0.007	0.105
Amygdala	1E-05	Cigarettes per day	-0.001	-0.006 to 0.003	0.552
Amygdala	1E-05	Drinks per week	0.002	-0.001 to 0.005	0.142
Amygdala	1E-05	Alcohol dependency	0.003	-0.004 to 0.011	0.400
Caudate	5E-08	Smoking initiation	-0.004	-0.009 to 0.002	0.190
Caudate	5E-08	Smoking cessation	0.001	-0.004 to 0.005	0.692
Caudate	5E-08	Cigarettes per day	2E-05	-0.006 to 0.006	0.993
Caudate	5E-08	Drinks per week	-0.001	-0.004 to 0.002	0.684
Caudate	5E-08	Alcohol dependency	0.004	-0.004 to 0.012	0.293
Hippocampus	5E-08	Smoking initiation	0.015	-0.008 to 0.039	0.227
Hippocampus	5E-08	Smoking cessation	0.002	-0.009 to 0.014	0.708
Hippocampus	5E-08	Cigarettes per day	0.003	-0.007 to 0.013	0.544
Hippocampus	5E-08	Drinks per week	0.009	0.004 to 0.015	0.003
Hippocampus	5E-08	Alcohol dependency	0.001	-0.018 to 0.019	0.939
Pallidum	5E-08	Smoking initiation	-0.003	-0.009 to 0.003	0.332
Pallidum	5E-08	Smoking cessation	0.003	-0.001 to 0.007	0.183
Pallidum	5E-08	Cigarettes per day	-0.003	-0.007 to 0.001	0.209
Pallidum	5E-08	Drinks per week	0.001	-0.002 to 0.004	0.561
Pallidum	5E-08	Alcohol dependency	0.004	-0.005 to 0.014	0.384
Putamen	5E-08	Smoking initiation	-0.001	-0.007 to 0.004	0.653
Putamen	5E-08	Smoking cessation	0.002	-0.002 to 0.007	0.335
Putamen	5E-08	Cigarettes per day	-2E-03	-0.006 to 0.002	0.430
Putamen	5E-08	Drinks per week	2.27E-04	-0.003 to 0.004	0.894
Putamen	5E-08	Alcohol dependency	-0.003	-0.014 to 0.009	0.670

Thalamus	5E-08	Smoking initiation	0.007	-0.196 to 0.210	0.222
Thalamus	5E-08	Smoking cessation	0.006	-0.005 to 0.016	0.312
Thalamus	5E-08	Cigarettes per day	-0.002	-0.016 to 0.012	0.762
Thalamus	5E-08	Drinks per week	-0.009	-0.014 to -0.004	0.002
Thalamus	5E-08	Alcohol dependency	-0.019	-0.047 to 0.008	0.195
Smoking initiation	5E-08	nucleus accumbens	-3E-05	-0.003 to 0.002	0.978
Smoking initiation	5E-08	Amygdala	-0.001	-0.003 to 0.001	0.457
Smoking initiation	5E-08	Caudate	-0.001	-0.004 to 0.002	0.377
Smoking initiation	5E-08	Hippocampus	-3E-04	-0.003 to 0.002	0.815
Smoking initiation	5E-08	pallidum	-0.002	-0.004 to 0.001	0.227
Smoking initiation	5E-08	Putamen	-0.001	-0.004 to 0.002	0.412
Smoking initiation	5E-08	Thalamus	-0.003	-0.005 to -1.7E-04	0.037
Drinks per week	5E-08	Nucleus accumbens	7E-04	-0.002 to 0.004	0.657
Drinks per week	5E-08	Amygdala	0.001	-0.002 to 0.004	0.537
Drinks per week	5E-08	Caudate	0.001	-0.003 to 0.004	0.629
Drinks per week	5E-08	Hippocampus	0.001	-0.001 to 0.004	0.323
Drinks per week	5E-08	pallidum	0.003	6.5E-05 to 0.007	0.049
Drinks per week	5E-08	Putamen	0.001	-0.002 to 0.005	0.431
Drinks per week	5E-08	Thalamus	0.001	-0.002 to 0.004	0.459
Alcohol dependence	5E-08	nucleus accumbens	0.016	-0.010 to 0.041	0.258
Alcohol dependence	5E-08	Amygdala	0.007	-0.012 to 0.026	0.468
Alcohol dependence	5E-08	Caudate	-0.027	-0.057 to 0.003	0.117
Alcohol dependence	5E-08	Hippocampus	0.023	0.001 to 0.045	0.080
Alcohol dependence	5E-08	pallidum	0.028	-0.004 to 0.061	0.129
Alcohol dependence	5E-08	Putamen	-0.009	-0.037 to 0.019	0.548
Alcohol dependence	5E-08	Thalamus	0.021	-0.005 to 0.048	0.150
Cigarettes per day	5E-08	NAc ever smokers	-0.396	-0.966 to 0.174	0.179
Cigarettes per day	5E-08	Amygdala ever smokers	0.175	-1.078 to 1.428	0.785
Cigarettes per day	5E-08	Caudate ever smokers	2.097	-0.170 to 4.363	0.076
Cigarettes per day	5E-08	Hippocampus ever smokers	0.633	-1.524 to 2.790	0.568
Cigarettes per day	5E-08	pallidum ever smokers	-0.597	-2.334 to 1.140	0.504
Cigarettes per day	5E-08	Putamen ever smokers	-3.166	-6.200 to -0.132	0.046
Cigarettes per day	5E-08	Thalamus ever smokers	0.169	-2.798 to 3.137	0.911
Cigarettes per day	5E-08	NAc never smokers	0.349	-0.109 to 0.807	0.142
Cigarettes per day	5E-08	Amygdala never smokers	0.275	-0.754 to 1.303	0.603
Cigarettes per day	5E-08	Caudate never smokers	1.263	-0.858 to 3.385	0.249
Cigarettes per day	5E-08	Hippocampus never smokers	-1.093	-2.778 to 0.592	0.210

Cigarettes per day	5E-08	pallidum never smokers	0.021	-1.564 to 1.606	0.979
Cigarettes per day	5E-08	Putamen never smokers	0.290	-2.392 to 2.972	0.833
Cigarettes per day	5E-08	Thalamus never smokers	1.029	-1.578 to 3.635	0.443

Note that the reported intercept is taken from SIMEX-corrected analyses if the IGX2 value was 0.6-0.9

Supplementary table 6. Cochran's Q statistic as a measure of heterogeneity between individual SNP-effects.

Exposure	**Threshold**	**Outcome**	**Q**	**df**	**p**
nucleus accumbens	5E-08	Smoking initiation	25.63	12	0.012
nucleus accumbens	5E-08	Smoking cessation	12.91	12	0.376
nucleus accumbens	5E-08	Cigarettes per day	7.81	12	0.800
nucleus accumbens	5E-08	Drinks per week	57.11	12	7.56E-08
nucleus accumbens	5E-08	Alcohol dependency	12.15	12	0.434
Amygdala	5E-08	Smoking initiation	3.18	1	0.074
Amygdala	5E-08	Smoking cessation	3.39	1	0.066
Amygdala	5E-08	Cigarettes per day	4.19E-03	1	0.948
Amygdala	5E-08	Drinks per week	0.06	1	0.807
Amygdala	5E-08	Alcohol dependency	0.10	1	0.755
Amygdala	1E-05	Smoking initiation	72.87	39	0.001
Amygdala	1E-05	Smoking cessation	61.81	38	0.009
Amygdala	1E-05	Cigarettes per day	62.06	39	0.011
Amygdala	1E-05	Drinks per week	63.28	39	0.008
Amygdala	1E-05	Alcohol dependency	34.73	38	0.621
Caudate	5E-08	Smoking initiation	127.77	52	2.56E-08
Caudate	5E-08	Smoking cessation	125.64	52	4.94E-08
Caudate	5E-08	Cigarettes per day	126.58	52	3.69E-08
Caudate	5E-08	Drinks per week	159.65	51	4E-13
Caudate	5E-08	Alcohol dependency	56.35	52	0.316
Hippocampus	5E-08	Smoking initiation	97.88	18	5.40E-13
Hippocampus	5E-08	Smoking cessation	35.01	18	0.009
Hippocampus	5E-08	Cigarettes per day	32.87	18	0.017
Hippocampus	5E-08	Drinks per week	58.41	18	3.69E-06
Hippocampus	5E-08	Alcohol dependency	10.09	18	0.929
pallidum	5E-08	Smoking initiation	62.99	24	2.39E-05
pallidum	5E-08	Smoking cessation	37.16	24	0.042
pallidum	5E-08	Cigarettes per day	31.09	24	0.151
pallidum	5E-08	Drinks per week	69.97	24	2.21E-06
pallidum	5E-08	Alcohol dependency	24.70	24	0.422

Putamen	5E-08	Smoking initiation	132.44	34	1.49E-13
Putamen	5E-08	Smoking cessation	81.37	34	9.28E-06
Putamen	5E-08	Cigarettes per day	48.55	34	0.051
Putamen	5E-08	Drinks per week	76.25	33	2.82E-05
Putamen	5E-08	Alcohol dependency	63.09	35	0.002
Thalamus	5E-08	Smoking initiation	44.15	11	6.85E-06
Thalamus	5E-08	Smoking cessation	26.80	12	0.008
Thalamus	5E-08	Cigarettes per day	37.88	11	8.21E-05
Thalamus	5E-08	Drinks per week	27.20	12	0.007
Thalamus	5E-08	Alcohol dependency	21.90	12	0.039
Smoking initiation	5E-08	NAc	555.36	345	4.71E-12
Smoking initiation	5E-08	Amygdala	493.82	345	2.40E-07
Smoking initiation	5E-08	Caudate	730.97	345	7.35E-30
Smoking initiation	5E-08	Hippocampus	562.30	345	1.21E-12
Smoking initiation	5E-08	pallidum	543.66	345	4.39E-11
Smoking initiation	5E-08	Putamen	809.26	345	2.56E-39
Smoking initiation	5E-08	Thalamus	541.70	344	5.02E-11
Drinks per week	5E-08	NAc	163.45	91	4.85E-06
Drinks per week	5E-08	Amygdala	129.55	91	0.005
Drinks per week	5E-08	Caudate	198.05	91	6.42E-10
Drinks per week	5E-08	Hippocampus	115.61	91	0.042
Drinks per week	5E-08	pallidum	185.08	91	2.18E-08
Drinks per week	5E-08	Putamen	225.19	91	2.28E-13
Drinks per week	5E-08	Thalamus	136.94	91	0.001
Alcohol dependence	5E-08	NAc	7.30	9	0.605
Alcohol dependence	5E-08	Amygdala	3.33	9	0.950
Alcohol dependence	5E-08	Caudate	11.87	9	0.221
Alcohol dependence	5E-08	Hippocampus	6.48	9	0.691
Alcohol dependence	5E-08	pallidum	14.79	9	0.097
Alcohol dependence	5E-08	Putamen	8.22	9	0.513
Alcohol dependence	5E-08	Thalamus	8.41	9	0.493
Cigarettes per day	5E-08	NAc ever smokers	67.16	48	0.035
Cigarettes per day	5E-08	Amygdala ever smokers	57.30	48	0.168
Cigarettes per day	5E-08	Caudate ever smokers	67.99	48	0.030
Cigarettes per day	5E-08	Hippocampus ever smokers	51.00	48	0.357
Cigarettes per day	5E-08	pallidum ever smokers	115.85	48	1.55E-07
Cigarettes per day	5E-08	Putamen ever smokers	83.74	48	0.001

Cigarettes per day	5E-08	Thalamus ever smokers	63.19	48	0.070
Cigarettes per day	5E-08	NAc never smokers	67.71	48	0.032
Cigarettes per day	5E-08	Amygdala never smokers	60.77	48	0.102
Cigarettes per day	5E-08	Caudate never smokers	89.23	48	2.79E-04
Cigarettes per day	5E-08	Hippocampus never smokers	47.93	48	0.476
Cigarettes per day	5E-08	pallidum never smokers	155.19	48	3.19E-13
Cigarettes per day	5E-08	Putamen never smokers	96.92	48	3.73E-05
Cigarettes per day	5E-08	Thalamus never smokers	77.82	48	4.16E-03

df=degrees of freedom

table S7. MR-PRESSO distortion test and outlier corrected IVW estimates

exposure	**threshold**	**outcome**	**nb distribution**	**n outliers**	**MR-PRESSO dis-rtion test beta**	**n p**	**outlier-corrected IVW SNPs**	**beta**	**95% CI**	**p**
accumbens	5E-08	*SI*	1000	1	47266.3	0.001	13	0.00	-0.05-0.05	0.998
amygdala	1E-05	*SI*	1000	2	-102.99	0.391	41	0.02	-0.01-0.04	0.242
caudate	5E-08	*SI*	10000	2	-286.21	0.144	51	0.00	-0.04-0.03	0.778
hippocampus	5E-08	*SI*	1000	2	195.84	0.193	17	-2E-02	-0.08-0.04	0.523
pallidum	5E-08	*SI*	1000	2	15.21	0.718	24	-0.04	-0.07--0.01	0.014
putamen	5E-08	*SI*	1000	5	47.03	0.905	31	-0.01	-0.03-0.02	0.712
thalamus	5E-08	*SI*	1000	1	-377.30	0.097	16	-0.01	-0.07-0.05	0.767
accumbens	5E-08	*SC*	1000	-	-	-	-	-	-	-
amygdala	1E-05	*SC*	1000	-	-	-	-	-	-	-
caudate	5E-08	*SC*	10000	2	686.98	0.041	51	0.00	-0.03-0.03	0.944
hippocampus	5E-08	*SC*	1000	-	-	-	-	-	-	-
pallidum	5E-08	*SC*	1000	-	-	-	-	-	-	-
putamen	5E-08	*SC*	1000	1	-53.03	0.598	35	0.02	-0.01-0.06	0.163
thalamus	5E-08	*SC*	1000	-	-	-	-	-	-	-
accumbens	5E-08	*CPD*	1000	-	-	-	-	-	-	-
amygdala	1E-05	*CPD*	1000	1	-1053.53	0.041	42	0.00	-0.03-0.03	0.956
caudate	5E-08	*CPD*	10000	4	95.27	0.723	49	-0.01	-0.04-0.02	0.382

hippocampus	5E-08	*CPD*	1000	1	-34.13	0.666	18	0.04	-5E-3-0.08	0.100
pallidum	5E-08	*CPD*	1000	-	-	-	-	-	-	-
putamen	5E-08	*CPD*	1000	-	-	-	-	-	-	-
thalamus	5E-08	*CPD*	1000	1	-40.71	0.617	16	6E-02	-0.01-0.12	0.095
accumbens	5E-08	*ALC*	1000	3	38534.15	<0.001	11	0.00	-0.03-0.03	0.998
amygdala	1E-05	*ALC*	1000	-	-	-	-	-	-	-
caudate	5E-08	*ALC*	10000	3	240.19	0.133	49	0.00	-0.02-0.02	0.821
hippocampus	5E-08	*ALC*	1000	1	645.11	0.080	18	2E-03	-0.03-0.03	0.916
pallidum	5E-08	*ALC*	1000	2	16.46	0.835	24	-0.01	-0.03-0.01	0.235
putamen	5E-08	*ALC*	1000	1	37.54	0.770	34	-0.01	-0.03-0.01	0.375
thalamus	5E-08	*ALC*	1000	1	50.91	0.513	16	-2E-02	-0.05--10E-4	0.059
accumbens	5E-08	*AD*	1000	-	-	-	-	-	-	-
amygdala	1E-05	*AD*	1000	-	-	-	-	-	-	-
caudate	5E-08	*AD*	1000	-	-	-	-	-	-	-
hippocampus	5E-08	*AD*	1000	-	-	-	-	-	-	-
pallidum	5E-08	*AD*	1000	-	-	-	-	-	-	-
putamen	5E-08	*AD*	1000	-	-	-	-	-	-	-
thalamus	5E-08	*AD*	1000	-	-	-	-	-	-	-
SI	5E-08	*accumbens*	10000	4	13.18	0.793	359	-0.05	-0.10--5E-03	0.033
SI	5E-08	*amygdala*	10000	1	-7.66	0.872	362	-0.05	-0.10-1E-03	0.057
SI	5E-08	*caudate*	10000	9	22.91	0.969	354	0.00	-0.05-0.06	0.895
SI	5E-08	*hippocampus*	10000	2	63.02	0.786	361	-0.02	-0.07-0.03	0.449
SI	5E-08	*pallidum*	10000	4	-2.02	0.972	359	-0.05	-0.10-0.01	0.080
SI	5E-08	*putamen*	10000	5	331.47	0.129	358	-0.01	-0.06-0.05	0.794
SI	5E-08	*thalamus*	10000	3	-46.64	0.471	359	-0.02	-0.08-0.03	0.363
ALC	5E-08	*accumbens*	10000	-	-	-	-	-	-	-
ALC	5E-08	*amygdala*	10000	-	-	-	-	-	-	-
ALC	5E-08	*caudate*	10000	5	34.60	0.320	89	0.14	-0.02-0.29	0.083

ALC	5E-08	*hippocampus*	10000	-	-	-	-	-	-	-
ALC	5E-08	*pallidum*	10000	2	26.62	0.628	92	-0.19	-0.36--0.03	0.026
ALC	5E-08	*putamen*	10000	3	68.00	0.816	91	-0.05	-0.22-0.13	0.595
ALC	5E-08	*thalamus*	10000	-	-	-	-	-	-	-
AD	5E-08	*accumbens*	1000	-	-	-	-	-	-	-
AD	5E-08	*amygdala*	1000	-	-	-	-	-	-	-
AD	5E-08	*caudate*	1000	-	-	-	-	-	-	-
AD	5E-08	*hippocampus*	1000	-	-	-	-	-	-	-
AD	5E-08	*pallidum*	1000	-	-	-	-	-	-	-
AD	5E-08	*putamen*	1000	-	-	-	-	-	-	-
AD	5E-08	*thalamus*	1000	-	-	-	-	-	-	-
CPD	5E-08	*accumbens ever*	10000	-	-	-	-	-	-	-
CPD	5E-08	*amygdala ever*	1000	-	-	-	-	-	-	-
CPD	5E-08	*caudate ever*	10000	-	-	-	-	-	-	-
CPD	5E-08	*hippocampus ever*	1000	-	-	-	-	-	-	-
CPD	5E-08	*pallidum ever*	10000	1	-11.64	0.875	50	-11.65	-35.78-12.49	0.349
CPD	5E-08	*putamen ever*	10000	1	-8.21	0.774	50	-61.73	-115.56--7.90	0.029
CPD	5E-08	*thalamus never*	1000	-	-	-	-	-	-	-
CPD	5E-08	*accumbens never*	10000	-	-	-	-	-	-	-
CPD	5E-08	*amygdala never*	1000	-	-	-	-	-	-	-
CPD	5E-08	*caudate never*	10000	1	45.18	0.811	50	-14.64	-48.43-19.16	0.400
CPD	5E-08	*hippocampus never*	1000	-	-	-	-	-	-	-
CPD	5E-08	*pallidum never*	10000	2	2.20	0.971	49	-18.38	-37.69-0.93	0.068
CPD	5E-08	*putamen never*	10000	2	-17.49	0.352	49	-59.20	-96.78--21.62	-
CPD	5E-08	*thalamus neve*	10000	3	6.70	0.825	48	-45.93	-81.55--10.30	0.015

CPD	5E-08	*caudate never*	10000	1	141.93	0.106	50	-4.37	-40.04-31.30	0.811
CPD	5E-08	*hippocampus never*	1000	0	-	-	-	-	-	-
CPD	5E-08	*pallidum never*	10000	2	-2.71	0.948	49	-23.97	-43.83--4.10	0.022
CPD	5E-08	*putamen never*	10000	2	-16.33	0.365	49	-62.37	-101.79--22.95	0.003
CPD	5E-08	*thalamus neve*	10000	2	-3.85	0.918	49	-39.87	-81.19-1.46	0.065

for all unstable calculations due to too low nbdistribution with nbdistribution =1000, we repeated calculations with nbdistribution 10000.

Steiger method	Exposure	Thres hold	Outcome	SNP n	IVW* beta	IVW* 95% CIs	IVW* p	weighted median beta	weighted median 95% CIs	weighted median p	weighted mode beta	weighted mode 95% CIs	weighted mode p	MR-Egger# beta	MR-Egger# 95% CIs	MR-Egger# p
p<0.05	amygdala	1.00E-05	SI	39	0.00	-0.03 to 0.03	0.958	0.00	-0.04 to 0.04	0.857	0.00	-0.04 to 0.04	0.947	0.04	-0.01 to 0.09	0.138
p<0.05	amygdala	1.00E-05	AD	31	0.06	-0.02 to 0.14	0.150	0.10	-0.01 to 0.21	0.073	0.11	-0.02 to 0.24	0.105	0.00	-0.15 to 0.14	0.949
p<0.05	caudate	5.00E-08	AD	50	0.02	-0.04 to 0.07	0.565	0.00	-0.08 to 0.08	0.953	0.01	-0.13 to 0.15	0.915	-0.05	-0.21 to 0.12	0.576
p<0.05	Pallidum	5.00E-08	AD	24	0.02	-0.05 to 0.09	0.506	-0.05	-0.15 to 0.05	0.354	-0.04	-0.16 to 0.07	0.470	-0.02	-0.18 to 0.13	0.775
p<0.05	Putamen	5.00E-08	AD	33	0.02	-0.06 to 0.10	0.590	0.03	-0.07 to 0.14	0.508	0.04	-0.06 to 0.15	0.429	0.05	-0.14 to 0.24	0.597
without p-value threshold	SI	5.00E-08	accumbens	305	-0.06	-0.11 to -0.02	0.008	-0.03	-0.10 to 0.04	0.371	-0.01	-0.14 to 0.11	0.826	-	-	-
p<0.05	SI	5.00E-08	accumbens	61	-0.03	-0.19 to 0.14	0.473	-0.02	-0.12 to 0.08	0.698	-0.06	-0.22 to 0.11	0.495	0.00	-0.20 to 0.20	0.984
without p-value threshold	SI	5.00E-08	Amygdala	302	-0.06	-0.10 to -0.01	0.013	0.00	-0.07 to 0.07	0.996	0.02	-0.11 to 0.15	0.815	-	-	-
p<0.05	SI	5.00E-08	Amygdala	58	0.01	-0.07 to 0.08	0.862	0.01	-0.11 to 0.13	0.865	0.01	-0.14 to 0.17	0.860	-0.07	-0.33 to 0.19	0.530
without p-value threshold	SI	5.00E-08	Caudate	289	0.00	-0.04 to 0.05	0.837	-0.02	-0.11 to 0.06	0.042	-0.04	-0.18 to 0.10	0.565	-	-	-
p<0.05	SI	5.00E-08	Caudate	54	0.03	-0.05 to 0.11	0.464	-0.04	-0.16 to 0.09	0.568	-0.06	-0.23 to 0.11	0.475	-	-	-
without p-value threshold	SI	5.00E-08	Hip	304	-0.02	-0.07 to 0.03	0.400	-0.02	-0.09 to 0.06	0.682	0.02	-0.12 to 0.15	0.787	-	-	-
p<0.05	SI	5.00E-08	Hip	51	0.01	-0.08 to 0.10	0.762	0.03	-0.08 to 0.15	0.577	0.04	-0.16 to 0.24	0.716	-	-	-
without p-value threshold	SI	5.00E-08	Pallidus	297	-0.02	-0.07 to 0.03	0.365	-0.02	-0.09 to 0.06	0.682	0.02	-0.12 to 0.15	0.787	-	-	-
p<0.05	SI	5.00E-08	Pallidus	54	0.00	-0.09 to 0.08	0.933	-0.01	-0.13 to 0.11	0.876	0.00	-0.20 to 0.20	0.990	-	-	-

without p-value threshold	SI	5.00E-08	Putamen	297	-0.02	-0.07 to 0.02	0.365	-0.02	-0.09 to 0.06	0.667	-0.02	-0.17 to 0.13	0.791	-	-	-
p<0.05	SI	5.00E-08	Putamen	48	-0.03	-0.11 to 0.06	0.544	-0.04	-0.16 to 0.09	0.578	-0.04	-0.20 to 0.12	0.630	-	-	-
without p-value threshold	SI	5.00E-08	Thalamus	281	0.04	-0.01 to 0.09	0.090	0.00	-0.07 to 0.08	0.922	-0.05	-0.19 to 0.09	0.489	-	-	-
p<0.05	SI	5.00E-08	Thalamus	62	0.01	-0.07 to 0.09	0.842	-0.02	-0.13 to 0.09	0.728	-0.08	-0.29 to 0.13	0.454	-	-	-
without p-value threshold	ALC	5.00E-08	accumbens	63	-0.07	-0.21 to 0.07	0.297	-0.17	-0.39 to 0.04	0.105	-0.18	-0.40 to 0.04	0.110	-0.25	-0.48 to -0.02	0.038
p<0.05	ALC	5.00E-08	accumbens	9	-0.17	-0.37 to 0.02	0.082	-0.18	-0.42 to 0.06	0.133	-0.22	-0.51 to 0.06	0.167	-0.28	-0.60 to 0.04	0.132
without p-value threshold	ALC	5.00E-08	Amygdala	65	-0.14	-0.28 to 0.00	0.043	-0.25	-0.46 to -0.04	0.021	-0.29	-0.56 to -0.01	0.046	-0.15	-0.38 to 0.08	0.214
p<0.05	ALC	5.00E-08	Amygdala	6	-0.14	-0.36 to 0.07	0.198	-0.25	-0.52 to 0.02	0.072	-0.30	-0.59 to -0.01	0.097	-0.35	-0.70 to -0.01	0.112
without p-value threshold	ALC	5.00E-08	Caudate	66	0.07	-0.07 to 0.21	0.353	0.17	-0.05 to 0.39	0.121	0.11	-0.13 to 0.35	0.365	0.06	-0.17 to 0.29	0.592
p<0.05	ALC	5.00E-08	Caudate	6	0.08	-0.14 to 0.30	0.481	0.09	-0.16 to 0.34	0.471	0.18	-0.10 to 0.45	0.264	0.22	-0.13 to 0.57	0.286
without p-value threshold	ALC	5.00E-08	Hip	65	-0.07	-0.20 to 0.07	0.345	-0.17	-0.38 to 0.04	0.115	-0.23	-0.47 to 3.41E-03	0.058	-0.22	-0.46 to 0.02	0.072
p<0.05	ALC	5.00E-08	Hip	8	-0.19	-0.39 to 0.02	0.070	-0.23	-0.47 to 0.02	0.068	-0.26	-0.55 to 0.02	0.116	-0.38	-0.72 to -0.05	0.063
without p-value threshold	ALC	5.00E-08	Pallidum	58	-0.14	-0.28 to 0.00	0.048	-0.28	-0.51 to -0.05	0.019	-0.33	-0.58 to -0.09	0.010	-0.34	-0.58 to -0.10	0.007
p<0.05	ALC	5.00E-08	Pallidum	4	0.17	-0.07 to 0.41	0.013	-0.30	-0.57 to -0.04	0.025	-0.31	-0.60 to -0.01	0.135	-0.30	-0.65 to 0.05	0.237
without p-value threshold	ALC	5.00E-08	Putamen	57	-0.08	-0.22 to 0.07	0.308	-0.07	-0.30 to 0.16	0.537	-0.05	-0.33 to 0.23	0.722	-0.13	-0.37 to 0.11	0.299
p<0.05	ALC	5.00E-08	Putamen	4	-0.15	-0.37 to 0.08	0.194	-0.07	-0.33 to 0.19	0.593	-0.01	-0.31 to 0.29	0.942	0.09	-0.27 to 0.46	0.664

without p-value threshold	ALC	5.00E-08	Thalamus	67	-0.09	-0.22 to 0.05	0.216	-0.26	-0.49 to -0.03	0.028	-0.29	-0.54 to -0.04	0.028	-0.25	-0.48 to -0.01	0.043
p<0.05	ALC	5.00E-08	Thalamus	6	-0.27	-0.49 to -0.05	0.015	-0.26	-0.52 to -0.01	0.045	-0.29	-0.57 to -0.01	0.102	-0.28	-0.64 to 0.07	0.192
without p-value threshold	CPD	5.00E-08	Nac ever smokers	32	4.76	-4.30 to 13.81	0.303	-1.34	-13.86 to 11.19	0.834	0.54	-11.14 to 12.22	0.928	1.90	-12.26 to 16.06	0.794
p<0.05	CPD	5.00E-08	Nac ever smokers	9	2.96	-6.88 to 12.80	0.555	-1.51	-14.80 to 11.79	0.824	-2.20	-19.80 to 15.39	0.812	12.35	-22.14 to 46.83	0.505
without p-value threshold	CPD	5.00E-08	Amygdala ever smokers	36	9.17	-11.79 to 30.14	0.391	12.83	-16.13 to 41.80	0.385	7.88	-20.80 to 36.55	0.594	-0.72	-32.47 to 31.03	0.965
p<0.05	CPD	5.00E-08	Amygdala ever smokers	10	7.18	-15.50 to 29.86	0.535	12.78	-16.88 to 42.45	0.398	13.47	-26.08 to 53.02	0.521	-20.04	-94.21 to 54.13	0.611
without p-value threshold	CPD	5.00E-08	Caudate ever smokers	36	-31.07	-67.29 to 5.16	0.093	-23.26	-70.83 to 24.31	0.338	-34.99	-79.85 to 9.86	0.135	-31.33	-86.60 to 23.95	0.274
p<0.05	CPD	5.00E-08	Caudate ever smokers	7	-27.52	-68.27 to 13.23	0.186	-23.77	-74.74 to 27.21	0.361	-15.72	-85.27 to 53.82	0.673	37.25	-174.40 to 248.91	0.744
without p-value threshold	CPD	5.00E-08	Hip ever smokers	40	-92.97	-131.12 to -54.81	0.000	-121.00	-170.28 to -71.72	0.000	-114.30	-160.57 to -68.04	0.000	-117.85	-175.19 to -60.51	0.000
p<0.05	CPD	5.00E-08	Hip ever smokers	10	-109.37	-151.05 to -67.70	0.000	-121.85	-174.34 to -69.35	0.000	-122.04	-182.34 to -61.74	0.003	-142.64	-288.50 to 3.21	0.092
without p-value threshold	CPD	5.00E-08	Pallidus ever smokers	36	-10.64	-31.14 to 9.86	0.309	-21.24	-50.20 to 7.72	0.151	-23.42	-49.05 to 2.21	0.082	-21.77	-53.51 to 9.97	0.188
p<0.05	CPD	5.00E-08	Pallidus ever smokers	9	-18.18	-40.68 to 4.32	0.113	-24.97	-55.23 to 5.28	0.106	-28.35	-65.07 to 8.37	0.169	59.59	-23.59 to 142.76	0.203
without p-value threshold	CPD	5.00E-08	Putamen ever smokers	31	-35.17	-79.71 to 9.36	0.122	-55.40	-114.22 to 3.43	0.065	-48.13	-103.20 to 6.94	0.097	-54.52	-123.50 to 14.46	0.132
p<0.05	CPD	5.00E-08	Putamen ever smokers	10	-42.72	-90.07 to 4.63	0.077	-56.47	-118.30 to 5.36	0.073	-63.05	-136.78 to 10.67	0.128	-39.12	-188.87 to 110.64	0.623

without p-value threshold	CPD	5.00E-08	Thalamus ever smokers	40	12.79	-34.57 to 60.16	0.596	27.86	-36.87 to 92.60	0.399	9.08	-44.39 to 62.54	0.741	-19.23	-88.53 to 50.07	0.590
p<0.05	CPD	5.00E-08	Thalamus ever smokers	10	0.02	-51.08 to 51.11	1.000	27.91	-37.50 to 93.33	0.403	31.95	-59.33 to 123.23	0.510	16.40	-145.21 to 178.01	0.847
without p-value threshold	CPD	5.00E-08	Nac never smokers	40	-9.69	-16.82 to -2.55	0.008	-11.82	-21.61 to -2.03	0.018	-13.58	-22.27 to -4.88	0.004	-15.34	-26.23 to -4.46	0.009
p<0.05	CPD	5.00E-08	Nac never smokers	11	-11.69	-19.36 to -4.02	0.003	-12.35	-12.42 to -12.28	0.011	-13.71	-25.22 to -2.21	0.042	-30.81	-55.12 to -6.50	0.035
without p-value threshold	CPD	5.00E-08	Amygdala never smokers	39	-13.44	-30.00 to 3.11	0.111	-20.17	-43.28 to 2.94	0.087	-22.24	-41.92 to -2.56	0.033	-27.88	-53.07 to -2.69	0.037
p<0.05	CPD	5.00E-08	Amygdala never smokers	10	-18.90	-36.94 to -0.86	0.040	-21.04	-43.40 to 1.32	0.065	-20.74	-47.14 to 5.66	0.158	-49.23	-101.43 to 2.96	0.102
without p-value threshold	CPD	5.00E-08	Caudate never smokers	36	-23.21	-51.83 to 5.41	0.112	-9.16	-53.95 to 35.63	0.688	-8.07	-46.52 to 30.38	0.683	-38.91	-82.72 to 4.89	0.091
p<0.05	CPD	5.00E-08	Caudate never smokers	12	-26.13	-60.64 to 8.39	0.138	-8.97	-53.88 to 35.93	0.695	11.32	-52.95 to 75.60	0.736	-74.66	-162.83 to 13.51	0.128
without p-value threshold	CPD	5.00E-08	Hip never smokers	42	-26.95	-57.50 to 3.60	0.084	-26.55	-67.99 to 14.90	0.209	-23.06	-58.09 to 11.98	0.204	-21.35	-67.42 to 24.72	0.369
p<0.05	CPD	5.00E-08	Hip never smokers	10	-27.01	-60.51 to 6.49	0.114	-25.20	-67.16 to 16.76	0.239	-17.34	-67.40 to 32.73	0.514	0.54	-101.08 to 102.16	0.992
without p-value threshold	CPD	5.00E-08	Pallidus never smokers	41	-15.75	-32.59 to 1.09	0.067	-35.11	-60.02 to -10.20	0.006	-28.30	-48.40 to -8.20	0.009	-25.18	-50.42 to 0.06	0.058
p<0.05	CPD	5.00E-08	Pallidus never smokers	10	-19.09	-41.83 to 3.66	0.100	-37.34	-63.22 to -11.46	0.005	-44.62	-79.27 to -9.97	0.033	-23.05	-99.22 to 53.12	0.569
without p-value threshold	CPD	5.00E-08	Putamen never smokers	38	-65.36	-99.50 to -31.22	0.000	-69.85	-121.15 to -18.55	0.003	-63.46	-104.85 to -22.06	0.005	-78.44	-129.74 to -27.14	0.005
p<0.05	CPD	5.00E-08	Putamen never smokers	13	-71.28	-107.57 to -34.99	0.000	-63.38	-110.73 to -16.04	0.009	-61.83	-109.60 to -14.06	0.026	-83.17	-182.97 to 16.63	0.131

without p-value threshold	CPD	5.00E-08	Thalamus never smokers	38	-45.24	-82.52 to -7.95	0.017	-67.14	-117.91 to -16.37	0.010	-57.32	-100.30 to -14.33	0.013	-67.19	-124.39 to -10.00	0.027
p<0.05	CPD	5.00E-08	Thalamus never smokers	11	-52.79	-92.79 to -12.78	0.010	-67.31	-119.07 to -15.55	0.011	-71.00	-134.55 to -7.44	0.053	-45.04	-171.86 to 81.78	0.504

There are two approaches to steiger filtering, the first and most commonly used is where all SNPs that explain more variance in the exposure than in the oucome are retained, while for the second only SNPs that explain significantly (p<0.05) more variance in the exposure than in the outcome are retained. The latter is considered quite a stringent approach. Note that results are only shown for relationships where Steiger filtering identified SNPs to exclude, if all SNPs in the instruments where valid results remained the same as in the main tables in the manuscript

AD=alcohol dependence, SI= smoking initiation, CPD=cigarettes per day, NAc=nucleus accumbens

*IVW shows Wald ratio for intruments with 1 SNP

MR-Egger reports its SIMEX-corrected value if the IXG2 statistic was below 0.9, or was not reported if the I2 statistic was below 0.6.

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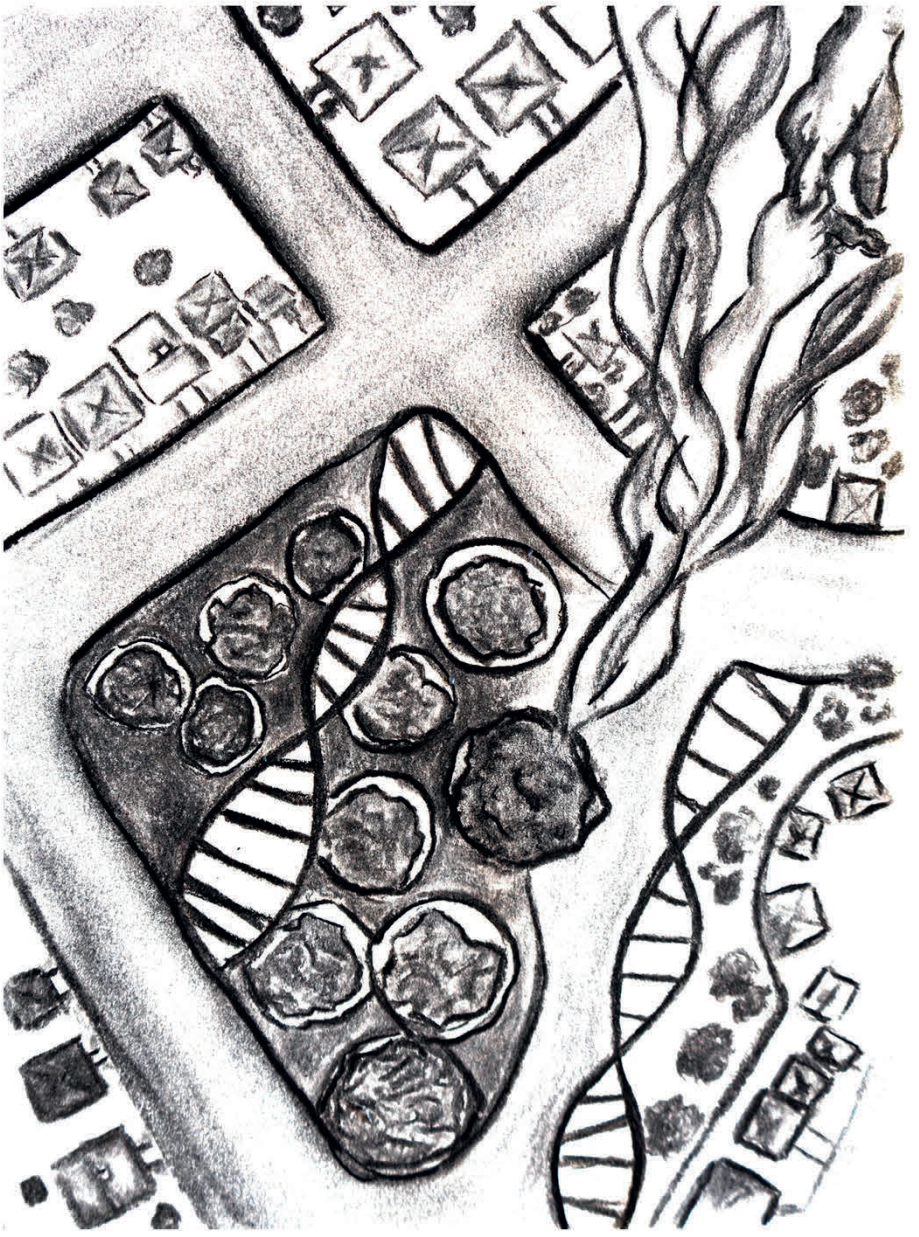

GENE-ENVIRONMENT INTERPLAY

PART 3

CHAPTER 9

Systematic review of polygenic gene-environment interaction in tobacco, alcohol, and cannabis use

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Behavior Genetics, 49, 201

Abstract

Studies testing the effect of single genetic variants on substance use have had modest success. This paper reviewed 39 studies using polygenic measures to test interaction with any type of environmental exposure (GxE) in alcohol, tobacco, and cannabis use. Studies using haplotype combinations, sum scores of candidate-gene risk alleles, and polygenic scores (PS) were included. Overall study quality was moderate, with lower ratings for the polygenic methods in the haplotype and candidate-gene score studies. Heterogeneity in investigated environmental exposures, genetic factors, and outcomes was substantial. Most studies (N=30) reported at least one significant GxE interaction, but overall evidence was weak. The majority (N=26) found results in line with differential susceptibility and diathesis-stress frameworks. Future studies should pay more attention to methodological and statistical rigor, and focus on replication efforts. Additional work is needed before firm conclusions can be drawn about the importance of GxE in the etiology of substance use.

Introduction

The use of tobacco, alcohol, and cannabis continues to be widespread. Global smoking prevalence in individuals above age 15 is around 23% (World Health Organization 2016). On average, people drink a glass of alcohol per day, with higher estimates for America and Europe (World Health Organization 2014). Lifetime prevalence of cannabis use is 26% in the European Union and up to 44% in the United States (European Monitoring Centre for Drugs and Drug Addiction 2017; U.S. Department of Health and Human Services 2016). Risk factors on biological, social, and psychological level have been found to contribute to individual differences in substance use behaviors.

Genetic vulnerability is an important risk factor. Traditionally, this factor has been investigated using family and twin designs to determine how much variance in a trait is explained by genetic factors (Boomsma et al. 2002). Heritability estimates for substance use, abuse and dependence derived from these types of studies are moderate to high (about 30%-75%; Ducci and Goldman 2012; Vink 2016).

Genetic molecular studies have tried to identify specific genetic variants underlying this heritability. In early studies the focus was on candidate-genes, selected based on their proposed biological function. In recent years, researchers have tried to identify genetic variants in a hypothesis-free manner in genome-wide association studies (GWASs), thereby focusing on single-nucleotide polymorphisms (SNPs). Although GWASs have had more success than candidate-gene studies, results are modest: only a handful of variants have been identified for substance use.

To increase power and because behavior is highly polygenic, studies have tried to test the effect of multiple genetic variants simultaneously. Some studies have used combinations of variants that are strongly related (i.e., are in high linkage disequilibrium (LD)) and are transmitted to offspring together in so-called LD blocks. The exact combination of alleles a person has on the variants in such a block is called a haplotype. It has been assumed that the effects of haplotypes are larger and thus easier to detect than those of single variants. Other studies have sought to increase power by combining several (unrelated) candidate-gene variants in a single sum score. A newer method uses summary statistics of GWASs to create weighted sums of the number of risk variants an individual carries, often called polygenic scores (PS). Research using haplotypes, candidate-gene sum scores, or PS has had some success in predicting substance use phenotypes. However, for all approaches, explained variance is still much smaller than expected based on the heritability estimates from twin research.

Possibly, explained variance can be increased by taking into account the interplay between genetic factors and the environment. In gene-environment interaction (GxE), the effect of a genetic factor depends on the presence of an environmental factor. The premise is that genetic factors underlie biological mechanisms (e.g., stress system responsivity) that make a person more or less vulnerable to environmental circumstances (Belsky and Pluess, 2009). Indeed, twin research has shown that the extent to which genetic risk contributes to substance use can depend on environmental factors (Dick 2011). Early molecular genetic studies investigated GxE using single candidate-genes. For example, it was found that childhood maltreatment increased chances of early alcohol initiation more in carriers of the s-allele of the 5-HTTLPR polymorphism of the serotonin transporter gene than in non-carriers (Kaufman et al. 2007). This finding is one of many in line with the diathesis-stress model, stating that adverse environmental circumstances enhance the chance that genetic vulnerability comes to expression (Monroe and Simons 1991). Other GxE frameworks include the differential susceptibility model, posing that genetic predisposition might enhance the effect of adverse, but also of positive environmental factors (Belsky and Pluess 2009). Less commonly it has been predicted that more adverse outcomes arise when genetic plasticity is high and environmental risk is *either high or low,* as both might lead to high stress reactivity (Boyce and Ellis 2005).

Studies using single candidate-genes to test GxE in substance use and other complex phenotypes have yielded mixed findings (see e.g. Do and Maes 2016; Milaniak et al. 2015 for recent reviews). Non-replication and contradicting results seem the rule rather than the exception. The merits of the different theoretical GxE models remain unclear. Low powered study designs and publication bias are likely to have contributed to these mixed findings (Duncan and Keller 2011). To increase power, the logical next step has been to use polygenic rather than single-variant measures in GxE designs.

Whereas previous reviews focused on GxE with single (candidate) genes, this review presents a summary of GxE studies that used a polygenic measure, including haplotype-based measures, sum scores of risk alleles in candidate-genes, and PS based on SNPs identified in GWASs. We focused on (ab)use of and dependence on tobacco, alcohol, and cannabis, as these are the most frequently used substances, and most literature was available for these substances. No previous studies to our knowledge have attempted a review of GxE with polygenic measures, or developed a method to systematically review study quality. Because this field is relatively new, we included all GxE studies, regardless of the type of environmental exposure under investigation, ranging from cohort effects to childhood trauma. Based on our findings, methodological and theoretical recommendations for future research were formulated.

Methods

For this review, PRISMA guidelines were used (Moher et al. 2009). The study method was preregistered in PROSPERO (CRD42017057478).

Search strategy

Literature searches were conducted in Web of Science, PubMed and Google Scholar, and based on title and abstract potentially relevant articles were added. Only articles published in peer-reviewed journals were considered. Keywords included substance use, gene-environment interaction, and polygenic risk. The exact keyword combinations used can be found in supplemental Table SI. Reference lists were checked for additional articles. The last search was conducted February 1st, 2018.

Study eligibility

Inclusion criteria were met if a) the study included human subjects; b) the outcome was some form of tobacco, alcohol, or cannabis use, or a combination thereof; c) the study was an original research report; d) the measure of genetic risk comprised a combination of multiple risk variants (i.e., no single variant designs); and e) an interaction with an environmental variable was tested statistically. Criterion d allowed for studies that looked at multiple variants within one gene. Although this kind of study does not meet the strict definition of 'polygenic', it might be more powerful than studies looking at only one variant (Oroszi et al. 2009). Earlier reviews of candidate-gene studies have not explicitly investigated the merits of this method. Criterion e allowed for any demographic/environmental factor that has been investigated in this context, including for example birth cohort and as specific as roommate's alcohol use levels in high school.

Assessment of study quality

For each study, quality characteristics were assessed. Important hallmarks included study design, sample size, power (sample sizes necessary to achieve different levels of power are described in Supplementary Table SII), the method for controlling for confounders (sex, age, population stratification/ ethnicity, gene-environment correlation), and phenotype measurement. The quality of the operationalization of the polygenic measure was assessed separately for the haplotype, candidate-gene score, and PS studies.

As no scale exists for assessing the characteristics of this specific type of study, study quality was visualized using symbols (-, +-, +). Symbol allocations for study characteristics are summarized in Table I. Although literature was consulted for handholds (Table I),

Table I. Symbol allocation for quality characteristics of the GxE studies

method	characteristic	-	-+	+	not applicable
all	study type	correlational	case control	randomized	
	sample size	<1,000	1,000-2,500	>2,500	
	power calculation	no	-	yes	
	control for age and sex[a]	none	descriptive	statistical	homogenous sample/ age as predictor or outcome
	control for ethnicity[b]	none	descriptive	statistical	homogenous sample
	control for rGE[c]	none	descriptive	statistical	interventions/ cohort effects
	phenotype measures	self-developed short survey	validated survey/ interview	biological/combined measures	interventions/ cohort effects
haplotype	# of blocks[d]	1-4	-	>4	
	# of genes[d]	1-3	-	>3	
	# of variants[d]	<5	5-10	>10	
	rationale for risk haplotype[e]	debatable	-	solid	
candidate	# of genes[d]	1-3	-	>3	
	# of variants[d]	<5	5-10	>10	
	rationale for risk allele	debatable	-	solid	
polygenic score (PS)	based on	overlapping sample GWAS	-	independent GWAS	
	discovery sample size	<10,000	10,000-25,000	>25,000	
	p-value threshold[f]	*p*<.0001	-	*p*>=.0001	
	correspondence phenotypes[g]	weak	moderate	strong	

[a] genetic associations may vary in different age and sex groups (Kendler et al. 2008; The Wellcome Trust Case Control Consortium 2007).

[b] population stratification resulting from ancestry differences can distort genetic association results (Price et al. 2006); statistical control using principal component analysis is preferable to control for these effects.

[c] in gene-environment correlation (rGE) genetic make-up influences to what environment an individual is exposed (only possible in non-randomized studies). These effects can muddle GxE findings (Rathouz et al. 2008).

[d] inclusion of more genetic factors in the aggregate predictor was considered better. Cut-offs were based on commonly chosen numbers of variants for these studies.

[e] the rationale for defining which haplotype or allele was the active (risk/protective) allele was deemed less strong when it was based on the results of the main analyses in the same sample, rather than on theory or results from independent samples.

[f] this threshold most commonly concerns the *p*-value for the association between the SNPs and the phenotype in the original GWAS. The lower this value, the fewer SNPs are included in the PS. We considered PS including only a few SNPs as less strong than PS including more SNPs, although the exact optimal threshold depends on several other study characteristics (Chatterjee et al. 2013; Dudbridge 2013).

[g] the more similar the outcome variable is to the original GWAS phenotype on which the PS was based, the better the predictive value (Wray et al. 2014).

quality cut-offs had to be chosen without objective reference points. Assessment of study quality was done in duplicate (JP, KV); any disagreement was solved through discussion with a third assessor (JV).

Data extraction and evaluation of results

The studies were categorized according to a) the measure of genetic risk (haplotype, candidate-gene score, or PS), and b) the nature of the environmental exposure (intervention or other, e.g., traumatic experiences). Further categorization could not be realized due to heterogeneity in environmental factors, outcomes, and study designs. No meta-analysis nor formal publication bias assessment could be attempted because of study heterogeneity, inconsistent statistical reporting, and absence of report of (standardized) effect sizes.

As most studies did report *p*-values for the GxE analysis, a *p*-curve analysis could be conducted (Simonsohn, Nelson, & Simmons, 2014) to give an indication of the strength of the evidence and of the probability that *p*-hacking occurred in the included studies. The assumption of the *p*-curve method is that if the investigated effect is real, there should be more small *p*-values than large *p*-values reported in the literature. If there are more large than small *p*-values, this might be seen as evidence for *p*-hacking or selective reporting; it is more likely that investigators have been conducting tests until they reached a *p*-value just below .05. Supplementary Table SIII summarizes what GxE test statistics were selected from each study. The analysis was conducted twice, once using all the reported GxE *p*-values in each study, and once using only the first reported *p*-value in each study. If a study only reported that the *p*-value was smaller than some threshold (e.g., $p<.05$, $p<.001$), we included this threshold minus one decimal (e.g., $p=.049$, $p=.00099$) as the estimated *p*-value in the analysis.

Results

Selection

The study selection process is summarized in the flow chart in Figure 1. In total, 34 articles describing 39 studies were left for inclusion in the systematic review. These studies described results from 27 independent samples.

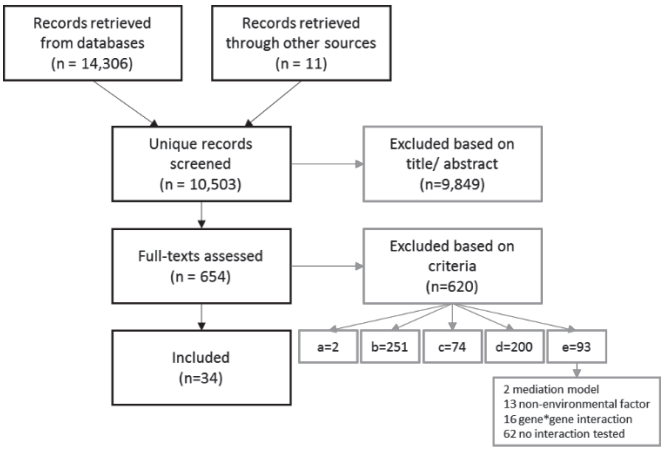

Figure 1. Flow-chart of study selection for inclusion in the review. Exclusion criteria: a) non-human subjects; b) no substance use outcome; c) no original research; d) no polygenic risk predictor; e) no statistical test of interaction with environmental

Study description

In Table IIa-IIc key features and GxE findings are summarized separately for studies using (a) haplotype, (b) candidate-gene score, and (c) PS measures. Symbols are used to annotate what studies used data from overlapping samples.

Sample characteristics for each study are given in Supplementary Table SIV. Samples consisting of only European descent individuals were overrepresented (51%). Studies included clinical (N=8), clinically ascertained (N=5), and general populations (N=26). Eleven studies included family-related individuals. Studies comprised various age ranges starting from adolescence, with 19 studies specifically focusing on adolescents or young adults and two on older adults. There was an approximately equal representation of female and male subjects within the studies. Sample sizes ranged from N=81 to N=11,423, with an average of *N*=1,865.

Fifteen studies used some form of correlational design (7 longitudinal), 12 were case-control studies, 11 were RCTs and 1 was a randomized longitudinal design. Twenty

studies included alcohol and 16 included tobacco outcomes (among others), 4 focused on combined phenotypes (e.g., substance use disorder), and only 3 included cannabis outcomes. There were 11 intervention studies, 12 studies that included measures of trauma-like experiences, and 16 that focused exclusively on typical environmental exposures in for example the family or peer context. Most haplotype studies focused on interventions or psychological trauma as environmental exposures, whereas the candidate-gene score and PS studies more often focused on common environmental factors.

Table IIa. Summary of GxE studies using haplotypes as a measure for polygenic risk (G). Top rows for studies testing intervention/ prevention as environmental exposure (E). Only first author of the respective papers is mentioned.

ID	year	1st author	genes	environmental exposure	outcome	quality[a]	finding
				INTERVENTION			
1*	2007	Berrettini	COMT	pharmacotherapy vs placebo	7-day smoking abstinence	+-	3 out of 4 haplotypes predicted abstinence more strongly for individuals randomized to a bupropion intervention. Weakened at 6-month follow-up.
2*	2007	Berrettini	COMT	pharmacotherapy vs placebo	7-day smoking abstinence	+-	2 out of 4 haplotypes predicted abstinence more strongly for individuals randomized to a bupropion intervention. Not retained at 6-month follow-up.
3	2009	Oroszi	OPRM1	pharmacotherapy vs placebo	alcochol abstinence or moderate drinking	-	Patients treated with naltrexone who had 1 haplotype out of 3 combinations had better outcomes than those treated with placebo or other haplotypes . No interaction effect for haplotypes in other block.
4	2012	Chen	CHRNA5 CHRNA3 CHRNB4	pharmacotherapy vs placebo	smoking cessation	+	1 haplotype predicted failed abstinence only for individuals randomized to placebo and not for those treated with nicotine patch, nicotine lozenge, or bupropion
5+	2013	Brody	DRD2 ANKK1 GABRG1 GABRA2	prevention trial	alcohol use	+	risk haplotypes in 3 out of 5 blocks predicted only predicted increased alcohol use for individuals randomized to control, not for individuals in the prevention group
6	2015	Tyndale	CHRNA5 CHRNA3 CHRNB4	pharmacotherapy vs placebo	smoking cessation	+	no interaction

				OTHER				
7[%]	2006	Lerer	HTR6 HTR1B	trauma exposure	smoking initiation & nicotine dependence	-	no interactions	
8[%]	2007	Segman	DAT1	trauma exposure	smoking initiation & nicotine dependence	+-	in 1 of 2 blocks, 1 of 3 haplotypes protected for the effect of trauma on nicotine dep	Not replicated for smok init
9	2008	Ducci	MAOA MAOB	childhood sexual abuse	alcoholism	--	in 1 of 2 blocks, 1 of 5 haplotypes predicted alcoholism, but only in sexually abused individuals	
10	2010	Nelson	CRHR1	childhood sexual abuse	alcohol use & alcohol dependence	-	1 of 2 haplotype combinations protected for the effect of childhood sexual abuse on alc consumption and dep	
11	2010	Enoch	GABRA2	childhood trauma	alcohol dep & substance dependence	-	in 1 of 2 blocks, 1 of 4 haplotypes was protective for the effects of childhood trauma on subst dep	not replicate for alc dep
12[&]	2011	Kranzler	CRHR1	childhood adverse events	alcohol dependence	-	no interaction	
13[&]	2011	Kranzler	CRHR1	childhood adverse events	alcohol dependence	-	no interaction	
14	2013	Ray	CRHR1	trauma exposure	alcoholism	+-	in block 1 there was 1 out of 3 and in block 2 there were 2 out of 7 haplotypes that were a risk factor in non-trauma-exposed individuals, but were protective in trauma-exposed individuals	
15	2015	Handley	FKBP5	childhood maltreatment	marijuana dependence	-	childhood maltreatment only predicted marijuana dep in 1 of 2 haplotype combinations	
16	2017	Handley	FKBP5	childhood maltreatment	problem drinking	+-	the internalizing pathway from maltreatment to alc problems was only present for haplotype carriers	no interaction for externalizing pathway

Green=reinforcing, dark green=reinforcing such that G only has effect in one E, blue=E has only effect for one level of G, orange=G's effect is reversed by E, gray=no evidence for GxE.

*+%&$@#" Studies denoted with the same symbol used data from identical or overlapping samples.

[a] Quality ratings based on characteristics from Supplementary Tables SVa-SVc.

Table IIb. Summary of GxE studies using candidate-gene scores as a measure for polygenic risk (G). Top rows for studies testing intervention/ prevention as environmental exposure (E). Only first author of the respective papers is mentioned.

ID	year	1st author	genes	environmental exposure	outcome	quality[a]	finding
				INTERVENTION			
17	2012	McGeary	ANKK1 DRD4 DAT1 COMT	pharmacotherapy vs placebo	smoking cessation	--	no interaction
18[+]	2013	Brody	DRD2 GABRG1 GABRA2	prevention trial	alcohol use	+-	score predicted alcohol use, but only for the individuals randomized to control and not to prevention trial
19	2013	David	ANKK1 COMT DRD4 DAT1	pharmacotherapy vs placebo	smoking cessation	+	score predicted time to first lapse only for individuals randomized to placebo; Not replicated for abstinence at end of treatment
				OTHER			
20	2015	Guo	DRD2 MAOA LMO3 and others	roommate's binge drinking in high school	binge drinking in college	+-	medium propensity predicted higher levels of binge drinking when the roommate was a drinker than high/ low propensity. Similar results for larger SNP subsets.
21[$]	2015	Guo	DRD2 MAOA LMO3 and others	roommate's binge drinking in high school	binge drinking in college	+-	medium propensity predicted higher levels of binge drinking when the roommate was a drinker than low/ high propensity. Similar results for larger SNP subsets.
22	2016	Bountress	DRD2 ADH4 CNR1 GABRA2 PDYN OPRM1	parental knowledge & peer substance use	substance use disorders	+-	highest level of score predicted substance use disorder more strongly when parental knowledge was low and peer substance use was high
23[$]	2016	Stogner	DAT1 DRD2 DRD4 SERT MAOA	parental rejection	alcohol use initiation	+-	score was a risk factor for alcohol use when parental rejection was high, but a protective factor when rejection was low
24[@]	2017	Pasman	DAT1 DRD4 DRD2 OPRM1	parental education level	polysubstance use	+-	no interaction
25	2017	Pasman	DAT1 DRD4 DRD2 OPRM1	(parental) education level	polysubstance use	+-	score marginally predicted lower polysubstance use for people with a low education level

26	2017	Coley	DAT1 DRD2 DRD4 DRD5 COMT MAOA	parent and peer alcohol use & stressful events	alcohol use, intoxication & use disorders	+	no interactions

Green=reinforcing, dark green=reinforcing such that G only has effect in one E, blue=E has only effect for one level of G, orange=G's effect is reversed by E, gray=no evidence for GxE.

*+%&$@#" Studies denoted with the same symbol used data from identical or overlapping samples.

a Quality ratings based on characteristics from Supplementary Tables SVa-SVc.

Table IIc. Summary of GxE studies using polygenic scores (PS) as a measure for polygenic risk. Top rows for studies testing intervention/ prevention as environmental exposure (E). Only first author of the respective papers is mentioned.

ID	year	1st author	PS phenotype	environmental exposure	outcome	quality[a]	finding	
				INTERVENTION				
27[#]	2015a	Musci	smoking cessation	prevention trial	age smoking initiation	+-	higher PS predicted stronger effect of the intervention on age of smok init	
28[#]	2016	Musci	smoking cessation	prevention trial	age cannabis initiation	-	higher PS predicted stronger effect of the intervention	
				OTHER				
29	2012	Vrieze	smoking heaviness	age cohort	smoking heaviness & alcohol use	+	PS predicted smok more strongly in the older cohorts than in the younger cohorts	PS predicted *less* alc use more strongly in the older cohorts than in the younger cohorts
30	2013	Meyers	smoking heaviness	neighborhood cohesion & trauma	smoking heaviness	+	higher PS predicted smok more strongly for high levels of trauma exposure and less so individuals experiencing high neighborhood social cohesion	
31[”]	2014	Salvatore	alcohol problem	parental knowledge & peer deviance	alcohol problems	+-	PS had a stronger effect on alc problems when parental knowledge was low or peer deviance was high.	Not replicated for analyses using subset of top-SNPs
32[#]	2015b	Musci	smoking cessation	friend's substance use & parental monitoring	smoking frequency & cannabis frequency	+-	PS in combination with high parental monitoring and low friend’s substance use environmental risk predicted lower tobacco and marijuana use	
33[~]	2016	Domingue	smoking initiation	birth cohort	smoking initiation	+	marginal interaction such that influence of PS became more important over time	

34˜	2016	Schmitz	smoking initiation	veteran status & educational attainment	smoking initiation & smoking heaviness	+	PS predicted smoking initiation and heaviness more strongly in veterans than in non-veterans. Post-war educational attainment buffered for this effect	
35	2017	Li	alcohol dependence symptoms	close friend's substance use	heavy episodic drinking	-	no interaction	
36@	2017	Treur	smoking heaviness	childhood smoke exposure	smoking heaviness & smoking inititation	+	PS marginally predicted smok heaviness for individuals exposed to smoke in childhood and not for unexposed individuals	not replicated for smok init
37@	2018	Mies	alcohol use	stress at home & life satisfaction	alcohol use & alcohol problem		no interaction	
38	2018	Polimanti	bipolar disorder, major depressive disorder & schizophrenia	trauma exposure	alcohol misuse & nicotine dependence	+	bipolar disorder PS predicted more alcohol misuse for individuals exposed to trauma and less for unexposed individuals.	no interaction for bipolar disorder and schizophrenia PS on nicotine dependence. Inconsistent marginal effects for other GxE analyses.
39“	2018	Salvatore	alcohol problems	romantic relationship	alcohol intoxication	+	No interaction	

Green=reinforcing, dark green=reinforcing such that G only has effect in one E, blue=E has only effect for one level of G, orange=G's effect is reversed by E, gray=no evidence for GxE

*+%&$@#” Studies denoted with the same symbol used data from identical or overlapping samples.

a Quality ratings based on characteristics from Supplementary Tables SVa-SVc.

Quality

General quality characteristics per study type are summarized in Table IIIa. Quality of the implementation of the polygenic method is summarized in Table IIIb. Full details on quality characteristics per study are given in Supplementary Tables SVa-SVc.

Haplotype method

Haplotype studies (N=16) were on average published 7 years before the date of inclusion in this review. They used a strong experimental design (i.e., RCT or case control) more often than both other study types. Sample sizes were quite low given the expected small effects, with an average of $\widehat{N}$=771 and almost half of the studies using a sample of less than N=500 individuals. In many cases, exact sample sizes were only reported for the main effects analysis and not for the GxE analysis. Power calculations were not reported in 10 out of 16 studies.

Only 3 studies controlled statistically for both age and sex, although other studies often reported that outcomes and predictors did not vary for different sexes or age groups ('descriptive' control). In a few cases, statistical control was unnecessary as the sample was sufficiently homogeneous (e.g., all female or all within the same 2-year age range). Control for ethnicity was absent or rudimentary in all but one study (16), for example consisting of self-reported 'white/non-white' racial background. In 9 studies the sample was reasonably ethnically homogeneous. In non-randomized studies (N=10) gene-environment correlation (rGE) might confound the GxE interaction results. Only 4 of the 10 studies reported on rGE and no studies controlled for these effects.

The quality of the application of the haplotype method was limited. Almost all studies tested haplotypes in one or two LD blocks, with the number of tested variants ranging between 2 and 18. Many studies looked at 1 or 2 blocks in a single gene or a few genes in high LD, so that they are hardly more 'polygenic' than single candidate-gene studies. Most studies did not formulate a literature-based directional prediction. In many cases, only haplotypes that showed a main effect on the outcome were included in the GxE analysis.

For severe outcomes (e.g., clinical diagnosis) or environmental exposures (e.g., traumatic experiences) interviews were used as a measurement instrument. In the smoking cessation trials, biological measures were employed to validate self-reported abstinence. Other outcomes and exposures were mostly measured using validated questionnaires or more crudely using short questionnaires that were developed for the purpose of this or an earlier study.

Most haplotypes investigated were located in genes involved in in (dopamine-related) reward and inhibition processes in the brain (e.g. *COMT, ANKK1, DRD2, DAT1, OPRM1, HTR6, HTR1B,* and *GABA*- and *MAO*-related genes). Other candidate-genes included *CRHR1* and *FKBP5*, related to the stress system. Genes such as the nicotine metabolism, cannabinoid receptor, or the alcohol dehydrogenase genes seem equally suitable candidates, but received much less research attention.

Summarizing, weaknesses of the haplotype studies included small sample sizes, low statistical control, and limitations in the implementation of the polygenic method. Strengths included the use of strong designs and phenotypical measures.

Candidate-gene method

Studies using candidate-gene scores were on average 3 years old at the time of inclusion in this review. The study designs were somewhat less strong than those used in the haplotype studies, with 4 out of 10 using some randomization procedure. Average sample size was larger than for the haplotype studies ($\widehat{N}$=2,141), although for 2 studies exact sample size for the GxE analyses were not reported and the average was boosted by one study with N=11,423. Three out of 10 studies reported power calculations.

Control for confounders was more stringent than in the haplotype studies, with 8 studies exerting statistical control for both age and sex. Five studies used some control for genetic ancestry and another 4 used a relatively homogenous sample. One of the 7 studies that did not use a randomization procedure statistically controlled for rGE effects and 3 studies reported on them.

All but one candidate-gene score study used an unweighted sum score of the number of risk alleles as a predictor. Sum scores were based on risk alleles in on average 7 variants. Almost all variants were located in previously investigated candidate-genes related to dopamine-signaling. The rationale for selecting the risk allele was debatable in 3 cases. Many studies did omitted a description of conflicting literature on the risk allele of the candidate-gene. Outcome and environmental exposure measures were generally of lower quality than those in the haplotype studies and mostly comprised self-developed short questionnaires.

Candidate-gene studies scored slightly better than the haplotype studies on sample sizes, control for confounding, and the implementation of the polygenic method.

Polygenic score method

Studies using PS were the newest, on average 2 years old at the time of inclusion. Study designs were more often simple correlational designs. Sample size was $\widehat{N}$=3,001 on average, with only two samples smaller than N=500. Five out of 13 studies reported power calculations.

Control for confounding was most rigorous in this type of studies, with 7 studies controlling statistically for both age and sex, and the rest using some procedure rendering statistical control less necessary. In PS studies it is possible to control statistically for ethnicity using ancestry-informative principal components. Eight of the 10 studies used this procedure, with an additional 2 using a more rudimentary approximation of this method and the final 3 using a reasonably homogenous sample. Of the 10 studies where rGE could have played a role, 1 exerted statistical control for rGE and 8 described the effects without controlling for them.

Half the studies constructed the PS based on results from larger GWASs (average discovery $\widehat{N}$=58,447, range N=31,266 -74,053), while the others based it on results from GWASs with limited sample size ($\widehat{N}$=3,140). One of the latter calculated the PS based on a GWAS in a sample that was genetically related to the target sample, which could have biased results (35). The similarity between the study outcome and the source GWAS phenotype was in most cases reasonably high. The specific score calculation method differed somewhat across studies, with 3 preselecting a subset of SNPs to include in the score. Most studies used the PLINK program to calculate the PS, using pruning or clumping to remove variants that were in high LD. One study used LDpred to calculate PS while accounting for this LD (36). The *p*-value thresholds for including SNPs in the PS varied widely from $p<1*10^{-8}$ (resulting in a score of only 2 variants) to $p=1$ (retaining all SNPs in the score), and 5 studies tested multiple PS with different thresholds.

Overall, the phenotype measures were of limited quality, with most studies using short self-developed questionnaires.

Overall, the PS studies scored higher than the other study types on sample size, control for confounding, and the implementation of the polygenic method, but similar or worse on study design and phenotypical measures.

In general, study quality appeared somewhat higher for studies that found a significant GxE result than for studies that did not. This might have been driven by the higher quality of the PS studies, that could also have yielded more significant findings because of higher power. Study quality did not seem to influence what kind of GxE pattern was found.

Table IIIa. Summary of general study quality per category, expressed in percentage of studies that met a criterion as specified and assigned with a -, +-, or + in Table I. For details per study, see Supplementary Tables SVa-Vc.

		haplotype	candidate-gene score	polygenic score (PS)
design	% correlational	0%	60.0%	69.2%
	% case control	62.5%	0%	15.4%
	% randomized	37.5%	40.0%	15.4%
	rating	+	+-	-
sample size	M (SD)	N=771 (658)	N=2,141 (3,159)	N=3,001 (3,439)
	% with N>=1,000	31.3%	60.0%	53.8%
	rating	-	-	+-
power	% reported	37.5%	30%	38.5%
	rating	-	-	-
control for confounders	% age statistically controlled[a]	60.0%	88.9%	100%
	% sex statistically controlled[a]	70.0%	100%	100%
	% ethnicity statistically controlled[a]	42.8%	83.3%	100%
	% rGE reported[b]	40.0%	50.0%	80.0%
	% rGE statistically controlled[b]	0%	16.7%	10%
	rating	-	+-	+
phenotype measures[c]	% self-developed short survey	12.5%	60.0%	72.7%
	% validated survey/ interview	62.5%	30.0%	27.2%
	biological/ combined measures	25%	10.0%	0%
	rating	+	-	-

[a] when sample was sufficiently homogeneous, the study was not considered for calculating this percentage
[b] when rGE could not be an issue (in the case of cohort effects or intervention studies) the study was not considered for calculating this percentage
[c] only percentages for the outcome measures are mentioned here. For details on environmental measures, see Supplementary Tables SVa-Vc.

Table IIIb. Summary of quality of the implementation of the polygenic method. Averages or counts are given per criterion. Shading indicates that most studies (or the study average) fell into this quality category, with the darker shading indicating the average quality category per study type. For details per study, refer to Supplementary Tables SVa-SVc.

		average quality		
		-	+-	+
haplotype	# of blocks[a] (M=1.6)	1-4	-	>4
	# of genes[a] (M=1.6)	1-3	-	>3
	# of variants[a] (M=6.2)	<5	5-10	>10
	rationale for risk haplotype[b] (50% solid)	debatable	-	solid
	overall quality			
candidate-gene score	# of genes[a] (M=8.8)	1-3	-	>3
	# of variants[a] (M=6.2)	<5	5-10	>10
	rationale for risk allele[b] (67% solid)	debatable	-	solid
	overall quality			
polygenic score (PS)	based on (92.3% independent sample)	overlapping GWAS	-	independent GWAS
	discovery sample size (M=58,447)	<10,000	10,000-25,000	>25,000
	p-value threshold[c] (7.7% *p*<.0001)	*p*<.0001	-	p≥.0001
	correspondence phenotypes[d] (84.6% weak)	weak	moderate	strong
	overall quality			

[a] inclusion of more genetic factors in the aggregate predictor was considered better. Cut-offs were based on commonly chosen numbers of variants for these studies.

[b] the rationale for defining which haplotype or allele was the risk/protective allele was deemed less strong when it was based on the results of the main analyses in the same sample, rather than on theory or results from independent samples.

[c] this threshold most commonly concerns the *p*-value for the association between the SNPs and the phenotype in the original GWAS. The lower this value, the fewer SNPs are included in the PS. We considered PS including only a few SNPs as less strong than PS including more SNPs, although the exact optimal threshold depends on several other study characteristics (Chatterjee et al. 2013; Dudbridge 2013).

[d] the more similar the outcome variable is to the original GWAS phenotype on which the PS was based, the better the predictive value (Wray et al. 2014).

P-curve analysis

To get an indication of the overall evidence for GxE in substance use, a *p*-curve analysis was conducted. The analysis was based on 82 *p*-values (34 significant) derived from 28 studies (see Supplementary Table SIII). The other 11 studies did not report *p*-values for the GxE term specifically (or for the simple effects in the case of cross-over interactions) or statistics by which these could be calculated. As can be seen in Figure 2, there were more small *p*-values than expected under the null hypothesis. The *p*-curve is flatter than expected if studies had at least 70% power, such that there were not many more small *p*-values than medium to large *p*-values. This indicates that there was evidential value, but this was not very strong. Moreover, if only the first *p*-value reported in each study was taken into account, results deteriorated, indicating that they were driven by a few studies that reported many small *p*-values (data not shown). Results did not change if non-exact *p*-values (e.g., $p<.05$) were excluded from analysis (results not shown). There was no clear evidence for *p*-hacking, which would be indicated by a substantially higher proportion of *p*-values just below 0.05.

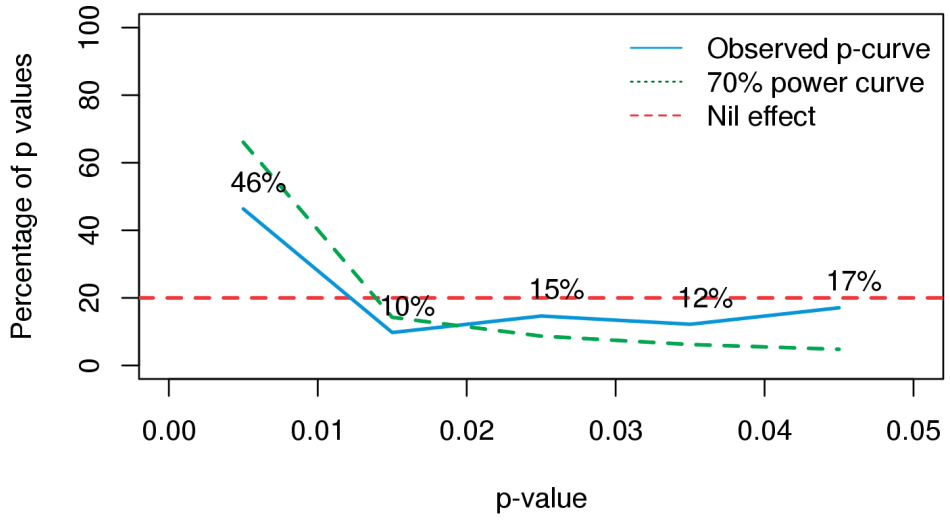

Figure 2. Percentage (y-axis) of reported p-values in the studies that fell in the range specified on the x-axis (p-curve), against the percentage that would be expected under the null hypothesis (nil effect) and under the alternative hypothesis given a power level of 70% (70% power curve).

GxE patterns

The GxE findings from each of the 39 studies were summarized in the last column of Table IIa-IIc. Thirty studies reported at least 1 significant GxE finding and 9 did not. Twenty-five of the significant GxE findings followed a pattern as depicted in Figure 3a (22 studies) or 3b (3 studies). The pattern in panel a indicates that environmental risk enhances the effect

of genetic risk, further increasing the chance of unfavorable outcomes. Or, likewise, a protective genetic predisposition might enhance the effects of a positive environment or counteract the effects of an adverse environment. Thus, in these cases, genetic and environmental factors *reinforce* each other's effects. In panel b the pattern is similar, only now a genetic factor that is a risk factor in one situation, is protective in the other situation, or likewise, an environmental exposure that is a risk factor for individuals with a certain genetic make-up is a protective factor for individuals with a different genetic make-up. Thus, genetic and environmental factors *reverse* each other's effects (cross-over interaction).

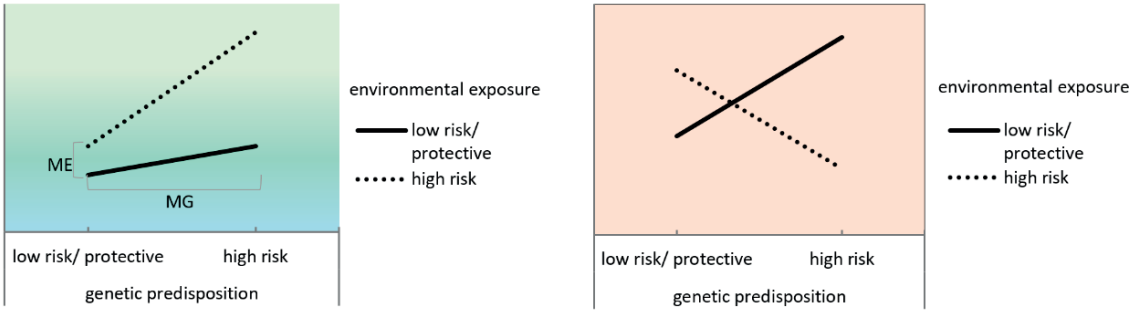

Figure 3. General pattern of GxE. **Panel a:** genetic factors and environmental factors reinforce each other (green-blue shades in Table Ia-c, N=21). ME represents the main effect of environmental exposure, MG that of the genetic factor. **Panel b**: the effect of a genetic factor is reversed as a function of an environmental factor (or vice versa; orange findings in Table Ia-c, N=2).

The colors in Table IIa-IIc correspond to specific patterns of results as summarized in Figure 3. The light green color indicates that the study interpreted the GxE effects such that the genetic and environmental factors reinforce each other (study 1-3, 21, 26-31, 33). For example, in study 22 a genetic risk factor (associated with substance use) enhances the effect of an adverse environmental factor (high peer substance use), yielding a negative outcome (substance use disorder).

Studies marked in darker green are similar, but did not find (or report) a main effect of environment ('ME' in Figure 3a) or a main effect of the genetic factor ('M' in Figure 3a; study 4-5, 9, 15-16, 18-19, 36). The interpretation in these cases is that genetic risk *only* has an adverse effect in an adverse situation (study 9, 15, 16, 36) or when there is no intervention to counteract it (study 4, 5, 18, & 19). Likewise, the blue studies find that environmental risk only has an adverse effect in the absence of a protective genetic factor.

The 3 studies finding this pattern are all haplotype studies where a specific combination of alleles protects for the effect of psychological trauma (studies 8, 10, 11).

Studies marked in orange (14, 23, & 38) showed that a genetic risk factor becomes a protective factor depending on the environment as depicted in Figure 3b. For example, in study 14, certain haplotype combinations were risk factors for alcoholism in controls, but protective factors in traumatized individuals.

The yellow studies find patterns that correspond with neither Figure 3a nor 3b. Study 20 and 21 find that *medium* levels of genetic risk predict adverse outcomes for high environmental risk. Study 25 reports that high genetic risk predicts *favorable* outcomes for an adverse environmental characteristic (low parental education). Study 29 found a PS for smoking heaviness to be related to less alcohol use for older cohorts, whereas it was related to more smoking in this group. Another cohort study (33) reported that genetic risk marginally predicted adverse outcomes more strongly for young cohorts, even though being in such a cohort is generally viewed as protective for substance use.

Patterns substances, genes, and environments

Patterns of GxE did not seem to differ depending on the substance under investigation. Fourteen of the 20 studies including alcohol outcomes, and 13 of the 16 studies including smoking outcomes found at least one significant GxE effect.

Results did not seem to differ depending on the kind of variants investigated. For example, studies looking at dopamine-related genes were not more likely to find significant GxE patterns than studies focusing on other candidate-genes. However, it was difficult to compare findings across gene-groups, as most studies used aggregates of genes from different groups.

Intervention studies and studies looking at trauma exposure seemed more likely to yield patterns corresponding to Figure 3a or 3b, but not more likely to show significant results. The 16 studies focusing on common environmental exposures yielded more diverse patterns. For example, all 5 yellow outcomes fell in this category, investigating birth cohort, peer substance use, and parental education level.

Discussion

The aim of this review was to provide an overview of all available studies (N=39) using measures of polygenic risk (haplotypes, candidate-gene scores, and polygenic scores) to investigate gene-environment interaction in substance use. There was some support for the existence of GxE in substance use, but the evidential value was weak.

Theoretical interpretation

Most GxE results followed the pattern as depicted in Figure 3a. These patterns nicely fit in the diathesis-stress framework (Monroe and Simons 1991), stating that individuals who are at risk genetically show higher levels of some adverse outcome when they are exposed to a risk environment. Although not stated in the original model, the same seems to apply for individuals who have a protective genetic predisposition in that they have more positive outcomes in beneficial environments. It is important to point out that this would fit equally well in the differential susceptibility framework (Belsky and Pluess 2009), but it is rarely found (or reported) within studies that the same genetic factor has a positive effect in one situation and a negative effect in the other. Only 3 studies report such an effect (14, 23, & 38), providing direct evidence for a 'genetic plasticity factor' yielding differential susceptibility.

Many studies did not provide a strong theoretical framework for predicting one GxE pattern rather than another. It seems that a fitting theoretical explanation can be found regardless of the pattern that was discovered. For example, findings that environmental factors have a stronger effect at *medium* levels of genetic risk have been explained lending from a 'social push' model framework, stating that a risk factor is overruled at particularly low or high levels of another risk factor (Guo et al. 2015). Researchers may be tempted to place their findings in a theoretical framework a posteriori, rather than formulating hypotheses beforehand. Pre-registering hypotheses might be a good way to overcome these caveats.

As heterogeneity in outcomes, genetic predictors, and environmental factors was substantial, it was difficult to discern patterns in the results. These did not appear to depend on gene group, environmental factor, or substance investigated. However, patterns were hard to discern, as there were many different (combinations of) factors investigated.

Limitations of included studies

Study quality differed within and between genetic risk assessment methods, and was often limited. For all study types, power calculation was mostly omitted, and many studies are likely to have been underpowered (see Table SII). Interaction effects usually require more power to be detected than main effects, and given that main effects of genetic predictors are often small this is especially relevant in GxE studies (Duncan & Keller, 2011). For example, if the effect size of a GxE effect would be R^2=0.5%, in order to achieve 80% power sample size would need to be N=2,185 (assuming 3 predictors and α=.05), and 31 out of 39 studies had smaller sample size than that (see Table SII). To put that in perspective, main effects of top SNPs in GWASs are often around 0.25%, and all measured SNPs together typically explain around 10% or less of the variance in substance use phenotypes (So et al., 2011). Also, control for gene-environment correlation was limited, and where it was tested, the test often entailed a simple correlation without controlling for the effects of covariates, or for interaction effects between the genetic predictor and covariates (Keller, 2014). This is problematic, as many environmental factors (such as parenting behaviors) are in fact influenced by genes themselves (Krapohl et al. 2017), and this covariation would decrease chances of detecting GxE and impede its interpretation (Rathouz et al. 2008). Over all study types, heterogeneous designs, lack of replication studies, and inconsistent statistical reporting made assessment of publication bias impossible. In candidate-gene GxE studies such bias to underreporting negative results has been demonstrated (Duncan and Keller 2011). As the number of *p*-values just below the significance threshold was not higher than the number of small *p*-values, we concluded that *p*-hacking did not seem to be an issue.

The haplotype method was limited as a measure of polygenic risk because many studies looked at a few variants in one gene, which is strictly speaking not ‘polygenic’ (but ‘polyvariant’) and will not capture much variation. The investigated genes were mostly plausible candidates for substance use because of their biological function. The benefits of this method compared to the traditional single candidate-gene method are modest. This might be reflected in the results, as more haplotype than other studies did not find a GxE interaction or found results that are difficult to interpret (i.e., did not follow a pattern as depicted in Figure 3).

Studies using candidate-gene score methods appeared of somewhat better quality than the haplotype studies. Sample sizes and the number of investigated variants still seem (too) low to detect small effects (Luan et al. 2001). A more fundamental drawback of the candidate-gene method in general is that the selection of variants and risk alleles by definition has to rely on a limited body of knowledge, that might or might not include information on the causally most important genetic variants (Zhu and Zhao 2007). As an

example, previous research has shown that candidate-genes for schizophrenia did not predict schizophrenia better than candidate-genes for an unrelated phenotype (diabetes; Johnson et al., 2017). Indeed, few of the proposed candidates in haplotype or candidate-gene score studies have actually been identified in hypothesis-free GWASs (e.g., Liu et al. 2019, Pasman et al. 2018, Walters et al. 2018). This might have added to the finding that studies using these methods more often yielded unexpected patterns.

Technical advances and decreasing costs have made it possible to consider the whole genome for risk prediction, and studies using such PS seem to become more popular than those using haplotype and candidate-gene methods. PS studies yielded the highest quality ratings, with sample sizes more adequate to capture small effects, although study design and phenotype measurements were less strong. It is important to note that PS studies are not necessarily appropriate for testing differential vulnerability hypotheses, as SNPs that operate through that mechanism would not necessarily have large main effects likely to be detected in a GWAS (Fox and Beevers 2016). It is an interesting possibility that different variants are important for interaction effects than for main effects, and this might contribute to the fact that GxE studies show disappointing results in comparison with GWASs. Furthermore, even the qualitatively better studies reviewed here show only small effects.

Recommendations for future studies

Following from the limitations of the included studies, important recommendations for future research can be made. A roadmap for future research is summarized in Figure 4. First, more attention should be given to hypothesis selection. Although addiction research would be advanced by a further expansion of the scope of research, direct replication attempts might be even more important at this stage (Duncan and Keller 2011). Replication and original studies alike should focus on formulating and pre-registering sharp predictions and give attention to the exact direction of the GxE effects (Belsky et al. 2013; Munafò et al. 2017).

Second, high quality study methods should be used. Despite having limitations, the PS studies yielded more consistent results than the other two study types. GWASs with substantial sample sizes for alcohol (N≈941,000) and tobacco use (N≈1,232,000; Liu et al. 2019), and cannabis use (Pasman et al., 2018; N≈184,000) are increasingly available, enhancing the predictive power of PS.

It is interesting to note that studies are emerging testing SNP by environment interactions in GWASs, making it possible to explore GxE in a hypothesis-free manner. This would circumvent the difficulty that SNPs captured in GWASs do not necessarily measure

differential susceptibility. For instance, Polimanti and colleagues (2017) showed a SNP by trauma exposure interaction on the risk of alcohol misuse. As the multiple testing burden for this kind of design is substantial, large sample sizes are needed to test GxE on a genome-wide level. However, as these samples are becoming increasingly available, the merits of this method might be further explored. Other important characteristics of high quality study methods include using better phenotypical measures, using large discovery and target sample sizes, controlling for covariates and taking into account possible rGE. Authors should report on rGE analysis (that controlled for covariates), and ideally the GxE analysis should control for the effects (for example using structural equation modeling).

Third, future studies should report more completely and transparently on statistics, such as effect size and achieved power level. Also, more attention should be given to null results, so that in future meta-analyses unbiased effect sizes can be estimated (see Figure 4).

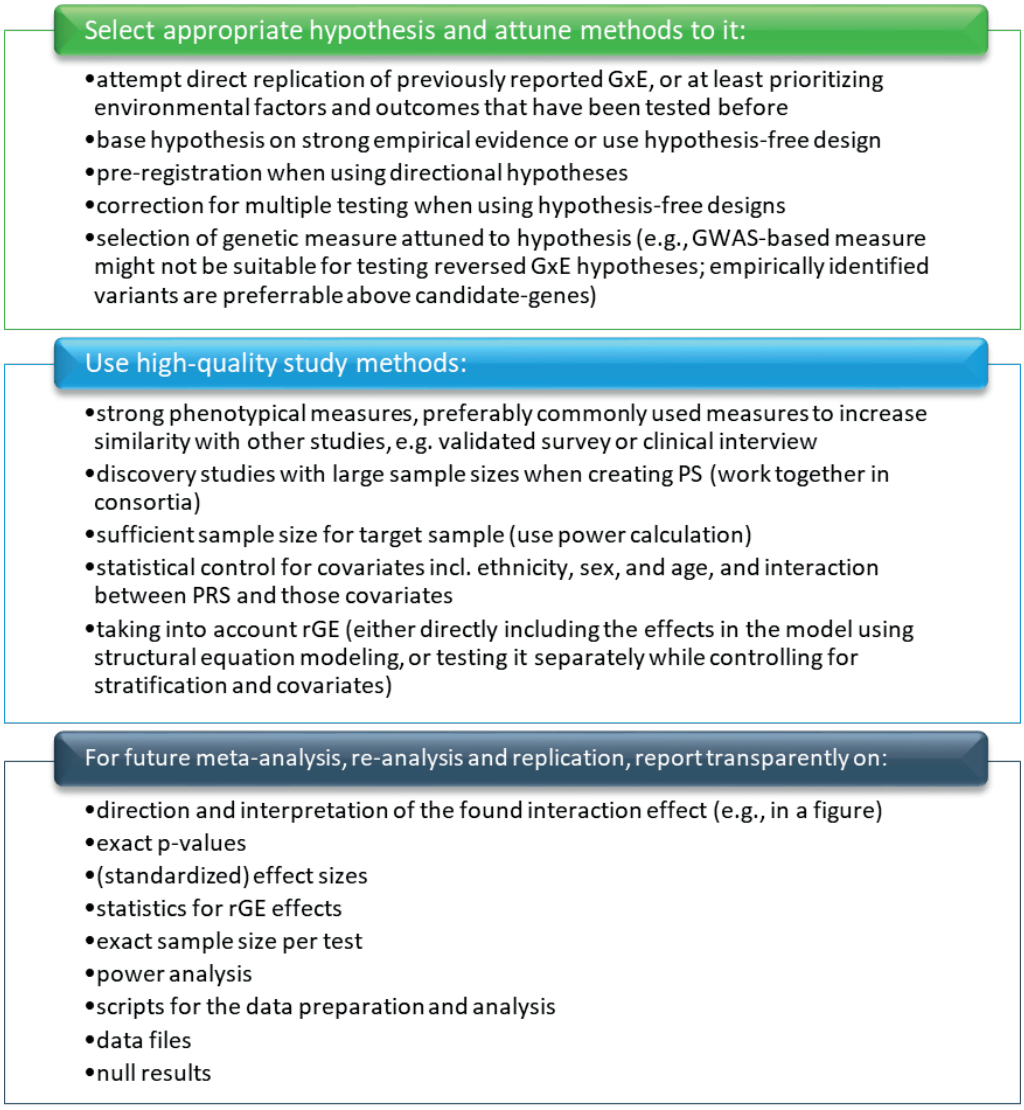

Figure 4. Road map for future studies with recommended steps for improving the stance of the substance use GxE literature.

Strengths and limitations

This is the first review focusing on and comparing multiple polygenic methods for assessing GxE in multiple substance use outcomes. Patterns of results could be compared across different methods, outcomes, and predictors. The quality assessment provided insight in important lacunas in study methodology and gave some suggestion that study quality influences the patterns of results.

The heterogeneity of the included studies introduced some important constraints for the review. No meta-analysis could be attempted and we had to devise our own method to visualize study quality. As we tried to integrate all findings in one comprehensive interpretation, some detail was inevitably lost. Another limitation lies in the fact that some of the studies did not set out to test GxE, but rather included it as a secondary analysis. This might have contributed to the fact that details on methods and results could sometimes be retrieved only with difficulty. Also, it may have biased results, as studies in some cases seemed to test an interaction with a variable that proved to have a main effect, rather than a GxE effect predicted based on the literature.

Conclusion

The current review summarized literature investigating if environmental and polygenic factors interact in influencing alcohol, tobacco, and cannabis use phenotypes. There are important limitations to the literature, concerning overall study quality, failure to formulate directional hypotheses, inconsistent reports of statistics (effect sizes), and a great lack of replication studies. It is likely that some publication bias exists.

Because of these limitations, it is difficult to draw conclusions about the existence of GxE effects in substance use. Before any substantive claims can be made, it is crucial that some steps are undertaken, such as using more sophisticated methods and direct replication attempts of GxE findings. Although still weak, there is some evidence that polygenic GxE effects are a factor in the etiology of substance use, with PS being the best measure of polygenic risk. Studies suggest that environmental factors can influence the effect of genetic predisposition, either by enlarging its (positive or negative) effects, or by reversing those. Additional work is needed before firm conclusions can be drawn about the importance of GxE in the etiology of substance use.

GxE research has the potential to give crucial insight in biopsychosocial mechanisms underlying substance use that might be leveraged for clinical applications. For example, polygenic scores (Musci et al. 2015) and even single genetic variants in the nicotinic receptor genes (Sarginson et al. 2011) can predict who will respond favorably to smoking interventions. In the future, well-conducted GxE studies have the potential to improve possibilities for clinical applications

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Supplementary Materials

Full supplementary materials can also be viewed online at:
Chapter 9 – Review of gene-environment interaction in substance use

Or copy this link into the browser:
https://drive.google.com/drive/folders/1UnjKXOxnwKmr0widCsk63NqnrJI8goi4

Supplementary information

Supplementary Table SI. Search term combinations for the systematic literature search

search terms (separated by AND)	substance use	gene-environment	polygenic risk
related terms (separated by OR)	substance abuse	genotype environment	genome*wide
	dependen*	GxE	GWA$
	addict*	gene$ environment	pathway genes
	alcohol	gene$ interaction	genetic candidate system
	ethanol	gene$ interplay	multilocus
	nicotine	gene$ moderat*	multi*locus
	smok*	genotype$ moderat*	gene*score
	cigarette*	genetic interaction	SNP*score
	tobacco	genetic interplay	haplotype*
	cannabis	differential susceptibility	PRS
	marijuana	diathesis*stress	
	marihuana	nature*nurture inter*	
	hash	gene inter*	

Supplementary Table SII. Power (in %) under different assumed effect size and sample size.

		R^2 (%)								
		0.1	**0.2**	**0.4**	**0.6**	**0.8**	**1**	**2.5**	**5**	**10**
sample size	**N=100**	5.6	6.2	7.4	8.6	9.9	11.3	22.5	42.5	74.2
	N=250	6.5	8.1	11.5	15.1	19.0	23.0	52.9	85.0	99.3
	N=500	8.0	11.5	19.1	27.2	35.6	43.7	85.3	99.3	>99.9
	N=1000	11.5	19.2	35.7	51.6	65.2	75.9	99.3	>99.9	>99.9
	N=2000	19.1	35.8	65.3	83.9	93.3	97.5	>99.9	>99.9	>99.9
	N=5000	44.0	76.1	97.5	99.8	>99.9	>99.9	>99.9	>99.9	>99.9

NB. To establish criteria for study sample size evaluation, power analysis was conducted. Effect sizes of individual genetic variants are commonly found to be between R^2 = 0.1 to 1% (shaded part of table; So et al., 2011). Studies included in the review tested any number of variants between 2 and thousands, so power was calculated between R^2=0.1 to 10%. In the analysis, a multiple regression model with at least 3 predictors (2 main and 1 interaction effect) and an alpha level of .05 was assumed. Note that most studies included in the review used more than 3 predictors and will probably have reached slightly lower power levels.

Supplementary Table SIII. Reported test statistics for GxE terms in each study. When *p* was not reported, it was calculated when possible from the test statistics.

ID	year	1st author	test	finding
1[*]	2007	Berrettini	bupropion treatment x hap on smoking cessation at end of treatment	$\chi^2(7)=20.6$, *p*=**.004**
			bupropion treatment x hap on smoking cessation at follow-up	$\chi^2(7)=16.1$, *p*=**.024**
2[*]	2007	Berrettini	bupropion treatment x hap on smoking cessation at end of treatment	$\chi^2(7)=22.7$, *p*=**.002**
			bupropion treatment x hap on smoking cessation at follow-up	$\chi^2(7)=8.88$, *p*=.260
3	2009	Oroszi	naltrexone treatment x hap block 1 on alcohol abstinence	*p*=**.030**
			naltrexone treatment x hap block 2 on alcohol abstinence	*p*=.070
4	2012	Chen	intervention x hap on smoking cessation	$\chi^2(2)=8.97$, *p*=**.020**
5[+]	2013	Brody	prevention x hap in DRD2 on alcohol use	*p*<**.001**
			prevention x hap in ANKK1 block 1 on alcohol use	*p*=**.008**
			prevention x hap in GABRG1 block 2 on alcohol use	*p*<**.001**
			prevention x hap in GABRA2 on alcohol use	*p*=**.001**
6	2015	Tyndale	intervention x hap on smoking abstinence	*NA*
7[%]	2006	Lerer	trauma x haplotype on smoking initiation	*NA*
			trauma x haplotype on nicotine dependence	*NA*
8[%]	2007	Segman	trauma x haplotype on nicotine dependence	$\chi^2(1)=6.22$, *p*=**.01**
			trauma x haplotype on smoking initiation	*NA*
9	2008	Ducci	childhood abuse x MAOA-B haplotype on alcoholism	$\chi^2(1)=4.47$, *p*=**.03**
			childhood abuse x MAOB-C haplotype on alcoholism	$\chi^2(1)=2.11$, *p*=.14
10	2010	Nelson	childhood sexual abuse x haplotype on alcohol use	t(1123)=-2.123, *p*<**.017**
			childhood sexual abuse x haplotype on alcohol dependence	OR=0.42, *p*=**.023**
11	2010	Enoch	trauma x haplotype on substance dependence	*NA*

			trauma x haplotype on alcohol dependence	*NA*
12[&]	2011	Kranzler	adverse events x haplotype on alcohol dependence in AA female	*p*=.17
			sample	*p*=.61
			adverse events x haplotype on alcohol dependence in AA male	
			sample	
13[&]	2011	Kranzler	adverse events x haplotype on alcohol dependence in EU female	*p*=.38
			sample	*p*=.13
			adverse events x haplotype on alcohol dependence in EU male	
			sample	
14	2013	Ray	trauma x block 1 haplotype 1 versus 2 on alcoholism[a]	
			haplotype	*p*=**.032**
			trauma	*p*<**.0001**
			trauma x block 1 haplotype 3 versus 2	
			haplotype	*p*=.43
			trauma	*p*<.0001
			trauma x block 2 haplotype1 versus 4 on alcoholism	
			haplotype	*p*=.051
			trauma	*p*<.0001
			trauma x block 2 haplotype2 versus 4 on alcoholism	
			haplotype	*p*=.43
			trauma	*p*<.0001
			trauma x block 2 haplotype3 versus 4 on alcoholism	
			haplotype	*p*=.43
			trauma	*p*<.0001
			trauma x block 2 haplotype5 versus 4 on alcoholism	
			haplotype	*p*=.99
			trauma	*p*<.0001
			trauma x block 2 haplotype6 versus 4 on alcoholism	

			haplotype	p=.74
			trauma	p<.0001
			trauma x block 2 haplotype7 versus 4 on alcoholism	
			haplotype	p=**.040**
			trauma	p<**.0001**
15	2015	Handley	childhood maltreatment x haplotype on marijuana dependence	z=2.00, p=**.04**
16	2017	Handley	childhood maltreatment x haplotype on alcohol problems via	*NA*
			internalizing	*NA*
			childhood maltreatment x haplotype on alcohol problems via	
			externalizing	
17	2012	McGeary	efficacy of bupropion x score on smoking cessation	*NA*
18[+]	2013	Brody	prevention x score on alcohol use	p<**.001**
19	2013	David	bupropion x score on time to first relapse in after smoking cessation	z=-2.4, p=**.016**
			bupropion x score smoking cessation at end of treatment	z=1.25, p=.213
20	2015	Guo	roommate's drinking x medium vs other score on binge drinking in 1st	*NA*
			semester	*NA*
			roommate's drinking x medium vs other score on binge drinking in 2nd	
			semester	
21[$]	2015	Guo	roommate's drinking x medium vs other score on drinking in past 2	*NA*
			weeks	*NA*
			roommate's drinking x medium vs other score on drinking in last year	
22	2016	Bountress	parental knowledge x score on emerging adult substance use	β=-0.46, p<**.05**
			disorder, mother report of parental knowledge	
			parental knowledge x score on emerging adult substance use	β=0.54, p<**.01**
			disorder, adolescent report of parental knowledge	
			peer substance use x score on emerging adult substance use	
			disorder, mother report of parental knowledge	β=-0.19, p<**.05**

			peer substance use x score on emerging adult substance use	β=0.51, *p*<.**01**
			disorder, adolescent report of parental knowledge	
23[$]	2016	Stogner	parental rejection on adolescent alcohol use	z=1.05, *p*=.294
			score on adolescent alcohol use[a]	z=0.29, *p*=.772
24[@]	2017	Pasman	education level x score on moderate polysubstance use sample 1	z=0.24, p.81
			education level x score on problematic polysubstance use sample 1	z=0.19, *p*=.85
25	2017	Pasman	education level x score on moderate polysubstance use sample 2	z=2.23, *p*=.**03**
			education level x score on problematic polysubstance use sample 2	z=1.90, *p*=.06
26	2017	Coley	parental drinking x score on alcohol drinking days	z=0.00, *p*=.951
			friend drinking x score on alcohol drinking days	z=-0.20, *p*=.779
			endogenous life events x score on alcohol drinking days	z=-0.17, *p*=.912
			exogenous life events x score on alcohol drinking days	z=?, *p*=.414
			parental drinking x score on alcohol intoxication	z=0.50, *p*=.668
			friend drinking x score on alcohol intoxication	z=-0.10, *p*=.946
			endogenous life events x score on alcohol intoxication	z=-0.10, *p*=.970
			exogenous life events x score on alcohol intoxication	z=0.44, *p*=.655
			parental drinking x score on alcohol use disorder	z=1.54, *p*=.134
			friend drinking x score on alcohol use disorder	z=-0.67, *p*=.480
			endogenous life events x score on alcohol use disorder	z=0.36, *p*=.720
			exogenous life events x score on alcohol use disorder	z=0.92, *p*=.338
27[#]	2015a	Musci	prevention x PS on age of smoking initiation	t(538)=-1.973, *p*=.**049**
28[#]	2016	Musci	prevention x PS on age of cannabis initiation	t(677)=-3.01, *p*=.**003**
29	2012	Vrieze	age cohort x PS on smoking heaviness	*NA*
			age cohort x PS on alcohol use	*NA*
30	2013	Meyers	neighborhood cohesion x PS on smoking heaviness	*p*<.**05**
			trauma x PS on smoking heaviness	*p*<.**05**
31[”]	2014	Salvatore	parental knowledge x PS on alcohol problems	t(1114)=2.27, *p*=.**020**
			peer deviance x PS on alcohol problems	t(1115)=2.11, *p*=.**040**

32[#]	2015b	Musci	environmental risk x PS on smoking frequency	OR=1.33, *p*=.**038**
			environmental risk x PS on cannabis use frequency	OR=0.10, *p*=.**046**
33[~]	2016	Domingue	birth cohort x PS on smoking initiation	*p*=.06
			birth cohort x PS on smoking initiation alternative statistical model	*p*=.05
34[~]	2016	Schmitz	veteran status x PS on smoking initiation	*NA*
			veteran status x PS on smoking heaviness	*NA*
35	2017	Li	friend's substance use x PS on heavy episodic drinking young age group male	z=0.00, *p*=1.00
			friend's substance use x PS on heavy episodic drinking young age group female	z=-0.38, *p*=.70
			friend's substance use x PS on heavy episodic drinking older age group male	z=0.63, *p*=.52
			friend's substance use x PS on heavy episodic drinking older age group female	z=1.32, *p*=.19
36[@]	2017	Treur	childhood smoke exposure x PS on smoking heaviness	OR=0.97-1.09, CI=0.83-1.26
			childhood smoke exposure x PS on smoking initiation	OR=1.05-1.44, CI=0.76-1.88
37[@]	2018	Mies	stress x PS on alcohol consumption	*p*=.963
			life satisfaction x PS on alcohol consumption	*p*=.406
			stress x PS on alcohol problems	*p*=.568
			life satisfaction x PS on alcohol problems	*p*=.392
38	2018	Polimanti	trauma x bipolar disorder PS on alcohol misuse[a]	*NA*
			trauma x major depressive disorder PS on alcohol misuse	z=-2.29, *p*=.**022**
			trauma x schizophrenia PS on alcohol misuse[a]	*NA*
			trauma x bipolar disorder PS on nicotine dependence[a]	*NA*
			trauma x major depressive disorder PS on nicotine dependence[a]	*NA*

			trauma x schizophrenia PS on nicotine dependence	z=1.45, *p*=.146
39[“]	2018	Salvatore	romantic relationship status x PS on alcohol intoxication	*NA*
			romantic relationship status x subset of PS on alcohol intoxication	*NA*

[*+%&$@#”] Studies denoted with the same symbol used data from identical or overlapping samples.
In bold *p*-values that were below the traditional two-tailed significance level of $p<.05$
Findings indicated with NA: *p*-value for the interaction term (or for the main effects in the case of reversed interaction) was not reported and could not be calculated
[a] These findings followed a reversed pattern; thus, the *p*-values for the main effects are included in the analysis

Supplementary Table SIV. Sample characteristics for all included studies.

ID	year	1st author	population	ethnicity	% female (cases|controls)	Mean age (cases|controls)
1*	2007	Berrettini	clinical adult	European American	54|55[b]	45|45[b]
2*	2007	Berrettini	clinical adult	African American	84|64[b]	45|48[b]
3	2009	Oroszi	clinical adult	European American	33|29[b]	45|45[b]
4	2012	Chen	clinical adult	European American	46 (total)	53 (total)
5+	2013	Brody	adolescent, families	African American	53-56 (total)[b]	11-17 (total)
6	2015	Tyndale	adult	European American	43 (total)	45 (total)
7%	2006	Lerer	college students	Jews (mixed)	100|100	20-30 (total)
8%	2007	Segman	college students	Jews (mixed)	100|100	24 (total)
9	2008	Ducci	adult	native American (mixed)	100|100	38 (total)
10	2010	Nelson	ascertained families with twins	European Australian	78|46	42 (total)
11	2010	Enoch	clinical adult	African American	0|0	46|34[b]
12&	2011	Kranzler	ascertained families	African American	44 (total)	42 (total)
13&	2011	Kranzler	ascertained families	European American	42 (total)	38 (total)
14	2013	Ray	clinical adult	European American	61|70[b]	38|39
15	2015	Handley	adolescent	American (mixed)	44 (total)	16 (total)

16	2017	Handley	young adult	African American	53|?	20|?
17	2012	McGeary	clinical adult	mixed American	16 (total)	50 (total)
18[+]	2013	Brody	families/ adolescent	African American	53-56 (total)[b]	11-17 (total)
19	2013	David	clinical adult	European American	52 | 52[b]	45 | 45[b]
20	2015	Guo	college student	mixed American	61[b]	not reported
21[$]	2015	Guo	high school student	mixed American	51[b]	23[b]
22	2016	Bountress	ascertained young adult	European American	53	21
23[$]	2016	Stogner	adolescent	mixed American	52	16
24[@]	2017	Pasman	families/ adult twins	European Dutch	63	35
25	2017	Pasman	families/ adolescent	European Dutch	49	33
26	2017	Coley	adolescent	mixed American	53[b]	28
27[#]	2015a	Musci	adolescent/ young adult	mixed American	46 (total)	±6-18 (total)
28[#]	2016	Musci	adolescent/ young adult	African American	47[b]	±6-18 (total)
29	2012	Vrieze	adolescent/ young adult twins	European American	52	20
30	2013	Meyers	adult	African American	52	48
31[”]	2014	Salvatore	families/ adolescent twin	European Finnish	53	14
32[#]	2015b	Musci	adolescent/ young adult	mixed American	46[b]	±12-22
33[~]	2016	Domingue	older adult	European American	58	±56-96
34[~]	2016	Schmitz	older adult	European American	0	±64-68 (total)

35	2017	Li	ascertained families/ adolescent	European American	57	23
36[@]	2017	Treur	families/ twin	European Dutch	67	±41
37[@]	2018	Mies	families/ twin	European Dutch	65	43
38	2018	Polimanti	adult soldiers	European American	9	23
39[“]	2018	Salvatore	young adult twins	European Finnish	54	22

[*+%&$@#”] Studies denoted with the same symbol used data from identical or overlapping samples. In case-control or RCT designs, percentage female and average age are given separately for cases and controls when reported or calculable. Otherwise, numbers for the total sample and/or ranges are used, as indicated with (total).

[b] True numbers and percentages might deviate, as not all individuals were included in the GxE analyses.

Table SVa. Quality characteristics for the GxE studies using haplotypes as measure of polygenic risk.

			design			control for			polygenic method				phenotype method		
ID	**year**	**1st author**	**type**	**N (cases)**	**power**	**age**	**sex**	**ethni**	**rGE**	**blocks**	**variants**	**genes**	**rationale risk allele**	**outcome measure**	**env measure**	**qual**
1*	2007	Berrettini	RCT	430 (235)[a]	yes	desc	stat	homo	NA	1	2	1	debatable	s-Q (1) B	NA	+-
2*	2007	Berrettini	RCT	81 (37)[a]	yes	desc	stat	homo	NA	1	2	1	debatable	s-Q (1) B	NA	+-
3	2009	Oroszi	RCT	306 (146)[a]	no	desc	desc	homo	NA	2	10	1	solid	s-Q (?) v-Q (50)	NA	-
4	2012	Chen	RCT	1073 (941)	no	stat	stat	homo	NA	1	2	3	solid	*unspecified Q* B	NA	+
5+	2013	Brody	long RCT	963-1,134 (?)[a]	yes	homo	stat	no	NA	5	13	4	debatable	e-Q (2)	NA	+
6	2015	Tyndale	RCT	654 (442)	yes	stat	stat	homo	NA	1	2	3	solid	*unspecified Q* B	NA	+

7[%]	2006	Lerer	case control	390 (242)[a]	yes	stat	NA	desc	no	2	8	2	debatable	s-Q (?) v-Q (6)	s-Q (?)	-
8[%]	2007	Segman	case control	390 (242)[a]	yes	stat	homo	desc	no	2	4	1	debatable	s-Q (?) v-Q (6)	s-Q (?)	+-
9	2008	Ducci	case control	187 (95)[a]	no	no	homo	desc	no	2	9	2	debatable	I	I	--
10	2010	Nelson	case control	1128 (156)	no	no	stat	homo	no	1	8	1	solid	I	I	-
11	2010	Enoch	case control	350 (72)[a]	no	stat	homo	rudim stat	desc	2	7	1	debatable	I	v-Q (28)	-
12[&]	2011	Kranzler	case control	1869 (634)	no	stat	sep	homo	desc	1	3	1	solid	I	s-Q (3)	-
13[&]	2011	Kranzler	case control	1,211 (330)	no	stat	sep	homo	desc	1	3	1	solid	I	s-Q (3)	-
14	2013	Ray	case control	2,533 (1,167)[a]	no	no	stat	homo	no	2	18	1	debatable	I	I	+-

15	2015	Handley	long case control	326 (179)	no	stat	desc	rudim stat	no	1	4	1	solid	I	records classification	-
16	2017	Handley	long case control	280 (163)	no	stat	desc	stat	desc	1	4	1	solid	s-Q (4)	I	-

*+%&$@#"Studies denoted with the same symbol used data from identical or overlapping samples. Sample size for the GxE analysis is given with the cases subsample in brackets for case-control and RCT designs. The 'power' column summarizes whether a power analysis was reported. The rGE column denotes NA when gene-environment correlation cannot be an issue because of the study design. The 'blocks' column provides the number of LD blocks included in the investigation, with 'variants' giving the total number of variants tested in these blocks. The 'measure' columns give the type of instrument used to measure phenotype, with the number of items provided in brackets for Qs.

[a] True numbers and percentages might deviate, as not all individuals were included in the GxE analyses.

Abbreviations: ethni=ethnicity; rGE=gene-environment correlation; env=environmental; qual=quality; RCT=randomized controlled trial; long=longitudinal; desc=description of differences without statistical control; se*p*=separate analyses for levels of confounder; stat=statistical control; rudim stat= rudimentary statistical control; homo=homogeneous sample; NA=not applicable; Q=questionnaire; I=interview; B=biological measure; v-=externally validated; s-=self-developed; e-=used earlier (but not validated).

Table SVb. Quality characteristics for the GxE studies using aggregates of candidate-genes as measure of polygenic risk.

			design			control for				polygenic method				phenotype method	
ID	**year**	**1st author**	**type**	**N (cases)**	**power**	**age**	**sex**	**ethni**	**rGE**	**gen predictor**	**variants**	**genes**	**rationale risk allele**	**outcome measure**	**env measure**	**qual**
17	2012	McGeary	RCT	90 (?)	no	no	stat	no	NA	sum	4	4	solid/ debatable	s-Q (?)	NA	--
18+	2013	Brody	long RCT	963-1,134[a]	yes	homo	stat	homo	NA	sum	3	3	debatable	e-Q (2)	NA	+-
19	2013	David	RCT	792 (410)[a]	no	stat	stat	homo	NA	sum	4	4	solid	*unspecified Q* B	NA	+
20	2015	Guo	randomized long	1,003	no	stat	stat	stat	NA	propensity levels	5/10	3/6	debatable	s-Q (2)	s-Q (2)	+-
21$	2015	Guo	long	1,612	no	stat	stat	stat	desc	propensity levels	5/27	3/21	debatable	s-Q (3)	s-Q (3)	+-
22	2016	Bountress	long corr	254	no	stat	stat	rudim stat	stat	sum	7	6	solid	I	e-Q (3) e-Q (6)	+-

23[$]	2016	Stogner	corr	1,495	no	stat	stat	rudim stat	desc	sum	5	5	solid	s-Q (1)	e-Q (3-5)	+-
24[@]	2017	Pasman	long corr	2,435	yes	stat	stat	homo	no	sum	3/7	3/7	solid	s/e/v-Qs	s-Q (1-3)	+-
25	2017	Pasman	long corr	1,173	yes	stat	stat	homo	no	sum	3/4	3/4	solid	s/e/v-Qs	s-Q (1-3)	+-
26[$]	2017	Coley	long corr	11,423	no	stat	stat	rudim stat	desc	sum	6	6	solid	s/e-Qs (13)	s-Q (24)	+

Studies denoted with the same symbol used data from identical or overlapping samples. Sample size for the GxE analysis is given with the cases subsample in brackets for case-control and RCT designs. The 'power' column summarizes whether a power analysis was reported. The rGE column denotes NA when gene-environment correlation cannot be an issue because of the study design. The 'gen predictor' column gives the type of aggregate of candidate-genes that was used, with sum meaning a sum score of the number of risk alleles. The 'measure' columns give the type of instrument used to measure phenotype, with the number of items provided in brackets for questionnaires.

[a] True numbers and percentages might deviate, as not all individuals were included in the GxE analyses.

Abbreviations: ethni=ethnicity; rGE=gene-environment correlation; env=environmental; qual=quality; RCT=randomized controlled trial; long=longitudinal; corr=correlational; desc=description of differences without statistical control; stat=statistical control; rudim stat= rudimentary statistical control; homo=homogeneous sample; NA=not applicable; env=environmental exposure; Q=questionnaire; I=interview; B=biological measure; v-=externally validated; s-=self-developed; e-=used earlier (but not validated).

Table SVc. Quality characteristics for the GxE studies using polygenic risk scores as measure of polygenic risk.

			design			control for				polygenic method				phenotype method		
ID	**year**	**1st author**	**type**	**N (cases)**	**power**	**age**	**sex**	**ethni**	**rGE**	**PRS basis**	**corresp pheno**	**discovery N**	**method**	**variants**	**outcome measure**	**env measure**	**qual**
27#	2015a	Musci	long RCT	539 (258)	no	NA	stat	rudim stat	NA	1-3 GWAS	moderate	1292-4696	weighted sum	*p*<.01	s-Q (1)	NA	+-
28#	2016	Musci	long RCT	678 (479)	no	NA	stat	rudim stat	NA	1-3 GWAS	weak	1292-4696	weighted sum	*p*<.01	s-Q (1)	NA	+-
29	2012	Vrieze	corr	3,231	no	stat	stat	stat	NA	MA of GWAS	strong	31266	weighted sum of SNPs in RoI	r^2>.07	s-/v-Q (1)	NA	+
30	2013	Meyers	corr	399	yes	stat	stat	stat	desc	MA of GWAS	strong	32389	weighted sum	*p*<5*10^-7	s-Q (1)	v-classification s-Q (5) s-Q (19)	+

31	2014	Salvatore	corr	1,162	no	homo	stat	homo	desc	1 GWAS	strong	4304	2 weighted sums	$p<.05$ $p\leq 0.0001$	I	e-Q (4) s-Q (4)	+-
32[#]	2015b	Musci	long	556	no	homo	stat	stat	desc	1-3 GWAS	moderate	1292-4696	quartiles weighted sum	$p<.01$	I/ Q (?)	s-Q (2) I	+-
33[~]	2016	Domingue	corr	8,904	yes	stat	stat	homo	desc	MA of GWAS	strong	74053	weighted sum	$p=1$	s-Q (1)	NA	+
34[~]	2016	Schmitz	case control	631 (198)	yes	stat	homo	stat	stat	MA of GWAS	strong	74053	weighted sum	$p=1$	s-Q (1) s-Q (1)	records ?	+
35	2017	Li	long	241	no	homo	sep	homo	desc	1 GWAS in related sample	moderate	1249	weighted sum	$p<.05$	I	I v-Q	-
36[@]	2017	Treur	corr	4,072	no	stat	stat	stat	desc	MA of GWAS	strong	~70200 (init) ~34200 (heaviness)	8 LDpred weighted sums	$p=$ 0.0001-1	s-Q (1)	s-Q (1)	+

37	2018	Mies	corr	6705	yes	stat	stat	stat	no	MA of GWAS	strong	~67,000	9 LDpred weighted sums	*p*=.0001 – *p*=1	s-Q (3) v-Q (10)	s-Q (1) v-Q (5)	++
38	2018	Polimanti	case control	10,732 (8,346) | 6,132 (4,938)	no	stat	stat	stat	desc for 1 PRS	MA of GWAS	weak	63,766 (BD) 18,759 (MD) 150,064 (SC)	7-9 weighted sums	*p*=1e-08 - 0.5	v-Q (13) v-Q (5)	v-Q (22)	+
39	2018	Salvatore	corr	1,170	yes	stat	stat	stat	desc	1 GWAS	strong	4,304	2 weighted sums, 2 selections of DNase sensitive sites	*p*=0.01 & *p*=0.05	s-Q (1)	s-Q (1)	+

Studies denoted with the same symbol used data from identical or overlapping samples. Sample size for the GxE analysis is given with the cases subsample in brackets for case-control and RCT designs. The 'power' column summarizes whether a power analysis was reported. The rGE column denotes NA when gene-environment correlation cannot be an issue because of the study design. The 'PRS basis' column gives the study type whose summary statistics were used to calculate the PRS. The 'corresp phen' indicates how strongly the phenotype is related to the original phenotype for which the summary statistics were calculated. The 'discovery N' gives the sample size for the study on which the PRS was based. The 'measure' columns give the type of instrument used to measure phenotype, with the number of items provided in brackets for questionnaires. Abbreviations: ethni=ethnicity; rGE=gene-environment correlation; env=environmental; qual=quality; RCT=randomized controlled trial; long=longitudinal; corr=correlational; desc=description of differences without statistical control; s*ep*=separate analyses for levels of confounder; stat=statistical control; rudim stat=rudimentary statistical control; homo=homogeneous sample; NA=not applicable; MA=meta-analysis; GWAS=genomewide association study; init=initiation; Q=questionnaire; I=interview (clinical); B=biological measure; v-=externally validated; s-=self-developed; e-=used earlier (but not validated); BD=bipolar disorder; MD=major depressive disorder; SC=schizophrenia.

CHAPTER 10

Substance use: Interplay between polygenic risk and neighborhood environment

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Abstract

Background: Tobacco, alcohol, and cannabis use are prevalent behaviors that pose considerable health risks. Genetic vulnerability and characteristics of the neighborhood of residence form important risk factors for substance use. Possibly, these factors do not act in isolation. This study tested the interaction between neighborhood characteristics and genetic risk (gene-environment interaction, GxE) and the association between these classes of risk factors (gene-environment correlation, rGE) in substance use.

Methods: Two polygenic scores (PGS) each (based on different discovery datasets) were created for smoking initiation, cigarettes per day, and glasses of alcohol per week based on summary statistics of different genome-wide association studies (GWAS). For cannabis initiation one PGS was created. These PGS were used to predict their respective phenotype in a large population-based sample from the Netherlands Twin Register (N=6,567). Neighborhood characteristics as retrieved from governmental registration systems were factor analyzed and resulting measures of socioeconomic status (SES) and metropolitanism were used as predictors.

Results: There were (small) main effects of neighborhood characteristics and PGS on substance use. One of the 14 tested GxE effects was significant, such that the PGS was more strongly associated with alcohol use in individuals with high SES. This was effect was only significant for one out of two PGS. There were weak indications of rGE, mainly with age and cohort covariates.

Conclusion: We conclude that both genetic and neighborhood-level factors are predictors for substance use. More research is needed to establish the robustness of the findings on the interplay between these factors.

1. Introduction

Use of tobacco, alcohol, and cannabis is prevalent in the Western world. Twenty percent of European and US individuals older than 14 smoke on a regular basis (WHO, 2016a) . The worldwide average daily intake of alcohol is 13.9 grams in this age group (about one glass; WHO, 2018a). In Europe, around 23% has ever used cannabis (in the age group ≥15 years) versus 52% in the US (age ≥16; EMCDDA, 2011). Smoking, alcohol use, and cannabis use can have deleterious health effects (WHO, 2016b; WHO, 2017; WHO, 2018a), making the etiology of these behaviors an important topic of study.

Heritability estimates for tobacco, alcohol, and cannabis use are substantial (Kendler et al., 2008), with even higher estimates for abuse and dependence (Ducci and Goldman, 2012; Mbarek et al., 2015; Verweij et al., 2010; Vink et al., 2005). Molecular genetic studies aim to identify specific genetic variants that increase risk for substance use. Hypothesis-free, large genome-wide association studies (GWASs) of smoking (Liu et al., 2019; The Tobacco and Genetics Consortium, 2010), alcohol use (Clarke et al., 2017; Liu et al., 2019), and lifetime cannabis use (Pasman et al., 2018) have had success in achieving this, but heritability estimates based on the accumulative effect of measured SNPs are still lower than estimates from twin and family studies. One of the causes of this 'missing heritability' might be the neglect of the interplay between the environment and genes (Manolio et al., 2009).

Neighborhood characteristics might increase risk for substance use, although results are often mixed. For instance, urbanicity has been associated with higher rates of smoking (Idris et al., 2007), cannabis use (Martino et al., 2008), and alcohol use (Atav and Spencer, 2002), but there is also evidence for associations in the opposite direction (Donath et al., 2011; Leatherdale et al., 2007; Lutfiyya et al., 2008). Some studies find that substance use is associated with a low average socioeconomic status (SES), but results seem to depend on study characteristics and the type of substance under investigation (for reviews, see Galea et al., 2004; Karriker-Jaffe, 2011). General measures of low neighborhood SES or deprivation have been shown to be positively associated to smoking (Stimpson et al., 2007). Alcohol and cannabis use might be more prevalent in high SES neighborhoods, but results have been mixed (Karriker-Jaffe, 2011).

Possibly, genetic vulnerability to substance use influences the relationship between these neighborhood characteristics and substance use. In the case of gene-environment interaction (GxE) adverse environmental circumstances may lead to deleterious outcomes only (or more strongly) in genetically vulnerable individuals ('contextual

triggering'), or reversely, a beneficial environment can protect against the effect of genetic vulnerability ('compensation,' Shanahan and Hofer, 2005). In other words, some individuals have a higher innate reactivity to environmental circumstances, meaning that there is 'differential susceptibility' (Belsky and Pluess, 2009). Previous studies into GxE have mainly used twin or candidate-gene methodology. For example, heritability of alcohol use was found to be higher when alcohol outlet density (selling points) was high in the neighborhood of residence than when the density was low (Slutske et al., 2018). Some twin studies have suggested that the genetic contribution to alcohol use and abuse is larger for people living in urban areas than for people living in rural areas (Davis et al., 2017; Legrand et al., 2008; Rose, 1998; Rose et al., 2001). Few studies have investigated GxE in the neighborhood using polygenic scores. A polygenic score (PGS) is a weighted count of risk alleles for a trait, where the weights are based on the SNP effect sizes in a GWAS. PGS might be the best available measure of genetic risk to date for use in GxE studies (Pasman et al., 2019). The only study to our knowledge that has used a PGS to test gene-neighborhood interaction focused on smoking and showed that more social cohesion in the neighborhood buffered against the effect of genetic risk (Meyers et al., 2013). No interaction effect was found for a measure of neighborhood poverty and disrepair.

Even less studied in this context is gene-environment correlation (rGE). In rGE, there is an association between genetic predisposition and the environment of a person, such that genetic factors are associated both with the outcome of interest and with the environmental context. For example, if lower intelligence is associated with both substance use and with living in low-SES environments (Fergusson et al., 2005) this can lead to rGE when a genetic measure for substance use includes variants that are also predictive of lower intelligence (i.e., variants that are pleiotropic). Also, 'evocative' rGE arises when genes contribute to some behavior (e.g., aggression) that elicits a response in the environment (e.g., rejection; Plomin et al., 1977). Few studies to our knowledge investigated rGE with neighborhood characteristics specifically. One study showed that a PGS for alcohol dependence was positively related to neighborhood social deprivation (Clarke et al., 2016). Another showed significant correlations between substance use PGS and Townsend neighborhood deprivation indices (Abdellaoui et al., 2019). Some GxE studies report the (uncorrected) correlation between their G and E factors (e.g., Meyers et al., 2013). Not accounting for rGE effects can lead to an overestimation of genetic or (shared) environmental variance (Blokland et al., 2013; Purcell, 2002), and to misinterpreted or even spurious GxE findings (Jaffee and Price, 2007).

The current study looked at the main effects of neighborhood characteristics and polygenic risk on substance use, and the interplay (interaction and correlation) between these factors.

2. Methods

2.1 Participants

We used cross-sectional data (survey 5, 6, 7, 8, and 10) collected between 2000 and 2014 from an ongoing longitudinal study in twin pairs and their family members registered at the Netherlands Twin Register (NTR; Willemsen et al., 2013). For the current study, a subsample of 6,567 Dutch ancestry participants was selected. We linked the most recent substance use data to neighborhood information as obtained from governmental registration systems that was closest in time (either from 2010 or 2004; CBS, 2012), using postal code at time of survey completion. Table 1 summarizes this data selection procedure and the resulting sample composition. There were small differences in distributions or mean values for the variables depending on what survey was used (Supplementary Table S1). These differences in predictor and outcome variables may stem from cohort effects (for example due to the economic crisis), age effects, or they may represent random fluctuations. However, results did not change when we controlled for the effects of measurement wave (results not shown).

About half (55%) of the sample consisted of twins. The sample included 65% females and 38% highly educated individuals (higher vocational education or university). Mean age at the time of completing the survey was M=45.3 years (SD=15.7; range 18-91 years). Average birth year was 1964 (for more details on the NTR sample, see e.g., Geels et al., 2013; Willemsen et al., 2013).

Table 1. Participant data from each measurement year (survey number) per phenotype.

		Phenotype			
	Year (survey)	Smoking initiation	Cigarettes/ day	Alcohol/ week	Cannabis initiation
N from survey	2014 (10)	3,958	666	2,509	3,059
	2010 (8)	814	1,408	1,457	1,645
	2004 (7)	146	328	671	not available
	2002 (6)	1,326	586	1,308	not available
	2000 (5)	227	108	229	972
	Total*	6,471	3,096	6,174	5,676

* For *N*=6,567 data were complete for at least one analysis

Shaded rows: for these participants, postal code data were linked to information on neighborhood characteristics available from 2010; for the others, these data were linked to information available from 2004.

2.2 Substance use outcomes

Substance use outcomes were based on self-report measures (Supplementary Table S2). For smoking initiation, participants were coded as ever smokers if they classified as current smokers at any survey (smoking at least weekly) or ex-smokers. When answers to these questions were incomplete or inconsistent, information was complemented with answers to different questions (Treur et al., 2016).

For tobacco use, we used an open-ended question asking how many cigarettes per day someone smoked at their heaviest period of smoking for survey 7, 8, and 10. For survey 5 and 6, cigarettes per day was available only for current smokers and was measured on an ordinal scale. For these surveys, the mid-point of each answering category was analyzed on a continuous scale.

For alcohol use, glasses of alcohol consumed per week was used as an outcome. If individuals drank less than 1 glass per week (N=1,297) their value was put to 0. Individuals who never drank alcohol (N=87) were excluded from analysis. We deemed it likely that a response of more than 70 glasses per week (N=7) represented an invalid answer rather than a true estimate; these responses were excluded. In survey 8 and 10 alcohol use was measured continuously; we used the midpoint of the answering categories in survey 7, 6, and 5.

For lifetime cannabis use, participants were asked if they had ever used cannabis (yes/no). This measure was only available for survey 5, 8, and 10.

2.3 Neighborhood characteristics

In the Netherlands postal codes exist of four digits, identifying areas at the level of neighborhoods, and two letters, identifying areas at the level of streets. We linked the four digits of the postal codes to registered neighborhood characteristics from governmental registration systems (CBS, 2012). Information was available on urbanicity in addresses/km2, housing values, percentage of non-western immigrants (% immigrants), monthly income, percentage of inhabitants receiving low income (% low income), percentage receiving high income (% high income), and percentage receiving governmental benefit payments (% benefits; Table 2). For some variables, there were large proportions of missing data on the neighborhood characteristics. We selected variables that had less than 30% missing data: urbanicity, % immigrants, housing values, and monthly income. We used the automatic multiple imputation procedure in SPSS to complete missing data in these variables. Five imputed datasets were created and merged back to one dataset by averaging the estimations of the missing data points. Because of the conceptual and statistical overlap between the variables we performed

principal component analysis (PCA) in SPSS with an oblimin rotation. The PCA yielded two factors (see Table 2) with Eigenvalues of 1.62 and 1.34. The first factor was defined by high urbanicity and a high percentage of non-western immigrants; we dubbed this factor metropolitanism. The second factor was defined by housing values and monthly income; this variable was called socioeconomic status (SES). The factor solution explained 74% of the variance in these variables.

Table 2. The neighborhood variables (with their components and measurement levels) for the main and exploratory analyses. For correlations between the original neighborhood variables, refer to Supplemental Table S3.

Analysis	Variable	Comprises original variables	Variable levels	Loadings
Main	Metropolitan factor	urbanicity: addresses/ km^2	*<500, 500-1000, 1001-1500, 1501-2500, >2500*	.83
		% non-western immigrants	<5%, 5-10%, 11-20%, 21-40%, >40%	.81
	SES factor	housing value	continuous	.90
		average monthly income	continuous	.89
Exploratory	SES index	housing value	continuous	
		average monthly income	continuous	
		% low income	continuous	
		% high income	continuous	
		% receiving governmental benefits payments	continuous	
	Urbanicity	*NA*	*<500, 500-1000, 1000-1500, 1500-2500, >2500*	
	% non-western immigrants	*NA*	<5%, 5-10%, 11-20%, 21-40%, >40%	

2.4 Polygenic scores

Genome-wide single-nucleotide polymorphism (SNP) data for NTR participants were obtained using several genotyping platforms over time (Lin et al., 2017; Willemsen et al., 2010). The genotyping, imputation, and quality control procedures have been described earlier (Abdellaoui et al., 2018; Nivard et al., 2014). PGS were generated with PLINK (version 1.9; Purcell et al., 2007), summing the one- or two risk allele effects of the weighted beta's for each set of summary statistics. The weighted beta's were calculated with LDpred, taking into account the LD structure in the European population to improve prediction (described in detail in Abdellaoui et al., 2018; Vilhjálmsson et al., 2015). PGS can be calculated for several expected fractions of causal genetic markers to further optimize prediction accuracy; we present results for the 30% fraction, which has shown good results in previous studies on complex behavioral traits (Hugh-Jones et al., 2016;

Vilhjálmsson et al., 2015). We used multiple source GWAS to extrapolate our results because the quality and predictive power of summary statistics can differ. Predictive power does not depend solely on sample size, but for example also on the SNP-based heritability, which is the variance explained in the phenotype by the SNP effects in the GWAS (Dudbridge, 2013). For smoking, PGS were created for smoking initiation and for cigarettes per day. The first set was based on GWAS summary statistics from The Tobacco and Genetics Consortium (2010; excluding the NTR, NESDA and GAIN samples) with N=69,207 and a SNP-based heritability of h2SNP=12% for smoking initiation and N=35,173, h2SNP=6% for cigarettes per day. The second set was based on GSCAN summary statistics (excluding NTR; N=1,224,825, h2SNP=8% for smoking initiation and N= 334,609, h2SNP=8% for cigarettes per day; Liu et al.,2019). For alcohol use, PGS were based on a 2017 GWAS on alcohol consumption in glasses per week (N=112,117, h2SNP=13%; Clarke et al.,2017), and the GSCAN GWAS on the same phenotype (N=936,196, h2SNP=4%; Liu et al., 2019). For cannabis initiation, the PGS was created based on GWAS data on lifetime cannabis use excluding NTR participants, (N=157,664, h2SNP=11%; Pasman et al., 2018).

2.5 Covariates

Sex and age were included as covariate. The participants' birth year had a tri-modal distribution due to recruitment of different age groups. Therefore, we created two cohort dummy variables (for 1960-<1980 and ≥1980, with <1960 as the reference category) to correct for cohort effects. To control for stratification within the Dutch population, ten principal components (PCs) based on systematic ancestry differences were included in each analysis that included genetic predictors (models 2 and 3; Abdellaoui et al., 2013). As over time different genotyping platforms were used, dummy variables were included to control for genotype platform stratification (Boomsma et al., 2013).

2.6 Statistical analyses

We tested the effects of the neighborhood factors, PGS, and their interactions using the generalized estimating equations (GEE) procedure in SPSS, controlling for family relatedness. For the binary outcomes, binary logistic GEE was used. Groups of variables were entered in four blocks. We first regressed the substance use outcomes on sex, age, and cohort (model 0), then added neighborhood characteristics (model 1), and then genetic predictors (model 2). In model 3 we added the interaction terms. In a separate GEE analysis, we used the PGS as outcome and the neighborhood variables as predictors to test rGE while controlling for sex, age, cohort, batch (genotyping platform), and principal components. In all analyses we used standardized predictors. We applied a Bonferroni correction for four independent tests for the four outcomes (the PGS based on

different discovery GWAS not being strictly ‘independent’) resulting in a significance threshold of .0125.

3. Results

45.3% of the participants had ever smoked. Current smokers smoked on average 13.9 cigarettes per day and ex-smokers smoked 14.2 cigarettes per day in their period of most heavy smoking. Individuals drank on average 6.1 standard glasses of alcohol per week and 19.3% of the participants had ever used cannabis. The correlation between the metropolitan factor and SES factor was small (r=-0.05, p<.001).

3.1 Main effects

Sex, age, and cohort were entered in model 0 (see Supplementary Table S4). Effects of sex were significantly positive for all substances, indicating higher (chance of initiation of) use for males. The association with age was positive for alcohol per week, negative for lifetime cannabis use and not significant for smoking. Younger cohorts were less likely to have smoked and more likely to have used cannabis compared to the cohort born before 1960. The youngest cohort smoked more cigarettes per day than the oldest cohort (although in model 0 this did not survive correction for multiple testing). In model 1 to 3 there was an indication that the intermediate cohort drank less alcohol than the oldest cohort, but no such effects were observed for the youngest cohort. Variance explained by age, cohort, and sex ranged from 2.4% for cigarettes per day to 13.6% for lifetime cannabis use.

The influence of the neighborhood factors on substance use outcomes differed per substance outcome and neighborhood predictor (model 1, Supplementary Table S4). Living in a metropolitan area was associated with higher chances of smoking initiation and higher levels of alcohol consumption, but not with cigarettes per day or cannabis use. Higher SES was related to smoking more cigarettes per day and higher chances of lifetime cannabis use. SES also showed a positive association with smoking initiation, but only in the models that included the genetic predictors (2-3, see Table 3). Variance explained by the neighborhood variables ranged from 0.3% for smoking initiation to 3.4% for lifetime cannabis use.

In model 2, the effects of the PGS and genetic covariates were added to the model (Table 3 and Supplementary Table S4). The PGS for smoking initiation, cigarettes per day, alcohol per week, and lifetime cannabis use significantly predicted their respective phenotypes, explaining 0.2% (TAG smoking initiation and GSCAN alcohol per week) to 1.1% (lifetime cannabis use) of the variance (Table 3).

Table 3a. Results of covariates, neighborhood predictors, genetic predictors, and gene-environment interaction terms (model 3) for the smoking phenotypes using the PGSs based on the GWAS from Tobacco and Genetics Consortium (TAG, 2010), and Liu et al. (GSCAN, 2019). The full results including model 0 (effects of covariates sex, age, and cohort, model 1 (covariates plus neighborhood predictors), and model 2-3 including the parameters for the genetic covariates (batch and 10 PCs) are given in Supplementary Table S4a.

		Smoking initiation *TAG* (*N*=6,471)		**Smoking initiation *GSCAN* (*N*=6,471)**		**Cigarettes/ day *TAG* (*N*=3,096)**		**Cigarettes/ day *GSCAN* (*N*=3,096)**	
Model		**OR (SE)**	***p***	**OR (SE)**	***p***	**B (SE)**	***p***	**B (SE)**	***p***
2	Sex[a]	1.39 (0.32)	<.001**	1.44 (0.33)	<.001**	2.41 (0.33)	<.001**	2.49 (0.33)	<.001**
	Age	0.99 (0.01)	.385	1.00 (0.02)	.391	0.02 (0.02)	.407	0.01 (0.02)	.558
	Cohort 1960-<1980[a]	0.34 (0.15)	<.001**	0.33 (0.15)	<.001**	-0.74 (0.62)	.234	-0.97 (0.61)	.111
	Cohort ≥1980[a]	0.16 (0.12)	<.001**	0.16 (0.12)	<.001**	-2.59 (1.02)	.011*	-2.82 (1.00)	.005**
	Metropolitan factor	0.90 (0.10)	.001**	0.91 (0.10)	.001**	0.28 (0.19)	.142	0.32 (0.19)	.089
	SES factor	1.06 (0.12)	.050*	1.06 (0.12)	.030*	0.67 (0.17)	<.001**	0.71 (0.17)	<.001**
	PGS	1.14 (0.13)	<.001**	1.42 (0.17)	<.001**	0.43 (0.17)	.008**	1.37 (0.17)	<.001**
	Model R^2	.125, Δ=.008[b] (Δ_{PGS}=.004)[b]		.152, Δ=.038[b] (Δ_{PGS}=.004)[b]		.040, Δ=.007 (Δ_{PGS}=.002)		.060, Δ=.027 (Δ_{PGS}=.003)	
3	Sex[a]	1.39 (0.32)	<.001**	1.44 (0.33)	<.001**	2.40 (0.33)	<.001**	2.49 (0.33)	<.001**
	Age	0.99 (0.01)	.388	1.00 (0.01)	.389	0.02 (0.02)	.399	0.01 (0.02)	.591
	Cohort 1960-<1980[a]	0.34 (0.15)	<.001**	0.33 (0.15)	<.001**	-0.73 (0.62)	.238	-0.99 (0.61)	.103
	Cohort ≥1980[a]	0.16 (0.12)	<.001**	0.16 (0.12)	<.001**	-2.57 (1.02)	.011**	-2.84 (0.99)	.004**
	Metropolitan factor	0.90 (0.10)	<.001**	0.91 (0.10)	.001**	0.28 (0.19)	.148	0.32 (0.19)	.082
	SES factor	1.06 (0.12)	.049*	1.07 (0.12)	.030*	0.67 (0.17)	<.001**	0.72 (0.17)	<.001**
	PGS	1.14 (0.13)	<.001**	1.42 (0.17)	<.001**	0.43 (0.16)	.009**	1.38 (0.17)	<.001**
	PGS* metropolitan	0.98 (0.11)	.567	1.00 (0.12)	.984	0.04 (0.15)	.802	0.31 (0.17)	.068
	PGS*SES	0.98 (0.10)	.582	0.98 (0.11)	.475	0.19 (0.16)	.214	0.23 (0.17)	.172
	Model R^2	.125 (Δ<.001)[b]		.153 (Δ=.001)[b]		.040 (Δ<.001)		.061 (Δ=.001)	

[a] Reference category for sex was female and for both cohort variables the reference category was <1960.

[b] For dichotomous outcomes (smoking and cannabis initiation) the Nagelkerke's Pseudo R^2 is reported.

*<.05; **<.0125 (significant after Bonferroni correction)

Δ=increase in variance explained compared to the previous model; Δ_{PGS}=additional variance explained (with respect to model 1) by the PGS alone; PC=genetic principal components; PGS=polygenic score for the respective substance use outcome

Table 3b. Results of covariates, neighborhood predictors, genetic predictors, and gene-environment interaction terms (model 3) for the alcohol phenotypes using the PGSs based on the GWAS from Tobacco and Genetics Consortium (TAG, 2010), and Liu et al. (GSCAN, 2019) and the cannabis phenotype from the International Cannabis Consortium (ICC). The full results including model 0 (effects of covariates sex, age, and cohort, model 1 (covariates plus neighborhood predictors), and model 2-3 including the parameters for the genetic covariates (batch and 10 PCs) are given in Supplementary Table S4b.

		Alcohol/ week *Clarke* (*N*=6,174)		Alcohol/ week *GSCAN* (*N*=6,174)		Cannabis initiation *ICC* (*N*=5,676)	
Model		**B (SE)**	***p***	**B (SE)**	***p***	**OR (SE)**	***p***
2	Sex[a]	4.17 (0.21)	<.001**	4.19 (0.20)	<.001**	1.65 (0.51)	<.001**
	Age	0.06 (0.01)	<.001**	0.05 (0.01)	<.001**	0.96 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.90 (0.37)	.017*	-0.96 (0.37)	.010**	1.66 (1.09)	.002**
	Cohort ≥1980[a]	0.36 (0.55)	.516	0.22 (0.55)	.691	1.55 (1.47)	.062
	Metropolitan factor	0.46 (0.11)	<.001**	0.47 (0.11)	<.001**	1.07 (0.16)	.070
	SES factor	0.13 (0.09)	.167	0.12 (0.09)	.177	1.49 (0.23)	<.001**
	PGS	0.52 (0.09)	<.001**	0.78 (0.09)	<.001**	1.26 (0.20)	<.001**
	Model R^2		.130, Δ=.008 (Δ_{PGS}=.006)		.137, Δ=.015) (ΔPGS=.002)		.189, Δ=.019[b] (Δ_{PGS}=.011)[b]
3	Sex[a]	4.18 (0.21)	<.001**	4.20 (0.20)	<.001**	1.65 (0.51)	<.001**
	Age	0.06 (0.01)	<.001**	0.05 (0.01)	<.001**	0.96 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.90 (0.37)	.016*	-0.97 (0.37)	.010*	1.67 (1.10)	.002**
	Cohort ≥1980[a]	0.35 (0.55)	.517	0.22 (0.55)	.684	1.57 (1.49)	.057
	Metropolitan factor	0.46 (0.11)	<.001**	0.47 (0.11)	<.001**	1.06 (0.17)	.113
	SES factor	0.12 (0.09)	.180	0.12 (0.09)	.182	1.49 (0.23)	<.001**
	PGS	0.52 (0.09)	<.001**	0.77 (0.09)	<.001**	1.26 (0.20)	<.001**
	PGS* metropolitan	-0.03 (0.10)	.798	-0.03 (0.09)	.714	1.05 (0.16)	.182
	PGS*SES	0.23 (0.08)	.005**	0.14 (0.08)	.079	1.01 (0.15)	.836
	Model R^2		.137 (Δ<.001)		.137 (Δ<.001)		.190 (Δ=.001)[b]

[a] Reference category for sex was female and for both cohort variables the reference category was <1960.

[b] For dichotomous outcomes (smoking and cannabis initiation) the Nagelkerke's Pseudo R^2 is reported.

*<.05; **<.0125 (significant after Bonferroni correction)

Δ=increase in variance explained compared to the previous model; Δ_{PGS}=additional variance explained (with respect to model 1) by the PGS alone; PC=genetic principal components; PGS=polygenic score for the respective substance use outcome

Table 4a. Gene-environment correlation tests with the polygenic scores as outcome and the neighborhood characteristics as predictors. PGS based on TAG (2010) and GSCAN (2019) for the smoking phenotypes, Clarke (2017) and GSCAN (2019) for alcohol, and ICC (2018) for cannabis. Covariates sex, age, cohort, batch, and PCs were included (parameter estimates for batch variables and principal components are given in Supplementary Table S5). Explained variance is given for the 2 neighborhood variables (Neigh R^2), the genetic covariates (Gen R^2), and the total model (incl. sex, age and cohort; Model R^2).

		Sex[a]	Age	Cohort2[a]	Cohort3[a]	Metro	SES fac	Neigh R^2	Gen R^2	Model R^2
PGS smoking initiation *TAG* (*N*=6,471)	**B**	-0.01	>-0.01	-0.13	-0.16	-0.02	-0.01	<.001	.070	.071
	(SE)	(0.03)	(<0.01)	(0.05)	(0.08)	(0.01)	(0.01)			
	p	.843	.016*	.015*	.049*	.197	.515			
PGS smoking initiation *GSCAN* (*N*=6,471)	**B**	-0.06	>-0.01	-0.10 (0.06)	-0.16 (0.09)	-0.03 (0.01)	-0.02 (0.01)	.001	.005	.007
	(SE)	(0.03)	(<0.01)							
	p	.031*	.258	.089	.076	.066	.150			
PGS cigarettes/ day *TAG* (*N*=3,096)	**B**	-0.08	<0.01	-0.03	0.05	<0.01	<0.01	<.001	.009	.009
	(SE)	(0.04)	(<0.01)	(0.08)	(0.14)	(0.02)	(0.02)			
	p	.039*	.919	.751	.697	.894	.923			
PGS cigarettes/ day *GSCAN* (*N*=3,096)	**B**	-0.09	0.01 (<0.01)	0.16	0.19	-0.03 (0.02)	-0.03 (0.02)	<.001	.009	.009
	(SE)	(0.04)		(0.08)	(0.14)					
	p	.025*	.104	.034*	.172	.170	.088			
PGS alcohol/ week *Clarke* (*N*=6,174)	**B**	0.03	<0.01	0.04	0.07	0.02	-0.03	.001	.007	.008
	(SE)	(0.03)	(<0.01)	(0.06)	(0.09)	(0.01)	(0.01)			

	p	.364	.466	.546	.457	.130	.069			
PGS alcohol/ week *GSCAN* (*N*=6,174)	**B**	-0.01	0.01 (<0.01)	0.11	0.23	<0.01	-0.01	.001	.007	.008
	(SE)	(0.03)		(0.06)	(0.09)	(0.01)	(0.01)			
	p	.673	.016*	.068	.010**	.940	.379			
PGS cannabis initiation *ICC* (*N*=5,676)	**B**	-0.01	>-0.01	-0.04	-0.09	0.01	0.01	<.001	.004	.004
	(SE)	(0.03)	(<0.01)	(0.09)	(0.09)	(0.01)	(0.02)			
	p	.704	.254	.496	.316	.481	.753			

[a] Reference category for sex was female and for both cohort variables the reference category was cohort1 (born before <1960).

*p<.05, **p<.0125 (significant after Bonferroni correction)

Cohort2=born between 1960-<1980, Cohort3=born ≥1980; Gen=genetic covariates (batch and genetic principal components); PGS=polygenic score; Metro=metropolitan factor; SES=socioeconomic status factor

3.2 Gene-environment interaction and correlation

One significant GxE effect was observed (model 3, Table 3), between the alcohol PGS based on Clarke et al. (2017) and the SES factor on alcohol per week. SES did not have a main effect on alcohol use. The slope for low SES (-1 SD) was not significantly different from zero (B=-0.20, p=.179), but the slope for high SES (+1 SD) was (B=0.27, p=.007), indicating that the PGS only had an effect on alcohol use for individuals with a high SES factor.

There was no significant rGE with the neighborhood variables (Table 4). Furthermore, there was unexpected rGE of sex, age, and cohort with different PGS. The positive rGE between cohort and the GSCAN PGS for alcohol per week survived correction for multiple testing.

4. Discussion

We found a negative association between the metropolitan factor and smoking initiation, indicating that chances of smoking initiation were lower in metropolitan areas. This finding follows patterns of higher smoking prevalence in rural areas as reported in some studies (Li et al., 2009) but contradicts those in others (Idris et al., 2007). Possibly, the urban-rural distinction means something different in different studies. For example, what constitutes a rural area in the Netherlands is quite different from that in countries with a lower population density. For cigarettes per day, in turn, there was a positive association with the metropolitan factor, suggesting that smokers in metropolitan areas smoke on average more cigarettes. Possibly, only individuals with a high vulnerability to becoming addicted start smoking in these areas, so that the average amount of smoked cigarettes becomes higher. Urban stress might contribute to these higher smoking levels (Idris et al., 2007). The SES factor showed small positive associations with both smoking variables, which is opposite to the pattern that is commonly reported (e.g., Chuang et al., 2005). This finding might be spurious or might be due to some unique feature of the research population, such as its relatively high age.

Alcohol use was higher in metropolitan areas, which may be due to a higher alcohol outlet density (Kuntsche et al., 2008). In contrast to studies showing positive (Galea et al., 2007) or negative (Karriker-Jaffe et al., 2013) association with neighborhood SES, we did not find an effect of our SES factor on alcohol use. This might be due to our use of an aggregate measure of alcohol consumption. One recent study showed that alcohol use frequency (how often someone drinks alcohol) was positively genetically correlated with SES measures, whereas alcohol use quantity (how much alcohol is consumed per occasion) was genetically negatively correlated with SES, suggesting these phenotypes represent distinct underlying vulnerabilities (Marees et al., 2019). In a similar vein, we only considered alcohol consumption levels ≤70 glasses per week, with most participants showing moderate alcohol use (M=6.0 glasses per week). Association patterns for measures of more extreme forms of alcohol use might be quite different (Karriker-Jaffe et al., 2018).

For lifetime cannabis use, there was a significant positive effect of the SES factor, which is in line with some previous findings (Galea et al., 2007) but in contrast with a study in cannabis use disorder (Buu et al., 2009). It appears that different cannabis use phenotypes show different associations with SES measures. Indeed, experimentation with cannabis is higher among people with higher education levels (at least in the Netherlands, CBS, 2010).

We confirmed that substance use can be predicted by PGS created based on an independent sample, but the PGS explained only 0.2-1.1% of the variance in their respective phenotype. Variance explained by PGS is often small, because PGS contain the sum of both true effects and error components. Also, their effect depends on the heritability of the trait, which is somewhat modest in the case of substance use. The PGS in this study were based on discovery GWAS with varying sample sizes. In general, it is expected that PGS based on larger GWAS would be more powerful (Dudbridge, 2013). Therefore, it is remarkable that the use of a larger discovery GWAS (GSCAN) hardly increased the predictive power of the PGS. It must be noted that the PGS were based on partly overlapping discovery samples; results of PGS based on other independent samples might be different. This suggests that future GWAS should not focus solely on increasing sample sizes, but should for example also focus on using homogeneous, reliable phenotype measures (Dudbridge, 2013; Manolio et al., 2009; Wainschtein et al., 2019).

There was an interaction between the PGS based on Clarke et al. (2017) and SES on alcohol use, such that genetic risk only came to expression when neighborhood SES was high. As there was no main effect of SES it is difficult to interpret this finding. Assuming that high SES generally acts as a risk factor for alcohol use (Galea et al., 2007), our GxE finding is in line with diathesis-stress or differential susceptibility frameworks, stating that individuals that are already at risk genetically will react more strongly to environmental risk (Belsky and Pluess, 2009). The only previous study that used PGS to test GxE with neighborhood factors in substance use found indications for GxE in the same direction (Meyers et al., 2013).

However, it needs to be pointed out that this was only one of the 14 tested interactions, and it explained a very small amount of variance in alcohol use (less than 0.1%). The same interaction did not reach significance when using the GSCAN PGS. This difficulty detecting GxE might be due to the fact that only SNPs that had a main effect on substance use in the GWAS ended up in the PGS, whereas potentially more relevant SNPs for GxE may be those that have an effect on differential susceptibility rather than on substance use per se (Fox and Beevers, 2016). It is also a possibility that GxE effects are different in other (earlier) developmental periods than during one's late forties, which was the average age of our sample (Kendler et al., 2011; Samek et al., 2017). Although we controlled for age and cohort effects, we deemed sample size insufficient to test such three-way interactions. For main and two-way GxE effects power seemed reasonable: assuming an effect size of $f2 = 0.005$, power was estimated to range between 66-98% (see Supplementary Table S6), but it is possible that true effects are even smaller. If that is so, GxE might not be as

important in the etiology of substance use as has traditionally been predicted. Indeed, a recent review of studies that used polygenic measures of genetic risk showed that the evidence for GxE in substance use is still weak (Pasman et al., 2019). More studies will be needed to establish the robustness of GxE effects in this context.

There was no strong evidence for gene-environment correlation (rGE), although there were some interesting patterns. First, there was small non-significant rGE between SES and the PGS for alcohol use based on Clarke et al. (2017; p=.069), which is potentially relevant as there was also gene-environment interaction (GxE) between these variables in the alcohol use analysis. Secondly, there were some unexpected rGE relationships between the covariates and PGS. Although they did not survive correction for multiple testing, there was a pattern of rGE between age/ cohort and the different smoking PGS. These effects might be due to genetic overlap between smoking phenotypes and educational attainment, as education level was higher in the later cohorts ($\chi 2[16]=2,409$, $p<.001$) and for lower ages ($b=-0.60$, $SD=.07$, $p<.001$). It might also be the case that the PGS constituted a better measure for risk for smoking behavior in the older cohorts, as they were largely based on GWAS with earlier born participants (Tobacco and Genetics Consortium, 2010; Liu et al., 2019). The negative rGE between sex and the smoking PGS might be spurious or represent an actual gender difference in the genetic architecture of this trait (Gilks et al., 2014). The only rGE that survived correction for multiple testing was between the GSCAN PGS for alcohol per week and cohort, such that being born in 1980 or later was associated with a higher PGS as compared to being born before 1960. Speculatively, this might be due to decreasing alcohol use in western countries in recent years (World Health Organization, 2018). It might be the case that among younger cohorts only vulnerable individuals consume alcohol, which increases the genetic contribution to this phenotype and would result in higher PGS in this group. Regardless of the interpretation, these findings show that rGE might exist, and that these effects have to be taken into account when studying GxE.

4.1 Conclusions

The current study confirmed that substance use was associated with genetic risk and characteristics of the neighborhood. We found some indication for GxE, such that the effect of genetic risk for substance use could be augmented by environmental risk. Furthermore, there were weak indications of rGE effects. More research into the relationships between neighborhood characteristics and substance use outcomes might help to select stronger neighborhood predictors, increasing the chance to detect GxE effects. Furthermore, more attention should be given to possible rGE effects. Knowledge of gene-environment interplay could help prevent genetic vulnerability from coming to

expression, providing clues on which people in which neighborhoods will need intervention the most.

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Supplementary Materials

Full Supplementary Materials can also be viewed online at:
Chapter 10 – Gene-environment interaction with neighborhood SES

or copy this link into the browser:
https://drive.google.com/drive/folders/1eUNcTao4bdTZHOZgSDjoUmtx3MzG-IRP

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Table S1. Descriptive statistics of predictor, outcome, and control variables per measurement wave.

		Survey 5 M(SD)/%	**Survey 6** M(SD)/%	**Survey 7** M(SD)/%	**Survey 8** M(SD)/%	**Survey 10** M(SD)/%	**ANOVA**[c] *p*-value	**Total**
predictors	Average monthly income	2333 (794)	2278 (786)	2257 (837)	2675 (878)	2750 (931)	<.001	2624 (913)
	Housing value (*10,000)	146 (76)	153 (76)	156 (71)	210 (102)	217 (103)	<.001	200 (101)
	% Immigrants[a]	0.61 (1.03)	0.69 (1.13)	0.81 (1.25)	0.87 (1.27)	0.95 (1.25)	<.001	0.88 (1.22)
	Urbanicity[b]	2.11 (1.41)	2.02 (1.45)	2.06 (1.38)	1.82 (1.39)	1.93 (1.37)	.006	1.95 (1.39)
Outcomes	Smoking initiation (% yes)	45	45	51	52	44	.001	45
	Cigarettes/ day	11 (8)	13 (8)	11 (9)	15 (9)	13 (9)	<.001	14 (9)
	Alcohol/ week	6 (8)	6 (7)	2 (5)	6 (7)	8 (7)	<.001	6 (7)
	Cannabis initiation (% yes)	28	-	-	10	22	<.001	20
Control	Sex (% female)	65	62	59	65	66	.097	65
	Age	28 (9)	36 (14)	43 (15)	48 (15)	49 (15)	<.001	45 (16)
	Cohort[d]	10/ 79	29/ 61	46/ 52	47/ 46	46/ 40	<.001	41/ 47

[a] 1=less than 5%, 2=5-10%, 3=11-20%, 4=20-40%, 5=more than 40%

[b] 1=less than 500, 2=500-1000, 3=1000-1500, 4=1500-2500, 5=more than 2500 addresses/ km^2

[c] For the categorical variables (smoking initiation, cannabis initiation, sex, and cohort), χ^2 tests were used to test differences across the measurement waves. No standard deviations are provided for these variables.

[d] For cohort the percentages of participants in the first 2 cohorts are given

Table S2. Measurement of the substance use outcomes.

Outcome	Operationalization	Survey	Answer
Smoking initiation	Ever smoking	10	Dichotomous[a]
	Ever smoking	8	Dichotomous
	Ever smoking	7	Dichotomous
	Ever smoking	6	Dichotomous
	Ever smoking	5	Dichotomous
Cigarettes per day	Maximum at heaviest period of smoking	10	Continuous
	Maximum at heaviest period of smoking	8	Continuous
	Maximum at heaviest period of smoking	7	Continuous
	Average amount for current and ex-smokers	6	<1, 1-5, 6-10, 11-20, 21-30, >30
	Average amount for current and ex-smokers	5	<1, 1-5, 6-10, 11-20, 21-30, >30
Alcohol use (N=6986)	Sum of beer, wine, and liquor per week in past year	10	Continuous
	Sum of glasses per day in past year	8	Continuous
	Glasses per week on average	7	<1, 1-5, 6-10, 11-15, 16-20, 21-40, >40
	Glasses per week on average	6	<1, 1-2, 3-5, 6-10, 11-20, 21-40, >40
	Glasses per week on average	5	<1, 1-2, 3-5, 6-10, 11-20, 21-40, >40

Cannabis initiation (N=5102)	Ever and past year use	10	Dichotomous
	Ever experimental use	8	Dichotomous
	Ever experimental use	5	Dichotomous

[a] All dichotomous variables had the values 0 'No' or 1 'Yes'.
[b] Al continuous variables were open-ended.

Table S3a. Pearson correlations between standardized and multiple imputed variables that comprise the SES and metropolitan factors

	Housing value	Income	Urbanicity	%Immigrants
Housing value		.59**	-.12**	-.07**
Income	.59**		.06**	-.03*
Urbanicity	-.12**	.06**		.34**
%Immigrants	-.07**	-.03*	.34**	

Table S3b. Pearson correlations between original non-imputed neighborhood variables

	Housing value	Income	%Low inc	%High inc	%Benefits	Urbanicity	%Immigrants
Housing value		.54**	-.34**	0.25**	-.25**	-.12**	-.07**
Income	.54**		-.51**	.65**	-.27**	.08**	-.02
%Low income	-.34**	-.51**		-.16**	.38**	-.03	-.01
%High income	0.25**	.65**	-.16**		.07	.12**	-.00
%Benefits	-.25**	-.27**	.38**	.07		.02	.16**
Urbanicity	-.12**	.08**	-.03	.12**	.02		.34**
%Immigrants	-.07**	-.02	-.01	-.00	.16**	.34**	

*p<.05, **p<.01, ***p<.001

Tabel S4a. Full results of the main and GxE effects on the smoking phenotypes.

		Smoking initiation *TAG* (N=7,312)		**Smoking initiation *GSCAN* (N=7,312)**		**Cigarettes per day *TAG* (N=3,421)**		**Cigarettes per day *GSCAN* (N=3,421)**	
Model	**variable**	**OR (SE)**	***p***	**OR (SE)**	***p***	**B (SE)**	***p***	**B (SE)**	***p***
0	Sex[a]	1.39 (0.32)	<.001**	1.39 (0.32)	<.001**	2.39 (0.34)	<.001**	2.39 (0.34)	<.001**
	Age	0.99 (0.02)	.084	0.99 (0.02)	.084	0.02 (0.02)	.354	0.02 (0.02)	.354
	Cohort 1960-<1980[a]	0.32 (0.40)	<.001**	0.32 (0.40)	<.001**	-0.54 (0.62)	.391	-0.54 (0.62)	.391
	Cohort ≥1980[a]	0.14 (0.10)	<.001**	0.14 (0.10)	<.001**	-2.45 (1.02)	.016*	-2.45 (1.02)	.016*
	Model R^2	.114[b]		.114[b]		0.024		0.024	
1	Sex[a]	1.39 (0.32)	<.001**	1.39 (0.32)	<.001**	2.35 (0.33)	<.001**	2.35 (0.33)	<.001**
	Age	1.00 (0.02)	.296	1.00 (0.02)	.296	0.02 (0.02)	.444	0.02 (0.02)	.444
	Cohort 1960-<1980[a]	0.33 (0.15)	<.001**	0.33 (0.15)	<.001**	-0.72 (0.62)	.249	-0.72 (0.62)	.249
	Cohort ≥1980[a]	0.15 (0.11)	<.001**	0.15 (0.11)	<.001**	-2.68 (1.02)	.008**	-2.68 (1.02)	.008**
	Metropolitan factor	0.90 (0.10)	<.001**	0.90 (0.10)	<.001**	0.31 (0.20)	.116	0.31 (0.20)	.116

	SES factor	1.04 (0.12)	.144	1.04 (0.12)	.144	0.69 (0.17)	<.001**	0.69 (0.17)	<.001**
	Model R^2	.117, Δ=.003[b]		.117, Δ=.003[b]		.033, Δ=.009		.033, Δ=.009	
2	Sex[a]	1.44 (0.33)	<.001**	1.44 (0.33)	<.001**	2.41 (0.33)	<.001**	2.49 (0.33)	<.001**
	Age	1.00 (0.02)	.385	1.00 (0.02)	.391	0.02 (0.02)	.407	0.01 (0.02)	.558
	Cohort 1960-<1980[a]	0.33 (0.15)	<.001**	0.33 (0.15)	<.001**	-0.74 (0.62)	.234	-0.97 (0.61)	.111
	Cohort ≥1980[a]	0.16 (0.12)	<.001**	0.16 (0.12)	<.001**	-2.59 (1.02)	.011**	-2.82 (1.00)	.005**
	Metropolitan factor	0.91 (0.10)	.001**	0.91 (0.10)	.001**	0.28 (0.19)	.142	0.32 (0.19)	.089
	SES factor	1.06 (0.12)	.050*	1.06 (0.12)	.030*	0.67 (0.17)	<.001**	0.71 (0.17)	<.001**
	PGS	1.42 (0.17)	<.001**	1.42 (0.17)	<.001**	0.43 (0.17)	.008**	1.37 (0.17)	<.001**
	Illumina 660[a]	1.11 (0.33)	.262	1.11 (0.33)	.152	0.17 (0.43)	.693	0.17 (0.17)	.541
	Illumina 1M[a]	0.90 (0.77)	.598	0.90 (0.77)	.633	0.49 (1.49)	.743	0.49 (1.48)	.741
	Perlegen-Affymetrix[a]	1.04 (0.35)	.834	1.04 (0.35)	.653	0.09 (0.47)	.846	0.02 (0.46)	.976
	GONL sequence data[a]	0.87 (0.52)	.346	0.87 (0.52)	.343	-1.29 (0.93)	.169	-1.29 (0.95)	.139
	PC1	0.05 (9.54)	.457	0.05 (9.54)	.264	-41.91 (14.52)	.004**	-33.57 (14.49)	.021*
	PC2	31.20 (9275.32)	.174	31.20 (9275.23)	.236	35.47 (15.36)	.021*	32.62 (15.06)	.030*
	PC3	5214.80 (>10000)	.004**	5105.12 (>1000000)	.004**	2.42 (16.26)	.882	-0.13 (15.97)	.993
	PC4	0.14 (50.04)	.551	0.14 (50.04)	.510	46.74 (18.27)	.011*	37.29 (17.81)	.036*
	PC5	9.60 (4774.71)	.412	9.60 (4774.69)	.475	-1.23 (17.45)	.944	-7.46 (17.08)	.662
	PC6	0.56 (252.93)	.650	0.56 (252.94)	.852	1.85 (16.90)	.913	1.30 (16.59)	.938
	PC7	1.13 (474.38)	.908	1.13 (474.38)	.968	-4.34 (17.45)	.803	-2.60 (17.17)	.880
	PC8	3.03 (1396.09)	.687	3.03 (1396.08)	.723	4.05 (17.97)	.822	6.21 (17.63)	.725
	PC9	1.75 (781.05)	.857	1.75 (781.05)	.858	-40.79 (17.86)	.022*	-33.19 (17.69)	.061
	PC10	1.82 (843.10)	.923	1.82 (843.10)	.849	-7.42 (17.72)	.676	-10.51 (17.39)	.546
	Model R^2	.125, Δ=.008[b] (Δ_{PGS}=.004)[b]		.152, Δ=.038[b] (Δ_{PGS}=.004)[b]		.040, Δ=.007 (Δ_{PGS}=.002)		.060, Δ=.027 (Δ_{PGS}=.003)	

3	Sex[a]	1.44 (0.33)	<.001**	1.44 (0.33)	<.001**	2.40 (0.33)	<.001**	2.49 (0.33)	<.001**
	Age	1.00 (0.01)	.388	1.00 (0.01)	.389	0.02 (0.02)	.399	0.01 (0.02)	.591
	Cohort 1960-<1980[a]	0.33 (0.15)	<.001**	0.33 (0.15)	<.001**	-0.73 (0.62)	.238	-0.99 (0.61)	.103
	Cohort ≥1980[a]	0.16 (0.12)	<.001**	0.16 (0.12)	<.001**	-2.57 (1.02)	.011*	-2.84 (0.99)	.004**
	Metropolitan factor	0.91 (0.10)	.001**	0.91 (0.10)	.001**	0.28 (0.19)	.148	0.32 (0.19)	.082
	SES factor	1.07 (0.12)	.049*	1.07 (0.12)	.030*	0.67 (0.17)	<.001**	0.72 (0.17)	<.001**
	PGS	1.42 (0.17)	<.001**	1.42 (0.17)	<.001**	0.43 (0.16)	.009**	1.38 (0.17)	<.001**
	PGS* metropolitan	1.00 (0.12)	.567	1.00 (0.12)	.984	0.04 (0.15)	.802	0.31 (0.17)	.068
	PGS*SES	0.98 (0.11)	.582	0.98 (0.11)	.475	0.19 (0.16)	.214	0.23 (0.17)	.172
	Illumina 660[a]	1.11 (0.33)	.262	1.11 (0.33)	.149	0.16 (0.43)	.701	0.29 (0.42)	.492
	Illumina 1M[a]	0.91 (0.77)	.615	0.91 (0.77)	.640	0.48 (1.49)	.746	0.59 (1.48)	.688
	Perlegen-Affymetrix[a]	1.04 (0.35)	.844	1.04 (0.35)	.658	0.09 (0.47)	.848	0.05 (0.46)	.915
	GONL sequence data[a]	0.87 (0.52)	.346	0.87 (0.52)	.342	-1.28 (0.94)	.172	-1.40 (0.95)	.140
	PC1	0.05 (9.36)	.436	0.05 (9.36)	.263	-41.70 (14.51)	.004**	-33.47 (14.47)	.021*
	PC2	31.47 (9404.93)	.177	31.47 (9404.93)	.240	35.66 (15.38)	.020*	33.12 (15.01)	.027*
	PC3	5265.86 (>10000)	.004**	5265.86 (>100000000)	.004**	2.85 (16.90)	.861	-1.67 (15.94)	.917
	PC4	0.14 (49.20)	.558	0.14 (49.20)	.506	46.60 (18.26)	.011**	36.33 (17.80)	.041*
	PC5	9.37 (4675.05)	.413	9.37 (4675)	.480	-1.51 (17.43)	.931	-7.22 (17.02)	.671
	PC6	0.56 (252.65)	.643	0.56 (252.65)	.851	2.17 (16.90)	.898	1.28 (16.60)	.671
	PC7	1.06 (446.30)	.905	1.06 (446.30)	.985	-3.63 (17.39)	.835	-2.16 (17.17)	.900
	PC8	3.12 (1440.86)	.692	3.12 (1440.86)	.716	4.39 (17.99)	.807	6.72 (17.58)	.702
	PC9	1.72 (771.24)	.874	1.72 (771.24)	.863	-40.68 (17.87)	.023*	-34.07 (17.72)	.055
	PC10	1.80 (840.50)	.925	1.80 (840.50)	.851	-7.18 (17.70)	.685	-10.68 (17.37)	.539
	Model R^2	.125 (Δ<.001)[b]		.153 (Δ=.001)[b]		.040 (Δ<.001)		.061 (Δ=.001)	

[a] Reference category for sex was female, for both cohort variables the reference category was <1960, for batch it was Affymetrix 6.0

Tabel S4b. Full results of the main and GxE effects on the the alcohol and cannabis phenotypes.

		Alcohol per week (N=6,851)		Alcohol per week (N=6,851)		Cannabis initiation (N=5,099)	
Model	**variable**	**B (SE)**	***p***	**B (SE)**	***p***	**OR (SE)**	***p***
0	Sex[a]	4.17 (0.21)	<.001**	4.17 (0.21)	<.001**	1.66 (0.50)	<.001**
	Age	0.06 (0.01)	<.001**	0.06 (0.01)	<.001**	0.95 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.62 (0.38)	.100	-0.62 (0.38)	.100	1.30 (1.13)	<.001**
	Cohort ≥1980[a]	0.84 (0.54)	.121	0.84 (0.54)	.121	1.05 (1.47)	.030*
	Model R^2		0.118		0.118		.136[b]
1	Sex[a]	4.18 (0.21)	<.001**	4.18 (0.21)	<.001**	1.63 (0.50)	<.001**
	Age	0.05 (0.01)	<.001**	0.05 (0.01)	<.001**	0.96 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.81 (0.38)	.032*	-0.81 (0.38)	.032*	1.61 (1.06)	.004**
	Cohort ≥1980[a]	0.49 (0.55)	.379	0.49 (0.55)	.370	1.46 (1.37)	.104
	Metropolitan factor	0.46 (0.11)	<.001**	0.46 (0.11)	<.001**	1.07 (0.16)	.092
	SES factor	0.09 (0.09)	.294	0.09 (0.09)	.294	1.49 (0.23)	<.001**
	Model R^2		.122, Δ=.004		.122, Δ=.004		.170, Δ=.034[b]
2	Sex[a]	4.19 (0.20)	<.001**	4.17 (0.21)	<.001**	1.65 (0.51)	<.001**
	Age	0.05 (0.01)	<.001**	0.06 (0.01)	<.001**	0.96 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.96 (0.37)	.010**	-0.90 (0.38)	.017*	1.66 (1.09)	.002**
	Cohort ≥1980[a]	0.22 (0.55)	.691	0.36 (0.55)	.516	1.55 (1.47)	.062
	Metropolitan factor	0.47 (0.11)	<.001**	0.46 (0.11)	<.001**	1.07 (0.16)	.070
	SES factor	0.12 (0.09)	.177	0.13 (0.09)	.167	1.49 (0.23)	<.001**
	PGS	0.78 (0.09)	<.001**	0.52 (0.09)	<.001**	1.26 (0.20)	<.001**
	Illumina 660[a]	-0.54 (0.23)	.017*	-0.54 (0.23)	.018*	0.97 (0.42)	.751
	Illumina 1M[a]	0.50 (0.62)	.418	0.47 (0.64)	.466	1.00 (1.16)	.992
	Perlegen-Affymetrix[a]	-0.09 (0.29)	.753	-0.06 (0.29)	.828	0.86 (0.45)	.271
	GONL sequence data[a]	-0.96 (0.49)	.049*	-0.92 (0.50)	.063	0.67 (0.64)	.094
	PC1	19.52 (8.41)	.020*	17.08 (8.48)	.044*	0.38 (582.89)	.795
	PC2	5.66 (8.44)	.502	3.64 (8.49)	.668	0.30 (414.88)	.742
	PC3	16.13 (9.43)	.087	15.74 (9.50)	.097	190.76 (>10000)	.198

	PC4	10.83 (9.44)	.251	11.43 (9.47)	.227	684196.20 (>10000)	.001**
	PC5	3.48 (9.26)	.708	2.33 (9.28)	.802	0.03 (182.91)	.436
	PC6	9.41 (9.18)	.305	10.07 (9.28)	.278	0.01 (64.91)	.320
	PC7	2.97 (9.19)	.747	1.91 (9.24)	.836	0.01 (35.80)	.250
	PC8	12.87 (9.05)	.155	14.26 (9.13)	.118	321.50 (>10000)	.172
	PC9	-0.15 (9.99)	.988	-0.60 (10.05)	.952	15382.72 (>10000)	.026*
	PC10	-2.83 (9.37)	.763	-3.16 (9.48)	.739	27.77 (>10000)	.443
	Model R^2		.137, Δ=.015) (Δ_{PGS}=.002)		.130, Δ=.008 (Δ_{PGS}=.006)		.189, Δ=.019[b] (Δ_{PGS}=.011)[b]
3	Sex[a]	4.20 (0.20)	<.001**	4.20 (0.20)	<.001**	1.65 (0.51)	<.001**
	Age	0.05 (0.01)	<.001**	0.05 (0.01)	<.001**	0.96 (0.02)	<.001**
	Cohort 1960-<1980[a]	-0.97 (0.37)	.010**	-0.97 (0.37)	.010**	1.67 (1.10)	.002**
	Cohort ≥1980[a]	0.22 (0.55)	.684	0.22 (0.55)	.684	1.57 (1.49)	.057
	Metropolitan factor	0.47 (0.11)	<.001**	0.47 (0.11)	<.001**	1.06 (0.17)	.113
	SES factor	0.12 (0.09)	.182	0.12 (0.09)	.182	1.49 (0.23)	<.001**
	PGS	0.77 (0.09)	<.001**	0.77 (0.09)	<.001**	1.26 (0.20)	<.001**
	PGS* metropolitan	-0.03 (0.09)	.714	-0.03 (0.09)	.714	1.05 (0.16)	.182
	PGS*SES	0.14 (0.08)	.079	0.14 (0.08)	.079	1.01 (0.15)	.836
	Illumina 660[a]	-0.55 (0.23)	.016*	-0.55 (0.23)	.017*	0.96 (0.42)	.739
	Illumina 1M[a]	0.50 (0.62)	.421	0.46 (0.64)	.477	1.01 (1.17)	.969
	Perlegen-Affymetrix[a]	-0.09 (0.29)	.766	-0.03 (0.10)	.803	0.86 (0.46)	.272
	GONL sequence data[a]	-0.97 (0.49)	.047*	-0.94 (0.50)	.057	0.67 (0.65)	.093
	PC1	19.43 (9.40)	.021*	16.83 (8.46)	.047*	0.35 (541.13)	.778
	PC2	5.92 (8.44)	.483	4.57 (8.49)	.591	0.29 (408.30)	.741
	PC3	16.24 (9.43)	.085	15.53 (9.48)	.102	175.91 (>10000)	.205
	PC4	10.68 (9.43)	.257	11.57 (9.44)	.220	663975.15 (>10000)	.001**
	PC5	3.40 (9.25)	.714	2.52 (9.25)	.785	0.03 (185.68)	.438
	PC6	9.41 (9.16)	.304	9.87 (9.25)	.286	0.01 (67.42)	.324
	PC7	2.98 (9.18)	.745	2.06 (9.24)	.824	0.01 (33.28)	.243

PC8	12.62 (9.04)	.163	14.16 (9.11)	.120	308.89 (>10000)	.175
PC9	-0.09 (9.98)	.993	-0.79 (10.03)	.937	14764.78 (>100000)	.027*
PC10	-2.99 (9.38)	.750	-3.60 (9.47)	.704	27.49 (>10000)	.444
Model R^2	.137 (Δ<.001)		.137 (Δ<.001)		.190 (Δ=.001)[b]	

[a] Reference category for sex was female, for both cohort variables the reference category was <1960, for batch it was Affymetrix 6.0

Table S5. Parameters for the batch variables and principal components for population stratification as included in the rGE analyses.

		Illumina 660[a]	Illumina 1M[a]	Perl Affym[a]	GONL seq[a]	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PGS smoking initiation *TAG*	**B**	0.03	-0.03	0.05	0.16	-23.19	2.43	-2.12	3.99	-1.02	-0.26	1.06	-0.59	-2.38	-1.16
	(SE)	(0.03)	(0.11)	(0.04)	(0.09)	(1.44)	(1.46)	(1.58)	(1.60)	(1.62)	(1.58)	(1.63)	(1.57)	(1.57)	(1.64)
	p	.352	.823	.266	.082	<.001**	.096	.180	.013*	.527	.867	.516	.710	.129	.477
PGS smoking initiation *GSCAN*	**B**	-0.04	-0.06	-0.04	0.08	5.26	2.50	-0.65	2.14	1.10	-2.43	-0.94	0.69	-0.93	-2.65
	(SE)	(0.04)	(0.12)	(0.04)	(0.08)	(1.45)	(1.46)	(1.56)	(1.67)	(1.68)	(1.66)	(1.65)	(1.64)	(1.68)	(1.67)
	p	.229	.617	.364	.361	<.001**	.086	.678	.200	.512	.145	.570	.675	.580	.113
PGS cigarettes/ day *TAG*	**B**	0.01	-0.1	-0.07	0.18	2.41	5.14	2.75	-1.57	-5.01	1.12	0.99	-7.63	0.80	0.78
	(SE)	(0.05)	(0.11)	(0.06)	(0.10)	(1.85)	(1.84)	(1.97)	(2.21)	(2.20)	(2.19)	(2.17)	(2.15)	(2.08)	(2.15)
	p	.804	.404	.223	.077	.193	.005**	.164	.478	.023*	.608	.650	<.001**	.702	.717
PGS cigarettes/ day *GSCAN*	**B**	-0.06	-0.03	0.03	-0.03	-5.33	3.68	2.72	6.40	2.98	0.75	-0.96	-3.96	-5.30	2.50
	(SE)	(0.05)	(0.15)	(0.05)	(0.15)	(1.81)	(2.00)	*1.96)	(2.15)	(2.14)	(2.03)	(2.13)	(2.26)	(2.16)	(2.15)
	p	.182	.842	.182	.842	.003**	.066	.165	.003	.163	.711	.651	.080	.014*	.244
PGS alcohol/ week *Clarke*	**B**	<0.01	0.09	-0.08	-0.16	4.00	0.97	1.57	1.29	5.20	0.85	3.00	1.23	-3.24	-0.15
	(SE)	(0.04)	(0.11)	(0.04)	(0.07)	(1.47)	(1.58)	(1.62)	(1.70)	(1.76)	(1.67)	(1.66)	(1.67)	(1.69)	(1.68)
	p	.959	.401	.071	.029*	.006**	.537	.331	.445	.003**	.611	.071	.462	.055	.930

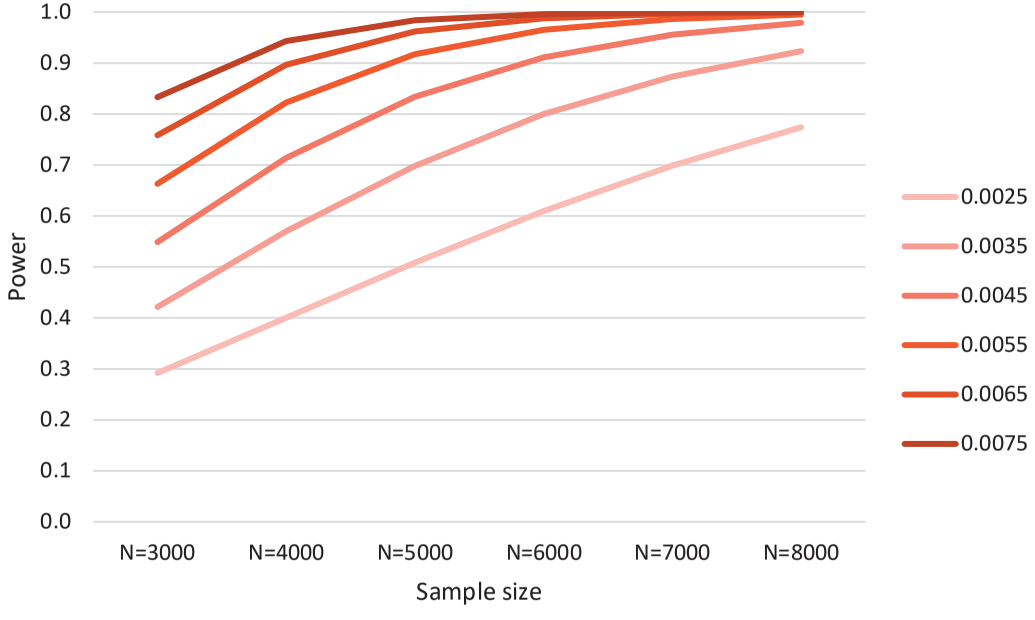
PGS alcohol/ week	**B**	<0.01	0.01	-0.02	-0.06	-0.48	-1.96	0.55	1.64	1.99	1.43	0.64	2.62	-2.74	-0.52
	(SE)	(0.04)	(0.12)	(0.04)	(0.09)	(1.43)	(1.52)	(1.58)	(1.70)	(1.66)	(1.67)	(1.66)	(1.65)	(1.67)	(1.72)
GSCAN	***p***	.940	.908	.705	.511	.738	.197	.728	.336	.230	.393	.702)	.112	.101	.761
PGS cannabis initiation	**B**	0.03	-0.05	-0.01	0.03	-2.4	-0.42	0.65	6.25	-2.98	-1.34	-0.09	2.97	-1.02	2.01
	(SE)	(0.04)	(0.13)	(0.05)	(0.11)	(1.57)	(1.68)	(1.66)	(1.71)	(1.74)	(1.74)	(1.68)	(1.77)	(1.77)	(1.72)
ICC	***p***	.426	.729	.814	.760	.127	.802	.694	<.001**	.087	.444	.955	.092	.565	.243

[a] Reference category for batch was Affymetrix 6.0
*p<.05; **p<.0125 (significant after Bonferroni correction)

Supplementary Table S6. Power levels achieved under different assumed effect sizes and sample sizes. Assuming the maximum 20 predictors (model 3) and an alpha level of .05. Estimated using post hoc power analysis in G*Power.

	Effect size f^2					
Sample size	*0.0025*	*0.0035*	*0.0045*	*0.0055*	*0.0065*	*0.0075*
N=3000	0.29	0.42	0.55	0.66	0.76	0.83
N=4000	0.40	0.57	0.71	0.82	0.90	0.94
N=5000	0.51	0.70	0.83	0.92	0.96	0.98
N=6000	0.61	0.80	0.91	0.96	0.99	1.00
N=7000	0.70	0.87	0.96	0.99	1.00	1.00
N=8000	0.77	0.92	0.98	1.00	1.00	1.00

Supplementary Figure S1. Plot of power as a function of sample size and effect size.



CHAPTER 11

Genetics and parenting interplay in adolescence and substance use in young adulthood: a TRAILS study

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Abstract

During adolescence many youth start using tobacco, alcohol and cannabis, a pattern that can persist into adulthood and can have deleterious health consequences. Genetic vulnerability, parent characteristics in young adolescence, and interaction (GxE) and correlation (rGE) between these factors can contribute to the development of substance use.

Using prospective data from the TRacking Adolescent Individuals' Lives Survey (TRAILS, N=1,649), we model latent parent characteristics (involvement, substance use, and the parent-child relationship) in young adolescence to predict young adult substance use. Polygenic scores (PGS) are created based on GWAS summary statistics for smoking, alcohol use, and cannabis use. Using Structural Equation Modelling (SEM) we model the direct, GxE, and rGE effects of parent factors and PGS on young adult smoking, alcohol use, and cannabis initiation.

High PGS, low parental involvement, high parental substance use, and low parent-child relationship quality predicted smoking. There was GxE such that a high PGS amplified the effect of parental substance on smoking. There was positive rGE between all parent factors and the PGS. Alcohol use was not significantly predicted by genetic or parent factors, nor by interplay between those. Cannabis initiation was predicted by the PGS and by high parental substance use, but there was no evidence for GxE or rGE.

We found evidence for the contribution of genetic risk and parent factors to smoking and cannabis initiation, and GxE and rGE for smoking. These findings can act as a starting point for identifying people at risk and formulating targets for prevention and intervention.

Keywords: gene-environment interaction; parenting; substance use; genetic nurturing

Introduction

Adolescence and young adulthood are periods marked by significant changes in youths' lives, and many youth start to experiment with substances (Arnett, 2000). Despite some decreases in recent years, many adolescents experiments with tobacco, alcohol, or cannabis, with substantial percentages progressing into regular use. Worldwide, about 15% of individuals below age 18 smoke (WHO, 2017). Over a quarter of adolescents aged 15-19 drink alcohol, and almost half of those engage in heavy episodic drinking (WHO, 2018). The annual prevalence of cannabis use among youths aged 15-16 is 14% in Europe and 12% in the Americas (UNODC, 2018). To some degree, these increases in substance use during adolescence and young adulthood can be understood as part of normal development, in which young people want to obtain a wide range of experiences before acquiring adult norms and behaviours (Arnett, 2000). However, among users of tobacco, alcohol, and cannabis, the chances of developing dependence may be as high as 67%, 23%, and 9%, respectively (Lopez-Quintero et al., 2011). Serious (mental) health risks have been associated with long-term use of these substances (Hall et al., 2016) and the associated disease burden is substantial (Degenhardt et al., 2013; Ezzati et al., 2002).

When trying to understand the emergence of substance use behaviour among young adults, research has proposed a dynamic cascade developmental model (Dodge et al., 2009). This model proposes that adolescent substance use develops through a complex of child and environmental factors that influence each other over the course of development. Important environmental risk factors that play a role during adolescence concern parental factors. Parents are significant role-models for their children, and indeed parental modelling of substance use predicts adolescent substance involvement (Li et al., 2002). Adolescence is a period marked by increased need for individuation and independence, which is associated with decreases in parental monitoring (Lionetti et al., 2019) as well as with temporary perturbations in parent-child relationships (De Goede et al., 2009). Both parental monitoring and the parent-child relationship may predict higher adolescent substance use. For example, having parents that are less inquisitive about the whereabouts of their child (i.e., low parental monitoring) predicts affiliation with deviant peers (Dodge et al., 2009) which in turn predicts substance use (Rai et al., 2003). Likewise, having parents that know less about the child's activities is associated with adolescent risk behavior, including alcohol use (Waizenhofer et al., 2004) and smoking (Harakeh et al., 2004). The parent-child relationship might directly and indirectly influence adolescents' substance use. One study reported that high parental support was related to lower adolescent substance use, and that this relationship was mediated by cognitive self-control (Wills et al., 2004). Also, a low quality parent-child relationship has been

associated with cannabis use (Creemers et al., 2011), smoking, and alcohol use (Simons-Morton et al., 2001; Visser et al., 2012).

There is evidence that if such parental risk factors are operating during adolescence, their effects on substance use can last well into young adulthood (for reviews, see Ryan et al., 2010; Stone et al., 2012). As an example, one study found that low parental monitoring, warmth, and high parental alcohol use in adolescence predicted binge drinking in early adulthood, seven years later (Donaldson et al., 2016). Many mechanisms seem to underlie such longitudinal associations. Parental warmth and monitoring have been found to prospectively influence substance use norms and beliefs, as well as increase self-regulation skills and decrease of susceptibility to peer influence (Baker & Hoerger, 2012; Lac et al., 2009; Ryan et al., 2010; Van Ryzin et al., 2012; Yang et al., 2013). As another example, exposure to parental alcohol use prospectively predicted more positive expectancies and attitudes toward alcohol (Smit et al., 2020), and being exposed to smoking in the household predicted lower perceived harm of tobacco a year later, which in turn predicted future smoking initiation (Rodriguez et al., 2007).

Genetic vulnerability also plays a role in the aetiology of substance use. Heritability estimates from family studies are moderate to high, with the exact estimate depending on developmental period (with lower estimates for youngsters) and whether the behaviour constitutes normative use or abuse/dependence (with higher estimates for the latter; Ducci & Goldman, 2012; Hopfer et al., 2003; Mbarek et al., 2015; Verweij et al., 2010; Vink et al., 2005). Molecular genetic studies have sought to trace these estimates back to specific genetic variants. Genome-wide association studies (GWAS) have identified many variants of small effect. The variance in a trait explained by all measured genetic variants together (SNP-based heritability) are not as high as heritability estimates based on twin research, with the most recent GWAS, for instance, showing a SNP-based heritability of 4% for alcohol use per week, 8% for cigarettes per day (Liu et al., 2019), and 11% for cannabis initiation (Pasman et al., 2018). Based on GWAS findings, polygenic scores (PGS) can be created to predict genetic risk of substance use in an independent group of individuals. Such scores count and weigh the number of risk alleles from each individual (by their effect estimates from GWAS), creating a personal genetic risk score.
Risk factors interact with each other on multiple levels (Dodge et al., 2009; Masten, 2006). In gene-environment interaction (GxE), genetic risk amplifies, diminishes, or even reverses the effect of environmental risk. Although there has been some research into GxE with parent factors in substance use, most have used the (single) candidate-gene method, which has been largely abandoned because most used underpowered designs and findings did not replicate in subsequent GWAS (Border et al., 2019; Duncan & Keller, 2011). Few PGS studies have been conducted to test GxE with parenting factors. One showed

that low parental knowledge was more likely to lead to alcohol problems when genetic risk was high (Salvatore, Aliev, Edwards, et al., 2014). Likewise, a PGS study testing externalizing behaviour (including substance use) showed that low parental monitoring predicted externalizing behaviour more strongly when genetic risk was high (Salvatore, Aliev, Bucholz, et al., 2014). Lastly, one study found that parental monitoring (in combination with low peer substance use) buffered for the effect of a smoking cessation PGS on smoking and cannabis use (Musci et al., 2015).

One explanation for the mixed findings in GxE research is the neglect of gene-environment correlation (rGE) effects, where an individual's genetic risk shows a relation to the level of exposure to environmental risk variables, thereby muddling GxE effects (Pasman et al., 2019). Parenting characteristics are likely to be influenced by the parents' genetic predisposition, and genetic factors important for parenting characteristics may to some extent overlap with genetic factors important for the trait under investigation in offspring (Kong et al., 2018). This is in line with older twin studies showing significant heritability for parenting and other family environment variables (Deater-Deckard et al., 1999; Elkins et al., 1997; Jang et al., 2001; Pérusse et al., 1994; Plomin et al., 1994). Also, one study found a genetic factor for substance use to be related to 'contextual risk' (including family functioning and the parent-child relationship; Hicks et al., 2013). These relationships might make it difficult to detect and interpret the presence of GxE. It has been demonstrated mathematically that rGE can lead to spurious GxE findings (Dudbridge & Fletcher, 2014).

The current study aims to expand knowledge of GxE and rGE mechanisms in the effects of genetic risk and parent environment on substance use, thereby using PGS as measures of genetic risk and incorporating GxE and rGE in a single model to assess their relative contribution. Investigating GxE and rGE is crucial, as these effects can confound the effects of both genetic and environmental factors. For example, if not explicitly modelled, GxE and rGE can present as main effects of G or E in twin research, leading to an overestimation of either effect (Purcell, 2002), and genetic association studies can pick up on environmental signal in the case of rGE (Selzam et al., 2019). Disentangling these mechanisms can provide directions for future intervention studies, for example showing the merits of intervening in parental behavior to prevent genetic vulnerability from coming to expression, or showing which genetic pathways are causally related to substance use independently from environmental confounders. Using prospective data from the TRacking Adolescents' Individuals Lives Survey (TRAILS), we study the joint effects of genetic risk and different parent factors during adolescence (parental involvement, parental substance use, parent-child relationship quality) on substance use in young adulthood (alcohol use, smoking, cannabis use).

Methods

This study's pre-registration can be found on Open Science Framework (https://osf.io/wv3kb). A description of the analyses scripts can be found in the Supplemental Note, and all scripts are published on GitHub (https://github.com/joellepasman/TRAILS_substanceuse/).

Participants

Data were derived from the ongoing TRacking Adolescents' Individual Lives Survey (TRAILS), which has been described in detail elsewhere (Oldehinkel et al., 2014). We used data from the first five waves, collected every two years from 2000 to 2013. For N=1,842 adolescents genetic were available. After genetic quality control and excluding individuals that had no data on parental characteristics, N=1,649 European-ancestry, unrelated individuals (47.1% female) remained. Average age at wave 1 was 11.1 years (SD=0.54, range 10.0-12.6) and at outcome 22.2 years (SD=0.66, range 20.7-24.1).

Genotyping

At wave 3, blood samples were collected in the adolescents. DNA was isolated and genotyped on a Golden Gate Illumina BeadStation 500 platform and using the HumanCytoSNP-12 BeadChip (Illumina Inc., San Diego, CA, USA). The genotype data (single-nucleotide polymorphisms, SNPs) were merged, checked for concordance for overlapping SNPs, and imputed against the 1000 Genomes Project Phase 3 global reference panel. All quality control steps were performed with PLINK v1.07 and v1.9 (Chang et al., 2015; Purcell et al., 2007). SNPs with a call rate below 95%, a minor allele frequency (MAF) below .05, missingness rates above 5%, and a Hardy-Weinberg disequilibrium p-value below 1E-06 were excluded. Individuals with more than 5% missingness on SNP data, individuals from non-European ancestry and family-related individuals (closer than 3rd degree) were removed. In order to control for population stratification effects, ten principal components for ancestry were created using multidimensional scaling. Alleles were aligned with 1000 Genomes, excluding SNPs that had MAFs deviating more than 0.15 from the reference set. Following these cleaning, quality control and selection procedures N= 7,781,794 SNPs and N=1,649 individuals remained.

Polygenic scores

For the genetic predictor variables, polygenic scores (PGS) were created. As source GWAS we used the largest studies available to date: from the Liu et al. (2019) GWAS we used summary statistics on having smoked on a regular basis (N= 1,232,091), cigarettes per day (N= 337,334), and alcohol consumption in glasses per week (N= 941,280); for cannabis we used summary statistics on lifetime cannabis use from Pasman et al., 2018 (excluding the TRAILS sample, N= 183,539). In order to use information on both smoking initiation and cigarettes per day for the smoking PGS we used multi-trait analysis of GWAS (MTAG). MTAG

jointly analyzes two or more genetically correlated traits, aggregating their signal and boosting power to detect genetic associations (Turley et al., 2018).

PGS are created by summing an individual's risk alleles per locus, weighted by the effect size as found in the source GWAS. However, these weights are not randomly distributed across the genome, due to interdependence between variants (linkage disequilibrium, LD). We used the GCTA-SBLUP tool (Robinson et al., 2017) to adjust the weights for the LD structure within the genome. As reference data for the LD structure we used a random sample of 10,000 European ancestry UK-Biobank participants, selecting a subset of high-quality HapMap 3 SNPs for computational efficiency. We used SNP-based heritability estimates retrieved from the original publications to estimate the model (4% for alcohol use and 11% for cannabis initiation; for the MTAG smoking phenotype we used 8%, which was the estimate for both smoking initiation and cigarettes per day). LD with SNPs more than 1Mb up- or downstream was ignored. In SBLUP it is not necessary to choose arbitrary p-value cut-offs or estimate what proportion of the genome should be considered in the PGS (as is necessary using in other methods); rather the whole genome is integrated in the score. In the final step the SBLUP-corrected variant weights were used to create individual-level PGS with the software tool PLINK (Chang et al., 2015).

Measures

Survey items used to measure all non-genetic variables are summarized in Table 1. The earliest measurement point of each variable was included as predictor variable. The parent predictors included measures of parental involvement, consisting of parental monitoring (control, solicitation, and child disclosure) and parental knowledge (Stattin & Kerr, 2000). Parental involvement variables were measured at wave 3 (age 16) and were all based on child-report. Parental substance use was measured at wave 1 (age 11) using parent-report and included measures of smoking, alcohol use, lifetime cannabis use, and addiction to any substance other than nicotine. Measures of the parent-child relationship at wave 1 (age 11) included child-reported warmth and rejection (subscales from the EMBU-C, Markus, 2003). If answers from (or about) both parents were available, these were averaged. All was scored in a hypothetically substance use increasing direction (see Table 1).

The child's substance use outcomes were measured in young adulthood at wave 5 (age 22). For smoking, we focused on daily smoking (yes/no), cigarettes per day, and nicotine dependence; for alcohol use we used glasses per week (in drinkers); for cannabis, we used cannabis initiation (yes/no). These outcomes were the most similar to the traits measured in the discovery GWAS that were used to create the PGS.

Analyses

We sought to summarize the parent variables within underlying constructs. Using exploratory factor analyses (EFA) in Mplus 8.3 (Muthén & Muthén, 1998-2017), it was tested whether the parental variables clustered in the hypothesized latent constructs (parental involvement, parent-child relationship quality, and parental substance use). For the smoking outcomes, we tested whether the three variables clustered in a single smoking factor. With the results from the EFAs, a measurement model was defined, which was used in the structural model.

Using Mplus, we created three separate structural equation models (SEMs) for the three substance use outcomes. We used Full Information Maximum Likelihood (FIML) using the Maximum Likelihood Estimator with robust standard errors (MLR) to control for missing data and non-normality. First, the direct effects of the parental factors and PGS on young-adult substance use were assessed (model 1, purple arrow in Figure 1). Second, the moderating effects of the PGS (GxE) were added (model 2, blue arrow). The latent variable interactions between the parent factors and the PGS were computed using the XWITH statement. Significant interactions were followed up with simple slope analysis (Stride, 2015). Third, the gene-environment correlation pathways were added (rGE), while the moderating effects of the genetic factors were deleted (model 3, yellow arrow). Note that although these paths are called 'correlations,' we modelled them as a directional pathway (one-headed arrow), to investigate the effect of the PGS on parenting and not vice versa. Fourth, the GxE and rGE pathways were included in the same model, to assess their net effects (model 4). Control variables included age, sex, and ten genetic principal components (PCs) for ancestry. These latter variables measure population background, which was included to control for genetic similarities arisen because of subgroups of different ancestry within the Dutch population.

The fit of the four models was determined using commonly used model fit statistics, with acceptable fit defined as Root Mean Square Error of Approximation (RMSEA) <.08 (MacCallum et al., 1996), Comparative Fit Index (CFI) >.90, and Tucker Lewis Index (TLI) >.90 (Iacobucci, 2010). To compare the models, the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used, which are suitable for comparing non-nested models. AIC and BIC differences of >2 and >10, respectively, are thought to be a strong indication for model fit improvement (in case of a decrease) or deterioration (in case of an increase; Burnham & Anderson, 1998; Raftery, 1995). If AIC and BIC disagreed on what was the best fitting model, we prioritized BIC (Nylund et al., 2007). In the models including latent variable interactions, and models combining categorical indicators and categorical outcomes, only AIC and BIC, but not CFI, TLI, and RMSEA are computed. Moreover, in models combining categorical indicators with categorical outcomes, CFI, TLI, and RMSEA cannot computed in Mplus with the MLR estimator. In these models we used the WLSMV estimator to compute these fit indices. For individual

path parameters we adopted a conventional *p*-value threshold of $p<.05$. The separate tests for outcomes and parental predictors were not strictly independent and only models with adequate fit parameters were interpreted, foregoing the necessity of stringent correction for multiple testing.

Divergence from preregistration

There were several divergences from the plans as specified in the preregistration. Firstly, the plan was to use latent variables for all the substance use outcomes. Although model fit for the latent alcohol use factor was good, the use of dichotomous and continuous variables within the same model led to non-convergence in some of the models. For the cannabis outcome we wanted to use cannabis initiation as well as Cannabis Use Problems Identification Test (CUPIT) scores, but due to high missingness and floor effects we could not use the latter. As specified in the preregistration we resorted to using the outcomes that were most similar to those in the GWAS used for the computation of the PGS. Secondly, the preregistered power calculations were based on larger sample size (N=1,842) than we had available in the analyses, due to the exclusion of relatives, non-European ancestry individuals, and individuals with high levels of phenotype missingness (final N=1,649). Thirdly, we could not estimate the SEMs including all latent variable interactions in the same model because the model did not converge. We had taken this possibility into account in the preregistration and followed the plan to present the models separately per parent factor.

Results

Parent characteristics were reasonably normally distributed, although parental warmth was high on average, and only few parents reported recent cannabis use or lifetime substance addiction (Table 2). The quarter of the young adults that indicated to ever have smoked daily smoked 8 cigarettes per day on average in the past four weeks and had a low to moderate nicotine dependence score. Participants drank about 8 glasses of alcohol per week and almost 60% indicated to have used cannabis. There were high correlations between parent variables and substance use outcomes, and between the PGS and covariates and other traits (Supplementary Table S2).

Measurement model

The exploratory factor analysis of the parent variables showed that the best fitting solution included 3 factors (see Table 3). The 4-factor solution had better fit, but the parsimony and the interpretability of the structure decreased (i.e., there was a factor with only one indicator). Thus we selected the 3-factor solution which showed clustering in the hypothesized constructs of parental involvement (indicated by parental control, solicitation, and knowledge, and child disclosure), parental substance use (smoking initiation, cannabis initiation, and lifetime addiction), and the parent-child relationship (parental rejection and warmth). We constructed the latent parent-child relationship factor by constraining the two factor loadings to be equal to ensure model identification. Parental alcohol use had no loadings larger than 0.1 on any factor and was excluded from further analysis. Although parental cigarettes per day did load on the parental substance use factor, we excluded this variable because simultaneously using categorical and continuous indicators in one factor led to computational issues. Excluding these variables resulted in the solution presented in Table 4. This model showed good fit, RMSEA=0.05, CFI=0.97, TLI=0.91. Variance explained in the observed variables by the factors ranged from 21.4% (for parental knowledge) to 62.3% (for parental solicitation), with an average of 42.2%. All factor loadings were significant, although the loading of parental knowledge on the first factor was low and this variable also loaded on the second factor. Because of the theoretical similarity to the variables in the first factor we decided to keep this variable in the first factor in the subsequent analyses. One of the most frequently observed modification suggestions was to add the correlation between parental knowledge and child disclosure. Reasoning that these concepts should be related we added this correlation in all relevant models.

For the young adult latent smoking factor, there were three indicators. Thus, the only possible factor solution contained one factor. All indicators loaded significantly on the smoking factor in the EFA, with 0.97 for daily smoking, 0.70 for cigarettes per day, and

0.81 for nicotine dependence. Fit indices were not interpretable because the model was just identified.

Structural equation models

Smoking factor

The fit statistics per model (main, GxE, rGE, and full) are presented in Table 5 (refer to Supplementary Table S1 for parameter estimates for paths in the best fitting model). Model fit only reached acceptable levels when the parental factors were regressed on the covariates sex and age. We added these paths in all subsequent models for all outcomes (as the same was observed for alcohol per week and cannabis initiation). The effect of sex on smoking was not significant; the effect of age showed higher smoking levels in older individuals. The smoking PGS significantly predicted young adult smoking.

With *parental involvement* as predictor, the model excluding GxE and including rGE showed the best fit (model 3). There was a main effect of parental involvement in mid-adolescence (such that higher involvement led to lower smoking in young adulthood) and an rGE between the young adult's smoking PGS and parental involvement (such that high genetic risk was associated with low parental involvement). Variance explained in the smoking factor by these paths was 13%.

With *parental substance use*, the full model (including GxE and rGE; model 4) showed the best fit. Simple slope analysis suggested that parental substance use in early adolescence significantly predicted young adulthood smoking when the PGS was low (1SD below the mean; b=.07, SE=.02, *p*=.002, β=.18), but that this effect became stronger when the PGS was high (1SD above the mean; b=.19, SE=.09, *p*=.036, β=.48). It needs to be noted that although significant in the standardized model results, the GxE effect exceeded the *p*=.05 threshold in the unstandardized model results (due to a different computation of SE), suggesting this effect should be interpreted with caution. There was significant rGE between parental substance use and the smoking PGS. Together, these effects explained 14% of the variance in smoking.

With the *parent-child relationship*, again the full model showed the best fit (model 4). A worse parent-child relationship in early adolescence was associated with more smoking in young adulthood. The GxE suggested that this relationship might become stronger when the young adult had a high PGS, but this effect was not significant (β=.10, *p*=.057). There was significant rGE between the parent-child relationship and the young adult's PGS. All paths together explained 10% of variance in the smoking factor.

The three best fitting smoking models are presented in Figure 2a. Note that the analyses were conducted separately per parent factor but are summarized in one figure. Summarizing, there were significant positive main effects of the PGS and all parent factors

on smoking, there was significant positive rGE between the PGS and all parent factors, and GxE with parental substance use. GxE with the parent-child relationship did not reach significance.

Alcohol per week

With *parental involvement* and the *parent-child relationship* as predictors the main models showed the best fit (model 1). Although with *parental substance use* the full model including rGE and GxE showed superior fit (model 4), these paths were not significant. The alcohol PGS did not significantly predict young adult alcohol per week (p=.069-.108 in the main effect models; model 1). Also, none of the early or mid-adolescence parenting factors predicted young adult alcohol per week (p=.460-.850). The best fitting models for alcohol per week are summarized in Figure 2b. The variance explained in alcohol per week by all paths was 12% for all three models. Sex effects (β=.32-.34) might have contributed strongly to the explained variance, showing that males used significantly more alcohol than females. Age had no significant effect on alcohol per week.

Cannabis initiation

Cannabis initiation was significantly predicted by the cannabis PGS, see Figure 2c. For all parent factors, the main model excluding rGE and GxE were the best fitting models (model 1). Low parental involvement in mid-adolescence did not significantly increase chances for cannabis initiation in young adulthood (β=.08, OR=1.70. p=.064). Parental substance use in young adolescence did have a significant effect, such that it was associated with a higher chance of cannabis initiation. There was no effect of the parent-child relationship in young adolescence. No evidence for rGE or GxE was found. In the models with parental substance use and parent-child relationship there was a significant effect of sex, such that males had a higher chance of having used cannabis. In all models there was a positive effect of age.

Discussion

This 11-year longitudinal study investigated the effect of and interplay between genetic risk and parental factors during adolescence in predicting substance use in young adulthood. Results indicated that young adult substance use is driven by a complex interplay between genetic and parental factors during early and middle adolescence, especially for smoking. Smoking was predicted by genetic risk (PGS), parental involvement, parental substance use, and the parent-child relationship. The effect of parental substance use was further augmented by the PGS (GxE). Additionally, there was evidence of gene-environment correlation between the parent factors and the smoking PGS (rGE). Alcohol use per week was not predicted by genetic risk, parent factors, or their interplay. Cannabis initiation was predicted by genetic risk and parental substance use separately, but not by any interplay between those.

Main effects of genetic and parent factors

Polygenic scores

The PGS for smoking behavior based on smoking initiation and cigarettes per day was a significant predictor of a latent factor for smoking behavior in young adults. Likewise, the cannabis PGS significantly predicted its own phenotype. However, the alcohol PGS did not predict alcohol use. This might be due to the fact that the PGS was based on GWAS in older adults, whose data were collected some time ago (Liu et al., 2019). Alcohol consumption rates have been declining in Europe (World Health Organization, 2018) and attitudes toward alcohol seem to become slowly more negative in the Western world (Keyes et al., 2012; Livingston & Callinan, 2017; Looze et al., 2015). Alcohol consumption in current youth has become less normative than in youth two decades ago (van Laar, 2020), which would have resulted in changes in the genetic risk profile. Also, there are indications that the genetic contribution to alcohol use increases with age, and that environmental factors are more important for this behavior in adolescents and young adults (Hopfer et al., 2003; van Beek et al., 2012). Finally, the alcohol use GWAS found low SNP-based heritability (4% of the variance in alcohol use was explained by all GWAS SNPs). In general, PGS already tend to explain small proportions of variance; the low SNP-heritability could have further decreased the power to detect an effect.

Parental involvement and the parent-child relationship

Lower parental involvement (comprised by knowledge, control, solicitation, and child disclosure) in middle adolescence significantly predicted smoking behavior (comprised by daily smoking, cigarettes per day, and nicotine dependence) in young adulthood. This is in line with previous literature showing cross-sectional effects of low parental

monitoring (Rai et al., 2003) and low parental knowledge on the children's whereabouts (Harakeh et al., 2004). Likewise, a lower quality parent-child relationship (comprised by higher rejection and lower warmth) in young adolescence significantly predicted higher young adult smoking levels, while controlling for the effects of parental substance use. This is in line with some previous literature (Harakeh et al., 2004; Piko & Balázs, 2012). There are several possible explanations for these effects. Harakeh and colleagues (2004) reported that a good parent-child relationship led to negative smoking attitudes and high refraining self-efficacy regardless of parenting smoking status, and this in turn led to lower current and future smoking. A good parent-child relationship has been associated with better mental health and self-control (Ackard et al., 2006; Phythian et al., 2008). Also, adolescents with a good relationship with their parents might be more inclined to follow smoking rules set by their parents.

In contrast to some previous studies (Burdzovic Andreas et al., 2016; Ryan et al., 2010; Visser et al., 2012), we found no effect of parental involvement and the parent-child relationship on alcohol consumption and cannabis initiation. Possibly, parent behaviors during middle adolescence are less likely to exert effects across longer time-frames (i.e., in young adulthood) for these substances. Alcohol use might also be something that is less likely to be under strict parental control, as this represents more normative, socially acceptable behavior (Maciejewski et al., 2019). Furthermore, specific parenting practices, such as alcohol and cannabis rule setting, could be more important predictors for alcohol and cannabis use (Engels & Bot, 2006; Vermeulen-Smit et al., 2015).

Parental substance use

Higher levels of parental substance use in early adolescence (comprised by binary measures of current smoking, recent cannabis use, and lifetime addiction) significantly predicted higher levels of smoking and higher chances of cannabis initiation in young adulthood. These effects might be direct modelling effects, such that offspring imitate observed parental substance use, or indirect modelling effects, for example through attitude formation and rule setting (Engels & Bot, 2006). We did not find an effect of the parental substance use factor on alcohol use, presumably because this factor did not include parental alcohol use. Also, modeling effects might be less strong for alcohol which is predominantly used in the peer context, especially by older adolescents (Goncy & Mrug, 2013).

Age and sex

Considering covariates, it is interesting to see that age had a significant positive effect on cannabis initiation and smoking behavior, even though the age variability in the sample was low. This suggests that these years in young adulthood comprise a sensitive period in the development of substance use where much change is occurring. This is in line with

previous literature showing different trajectories of change and development in this period (Bachman et al., 2013). We observed that males consumed more alcohol and had higher chances of cannabis initiation, consistent with estimates in the general population (Centraal Bureau voor de Statistiek, 2020). We observed no sex differences in smoking after controlling for the other factors in the model, even though population statistics suggest such a difference exists (Leefstijlmonitor, 2020). This might suggest that eventual sex differences might be mediated by differences in parent factors. Interestingly, there were significant associations between parent factors and sex, such that males experienced lower parental involvement and a lower parent-child relationship quality, and higher levels of parental substance use in the cannabis initiation model (see Supplementary Table S3). This is in line with previous reports of small differences in parenting behavior towards sons versus daughters, that could be due to gender roles in society and gender stereotypes (Endendijk et al., 2016). Though outside of the scope of this study, future research could further explore these effects.

Gene-environment interaction (GxE)

One of nine tested GxE paths reached significance at a conventional $p<.05$ threshold. There was positive GxE between parental substance use and the PGS on smoking. Although the models containing GxE showed the best fit for the parent-child relationship on smoking and for parental substance use on alcohol per week, these GxE paths did not reach significance and the effects were small. Also, the negative direction of the GxE in the alcohol model is not in line with what has been reported previously (Pasman et al., 2019).

The effect of parental substance use on smoking was enlarged when genetic risk for smoking was high. This direction is in line with differential susceptibility frameworks, which state that the effect of an environmental factor can be amplified when genetic vulnerability is high (Belsky & Pluess, 2009). Such an effect would contribute to the likelihood that smoking becomes widespread in families and would suggest that especially individuals that are at risk genetically would benefit from prevention targeted at parental substance use. An alternative explanation might be that this effect is driven by the overlap in genetic risk for smoking between parents and offspring. However, we tested this by bringing the gene-environment correlation (rGE) between parental substance use and the offspring's smoking PGS into the model, and this did not change the GxE effect. Thus, parental substance use affected smoking and magnified the effect of genetic risk on smoking independently of genetic overlap with the young adult. Still, because the effect was small and was the only one to reach significance in the tested models caution must be taken in the interpretation.

Although it is possible that GxE effects are specific to smoking and parental substance use only, there are alternative explanations for the fact that only this GxE path was significant. The smoking analyses are likely to be the most powerful. We used a

multivariate, more informative approach to compute the smoking PGS. The smoking outcome likewise used information from multiple traits. Also, the parental substance use factor had the largest main effect (which is relevant in this case as the PGS augmented this main effect). If the parental substance use factor would have included a measure of alcohol use it might have been more likely to have an effect in the alcohol models. Although we conducted power analyses (see preregistration) and power was deemed sufficient to detect GxE also in the other models, it is possible that we were overly optimistic in choosing parameters for this analysis. This certainly seems likely for the alcohol analyses, where the PGS did not predict its own phenotype. Another explanation as to why GxE effects tested with PGS are generally difficult to detect is that GWAS only test direct associations between variants and outcomes, and would not detect variants that increase vulnerability to environmental circumstances per se (Fox & Beevers, 2016). Also, there is a possibility that individual variants included in the PGS interact or correlate with environmental exposures in different directions, cancelling out an overall interaction effect.

Gene-environment correlation (rGE)

For the smoking models, there was significant rGE between the PGS and all parent factors. rGE between the smoking PGS and parental substance use likely stems from genetic overlap between parent and offspring ('passive' rGE, Knafo & Jaffee, 2013; Plomin et al., 1977). Beside passive rGE driven by transmitted parental alleles, there can be evocative or reactive rGE, that could also arise from non-transmitted alleles ('genetic nurturing,' Kong et al., 2018). Possibly, the association between the smoking PGS and parental involvement and the parent-child relationship arises through such processes. For instance, certain SNPs are associated to smoking, which in turn leads to parental disapproval, lower parental involvement and lower relationship quality, giving rise to a correlation between the smoking SNPs and a negative parent environment. However, our longitudinal design can in part rule out this explanation, as we looked at young adult smoking, and if adolescents did not smoke (yet) at the time of measurement of the parent variables, such a process cannot explain the link. Alternatively, there may be pleiotropic smoking SNPs that influence some other behavior which in turn elicits a response in the parents. For instance, SNPs important for smoking have also been associated with attention deficit hyperactivity disorder (ADHD; Liu et al., 2019), and ADHD can elicit negative parenting behaviors, including lower parental warmth and less solicitation (Glatz et al., 2011). Indeed, pleiotropy is the rule rather than the exception for SNPs associated with complex behavior (Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2019). A combination of passive and evocative processes might also exist, for instance such that transmitted smoking SNPs give rise to ADHD-like behavior in the parent, resulting in ineffective parenting behaviors (Mokrova et al., 2010). Still, all of

these explanations are speculative; there might also be genetic overlap with some phenotype that would elicit an opposite response. Future genetic nurturing (Kong et al., 2018) or Mendelian randomization studies could further disentangle underlying causal mechanisms.

It needs to be noted that GxE and rGE effects hardly shared variance and adding them in one model did not change either effect. It is interesting to see that these effects operate independently, as previous research has cautioned for bias introduced by rGE when testing GxE (e.g., Pasman et al., 2019). By testing both effects simultaneously, it became clear that rGE is independent from and at least as prominent as GxE, and is as such deserving more research attention.

Strengths and limitations

This is the first PGS study to our knowledge to investigate the main effects and complex interplay between genetic and parental factors during adolescence to understand substance use in young adults. The advantage of our use of SEM was that we could model directional paths (which makes sense in the case of genetic predictors that cannot be influenced by other parameters in the model) and test the relative contributions of main, rGE, and GxE effects. Also, the use of latent factors enabled us to leverage the wealth of information that was present in the TRAILS dataset. Effects were compared across different parenting characteristics and different substance use outcomes. We employed powerful and up-to-date PGS methods and summary statistics from the largest GWAS available to date.

Limitations of this study include the computational constraints of SEM which made it impossible to include all parent factors in a single model, or similarly, to look at all substance use outcomes simultaneously. Thus, unique contributions to substance use and interdependency between parent factors and substance use outcomes could not be modeled. Also, due to model non-convergence, some variables (including parental alcohol use) could not be considered in the models. Further, although we conducted power analyses, effect sizes might have been smaller than anticipated. We only found GxE and rGE effects for smoking, which had the most powerful PGS (based on MTAG) and strongest outcome measure (latent factor with multiple smoking behavior indicators), suggesting that power might have been an issue in the other models. Indeed, the low SNP-based heritability in some of the source GWAS suggest that the power of the PGS may have been limited. Also, power might have been limited by selective attrition between baseline and wave 5 of participants of lower socioeconomic status and lower IQ (Ormel et al., 2012), factors that have previously been associated with substance use (Johnson et al., 2009; Patrick et al., 2012). As a more general limitation, it needs to be noted that we only included individuals of European ancestry in our genetic analyses; as discovery GWAS are still largely unavailable for other ethnic groups, currently PGS research can only reliably be conducted in European samples.

Conclusions and future directions

Summarizing, we found that high genetic risk, low parental involvement, high parental substance use, and a low-quality parent-child relationship predicted smoking and cannabis initiation, but not alcohol use.

For smoking, the effect of genetic risk was enlarged by parental substance use. Also, genetic risk for smoking was associated with lower parental involvement, higher parental substance use, and a lower quality parent-child relationship. In addition, we showed that rGE and GxE operated relatively independently from each other and are unlikely to be captured when not modeled explicitly. Our findings that parent behavior influences substance use both directly and through indirect genetic pathways suggest that parents are an important target point for intervention, especially for smoking behaviors. Future studies should aim to identify causal genetic pathways that operate independently from environmental circumstances, to provide clues for underlying biological mechanisms and potentially provide targets for pharmacogenetic interventions. Further elucidating pathways of genetic risk will provide more clues as to where prevention and intervention can be aimed to break the causal chain.

Supplementary Materials

There is a large number of supplementary materials associated with this paper. The full materials can be viewed online at:
Chapter 11 – Gene-environment interaction with parenting

or copy this link into the browser:
https://drive.google.com/drive/folders/1ZuhxaGELOHquT3WhFp-t1LNvoqC2T8_9

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Table 1. Measures of phenotypical predictors and outcomes (included in the models as observed variables or as indicators of latent variables).

latent construct	observed construct	level	informant	measure	wave (age)	direct	definition
parental involvement	parental control	continuous	child	Stattin & Kerr 2000	3 (16)	r	sum score of 5 items about parents' rule setting
	parental sollicitation	continuous	child	Stattin & Kerr 2000	3 (16)	r	sum score of 5 items about parents' asking about the child's behavior
	parental knowledge	continuous	child	Stattin & Kerr 2000	3 (16)	r	sum score of 5 items about parents' knowing of the child's behavior
	child disclosure	continuous	child	Stattin & Kerr 2000	3 (16)	r	sum score of 5 items about the child's telling the parent about his/ her behavior
parent-child relationship	rejection	continuous	child	EMBU-C	1 (11)	u	sum score of 17 items on perceived parental negative regard
	warmth	continuous	child	EMBU-C	1 (11)	r	sum score of 18 items on perceived parental positive regard
parental substance use	smoking	categorical	parent	TRAILS	1 (11)	u	at least one smoking parent
	cannabis	categorical	parent	TRAILS	1 (11)	u	at least one parent indicated past year use on at least one measure
	addiction	categorical	parent	TRAILS	1 (11)	u	at least one parent indicated to have been addicted to a substance
smoking	daily smoking	categorical	child	TRAILS	5 (22)	u	single item about ever having smoked on a daily basis
	cigarettes per day	continuous	child	TRAILS	5 (22)	u	single item average amount of cigarettes smoked per day in the past month
	nicotine dependence	continuous	child	FTND		u	sum score of 5 items on nicotine dependence

NA	alcohol per week	continuous	child	TRAILS	5 (22)	u	sum score for week- and weekend days or based on weekend days only (if weekdays is missing)
NA	cannabis initiation	categorical	child	TRAILS	5 (22)	u	indicated to have used cannabis at least once on at least one measure

Note. NA=not applicable because the model included the observed (rather than a latent) variable. Continuous=Likert response scale analyzed on a continuous scale (i.e. all questions had answering categories). Direct= direction; all predictors were coded such that it was hypothetically positively related to substance use; 'u' (unchanged) indicates the raw scores were used; 'r' (reversed) indicates where the scale was reversed

Table 2. Descriptive statistics for observed variables (before standardization and imputation). For the continuous variables minimum, maximum, M, and SD are given. For categorical variables the 'control' (reference) group, the 'case' group, and the percentage individuals belonging to the 'case' group are given.

observed construct	N	min/ controls	max/ cases	M/ %	SD
sex	1,649	female	male	52.1%	NA
age	1,649	20	24	21.7	0.70
parental control	1,568	0	4	2.2	0.95
parental sollicitation	1,568	0	4	1.2	0.70
parental knowledge	1,594	0	2	1.7	0.32
child disclosure	1,568	0	4	2.5	0.74
parental rejection	1,639	1	4	1.5	0.31
parental warmth	1,640	1	4	3.2	0.49
parental smoking	1,482	no	yes	49.5%	NA
parental cannabis	1,351	no	yes	4.4%	NA
parental addiction	1,576	no	yes	7.0%	NA
daily smoking	1,315	no	yes	24.2%	NA
cigarettes per day*	528	0	5	1.7	1.31
nicotine dependence*	539	0	10	1.8	2.18
alcohol per week	1,122	0	10	7.5	5.68
cannabis initiation	1,299	no	yes	58.8%	NA

Note. N=sample size before imputation, min= minimum value (for questionnaire scores, the minimum score that was possible to achieve), max=maximum value (for questionnaire scores, the maximum score that was possible to achieve), M=mean, %=percentage for cases, SD=standard deviation, NA=SD for dichotomous variable is not applicable.

*Reported only for current smokers. Cigarettes per day was categorized from 0=less than 1 cigarettes, 1=1-5 cigarettes, 2=6-10 cigarettes, 3=11-20 cigarettes, 4=21-30 cigarettes, and 5=more than 30 cigarettes.

Table 3. Results for the Exploratory Factor Analysis of the parenting variables. Fit indices per solution are provided. To the right side of the Table are the χ^2 for the difference between the models, with $p<.05$ indicating significant improvement with respect to the previous model with one factor less.

	CFI	TLI	RMSEA	χ^2 (df)	*p*-value
1-factor solution	.76*	.68*	.09*	NA	NA
2-factor solution	.86*	.74*	.08*	160.45 (8)	<.001
3-factor solution	.97	.91	.05	150.01 (7)	<.001
4-factor solution	1.00	1.00	.00	54.62 (6)	<.001

*Note.** indicates poor fit according to CFI/TLI<.90, RMSEA≥.08

Table 4. Factor loadings (standard errors) for the best factor solution for the parenting variables (3 factors) from the EFA.

Parental variables	F1 involvement	F2 substance use	F3 relationship
Knowledge	.24 (.05)	.24 (.04) *	
Disclosure	.51 (.04)		
Control	.67 (.04)		
Solicitation	.82 (.04)		
Smoking		.68 (.09)	
Addiction		.65 (.07)	
Cannabis		.78 (.11)	
Rejection			.52 (.05)
Warmth			.58 (.07)

Note. *This cross loading was removed in subsequent models; knowledge was forced to load on F1. The EFA indicated fit would improve further if the correlation between parental disclosure and knowledge in the first factor was allowed; this path was added in the subsequent SEM analyses. Presented here are significant loadings (p<.05) with a value >.20.

Table 5. Model fit indices for each of the model steps. In bold the best fitting model per outcome according to the AIC/ BIC.

		smoking factor			alcohol per week			cannabis initiation		
		F1	F2	F3	F1	F2	F3	F1	F2	F3
1 main	CFI	.89*[a]	.96[a]	.97[a]	.92	1.00[a]	.98	.92[a]	.99[a]	.97[a]
	TLI	.87*[a]	.95[a]	.96[a]	.90	1.00[a]	.97	.88*[a]	.98[a]	.95[a]
	RMSEA	.04 [a]	.02[a]	.02[a]	.03	.00[a]	.01	.03[a]	.01[a]	.02[a]
	AIC	21,790	8,419	14,117[b]	**19,714**	6,339	**12,008**	**18,333**	**4,951**	**10,631**
	BIC	21,990	8,582	14,273[b]	**19,881**	6,469	**12,133**	**18,495**	**5,075**	**10,750**
2 GxE	AIC	21,788	8,411	14,113	19,716	6,340	12,063	18,333	4,953	10,631
	BIC	21,993	8,578	14,275	19,888	6,475	12,187	18,500	5,082	10,755
3 rGE	CFI	.92[a]	.01[a]	.98	.92	1.00[a]	.98	.92[a]	.99[a]	.96[a]
	TLI	.90[a]	.99[a]	.97	.89*	1.00[a]	.96	.88*[a]	.99[a]	.93[a]
	RMSEA	.03[a]	.99[a]	.02	.04	.00[a]	.02	.03[a]	.01[a]	.02[a]
	AIC	**21,769**	8,388	14,111[b]	19,715	6,339	12,011	18,334	4,950	10,633
	BIC	**21,974**	8,556	14,273[b]	19,888	6,474	12,146	18,501	5,080	10,757

4 full	AIC	21,766	**8,379**	**14,107**	19,717	**4,852**	12,010	18,334	4,952	10,633
	BIC	21,977	**8,552**	**14,274**	19,896	**5,009**	12,145	18,507	5,087	10,763

Note. F1=parental involvement, F2=parental substance use, F3=parent-child relationship
CFI, TLI and RMSEA indices are available only for the main and rGE models, as they cannot be computed for models containing latent interactions.
*indicates poor fit according to CFI/TLI<.90, RMSEA≥.08

[a] For these estimates we used WSLMV as an estimator, because they are not available with MLR and categorical variables (indicators or outcome variables). AIC and BIC are based on the MLR estimator to allow for comparisons between the four different models.

[b] For these MLR models the model estimation reached a saddle point; however, model estimation (including standard errors) terminated normally, allowing for normal interpretation

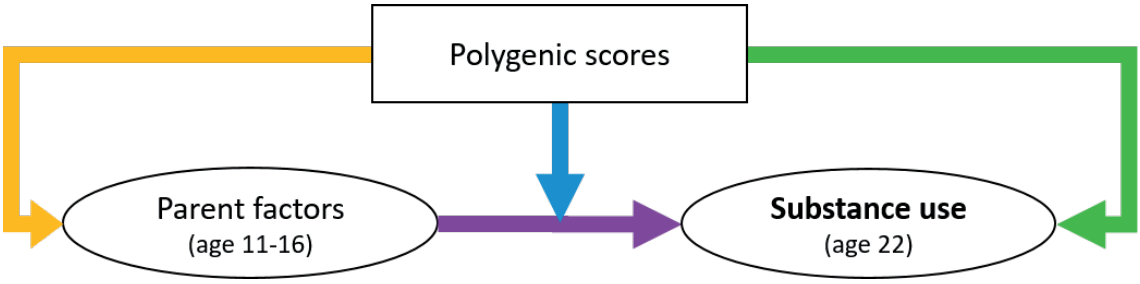
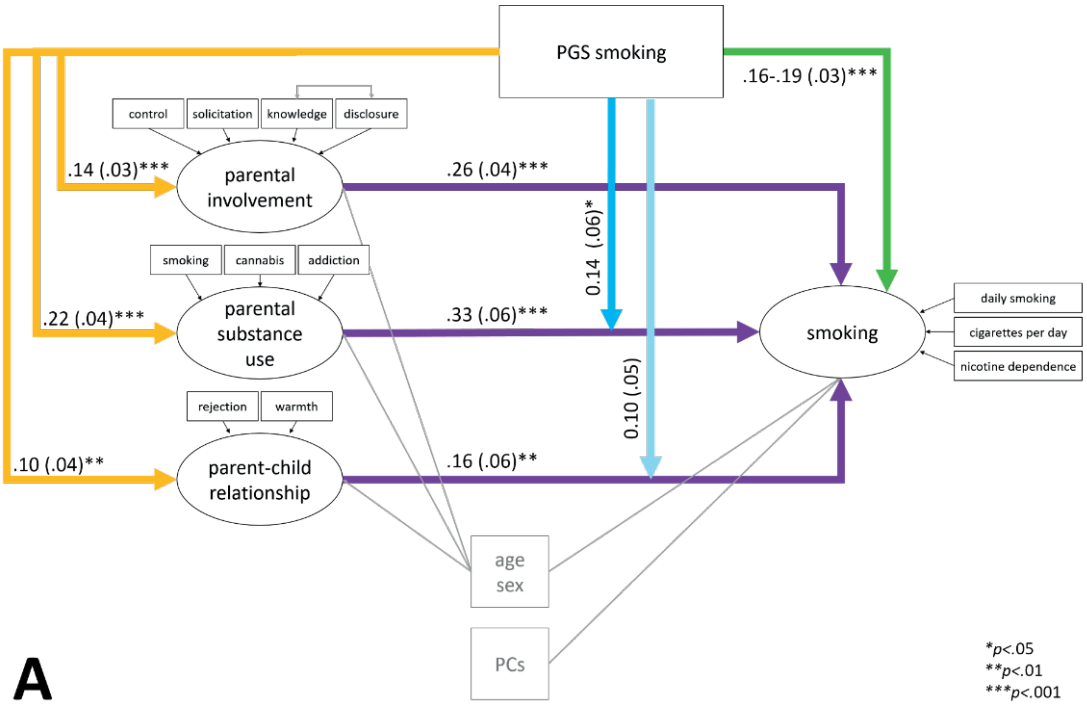
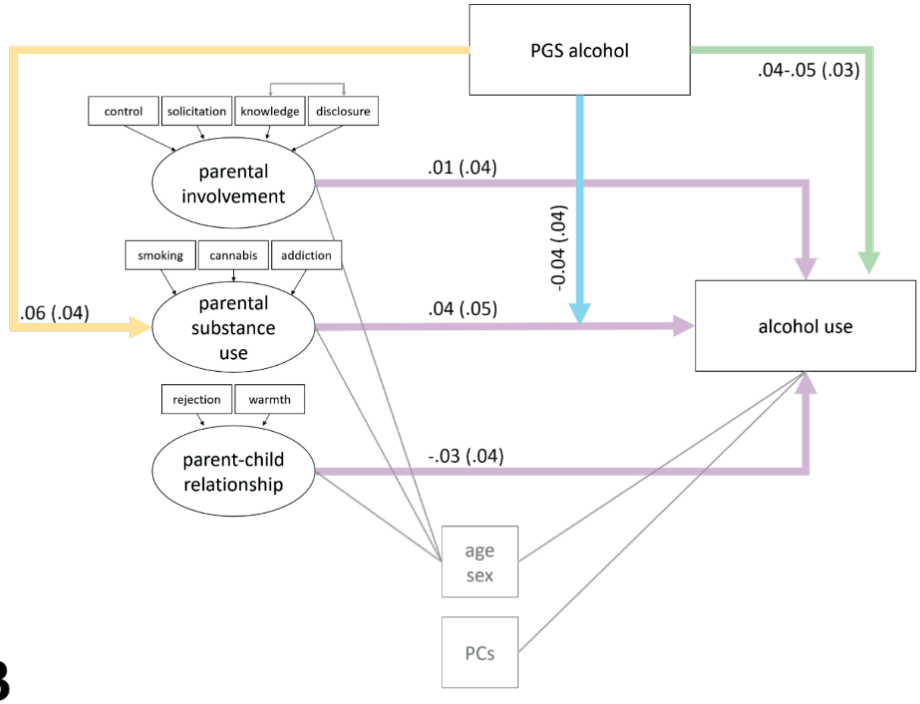


Figure 1. The conceptual model of the interplay between genetic and parent factors in the development of substance use, with the blue arrow indicating the gene-environment interaction path and the yellow indicating the gene-environment correlation path.


PGS smoking
.16-.19 (.03)***
control
solicitation
knowledge
disclosure
.14 (.03)***
parental involvement
.26 (.04)***
0.14 (.06)*
smoking
cannabis
addiction
.22 (.04)***
parental substance use
.33 (.06)***
daily smoking
cigarettes per day
nicotine dependence
smoking
0.10 (.05)
rejection
warmth
.10 (.04)**
parent-child relationship
.16 (.06)**
age
sex
PCs
A
*p<.05
**p<.01
***p<.001



B

*p<.05
**p<.01
***p<.001

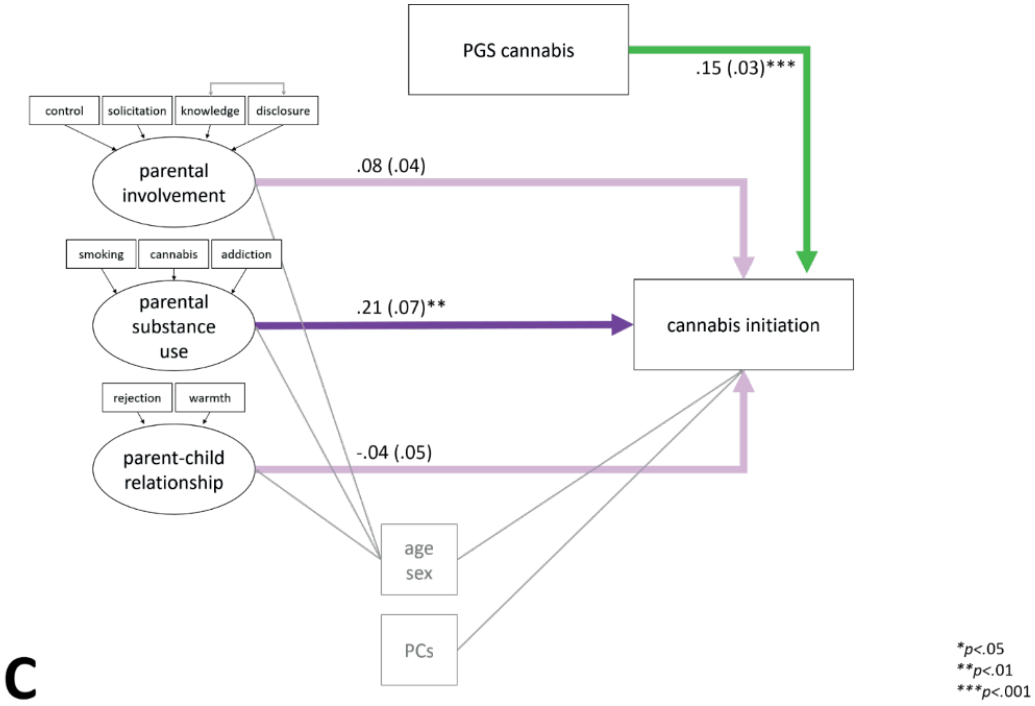


Figure 2. Standardized estimates β (with standard errors) from the best fitting Structural Equation Models of parent factors and PGS predicting **(A)** smoking; **(B)** alcohol per week; and **(C)** cannabis initiation. Note that the models presented in one figure were tested separately per parent factor; these figures are summaries of the separate analyses.

CHAPTER 12

Genetic risk for smoking: disentangling gene environment interplay with socioeconomic status

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submitted (*Behavior Genetics*)

Abstract

Background This study aims to disentangle the contribution of genetic liability, educational attainment (EA), and their overlap and interplay in lifetime smoking.

Methods We conducted genome-wide association studies (GWASs) in UK Biobank (N=394,718) to capture i) variants for lifetime smoking, ii) variants for EA, and iii) variants that contribute to lifetime smoking independently from EA ('smoking-without-EA'). Based on the GWAS results, three polygenic scores (PGSs) were created for individuals from the Netherlands Twin Register (NTR, N=17,805) and the Netherlands Mental Health Survey and Incidence Study-2 (NEMESIS-2, N=3,090). We tested the gene-environment (GxE) interactions between each PGS, neighborhood socioeconomic status (SES) and EA on lifetime smoking. To assess if the PGS effects were specific to smoking or had broader implications, we repeated the analyses with measures of well-being.

Results After subtracting EA effects from the smoking GWAS, the SNP-based heritability decreased from 9.2% to 7.2%. The genetic correlation between smoking and SES characteristics was reduced, whereas overlap with smoking traits was less affected by subtracting EA. The PGSs for smoking, EA, and smoking-without-EA all predicted smoking. For well-being, only the PGS for EA was a reliable predictor. There were suggestions for GxE for some relationships, but there were no clear patterns per PGS type.

Conclusion This study showed that the genetic architecture of smoking has an EA component in addition to other, possibly more direct components. PGSs based on EA and smoking-without-EA had distinct predictive profiles. This study shows how disentangling different models of genetic liability and interplay can contribute to our understanding of the etiology of smoking.

Introduction

Despite well-known health risks and a worldwide increase of discouragement policies, large proportions of the world's population continue to smoke (World Health Organization 2019). In the Netherlands, the promising decline in smoking seen in the past decades now seems to level off, especially among young adults (Bommelé and Willemsen 2020). Research into the etiology of smoking could shed new light on possible avenues for prevention and intervention. Both environmental and genetic factors play a role in smoking behavior (Sullivan and Kendler 1999).

Characteristics related to socioeconomic status (SES), with educational attainment (EA) as its core component, are important predictors for smoking (Hiscock et al. 2012). Individuals with lower SES (income and EA) are more likely to get exposed to tobacco smoke, start smoking in adolescence, smoke more heavily, and continue smoking. Such effects can be observed at the level of neighborhoods, with people living in more disadvantaged areas being more likely to smoke (Karriker-Jaffe 2013; Cambron et al. 2018). Reported effects are quite large for specific groups. For example, men have been reported to be two times more likely to smoke in a neighborhood marked by visible signs of disorder (e.g., vandalism and litter) than in a neighborhood low on these signs (Miles 2006). White residents of poor neighborhoods are 72% more likely to initiate smoking before age 25 than white residents in an affluent neighborhood (even after controlling for income and parental education; Kravitz-Wirtz 2016). However, estimated effect sizes vary widely and seem to be moderated by many individual-level SES and group attributes (Miles 2006; Kravitz-Wirtz 2016; Mathur et al. 2013; Cohen et al. 2011; Karriker-Jaffe et al. 2016).

Twin studies estimated that almost half of the individual differences in the population in smoking initiation can be attributed to genetic factors. The heritability estimate is even higher (around 75%) for nicotine dependence (Vink et al. 2005). Even though the prevalence of smoking seems to be declining, heritability estimates have remained stable (Vink and Boomsma 2011). Genome-wide association studies (GWASs) have identified specific genetic variants underlying smoking behavior (The Tobacco and Genetics Consortium 2010). The most recent smoking GWAS included more than a million participants, and all measured genetic variants could explain 8% of the variation in smoking initiation and 8% in the number of cigarettes smoked per day (Liu et al. 2019). Thus, part of the heritability as estimated by twin studies could not be traced back to common variation tested in this GWAS. There are several possible reasons for this commonly observed 'missing heritability', one of which might be interplay with environmental circumstances (Eichler et al. 2010).

It seems likely that socioeconomic and genetic factors do not operate in isolation in increasing risk for smoking. In the case of gene-environment interaction (GxE), the likelihood that a genetic risk (G) for smoking leads to smoking depends on environmental circumstances (E). Such GxE effects could contribute to the missing heritability phenomenon (Manolio et al. 2009). On the one hand, rGE and GxE effects (with shared environment) could inflate heritability estimates in twin research when not explicitly modeled (Verhulst and Hatemi 2013), and on the other hand they could deflate associations if the total effect of a SNP is canceled out due to different effects in different sub groups.

Twin studies have suggested that GxE effects exist for smoking (e.g., Timberlake et al. 2006; Boardman et al. 2011; Boardman et al. 2008; Dick et al. 2007). For example, educational attainment was found to moderate the heritability of smoking initiation (although the exact direction was difficult to establish due to strong gene-environment correlation effects; McCaffery et al. 2008). However, such studies do not provide any insight as to what genetic variants drive these GxE effects. More recently, studies have used smoking GWASs to create polygenic scores (PGSs) as a measure of genetic risk, and tested interaction between PGSs and environmental factors on smoking. For example, it was shown that a PGS for smoking initiation was associated with smoking heaviness only in individuals who had been exposed to tobacco smoke in childhood (Treur et al. 2018). Another study showed that a smoking PGS was more likely to contribute to smoking risk in individuals that had experienced trauma than in individuals who had not (Meyers et al. 2013). Similarly, it was found that a PGS for smoking predicted smoking more strongly in sample of war veterans than in non-veterans (Schmitz and Conley 2016). On the other hand, living in a neighborhood with high social cohesion buffered for genetic risk, such that the effect of the PGS on smoking was less strong for individuals living in such neighborhoods (Meyers et al. 2013). However, a recent study did not detect GxE with neighborhood-level SES and metropolitanism on smoking (Pasman et al. 2020). Overall, the evidence for GxE in PGS studies is somewhat mixed and still limited for smoking (Pasman et al. 2019). Also, given the small effect sizes of PGSs in general and the even smaller GxE effects, these studies have done little to solve the missing heritability.

GxE research has often been framed in terms of environmental exposures that moderate genetic risk factors. However, the distinction between ‘environmental’ exposures and other characteristics is often quite difficult to make. For instance, an interaction with sex could indicate biological differences in the chance that some genetic factor will come to expression, or could indicate an environmental effect of gender roles. Moreover, many environmental factors (e.g., the parenting and social environment, Vinkhuyzen et al.

2010) are actually heritable themselves, so that the environment and the genetic make-up become associated. This phenomenon is often referred to as gene-environment correlation (rGE). There are various mechanisms by which associations between an environmental exposure and genetic predisposition can arise. For example, given that parents and offspring share part of their genetic make-up, a correlation could arise between parenting behavior and offspring genes (passive rGE, Pasman under review; Kong et al. 2018; Plomin et al. 1977). Alternatively, a correlation between an individual's risk for smoking and the environment could arise because smoking elicits some response in other people (reactive rGE) or because smokers select different environments for themselves (active rGE; Plomin et al. 1977). Such rGE effects also exist for EA, which has a substantial genetic component. Both cognitive abilities (at the core of EA) as well as non-cognitive EA-traits and socioeconomic characteristics have been shown to be heritable traits (Marioni et al. 2014; Demange et al. 2020). Given the strong association between EA and smoking, this can give rise to rGE associations between genetic risk for smoking and EA.

Such rGE effects influence the interpretation of other genetic findings. First, they can lead to the detection of environmental signal in GWASs (Manolio et al. 2009; Shen and Feldman 2020). For example, GWAS will probably pick up on different variants for smoking in an environment that highly sanctions smoking (e.g., variants associated with risk taking and addiction-proneness) than in an environment where smoking is the norm (e.g., variants associated with social behavior), giving rise to rGE between smoking variants and social norms. Second, if there are rGE effects, this can change the interpretation of GxE effects, lower the chance that GxE will be detected, or lead to spurious GxE findings (Rathouz et al. 2008; Dudbridge and Fletcher 2014). There are indications for rGE effects in the smoking literature. Some twin studies have shown that peer behavior is associated with genetic risk for smoking in adolescents (Cleveland et al. 2005; Harden et al. 2008; Wills and Carey 2013). This has commonly been interpreted as showing that genetic risk for smoking somehow influences which friends adolescents select for themselves. One study using PGS to test rGE showed overlap between the parenting environment and a smoking PGS (Pasman under review). Another study showed rGE between a smoking PGS and neighborhood 'physical disorder' (i.e., disrepair and vacancy; Meyers et al. 2013). The plausibility that rGE exists in substance use has been widely acknowledged (Kong et al. 2018; Gage et al. 2016). Such effects imply that genetic associations from smoking GWAS have to be interpreted within the environmental context of the samples. Still, studies reporting rGE, especially those using PGS, are scarce.

The first aim of this study is to disentangle direct genetic effects on smoking from genetic effects that influence smoking through rGE with EA. That is to say, we model the genetic

predisposition for EA and subtract it from the total genetic liability for smoking. This way, we can assess the contribution of rGE with EA in the etiology of smoking, and compare it to a 'cleaner' genetic component of smoking effects that are independent from EA. Second, we aim to test if the PGS based on either these direct smoking variants ($PGS_{smok-noEA}$) or the EA variants (PGS_{EA}) pick up better on GxE effects, as compared to a general PGS based on all smoking variants taken together ($PGS_{allsmok}$). We test interactions with neighborhood quality and affluence. The GxE effects per PGS could go in different directions. On the one hand, if it is true that rGE effects dilute GxE effects, the PGS assessing direct smoking effects could be more sensitive for picking up GxE. In this case, individuals with a high $PGS_{smok-noEA}$ may react more strongly to an unfavorable neighborhood environment and have a higher chance to start smoking. On the other hand, it is also possible that individuals who are genetically liable for a high-risk environment react differently to that environment than people who are not. That is to say, individuals with a high PGS_{EA} may be vulnerable to the environment, whereas people with a high genetic risk for smoking (high $PGS_{smok-noEA}$) have a higher chance to start smoking regardless of the environment. Comparing GxE effects between PGSs for all-smoking, smoking-without-EA, and EA could contribute to formulating such competing hypotheses and shed more light on interplay between genetic and environmental vulnerability for smoking.

Methods

A number of different analysis steps were taken. First, using GWA analyses, we identified the genetic liability for smoking and EA. Second, using the results from these GWASs, EA effects were subtracted from smoking (using genetic structural equation modeling) to capture smoking-without-EA. Third, polygenic scores were created to conduct follow-up tests of GxE effects with measures of EA and neighborhood SES. The first two steps were conducted using data from the *UK Biobank*, the third step was conducted in two independent samples from the Netherlands Mental Health Survey and Incidence Study-2 (*NEMESIS-2*) and the Netherlands Twin Register (*NTR*).

Samples and measures: UK Biobank

The GWA analyses on smoking, EA, and smoking-without-EA were conducted in a sample from the *UK Biobank*. The *UK Biobank* contains phenotypic and genetic information from up to 500,000 inhabitants of the United Kingdom. It has received ethical approval from the National Health Service North West Center for Research Ethics Committee (reference: 11/NW/0382). Researchers can apply for access to this rich data set to conduct health-related studies. This study was conducted under project number 40310. For our analyses we selected N=394,718 individuals from European-ancestry for whom there was complete phenotypic and genotypic information. Mean age was M=56.8 (range 39-73, SD=8.0) and 54.2% of the sample was female.

To measure lifetime smoking in the *UK Biobank* we extracted information from all measurement instances of data fields 2867 and 2897 (age at smoking initiation), 2887 and 3456 (cigarettes per day), and 20116 (smoking initiation yes/no). People indicating on field 2887 or 3456 to (have) smoke(d) one or more cigarettes per day were classified as smokers. People indicating on field 20116 to never have been a smoker were classified as non-smokers. If field 2887 and 3456 were unavailable, but people indicated on field 20116, 2867, or 2897 to be an (ex-) smoker, they were classified as smokers. There were data for 272,943 (54.60%) never smokers and 226,795 lifetime smokers. To capture EA, we used the ISCED classification to transform reported educational levels from field 6138 to a standardized number of educational years (UNESCO Institute for Statistics 2011). We selected the highest reported completed educational level and classified 'none of the above' (N=90,360) as primary school only. Average years of education was M=14.93, SD=5.12, range=7-20, N= 451,800.

Samples and measures: NTR

In the second part of the study, we use data from two independent samples from the Netherlands. The Netherlands Twin Register (*NTR*) is an ongoing longitudinal study of

twins and their families which has been described in detail elsewhere (refer to Ligthart et al. 2019, also for a description of the genetic data). We included all available measures of smoking initiation that were collected between 1991 and 2019 (from 15 different surveys, see Supplementary Table S1). Part of the sample is followed longitudinally, and new participants have been recruited continuously. In order to maximize sample size, we selected the most recent available measurement of smoking status for all participants. For N=14,618 European ancestry adult individuals, there were genome-wide SNP and complete phenotypic data. For most participants, smoking data were collected between 2013-2016 (N=9,426) or in 2009 (N=1,361; see Table S1). At the time of phenotype measurement, mean age was M=43.31 (SD=17.12, range=18-94). Lifetime smoking was defined similarly as in the *UK Biobank*. Current and ex-smokers that (previously) smoked more than occasionally (1 cigarette per day or 7 per week) were classified as smokers. Occasional and never smokers were classified as non-smokers. The sample consisted of 63.1% females and 43.1% lifetime smokers.

To measure neighborhood SES (E in the GxE analysis) we focused on the average household income in the neighborhood of residence. We identified the first four digits of the postal code of the participant at the time of measurement of the smoking phenotype, corresponding to the residential area at the level of neighborhoods. These digits were coupled to governmental registration data on neighborhood-level income (Centraal Bureau voor de Statistiek (CBS) 2012). The CBS determined average monthly income per household before tax (rounded at hundreds) in 2004 and 2010. We used the neighborhood data that were closest in time to survey used to assess lifetime smoking. Data were available for N=12,584 participants, who on average lived in neighborhoods with a per-household monthly income of M=2678.64 (SD=934.40, winsorized at min=500 and max=10,000).

In the follow-up analyses we focused on satisfaction with life, which was available in 4 different surveys. It was measured using the translated Satisfaction With Life Scale (Arrindell et al. 1999), a survey with five 7-point Likert items on how happy people are with their life. The sum score on this scale was coupled to contemporaneous neighborhood income using similar procedures as before, prioritizing the measurements closest in time to the measure of neighborhood income. Average satisfaction with life was M=26.97 (SD=5.23, range=5-35, N=9,257).

Samples and measures: NEMESIS-2

The Netherlands Mental Health Survey and Incidence (*NEMESIS-2*) is a population sample of more than 6,500 individuals that were followed in four measurement waves spaced out between 2007 and 2018. The aim was to monitor the occurrence and course of common

mental disorders in the general population (De Graaf et al. 2010). For this study, we used data from the second wave (conducted in 2010-2012), where a measure of neighborhood quality was available. For a sub sample of N=3,090 European-ancestry individuals genetic and phenotypic data were available. About half of the sample was female (56.1%), and mean age at wave 2 was 47.2 (SD=12.5, range=21-71).

To assess smoking we used questionnaire items on smoking status. People were classified as smokers if they self-identified as current or ex-smokers; (former) occasional smokers were classified as never-smokers. A third of the sample classified as lifetime smokers (30.2%). To measure neighborhood quality, we used a sum score of 5 standardized Likert scale survey items, including appreciation of the neighborhood, frequency of noise from neighbors, traffic, or other sources in the neighborhood, frequency of feeling unsafe if walking alone in the neighborhood during the day, frequency of feeling unsafe if walking alone in the neighborhood during the night, and frequency of observing vandalism. Items were re-coded in the positive direction, such that a higher score means a higher neighborhood quality.

Since neighborhood quality was not measured at baseline, we used wave 2 data. There was some attrition from baseline (N=319), which incited us to employ the automatic multiple imputation procedure from SPSS to supplement wave 2 neighborhood quality. We used 32 unique sociodemographic measures as predictors (see Supplementary Table S2). Because each imputed value is subject to some random variation, we imputed 25 datasets and interpret the pooled results. In total, 10.3% of the neighborhood quality data were imputed using this procedure. Across analyses, we compared the pooled results with the results using the original data, and saw that differences were negligible. For the smoking outcome, we carried forward baseline data in case they were missing at wave 2. In follow-up analysis we looked at mental health as outcome. To measure this, we used a clinical rating if someone had met criteria for any DSM-IV axis-I disorder since the baseline measurement. If wave 2 data were unavailable while someone had met criteria for a disorder at baseline, we carried forward the baseline data (N=92 individuals). Remaining missingness (N=227) was imputed using the same baseline predictors as before. DSM-IV contains 18 disorder categories (including for example mood and psychotic disorders) with in total almost 300 different diagnoses. Diagnoses were made based on the Composite International Diagnostic Interview (CIDI) 3.0 by a trained professional (De Graaf et al. 2010). In total, 541 of the participants (17.5%) had recently met criteria for any disorder at wave 2 (since the last interview or in the past year for individuals who only had wave 1 data).

Smoking and EA GWAS in UK Biobank to model direct and rGE effects

In the first step, we ran GWASs to capture genetic associations for lifetime smoking and EA (yellow panel in Figure 1). We used the fast-GWA package from GCTA (Yang et al. 2011). GCTA makes use of a genetic relatedness matrix to account for relatedness in the sample. To reduce computational demand for subsequent analyses we limited the GWASs to 1.3 million HapMap3 SNPs (International HapMap 3 Consortium 2010). We filtered out SNPs with minor allele frequency below 1%, divergence from Hardy-Weinberg disequilibrium with $p_{HWE}<10^{-10}$, and call rate below 95%. We included genetic sex, standardized age, standardized year of birth, and 25 principal components (PCs) for genetic ancestry as covariates. These PCs were determined using PCA, as described in more detail in Abdellaoui et al. (2019).

In the next step, we used the summary statistics to fit a mediation model capturing genetic effects on smoking, EA, and smoking-without-EA in Genomic Structural Equation Modeling (Genomic SEM, Grotzinger et al. 2018). To obtain a smoking-without-EA GWAS, we regressed smoking on all genetic variants as well as on EA (blue panel, Figure 1). The model yielded two sets of GWAS results, one for SNP effects on smoking independent from EA ('smoking-without-EA', grey path) and one for SNP effects on EA (red path from SNP to EA). We inspected the GWAS results and performed post-processing analyses using FUMA on default settings to inspect the genetic architecture of the different traits (version v1.3.6a, Watanabe et al. 2017). We used LDscore regression (Bulik-Sullivan et al. 2015) to assess SNP-based heritability (the variance explained in the traits by all SNPs concurrently) and genetic correlations with other traits. SNPs and genes that were genome-wide significantly associated with one of our traits were looked up in the GWAS catalog from EMB-EBI (Buniello et al. 2019) to examine whether they were previously associated with other phenotypes.

Polygenic score analysis to test genetic and SES influences on smoking

PGS were created in *NTR* and *NEMESIS-2* based on the total smoking GWAS, EA, and smoking-without-EA summary statistics from the Genomic SEM model. A PGS can be created in a new sample by weighting variants by their GWAS effect size and aggregating them in a single score per individual. We used GCTA-SBLUP to take into account the linkage disequilibrium (LD) structure in the European population before creating the PGS, as this improves prediction accuracy (Yang et al. 2011; Robinson et al. 2017). An additional advantage of SBLUP is that no *p*-value threshold needs to be established for including SNPs in the PGS (as is the case for some other PGS computation methods); rather, the whole genome is weighted and integrated in the score. Using the SBLUP weighting scheme, the actual individual-level scores were computed with PLINK (Purcell et al. 2007) and merged to the phenotypical data in SPSS.

We tested the associations between these PGSs and lifetime smoking in *NTR* and *NEMESIS-2*. In order to compare the different PGS components, we first regressed the smoking outcome on the 'all smoking' PGS ($PGS_{allsmok}$; *model 1a*), and then on the PGS for EA (PGS_{EA}) and the PGS for smoking-without-EA ($PGS_{smok\text{-}noEA}$) together to assess their relative contribution (*model 1b*). All continuous variables were standardized. Covariates included in the model were age, sex, and the first ten principal components for genetic ancestry (PCs). In addition, in *NTR* we controlled for the genotyping batch, as several different SNP arrays have been used over the course of data collection. Also, because of the family structure in *NTR,* we used generalized estimating equations (GEE) to correct for clustering in this sample, whereas standard logistic regression could be employed in *NEMESIS-2*. Secondly, we included a measure of neighborhood quality to test its effect on smoking in the two different PGS models (*model 2a and 2b*). Third, we added neighborhood quality x PGS terms, comparing a model with the $PGS_{allsmok}$ (*model 3a*) with a model with the PGS_{SES} and the $PGS_{smok\text{-}noSES}$ (*model 3b*). Finally, we repeated these analyses with a measure of satisfaction with life (in *NTR*) and mental health (in *NEMESIS-2*) to see if the effects of PGS_{EA} and $PGS_{smok\text{-}noEA}$ are specific to smoking, or have a wider impact. If the $PGS_{smok\text{-}noEA}$ shows no relationship to mental health, this would be in support of our effort to 'regress out' EA effects, indicating that it captures genetic effects specific to smoking. To correct for multiple testing, we divided a conventional .05 *p*-value threshold by 8 independent tests (2 samples, 2 outcomes, 1 group of interdependent genetic predictors, and 2 neighborhood predictors) resulting in a threshold of $p<.006$. To compute R^2 of the individual PGSs, we regressed the outcomes on the PGS and the genetic covariates (genotyping batch and PCs; excluding the genetic covariates hardly added any explained variance, data not shown). As R^2 is not provided in GEE analyses, we were unable to control for family structure here.

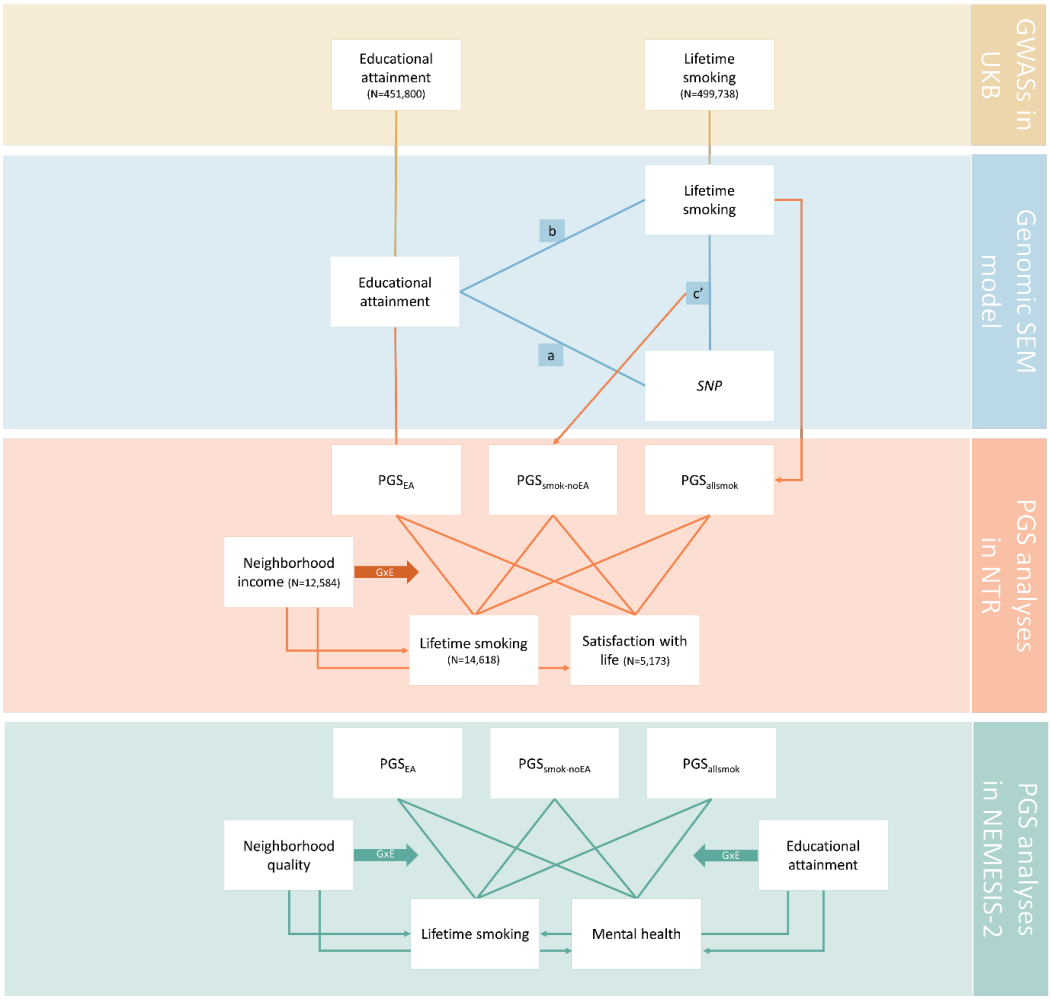

Figure 1. Flow-chart of the different analysis phases

Results

The results of the GWASs for smoking and educational attainment in *UK Biobank* can be found in the supplement. Supplementary Tables S3-4 show the independent risk loci for the traits (at R^2<0.1 and distance >250kb). In Supplementary Figures S1-2 Manhattan plots are presented. There were 112 independent variants identified for lifetime smoking, with the strongest association with a SNP in *NCAM1* on chromosome 11. SNP-based heritability (h^2) was 9.2% (SE=0.29). For EA the GWAS identified 276 independent significant loci (h^2=14.2%, SE=0.42), with the strongest SNP rs9372625 in *AL589740.1* on chromosome 6. The SNP h^2 for EA was 14.2%.

Modeling direct genetic effects and EA effects on smoking

Using Genomic SEM, we tested the model from Figure 1 based on the genetic correlation matrix between the summary statistics from the conducted GWASs. We tested a mediation model with the SNPs as the predictors, smoking as the outcome, and EA as the mediator. We were interested in path c', representing the genetic effects on smoking that remained after taking into account the effects that were mediated by EA. The summary statistics for c' thus constitute smoking-without-EA.

The GWAS for smoking-without-EA identified 47 genetic loci (Table S5 and Figure 2) and yielded a SNP-based heritability of 7.2% (SE=0.28). The top SNP was rs10891487, an intron variant in the *NCAM1* gene. This SNP and its LD partners have been associated with traits related to risk taking, substance use, cognitive ability, and socioeconomic status (Table S6). The strongest associations on the gene-level were found for *NCAM1* on chromosome 11 and *CADM2* on chromosome 3 (Table S7; Figure S3). *NCAM1* was already a top-gene for smoking before controlling for shared effects on EA (Figure S1), whereas the effect of *CADM2* was boosted after controlling for EA. Both genes have been implicated in numerous risk and substance use behaviors (Table S6), are highly brain expressed, and play a role in neuronal cell adhesion.

We performed sensitivity analyses to check if the Genomic SEM model succeeded in capturing smoking-without-EA by computing genetic correlations between smoking-without-EA and other traits (Table S8). Results are summarized in Figure 4. The genetic correlation between smoking-without-EA and the original smoking trait was r_g=.97, suggesting that the genetic architecture of smoking was only mildly affected by subtracting EA effects. The correlations with EA (*UK Biobank* summary statistics as well as GWAS summary statistics from an external, independent sample) were greatly reduced as compared to the original association (original: r_g=-.35; after subtraction: r_g=-.09), suggesting that we largely succeeded at subtracting EA effects. Genetic correlations

between smoking-without-EA with other SES-indicators (neighborhood deprivation and income) were similarly attenuated. The correlations with smoking-related traits (age at initiation, cigarettes per day, nicotine dependence, cessation, cannabis initiation, and risk-taking behavior) were also attenuated, but less so; this attenuation likely represents some power loss resulting from the subtraction.

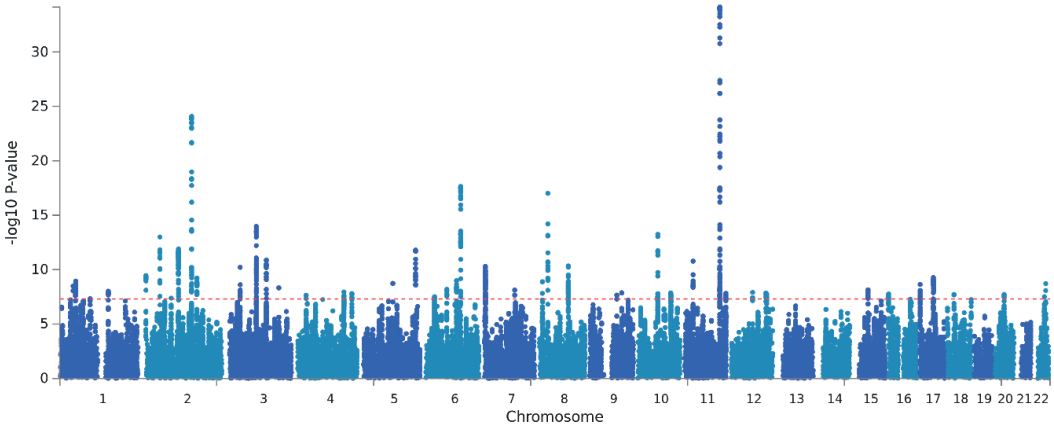

Figure 3. Manhattan plot for the GWAS on smoking-without-EA, where EA effects were subtracted from the smoking GWAS in Genomic SEM. The red line denotes the genome-wide significance threshold of *p*=5E-08.

Polygenic scores

Table 1 presents the results of the PGS analyses in *NTR* and *NEMESIS-2*, showing the association of the PGSs based on the EA and smoking-without-EA GWAS with lifetime smoking (parameter estimates for the full results including genetic covariates can be found in Table S9a and S10a). In all models, all PGSs significantly predicted lifetime smoking. Individually, the $PGS_{allsmok}$, PGS_{EA}, and $PGS_{smok\text{-}noEA}$ explained respectively 3.1%, 2.2%, and 0.5% of the variance in smoking in *NTR*, and 4.4%, 2.3%, and 0.8% in *NEMESIS-2*. Combined into the same model, the PGSs explained at total of 6.3% of the variance in smoking in *NTR* and 4.5% in *NEMESIS-2*. The effect of PGS_{EA} on smoking was negative, such that having a genetic predisposition for a higher EA was associated with lower chances of being a smoker.

In *NTR*, higher neighborhood income was associated with lower chances of smoking (R^2=1.9% for neighborhood only). There were no significant GxE interactions after correction for multiple testing, although the interactions with the $PGS_{allsmok}$ and PGS_{EA} added a minute amount of explained variance (about 0.1%; neighborhood-by- $PGS_{allsmok}$ p=.033, neighborhood-by-PGS_{EA} p=.026). In both cases the directions were such that the effect of the PGS was stronger for people living in a lower income neighborhood. The model with all effects combined (main, interactions and covariates) explained 18.5% of the variance in lifetime smoking. In *NEMESIS-2*, neighborhood quality was not a significant predictor of smoking. There were no interactions between neighborhood quality and any PGS. All effects combined explained 5.9% of the variance in lifetime smoking.

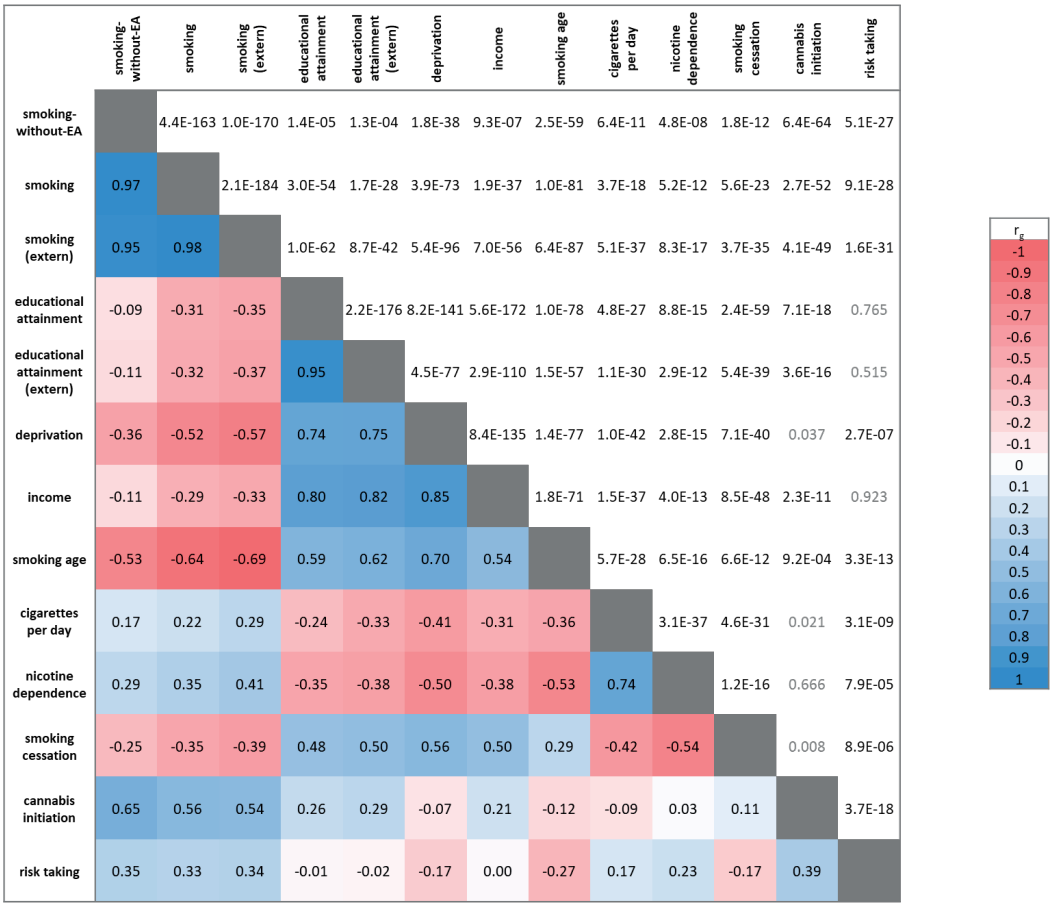

Figure 4. Heat map of genetic correlations between the smoking-without-EA GWAS and SES- and smoking related traits. Below the diagonal are the correlation estimates, with colors indicating the direction (red=negative; blue=positive) and strength (dark=strong; light=weak) of the association. Above the diagonal corresponding p-values are reported, with in grey those that were not significant after correcting for 13 traits (p=.05/13=.004). Trait description and sources can be found in Supplementary Table S6. (EA=educational attainment; extern= same trait but from independent GWAS source)

One of the aims of the $PGS_{smok\text{-}noEA}$ was to capture genetic variation that was less diluted by rGE. As a sensitivity analysis to test if this goal was achieved, we regressed EA and neighborhood-SES on the different PGSs (controlling for genetic covariates and sex and age; see Table 2). The $PGS_{allsmok}$ and PGS_{EA} significantly predicted educational attainment in both *NTR* and *NEMESIS-2.* In *NTR,* $PGS_{allsmok}$ and PGS_{EA} also showed rGE with neighborhood income. Crucially, the relationship between $PGS_{smok\text{-}noEA}$ and educational attainment was greatly reduced in both samples, and there was no rGE between the $PGS_{smok\text{-}noEA}$ and neighborhood-SES. Thus, it seems that we largely succeeded in excluding rGE effects by subtracting EA effects from the smoking PGS.

Because in *NEMESIS-2* there was no main effect of neighborhood quality, chances were low that a GxE effect would be detected. As a second sensitivity analysis, we repeated the PGS tests with baseline educational attainment (low, medium, high) as an alternative measure of SES (replacing neighborhood quality). We found a significant, negative association between educational attainment and lifetime smoking, but there were no significant GxE effects (p=.045-.164). The interactions did explain some variance in smoking (0.1-0.3%; Supplemental Table S11a), and followed a pattern such that PRS_{EA} only had effects on low to medium levels of education, whereas the effect of $PRS_{smok\text{-}noEA}$ and $PRS_{allsmok}$ were stronger at higher levels of education.

As a final sensitivity analysis, we repeated the analyses for measures of well-being (see Supplementary Table S9b, S10b and S11b). In *NTR*, higher PGS_{EA} and higher neighborhood income significantly predicted more satisfaction with life (R^2=1.0% and 2.0%, respectively); the effects of $PGS_{allsmok}$ and $PGS_{smok\text{-}noEA}$ were not significant (R^2=0.4% and 0.1%). There were patterns for GxE between neighborhood income and $PGS_{allsmok}$ and $PGS_{smok\text{-}noEA}$ (the latter surviving correction for multiple testing), such that the smoking PGSs had a negative effect on satisfaction with life only at high neighborhood income (both R^2<0.1%, p=.017 and p=.008*,* respectively). In *NEMESIS-2*, PGS_{EA} was significantly negatively related to the risk of having a recent diagnosis of a psychiatric disorder (R^2=1.2%), as was neighborhood quality (1.1%). The $PGS_{allsmok}$ predicted mental health less strongly than the PGS_{EA} (only reaching significance in the models including neighborhood quality, R^2=0.6%), and the effect of the $PGS_{smok\text{-}noEA}$ on mental health did not reach significance (R^2=0.4%). There were no GxE patterns for mental health in *NEMESIS-2.*

Table 1. Results of the polygenic score (PGS) analyses in the *NTR* and *NEMESIS-2* sample with *lifetime smoking* as outcome. Models include the effects of the PGS based on EA and the PGS based on smoking-without-EA, main effects of neighborhood environment (income in *NTR*; quality in *NEMESIS-2*), and interaction between PGSs and neighborhood. Covariates in all models 0-3b included age, sex, and the first 10 principal components for genetic ancestry (parameters estimates for the latter can be found in Supplementary Table S9a and S10a). Effects with p<.006 (corrected for 8 independent tests) are bold-faced. Explained variance of the total model is given, with the difference to the null model (Δ).

		Lifetime Smoking *NTR* (N= 12,584-14,618)[1]				**Lifetime Smoking *NEMESIS-2* (N=3,090)**			
		b	**SE**	**OR**	***p***	**b**	**SE**	**OR**	***p***
0	Age	.706	.021	2.03	**1.46E-252**	-.125	.083	.883	.133
	Sex[2]	-.291	.036	.748	**9.46E-16**	-.158	.042	.854	**2.07E-4**
				R^2=11.7%				R^2=1.9%	
1a	$PGS_{allsmok}$	0.389	0.019	1.476	**<1E-320**	0.354	0.042	1.424	**<1E-320**
	Age	0.652	0.022	1.919	**<1E-320**	-0.165	0.043	0.848	**1.38E-04**
	Sex	-0.273	0.037	1.314	**1.41E-13**	-0.141	0.084	0.869	0.095
				R^2=16.3% (Δ=4.6%)				R^2=5.4% (Δ=3.5%)	
1b	PGS_{EA}	-0.223	0.020	0.800	**<1E-320**	-0.462	0.059	0.630	**5.33E-15**
	$PGS_{smok\text{-}noEA}$	0.347	0.019	1.415	**<1E-320**	0.335	0.058	1.398	**8.36E-09**
	Age	0.663	0.022	1.941	**<1E-320**	-0.169	0.043	0.845	**9.49E-05**
	Sex	-0.272	0.037	1.313	**1.84E-13**	-0.138	0.084	0.871	0.100
				R^2=16.8% (Δ=5.1%)				R^2=4.9%(Δ=3.0%)	
2a	$PGS_{allsmok}$	0.396	0.022	1.486	**<1E-320**	0.354	0.042	1.425	**<1E-320**
	Neighborhood	-0.158	0.023	0.854	**1.77E-11**	0.071	0.042	1.073	0.091
	Age	0.798	0.028	2.222	**<1E-320**	-0.167	0.043	0.846	**1.19E-04**
	Sex	-0.273	0.043	1.314	**1.89E-10**	-0.148	0.084	0.863	0.080
				R^2=18.2% (Δ=6.5%)				R^2=5.6% (Δ=3.7%)	
2b	PGS_{EA}	-0.196	0.022	0.822	**<1E-320**	-0.461	0.059	0.631	**6.44E-15**
	$PGS_{smok\text{-}noEA}$	0.359	0.022	1.432	**<1E-320**	0.336	0.058	1.399	**7.91E-9**
	Neighborhood	-0.145	0.023	0.865	**5.32E-10**	0.064	0.042	1.067	0.123
	Age	0.804	0.028	2.235	**<1E-320**	-0.171	0.043	0.843	**8.40E-05**
	Sex	-0.272	0.043	1.312	**2.56E-10**	-0.145	0.084	0.865	0.086
				R^2=18.4% (Δ=6.7%)				R^2=5.2% (Δ=3.3%)	
3a	$PGS_{allsmok}$	0.396	0.022	1.485	**<1E-320**	0.354	0.042	1.425	**<1E-320**
	Neighborhood	-0.157	0.023	0.855	**1.39E-11**	0.070	0.041	1.073	0.091
	$PGS_{allsmok}$ * neigh	-0.044	0.021	0.956	0.033	-0.025	0.048	0.975	0.594

	Age	0.799	0.028	2.223	**<1E-320**	-0.167	0.043	0.846	**1.20E-04**
	Sex	-0.275	0.043	1.316	**1.52E-10**	-0.147	0.084	0.863	0.081
		R^2=18.2% (Δ=6.5%)				R^2=5.6% (Δ=3.7%)			
3b	PGS_{EA}	-0.195	0.022	0.823	**<1E-320**	-0.461	0.059	0.631	**7.99E-15**
	$PGS_{smok\text{-}noEA}$	0.358	0.022	1.430	**<1E-320**	0.336	0.058	1.399	**9.07E-09**
	Neighborhood	-0.152	0.023	0.859	**8.39E-11**	0.083	0.045	1.086	0.064
	PGS_{EA} * neigh	0.047	0.021	1.048	0.026	0.102	0.062	1.107	0.100
	$PGS_{smok\text{-}noEA}$ * neigh	-0.033	0.021	0.967	0.108	-0.038	0.057	0.963	0.505
	Age	0.805	0.028	2.236	**<1E-320**	-0.172	0.043	0.842	**7.51E-05**
	Sex	-0.272	0.043	1.313	**2.44E-10**	-0.141	0.084	0.868	0.093
		R^2=18.5% (Δ=6.8%)				R^2=5.5% (Δ=3.6%)			

[1] Due to missingness in the neighborhood measure, model 2 and 3 had a sample size of N= 12,584
[2] Sex was coded 1=male, 2=female
PGS=polygenic score; allsmok=all smoking; EA=educational attainment; smok-noEA=effects on smoking independent from EA; Neighborhood (neigh) = neighborhood characteristics, in NTR neighborhood-level income, in NEMESIS-2 neighborhood quality

Table 2. Relationships between the PGSs and measures of educational attainment and neighborhood-SES, controlled for genetic covariates (10 PCs in both samples as well as genotyping batch in *NTR*) and sex and age. The relationships were tested in separate models, so that these models do not control for overlap between the PGSs.

		NTR (N=8,989 for EA and N=12,584 for neighborhood)			***NEMESIS-2 (N=3,090)***		
		$PGS_{allsmok}$	**PGS_{EA}**	**$PGS_{smok\text{-}noEA}$**	**$PGS_{allsmok}$**	**PGS_{EA}**	**$PGS_{smok\text{-}noEA}$**
EA[1]	**b**	-0.059	0.221	-0.016	-0.08	0.228	-0.031
	SE	0.009	0.008	0.009	0.015	0.014	0.014
	p	**6.24E-12**	**2.12E-144**	0.070	**4.80E-08**	**2.28E-59**	0.030
	R^{2*}	2.3%	7.7%	1.8%	1.0%	8.1%	0.2%
Neighborhood[2]	**b**	-0.039	0.126	-0.011	0.027	0.001	0.025
	SE	0.010	0.0116	0.0104	0.08	0.019	0.018
	p	**1.63E-04**	**<5E-300**	.281	.147	.950	.177
	R^2	0.6%	2.0%	0.5%	0.3%	0.2%	0.3%

[1] Educational attainment. In *NTR,* 4-level variable with 1=primary school, 2=lower vocational/ lower secondary school, 3=intermediate vocational/ intermediate and high secondary school, and 4=higher vocational/ university; in *NEMESIS-2,* 3-level variable with 1=primary/ lower secondary, 2=higher secondary, 3=higher professional education.
[2]In *NTR,* a measure of neighborhood-level income; in *NEMESIS-2,* a survey-based measure of neighborhood quality
* $R2^2$ is given for the model excluding age and sex

Discussion

This study showed that the genetic signatures for educational attainment (EA) and smoking overlap substantially, but EA effects can be disentangled to some extent from smoking. After 'subtracting' EA effects from the genetic architecture of smoking, still 7.2% of the variance in smoking could be explained by SNP effects (as compared to 9.2% before subtracting). This suggests that the more 'direct' component of the genetic variance is important, and not all variance in smoking can be explained through gene-environment correlation (rGE) with EA. We showed that the genetic correlations of smoking with EA and SES-related traits were reduced after subtracting EA, whereas the correlations with smoking traits were less affected. Thus, our approach to subtracting the EA component from the genetic architecture of smoking was successful.

Polygenic scores (PGS) based on the regular smoking GWAS ('all-smoking'), the EA GWAS, and the GWAS for smoking independent from overlap with EA ('smoking-without-EA') all significantly predicted smoking in two independent samples. The PGS for all-smoking explained the largest amount of variance in smoking, followed by the PGS for EA. Thus, the 'smoking-without-EA' effects had lower predictive power, in spite of its substantial SNP-heritability and cleaner signal. This lower predictive ability could be simply due to loss of statistical power, or might indicate that genetic predisposition for EA actually contributes more strongly to smoking than direct genetic smoking effects. This suggestion aligns with research showing that genetic risk factors for smoking initiation are often of a more general behavioral nature, including for example genes associated with risk taking proneness, as compared to risk factors for smoking quantity and nicotine dependence, that are more related to the biological effects of smoking (Liu et al. 2019; Wang and Li 2010; Karlsson Linnér et al. 2019). However, it should be noted that it is likely that we also subtracted some 'real' smoking effects in our smoking-without-EA factor. For example, if a variant causes lifetime smoking, and smoking in turn causes lower EA (or vice versa; Gage et al. 2018; Gage et al. 2020), subtracting EA would eliminate the effect of that smoking variant. Such mechanisms may have contributed to the lower genetic signal in the smoking-without-EA GWAS, and the lower predictive power of its PGS.

For mental health we observed a contribution of genetic effects for EA, but no effects of the direct-smoking PGS, suggesting that these PGSs indeed captured what was purported. The variance explained by the EA PGS was higher than the variance explained by the all-smoking PGS, which captured both EA and smoking effects, which shows that taking into account genetic smoking effects diluted rather than strengthened the predictive power. This could indicate that previously observed (genetic) associations between smoking and mental health outcomes (Jang et al. 2020) could be explained in

part through genetic overlap between smoking and EA on the one hand and mental health and EA on the other. Overall, it seems that pleiotropy of genetic variants associated with EA play an important role in both smoking and mental health (Marees et al. 2020).

We further investigated the possibility that the different PGSs would show different profiles of GxE with environmental risk for smoking. If rGE between genetic effects on smoking and EA decreases the chance for detecting GxE, the PGS for smoking-without-EA ($PGS_{smok\text{-}noEA}$) should be more sensitive to detect GxE. Alternatively, there was a possibility that people with a genetic susceptibility for a lower EA (PGS_{EA}) would be more susceptible to environmental risk for smoking. Thus, we tested GxE of the $PGS_{smok\text{-}noEA}$ and PGS_{EA} with neighborhood quality. None of the interactions survived correction for multiple testing, but there were suggestive effects that contributed some explained variance. Specifically, in *NTR,* a high $PGS_{allsmok}$ was more likely to lead to smoking in lower-income neighborhoods, and a high PGS_{EA} was more likely to buffer against smoking in such neighborhoods. In *NEMESIS-2,* neighborhood quality had no main or interaction effects, so we used educational attainment as a proxy for SES. Here, a high $PGS_{smok\text{-}noEA}$ was more likely to result in smoking for people with a higher educational attainment, whereas there were no differences between smokers and non-smokers in $PGS_{smok\text{-}noEA}$ at low educational attainment. If neighborhood income and EA are regarded as aspects of the same underlying construct of SES, the GxE patterns in *NTR* and *NEMESIS-2* are incongruent (low SES amplified the PGS effects in *NTR* whereas high SES amplified PGS effects in *NEMESIS-2*). Furthermore, in *NTR* both smoking PGSs only had an effect on satisfaction with life at high neighborhood income, whereas no such GxE effects on mental health were observed in *NEMESIS-2*. These inconsistencies could suggest that GxE effects are specific to different aspects of the same environmental exposure (although alternative explanations, such as sample differences, are also possible). There were no clear patterns that could be discerned across samples and outcomes; the results did not clearly align with general models of differential susceptibility (Belsky and Pluess 2009) and did not show consistent differences between the type of PGS.

Important limitations of this study include the focus on smoking status rather than smoking quantity or nicotine dependence, which are more in-depth measures of smoking behavior and have been shown to be more heritable (Vink et al. 2005). However, given the need for statistical power we chose not to limit our analyses to sub samples of smokers, but rather used a general phenotype that was available for larger groups. Our use of the maximum sample size from the discovery sample (*UK Biobank*) and two independent target samples (*NTR* and *NEMESIS-2*) resulted in high power levels. Although the *NEMESIS-2* sample size was limited, it included high-quality measures (especially of mental health), making it a valuable addition. The self-reported neighborhood quality measure did not

predict smoking, which is not in line with previous literature. This could suggest that this measure does not reliably capture the neighborhood quality construct. Potentially, feelings toward the neighborhood constitute something inherently different than actual affluence (Wen et al. 2006; de Vries et al. 2020). Our use of different measures across the samples for SES (neighborhood-level income, self-reported neighborhood quality, and individual-level EA) and well-being (satisfaction with life and mental health) could be viewed as a limitation. It has certainly complicated the interpretation of the diverse GxE patterns that were observed. On the other hand, the use of different measures gives a more complete picture of the different aspects of the constructs of interest. It has alerted us to the presence of potential differences for specific (GxE) relationships tested. Another limitation includes the small effect sizes of the PGSs, which is a common limitation of the PGS method resulting from GWAS-identified genetic effects explaining only part of the trait. As a final limitation, our Genomic SEM model could not separate genetic effects on smoking that went *via* EA (i.e., were mediated by EA) from the total genetic effects for EA. Such 'mediation' variants might constitute a measure of vulnerability to EA circumstances, capturing the risk that a low EA would result in smoking behavior. A PGS based on such variants might be more likely to show interaction with environmental circumstances. Future research could aim to capture variants that increase *vulnerability to* an environmental exposure, rather than variants that simply increase the chance of being in such an environment.

The findings from this study have some important implications. We showed that, to some extent, genetic effects on EA could be subtracted from genetic effects on smoking, implying that besides overlap, there is also specificity in the genetic risk for EA and smoking. Focusing on specific genetic risk for smoking could improve precision of genetic prediction models and provide information on EA-independent etiological processes. This study has shown the feasibility and potential usefulness of dividing genetic predisposition in sub components, given that the components showed diverging patterns of overlap and their PGSs showed different main and interaction effects. This approach may be useful in other frameworks where it is important to tease apart pleiotropic and rGE effects, such as in Mendelian Randomization. The inconclusive GxE findings add to the mixed body of literature on GxE effects in substance use (Pasman et al. 2019). The fact that GxE effects did not reach significance and followed no clear pattern across different PGSs could be taken to suggest that GxE effects are small and specific to the individual relationships tested. The possibility that GxE effects are specific to the exact components that are in the PGS and in the environmental exposure opens up new lines for future research. Instead of reasoning from an overarching theoretical model (such as diathesis-stress or differential susceptibility) research could return to the drawing table and focus on testing interaction between specific genetic factors (e.g., 'clean' genetic risk factors,

controlled for environmental covariates) and specific environmental factors (e.g., housing value). Furthermore, given the evidence for rGE, it seems hardly accurate to continue speaking of interaction with the environment, since environmental circumstances are not actually something separate from the individual and their genetic make-up. Future research should be increasingly conscious about the meaning of statistical choices to model components as G, E, rGE, or GxE, and, preferably, test them concurrently.

Concluding, we show overlap and specificity in the genetic etiology of educational attainment and smoking. Gene-environment correlation plays an important role in the etiology of smoking. Evidence for gene-environment interaction was limited, but we showed the feasibility of the approach of modeling GxE using 'partitioned' genetic risk factors as a tool to investigate questions of overlap and interplay. Approaches such as those could contribute to further disentangling the knot of genetic and environmental factors in the etiology of smoking and other complex traits, while providing further insight into where they overlap and interact.

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Supplementary Materials

There is a large number of supplementary materials associated with this paper. Full materials can be viewed online at:
Chapter 12 – The role of socioeconomic status in smoking genetics

or copy this link into the browser:
https://drive.google.com/drive/folders/1OHx4FPnE1B7HGkwrvw20B92zSAp3Vtjs

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PART 4

DISCUSSION AND APPENDICES

CHAPTER 13

General discussion

Summary

In this thesis, I presented studies aimed at discovering genes for substance use and leveraging gene findings to answer new research questions on causal relationships and gene-environment interplay. The gene discovery studies took place in a context of rapidly developing technological and methodological advances in combination with expanding sample sizes of accessible data sets, that enabled the adoption of increasingly sophisticated methods. I started out with an adaptation of the traditional candidate-gene design, strengthening it by using larger samples, a within-study replication, an instrument that combined several variants in multiple genes, and a phenotype that summarized multiple substance use traits (chapter 2). Despite these efforts to increase power, no associations were detected between the candidate-gene instrument and substance use in my main analyses, nor in any of the sensitivity tests. These null findings, however, do not imply that genetic variants are irrelevant to substance use. Rather, the null results are likely due to the fact that this study did not tackle two crucial limitations of the candidate-gene design. First, candidate-genes are selected based on a body of literature that is full of false positives and has largely overlooked variants that we now know are important contributors. Second, candidate-gene studies have typically only looked at a few genetic variants, whereas their effects are likely so small that they cannot be detected with the employed sample sizes (Border et al., 2019; Duncan & Keller, 2011; Duncan, Ostacher, & Ballon, 2019; Johnson et al., 2017).

For my next study we therefore adopted a hypothesis-free design (genome-wide association study [GWAS], chapter 3), scanning the whole genome for associations between genetic variants and lifetime cannabis use. The sample size of this study was substantially larger than the one for my first study, with N=184,765. Indeed, this GWAS identified a number of variants and genes that were associated with the chance that someone had used cannabis. The top-gene was *CADM2,* a gene important for neural connectivity. In a next study we combined the results from this GWAS with those from other substance use GWASs to identify latent genetic factors underlying common substance use vulnerability (chapter 4), thereby increasing the power for discovery even further. We replicated and extended on gene findings for substance use traits from previous studies, and discovered new variants for more general substance use liability.

Findings from gene discovery studies have been and are being leveraged in a number of ways. First, follow-up research on specific variants and genes can be conducted. For example, our cannabis GWAS identified the *CADM2* gene as the main risk gene for cannabis use. This has led to numerous follow-up studies, including the 'reversed-candidate-gene studies' presented in chapter 5 and 6. In these studies we select the *CADM2* gene as a

candidate based on the results of the hypothesis-free scans of the whole genome instead of selective theoretical literature, and tested the association between this gene and a plethora of (risky) behavioral outcomes. Given that we already have some knowledge about the function of this gene, findings provide us with clues on the underlying biology of these traits. As an additional advantage, power levels are higher because we take the variants in one gene as our starting point rather than the whole genome, reducing the multiple testing burden. We showed the wide involvement of the *CADM2* gene in risk behavior and other (health) behaviors.

Second, GWAS results can be used to investigate the genetic overlap between traits. In chapter 3 we used this method to show that if someone is genetically vulnerable to start using cannabis, they are more likely to be genetically vulnerable to other kinds of substance use and mental health disorders. In chapter 4, we used these patterns of genetic overlap to identify latent genetic factors, including general vulnerability to substance dependence. Subsequently, we showed that the substance use dependence factor overlapped with genetic risk for a large number of unfavorable outcomes, including psychiatric disorders, physical health issues, and lower cognitive function. In chapter 7 we show genetic overlap between insomnia and substance use, and in chapter 8 between subcortical brain region volumes and substance use.

Third, variants identified in a GWAS can be used as instruments to test causal relationships. As genetic variants are randomly distributed across the population and they cannot be influenced by confounders, they are suitable instruments to test causality in a Mendelian Randomization design (MR). With MR studies we can use variants identified by GWASs as instrumental variables to test fundamental questions on what causes what, providing unique information on risk factors for and consequences of substance use. Using this design we showed a causal effect of schizophrenia liability on lifetime cannabis use (chapter 3), with only weak evidence for an effect of cannabis use risk on schizophrenia. In chapter 4 we found some support for causal effects of liability to substance dependence on ADHD and other psychiatric disorders. In the other direction, there was evidence that liability to ADHD and schizophrenia can cause substance dependence. For insomnia, I found that risk for insomnia causally affected smoking, alcohol dependence, and cannabis initiation, whereas in the other direction there was strong evidence only for a causal effect of smoking initiation risk on insomnia (chapter 7). In chapter 8 we found that genetic liability for alcohol dependence decreased amygdala and hippocampal volume, and that smoking liability decreased hippocampal volume. In the other direction, there was no strong evidence for causal effects of subcortical brain region volumes on substance use.

Finally, variants discovered in a GWAS can be taken together in a polygenic (risk) score. A polygenic score (PGS) counts and weights risk variants in an independent sample, resulting in an individual-level genetic risk profile. This approach can be used to investigate a myriad of interesting research questions. In chapter 9 I reviewed studies that have used polygenic scores to test gene-environment interplay. In gene-environment interaction (GxE), environmental circumstances moderate the effect of genetic predisposition, such that genetic predisposition comes to expression more strongly in certain circumstances. My review shows that a) modern PGS methods outperform traditional candidate-gene GxE methods, b) the quality of GxE studies can still be much improved, c) the evidence of GxE in substance use is still limited, and d) the possibility of gene-environment correlation (rGE) is insufficiently accounted for in these studies.

It is interesting to view the results from chapter 9 against the background of changing perspectives in the field of behavior genetics. When I started my PhD, much was expected from GxE research: it was viewed as one of the possible solutions to the missing heritability problem (Manolio et al., 2009) and was thought to play a key role in virtually all behavioral traits (Manuck & Mccaffery, 2014). However, in more recent years it has become clear that the power levels that we currently reach in our GWAS and PGS studies are often hardly sufficient to detect statistical interactions, which is apparent from the results from chapter 9. It seems that GxE effects are minuscule or even absent in many cases where they had been predicted to be widespread. Consequently, it seems that research attention for GxE has become more subdued. On the other hand, there has been a shift toward more attention for rGE. In 2018 a paper called ‘The nature of nurture’ sparked great research interest into what was called ‘genetic nurturing,’ or rGE with the parenting environment (Kong et al., 2018). A method was presented to distinguish between direct genetic and rGE effects from a PGS. The authors showed that parental genetic make-up for educational attainment influences offspring educational attainment not only through overlap with offspring genetic make-up, but also through shaping the (socioeconomic) environment of their offspring. These genetic nurturing effects were quite substantial and also contributed to smoking behavior in this study. Although an innovative and promising method, this modeling of parental genotypes does not (yet) integrate possible GxE effects.

Following these developments, in my next studies I aimed to simultaneously investigate the effects of rGE and GxE on substance use. In chapter 10 I investigated the interaction as well as the correlation between PGSs for substance use and the neighborhood environment. Results were somewhat mixed, showing little evidence for GxE and unexpected suggestions for correlations between the PGS and covariates such as age and the time period that someone grew up in. Though not providing conclusive evidence for

the occurrence of GxE, these findings prompted me to investigate rGE more, as it seemed to play some role. In chapter 11 I went a step further and tested rGE and GxE in the same model, to assess their relative contribution. I focused on the parent environment this time. In this context, rGE effects are likely to occur, for example through the overlap between parents' and offspring's genetic risk for substance use. In this study I showed that parenting behaviors influenced smoking directly, but were also correlated with the offspring's PGS for smoking. Moreover, parenting behaviors interacted with the smoking PGS such that they increased the chance that genetic risk led to smoking. For alcohol consumption and cannabis initiation there was little evidence for such direct, GxE, or rGE effects. An important conclusion from chapters 9 to 11 is that rGE effects are important in the etiology of substance use, and are deserving of more research attention. Also, rGE effects can impact both the chance of detecting GxE and the interpretation of GxE findings (Rathouz, Van Hulle, Rodgers, Waldman, & Lahey, 2008). I did not find evidence that controlling for rGE greatly increased the chance of detecting GxE, but rather that GxE and rGE can occur simultaneously for the same G and E factors. New insight is to be gained from these findings. Environmental influences do not operate independently from genetic factors, and show complex interplay in the etiology of substance use.

In my final chapter 12 I aimed to further disentangle this complex interplay between genetic and environmental factors. In all studies that I reviewed in chapter 9, the G factors used to test GxE effects were based on 'main effects' findings. In other words, the genetic variants used to measure G had all shown to have a direct relationship with substance use, either in a candidate-gene study or a GWAS. However, gene finding studies cannot discriminate in pathways through which a variant has an effect. GWAS results are diluted by signal for related traits (e.g., a GWAS on lung cancer will pick up variants associated with smoking) and signal for environmental circumstances (e.g., a smoking GWAS will pick up variants associated with the socioeconomic environment and level of education). Aiming to disentangle some of these effects for smoking, in chapter 12 I performed GWASs capturing genetic effects on smoking, on educational attainment, and on smoking *independently* from overlap with genetic effects on educational attainment. I showed that PGSs based on educational attainment and on smoking-without-education predicted smoking independently. To assess the possibility that either of those PGS effects were more likely to be augmented by environmental risk factors, I tested GxE with indicators of neighborhood socioeconomic status. I detected some hints for GxE effects, but these did not follow a distinct pattern per PGS type, nor were they consistent across different samples and measures. This paper showed the feasibility of partitioning the PGS for smoking into different components with distinct predictive profiles, although it remained unclear if this approach can help us to uncover reliable GxE mechanisms.

Strengths and limitations

The main strength of this dissertation lies in the flexibility and adaptability of my research questions and methods. In several ways I have reacted to shifts in the field of behavior genetics. I started out with a candidate-gene study when many drawbacks of the design were already known, but there was still hope that it could be redeemed by strengthening the design (using larger samples, combining variants, in-study replication). However, even with these improvements no associations were found in my first study. Originally, I had planned to follow-up on my first study by testing the same candidate-gene score on different phenotypes and in GxE designs. However, in the meantime new studies came out pointing out the problems associated with the very foundation of the candidate-gene method (Border et al., 2019; Duncan et al., 2019; Johnson et al., 2017), and I decided to abandon this approach.

I stepped into the field of the GWAS by joining the *International Cannabis Consortium*. GWASs were popping up for every thinkable phenotype with a speed uncommon to the field of the social sciences, where I was rooted. Our cannabis GWAS resulted in interesting gene discoveries and has become widely cited in the field. However, GWASs come with their own limitations, including the large multiple testing burden, dilution by environmental signal, and shallow phenotyping. An important strength of my thesis is that I circumvented or even exploited these limitations, while taking advantage of the invaluable information that GWASs provide. For example, based on the GWAS literature I could conduct empirical, 'reversed' candidate-gene studies. Given the fact that I conducted the trait-association tests gene-wide instead of genome-wide the multiple testing burden was greatly reduced, so that I could also include more in-depth phenotypes from smaller samples. Furthermore, I capitalized on multivariate GWAS methods to increase power for discovery. By combining different GWASs, signal strength could be increased while at the same time transcending the limitations from any specific source GWAS. Finally, I investigated dilution by environmental signal (rGE) and used it to answer research questions on gene-environment interplay, rather than simply viewing it as a limitation to the GWAS method.

All these studies rely heavily on another development that forms the backbone and major strength of the field: the increasing preparedness of governments, institutions, and scientists to share both their research findings and their data. The chief example is the publication of the UK-Biobank dataset, that I have used for 9 of the chapters in this thesis. The UK-Biobank is a governmentally funded dataset including ~500,000 individuals from the UK, providing a tremendous amount of biological and phenotypic data (Bycroft et al., 2018). It can be accessed by the international scientific community to investigate public

health questions, and has caused a true revolution in the field of behavior genetics. This increased availability of datasets suited for GWAS has led to a shift in the field. Instead of elementary GWAS analyses, that everyone in principle could now conduct, the focus is now more on in-depth research into a wide range of topics, of which my thesis chapters form only one example. Another dataset that I have gratefully mined is that from the Netherlands Twin Register (Ligthart et al., 2019; Willemsen et al., 2013), that includes genetic and longitudinal in-depth measures on a wealth of (mental) health and behavioral traits for over 20,000 twins and their family members. Given the small effect sizes that are the topic of our research, large samples are the prerequisite for conducting genetic studies. Without this preparedness to share in the mammoth efforts of collecting all these data and making it accessible, the majority of behavior genetics studies that have been published in the last decade could not have been conducted. We are all standing on the shoulders of giant(dataset)s.

Still, some limitations of the GWAS method are not easily evaded. For example, the GWASs that I used have only focused on common genetic variants while excluding rare and non-SNP variants. It has been suggested that including more participants and more genetic variants could solve the problem of missing heritability (Manolio et al., 2009). Indeed, an exciting paper has recently come out that demonstrates this for height and BMI, where virtually all the genetic contribution estimated by twin studies could be explained by sequencing the whole genome (about 47 million variants instead of a subset of ~10 million common SNPs as is usually done) in a sample of almost 22,000 people (Wainschtein et al., 2019). Thus, the exclusion of rare variants in the GWAS that I used indeed form an important limitation. Likewise, in none of my studies I have taken into account the possibility of interaction between genetic variants or pathways, which could be widespread across the genome (Mackay & Moore, 2014). However, such effects are likely to be minute and will probably not explain much of the differences in substance use behavior between individuals (Hivert et al., 2020).

Also, some of my studies focused on shallow phenotypes (e.g., chapters 3, 7), although in others I was able to include more in-depth traits (e.g., chapters 5, 6, 11). This limitation is common to many GWASs. In order to achieve samples as large as possible to maximize statistical power, GWAS often use measures from large population databases. The strength of such databases is that they include a plethora of measures that are potentially interesting for scientists from all thinkable fields, but to limit the burden for both researchers and participants the surveys cannot go into great depth for each single measure. Furthermore, many GWASs meta-analyze slightly different measures from several (smaller) samples, which can result in imprecise phenotypes. These issues have often been acknowledged, but so far increasing sample size is prioritized over deep

phenotyping (e.g., Howard et al., 2019; Liu et al., 2019). Although it seems that increasing sample size does help to reduce noise from imprecise phenotypes (Oexle, 2018), there is likely to be some trade-off between power and precision. Well-defined, deep phenotypes will result in smaller sample size and hence less statistical power to detect genetic associations in a GWAS, although they could still have a higher GWAS-based heritability than shallow phenotypes (e.g., Cabana-Domínguez, Shivalikanjli, Fernàndez-Castillo, & Cormand, 2019). Interestingly, my phenome-wide studies showed quite diverging patterns of associations of closely related phenotypes (e.g., different alcohol traits in chapter 6), suggesting that the merging of traits that is common in the field of behavior genetics can indeed introduce noise and lead to oversight of important differences in the etiology of seemingly similar traits.

Another crucial limitation that is common to the vast majority of studies in behavior genetics is the focus on European ancestry individuals. Findings from European GWASs cannot be generalized to other ethnic groups, due to systematic differences in the genetic make-up of individuals of different ancestry backgrounds. Say that a variant is more common in a certain ethnic group, and there is a certain phenotype that is also more common in that group, a GWAS would detect an association between that variant and that phenotype (population stratification). As an example, the association between a variant in the *DRD2* gene and alcoholism (that has often been reported in the candidate-gene literature) is likely largely due to differences in allele frequencies in different populations (Gelernter, Goldman, & Risch, 1993). Therefore, GWASs need to be conducted separately for different ethnic groups. However, there is a lack of genotyped and phenotyped individuals of a non-European background. This results in a shortage of GWASs in these groups, and down the line in the absence of PGS or MR studies. Although recent efforts to conduct multi-ethnic GWASs have been adorned, there is still a lot of room for improvement with regards to including specific ethnic groups and attaining larger sample sizes (Popejoy & Fullerton, 2016). My thesis results should be interpreted with these limitations in mind; they cannot be generalized directly to different ancestry populations.

It needs to be noted that although rGE effects were a focus of my research, I did not control for rGE effects in all of my studies, since they could not be modelled in a straightforward manner in most of them. For example, there was no control for environmental covariates in the cannabis GWAS. Any proportion of the effects that we detected could have been mediated by environmental circumstances, such as socioeconomic circumstances. This could be viewed as a limitation depending on the question one wants to answer using genetic studies. It would be a problem if the aim is to identify variants that have direct biological meaning for the trait (such as nicotine

receptor genes for smoking quantity). In my last chapter, I wanted to disentangle those environmentally mediated effects from those more 'direct' genetic effects. Still, I only separated the signal that went via socioeconomic variables. The remaining genetic variance could be diluted by other exposures, such as parenting characteristics, or genetic overlap with other traits. In chapter 11 I explicitly modeled rGE within the same model as main and GxE effects using structural equation modeling. In this study I can be reasonably sure that I actually grasped rGE and GxE in the parenting context. However, because I used PGS the results could not provide information on the specific genetic variants that drove direct, rGE, or GxE effects.

Summarizing, this thesis has adopted a versatile and flexible approach, capitalizing on the ever-increasing sample sizes of publicly available datasets and new methodological advances in the field. It has to be interpreted in the light of some important limitations, including those of the GWAS method itself. Still, these limitations must be placed into the perspective of the tremendous advances in the field. The GWAS method is only 15 years old, and although there is room for many improvements, it has taken the field unbelievable far in such a short time span. If the speed of the past developments is any indication, we can be optimistic that many of the limitations will be tackled soon. We can gratefully exploit the potential of the method as it is now (while being mindful of its limitations), and look forward to the unquestionably exciting new possibilities that lie ahead.

Behavior, genetics, and behavior genetics

The replication crisis

My work moves at the interface between the social sciences and molecular genetics. This has a number of advantages, including the possibility to employ a rich variation of research tools and methods within an interdisciplinary perspective. There are also disadvantages, however. For instance, both the replication crisis in psychology (Maxwell, Lau, & Howard, 2015) as well as the one in the field of candidate-gene studies (Border et al., 2019; Duncan & Keller, 2011; Johnson et al., 2017) are relevant to the field of behavior genetics. As described before, the replication crisis in genetics has fueled a rapid transformation of methodology, from hypothesis-driven to hypothesis-free, genome-wide research. The field of behavior genetics has been extremely quick and versatile in accepting the self-critique and embracing new techniques. The scientific movement towards more openness and transparency has also been taken up rather more quickly than in other fields, so that there is a high level of control of datasets, results, and methods by colleague scientists. In my PhD project I have followed these developments and taken

advantage of the new techniques. Although my studies have not been replicated directly, the use of GWAS results to test associations in new samples (e.g., with PGS) can be viewed as an external validation of the results. Results from large GWASs in general seem to replicate reasonably well across different sites and samples (Buniello et al., 2019). It seems that the replication crisis in behavior genetics has been largely averted.

In spite of important improvements, the replication crisis in psychology has not yet fully abated. A few years ago, it became apparent that many key findings from psychology failed to replicate (Ioannidis, 2005; Pashler & Wagenmakers, 2012). In my 'phenotypic' work I have observed this phenomenon, as well. For example, in chapter 10 and 12 I did not find strong support for a widely reported association between the socioeconomic characteristics of the neighborhood and different types of substance use. Different factors have been proposed to contribute to this replication crisis. For example, the incentive system in science in general seems to reward novel, positive findings more highly than null findings or replication efforts, which could lead to publication bias. Recently, there have been important improvements. Although the publication system is still largely in place, new protocols are developed to prevent publication bias. During my PhD project I have observed how pre-registration has been embraced by behavioral scientists, and has become ingrained in the new generation of researchers. An ever-increasing number of scientists publish hypotheses, methods, and analytic strategies online before viewing their data (Nosek, Ebersole, Dehaven, & Mellor, 2018). Pre-registration has gained status among scientists, creating a kind of alternative incentive system where good scientific conduct is rewarded regardless of the findings. I have pre-registered several studies, making use of public platforms, or simply by sharing a detailed analysis proposal among collaborators.

A second important cause of the replication crisis in psychology seems to lie in the abundance of different research methods and operationalizations used to study psychological phenomena and the lack of direct replication efforts. I observed this phenomenon in chapter 9, where I reviewed GxE studies with heterogeneous variables and measurements. The fact that my entire PhD project relied on existing datasets has two important implications in this context. For one thing, I have been able to make use of extremely large sample sizes, that could never have been attained if I had collected my own data. The advantage is that, mostly, there has been no need to aggregate multiple smaller samples (which would have introduced measurement heterogeneity). On the other hand, this implies that I have not been able to use my own operationalizations and had to rely on the measures that were collected. Sometimes, I have had to resort to shallow phenotypes for this reason. I have been conscious about measurement in my work and have taken effort to combine multiple sources of information (e.g., by extracting

information from multiple survey items or using multiple informants). Although my work contains no direct replication studies, part of it replicates previous studies with some adaptation or extension (conceptual replication), providing indirect corroboration of previous findings. For example, chapters 5 and 6 both investigated the relationship between *CADM2* and behavior, which had been implicated in several previous studies. Also, every study that uses a PGS could be viewed as an external validation test of the source GWAS: do these variants also predict the trait outside of the original study? Summarizing, using pre-registration, robust operationalization, and (partial) replication I have aimed to meet the challenges of the replication crisis in behavioral science.

Theoretical challenges for behavior genetics

Other issues common to both psychology and genetics as well as issues specific to behavior genetics remain that are more fundamental. For one thing, human behavior is notoriously difficult to study, given that theories about behavior are 'underdetermined'. This is to say, the possible causal chains that could have resulted in a particular behavior are so numerous as to be infinite, if it is even true that behavior can be exhaustively explained by such a causal chain of identifiable variables (Glymour & Sanchez-Romero, 2018). In a similar vein, a fundamental limitation lies in the difficulty of defining and quantifying something as complex and multifaceted as human behavior. The aim of psychological science and behavior genetics alike is to understand human behavior. Although all humans would agree that behavior is not something static or homogeneous (either within or between individuals) behavioral science does tend to focus on single time-point, unidimensional measures. This issue impacts both the field of psychology and behavior genetics. Given the need for large sample sizes, the opportunities for longitudinal, in-depth research are limited in behavior genetics. However, other possibilities that do more justice to the complexity of human behavior without requiring mammoth sample sizes have become available in psychology and could be employed in behavior genetics, as well. For example, dense-time measurement could provide insight into development and complex dynamic processes on the individual level (Van Geert, 2011). Also, results from large-scale, static-measure GWASs can be applied in smaller samples with longitudinal data. In chapter 11 I used GWAS-based PGSs to study substance use development, making use of in-depth, multi-rater, longitudinal assessments.

Another issue that is specific to the field of behavior genetics lies precisely in this bringing together of behavior and genetics, that operate on two fundamentally different levels of explanation. Something as physically real as a single-nucleotide change in the DNA is linked to a behavioral construct, that is difficult to define and demarcate. A behavioral trait is not something real in nature. Rather, it is 'emergent' on its natural properties, of which genetics form only one small aspect. That is to say, behavior is something more

than the sum of its parts, like a tornado is more than the sum of dust, wind, and debris (O'Connor, 2020). What happens in the causal chain between the genetic variant and the behavioral outcome is still largely unknown. A relatively straightforward pathway would be that a genetic variant changes the level of expression of a protein coding gene, and the changed protein levels impact the functioning of a certain brain region, which in turn influences behavior. To my knowledge, for no variant-outcome relationship such a causal chain has been fully mapped (although analogous efforts in medicine show that it can be done at least to some extent, e.g., Peltonen, Perola, Naukkarinen, and Palotie, 2006). For complex traits, efforts are made to investigate parts of the chain (e.g., with gene expression studies, chapter 3, or imaging genetics, chapter 8). Even if the full path can be mapped from genetic variant to gene expression, to brain functioning, to behavior, there is a plethora of biological and environmental factors that can correlate with, moderate or otherwise impact each link in the causal chain. Moreover, as large amounts of SNPs impact any given complex trait, not one such path, but hundreds of them need to be delineated. In even more complex scenarios, a variant identified in a GWAS has no known function (e.g., is in a non-coding region), impacts a gene of unknown function, or impacts an entirely different trait that has an unknown relationship to the behavioral outcome of interest. Unfortunately, it seems that these latter scenarios are currently more common than the more straightforward scenarios. Thus, there is a host of missing links between genes and behavior. This eventually amounts to a language problem: we have no means of translation between the genetic and the phenotypic level. No single component of the behavior that we study has a direct genetic substrate; the words we use to describe behavior have no translation in the language of genetics. The fields may be said to be incommensurable (Kuhn, 2012). Still, if behavior genetics and the related (molecular) biology fields continue the rapid rate of development they have in the past decades, we can expect to see this change. Future studies will each form a piece of the puzzle that will form a translation between genes and behavior.

These theoretical issues of underdetermination, static/ shallow phenotyping and incommensurability are important when thinking about behavior genetics. They have led me to believe that a paradigm shift will at some point become necessary in the field. Not only are the phenotypes we study inherently complex, it seems that at all levels of the causal chain there is overlap and interaction. An intricate network of genetic and environmental factors that show interplay on all levels lies at the heart of substance use. To put it in the winged words from the Netflix series Dark: "Alles ist miteinander verbunden". When the focus is to increase our understanding of human behavior, science needs to move away from simple x-y (genes-behavior) associations and start to theorize about these networks. My thesis can be viewed as a small step in the endeavor to

understanding how all the components cohere in the complex causal network underlying substance use.

Future perspectives

A future direction that immediately follows from these theoretical issues would be to start mapping causal chains between genes and behavior. With the level of knowledge which has now been achieved in behavior genetics, a sensible first step would be to zoom in on the gene findings and start exploring biological pathways. For example, the *CADM2* gene emerged for a wide range of substance use and risk behaviors. An interesting possibility would be to investigate the exact function of this gene in animal models. Such research can provide insight into how enhanced or reduced expression of this gene impacts physiological processes and, down the line, behavior. Similarly, imaging genetics studies could shed more light on how differential *CADM2* expression influences neurological processes, such as reactivity or connectivity in reward circuitry. Mapping (parts of) the biological causal pathway from *CADM2* to behavior would be a vital starting point for informing our thinking about how genes relate to behavior.

At the other end of the causal chain, future research can also start tapping into this perspective of complex interrelatedness. For example, an interesting idea would be to investigate transgenerational transmission of substance use through environmental effects, genetic effects, rGE, and GxE effects. By investigating parent-child triads, transmitted genetic variant alleles can be distinguished from non-transmitted alleles in an elegant design to disentangle such effects (Kong et al., 2018). This could be taken a step further by incorporating indirect genetic effects on the level of the neighborhood, focusing on the average level of genetic risk for substance use among neighborhood residents. In that way I could disentangle rGE from environmental effects at the neighborhood level. It has been found that socioeconomic characteristics cluster both on an observable as well as on a genetic level (Abdellaoui et al., 2019). This means that people living in the same area are more genetically similar than people from different areas, which could result in a similar kind of G, E, rGE, and GxE effects as those that occur in the parenting environment. The results of my thesis have prompted me to this widening view, to do more justice to the complex system of interrelated factors at different levels of explanation.

Beside this comprehensive indirect genetics approach, many other lines of inquiry could be explored based on the findings of this thesis. My studies have shown that with current methods it is hard to detect GxE effects, suggesting that they may be less prominent than has been predicted. My suggestion following this observation would be to focus more on

other types of interplay (i.e., rGE, that has received far less research attention) and to develop new methods to investigate GxE. Given the intrinsic links between G and E it seems a promising option to focus on both types of interplay in a single endeavor. An interesting opportunity might be to test GxE with variants that enhance vulnerability to environmental circumstances, instead of variants that are associated with substance use directly. Such environmental susceptibility variants could for example be identified by conducting a GWAS on resilience. Say that a group of people is exposed to the same environmental risk factors, but some develop substance use and others do not, this would constitute a measure of vulnerability to environmental risk. One could conduct a GWAS on this vulnerability trait and use the results to create a PGS, which in turn could be used to test GxE with environmental risk factors. Of course, recommendations for future research also include addressing the limitations outlined above, including making use of larger samples (also in non-Europeans), deep phenotyping, and including rare variants.

These venues for future research are exciting and could further our understanding of human behavior, but may still seem fundamental or abstract to many. The question that I was asked most often during my PhD project was: why is genetic research important, and does this information impact human well-being or society in any way? I would like to finish my thesis by describing the applications of current genetic research, and sketch potential lines for future research that hold promise for a substantial and wide-ranging impact.

Knowledge and impact: directions for disseminating results

The most concrete possibility for applying findings from genetic research that is available to us now is educating scientists, clinicians, policy makers, and the general public on the contribution of genetic factors to substance use behavior. Especially in the context of addiction, this kind of information can help reducing stigma. Social stigma may contribute to poorer outcomes for individuals suffering from addiction (Luoma, Kulesza, Hayes, Kohlenberg, & Larimer, 2014; Matthews, Dwyer, & Snoek, 2017). Viewing addiction as a neurobiological disease with a genetic basis can help to reduce stigma (Volkow & Koob, 2015), which could enhance societal support for governmental investments in access to extended treatment (Dackis & O'brien, 2005).

There is still much to gain in educating the public on the genetic contribution to substance use and other behavioral disease traits. Even among my fellow social scientists genetics knowledge is limited. During my PhD project, I observed that substance use genetics (and genetics in general) receives little attention in the social sciences. When asked, students at Radboud University students (mainly from social sciences) somewhat underestimate the heritability of addiction (N=83, estimated heritability=47%, own data collected within

student wellbeing project, PI Vink, unpublished result). It appears that individuals who have family members that are nicotine dependent, abuse alcohol (>15 glasses a week), or regularly use cannabis or other drugs estimate heritability to be somewhat higher (51%) than individuals who do not see substance abuse in their family (43%). Likewise, individuals with substance using family members estimate their own addiction proneness to be higher (34% versus 20%). Apparently, seeing genetics 'at work' in the own environment serves as an important information source. This insight could be leveraged in developing educational materials for the social sciences. Possibly a more relatable approach will help genetics dispense with the image of being notoriously complex and abstract.

Better science communication is also crucial for dissemination of substance use genetics studies for a wider public. When our cannabis GWAS was published, we wrote a press release with a full summary of our findings. However, one particular finding from the study received disproportional amounts of media attention. In our follow-up Mendelian Randomization analysis, we found evidence that liability to schizophrenia causes cannabis use initiation, whereas the evidence for a causal effect in the other direction was weaker. Media showed a tendency to present these results as if we falsified the claim that cannabis use could cause psychosis, or even that cannabis would help alleviate schizophrenia symptoms. This is just one example of how scientific findings can be magnified or taken out of context, a phenomenon society is becoming increasingly aware of with the spreading of sensational and fake news during the covid-19 pandemic and the election periods. Scientists need to be careful and conscious about their communication and work with communication professionals before releasing potentially controversial findings to the public. For example, a recent GWAS on same-sex sexual behavior (Ganna et al., 2019) has taken substantial effort to educate the public about the nuances and the implications of their findings by collaborating with advocacy groups and stakeholders. They released an impressive body of infographics, a short movie clip, a website, and interviews. Their research has been well received and has led to remarkably little controversy in the media worldwide. In my PhD I have mainly used a more small-scale channel for disseminating results: the RAD-blog, a weblog from our '*Substance use, addiction, and food lab'* group that presents research findings in layman terms (in Dutch). It is read by clinicians, researchers, and the wider public and is an ideal tool to communicate the importance of genetic research in an accessible manner. Besides this blog, I use twitter to gain attention for my research findings.

Future clinical applications

Beside this first purpose of educating the public, genetic research has many more possible applications. For one thing, knowledge on the genetic basis of substance use

disorders can inform clinicians and policy makers decide on their approach to intervention and prevention. For example, different interventions might be required for people with a family history of addiction (i.e., high genetic risk) than for people without. Indeed, one study showed that alcohol use disorder patients with a family history of alcohol dependence benefitted more from naltrexone treatment compared to placebo than patients without such a family history (Monterosso et al., 2001). Likewise, it would be effective and cost-efficient to target substance use prevention efforts to individuals whom you know are at risk; focusing on people with a family history of addiction seems a promising option (Valdez, Yoon, Qureshi, Green, & Khoury, 2010).

Both for educating the public and making treatment choices as outlined above, it would suffice to know someone's family history of substance use, rather than their complete genetic sequence. However, knowing someone's actual rather than predicted genetic predisposition for substance use has several advantages. For one thing, in the future prediction will be more precise: having a family history of substance use does not automatically lead to a high own genetic risk (although there is of course a greater chance that this is so). There are already excellent examples where basing treatment choice on a single genotyped variant makes treatment more efficient. For example, a variant in the *OPRM1* gene is such a good predictor of treatment response to naltrexone versus acamprosate for alcohol dependence, that it is likely to become cost-effective to genotype this variant (Sluiter et al., 2018). Likewise, for treating nicotine dependence, it was shown to be cheaper to genotype a variant in *DRD2* and base the choice for bupropion versus nicotine patches on that, than to simply prescribe either (Welton, Johnstone, David, & Munafò, 2008).

Unfortunately, there are few genetic variants with such strong and reliable effects. For future applications, we will have to rely on polygenic scores that summarize genetic risk from numerous variants across the genome. Right now, PGS are still imprecise and underpowered; they often explain only a few percent of the variance in the outcome trait. However, this is likely to change in the future. Deep phenotyping, whole genome sequencing, and increasing sample sizes are likely to contribute to the power of discovery in GWAS, resulting in more accurate SNP effects and better PGSs. When that has happened, we will be able to predict who is more likely to develop some form of substance use behavior (or disorder) or to benefit from a certain treatment, especially if we also take into account environmental risk factors that could overlap or interact with these PGSs (Murray et al., 2020). It needs to be noted that the applicability of PGS in clinical practice will always remain proportional to the level of heritability (PGS will have less precision for traits with a low heritability) and to the rareness of the investigated trait (precision will be lower for rare traits; Murray et al., 2020). Still, assuming that it will

become ever cheaper to sequence DNA and that an infinite number of PGSs can be computed for each sequence, it may become cost-effective to tailor prevention and intervention efforts to someone's PGSs. Even if genetic contribution to a substance use trait is not extremely large, the societal and personal costs of substance use often are. Consequently, every piece of information that can contribute to a more effective strategy to reduce substance use can result in substantial gains for public health.

Conclusions

This thesis forms a valuable contribution to the substance use genetics literature. My studies have identified several novel genes that are associated with these behaviors and have provided more insight into common and unique genetic factors underlying substance use traits. Furthermore, I have shown how gene findings can be leveraged to test overlap and causal relationships between psychiatric traits and substance use, and to provide insight in interplay between genetic risk and environmental circumstances. The main conclusion from my studies is that there are complex interrelationships on all levels of the gene-behavior association, or stated more poetically: "Alles ist miteinander verbunden". First, genetic vulnerability for any substance use trait overlaps with vulnerability for other substance use traits and other psycho-behavioral traits in general, although there are also variants with more unique effects for certain traits. This emphasizes the complexity of studying behavioral concepts, that are not clearly demarcated entities in nature. Second, genetic predisposition to substance use traits does not only overlap with, but is also causally predictive of other traits. Third, genetic vulnerability for substance use overlaps with genetic predisposition to certain environmental circumstances. Such effects seem to be widespread and were in my studies more pronounced than interaction effects, where the strength of a gene-behavior association depends on environmental exposures.

Future studies should work towards mapping these complex interrelationships, both at the level of gene-environment interplay, as well as on the smallest level of biological pathways from gene expression to behavior. As our knowledge of the precise details of such associations grows, it becomes increasingly difficult to bring together all pieces of the puzzle. Scientists from numerous different disciplines will have to work together to develop models of explanation, rather than simply describing phenomena in their own niche. Ultimately, the goal of behavior genetics should be to understand the human condition, which is only achievable by bringing together different sources of information and levels of explanation.

My studies contribute to the extensive groundwork that is being laid out for future clinical applications of genetic knowledge. Already, genetic research has an impact by forming the public opinion on and policy for substance use and addiction. To a modest extent, genetic knowledge is already being applied to identify people at risk and to guide prevention and intervention choice. In the future, applications such as these will become increasingly feasible, and it is my belief that these will have a substantial impact on how we view, prevent, and treat substance use. I am confident that over the course of my future career I will see these possibilities unfold, and it is my ambition to keep contributing to the knowledge required to make this happen.

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DUTCH SUMMARY

Nederlandse samenvatting

Het gebruik van alcohol, tabak, en cannabis kan schadelijke gevolgen hebben voor de fysieke en mentale gezondheid, en is ondanks recente afnames nog altijd wijdverspreid in de Westerse samenleving. Onderzoek naar de oorzaken van middelengebruik kan ons helpen om manieren te vinden om de persoonlijke en maatschappelijke kosten verbonden aan de gevolgen van middelgebruik te reduceren. Een scala aan risicofactoren voor middelengebruik is in de afgelopen decennia geïdentificeerd middels wetenschappelijk onderzoek. Zo is het duidelijk geworden dat genetische aanleg een belangrijke rol speelt, en is er een verscheidenheid aan omgevingsfactoren in kaart gebracht die een bijdrage leveren. In dit proefschrift beschrijf ik mijn zoektocht naar genetische risicofactoren en het samenspel tussen die factoren en omgevingsinvloeden in de etiologie van middelengebruik.

Mijn proefschrift is ruwweg onder te verdelen in drie hoofdthema's. In deel 1 beschrijf ik drie studies gericht op het ontdekken van genetische varianten die bijdragen aan middelengebruik (*gene finding*). Deel 2 omvat vier studies die laten zien hoe dergelijke gen-ontdekkingen kunnen worden toegepast om nieuwe vragen te beantwoorden (*leveraging gene findings*). In deel 3 van mijn proefschrift (*gene-environment interplay*) ga ik in vier verschillende studies in op het samenspel tussen genetische factoren en omgevingsinvloeden bij middelengebruik.

Deel 1: genen voor middelengebruik
De genetische aanleg voor een bepaald kenmerk kan onderzocht worden met behulp van tweelingdata. Als eeneiige tweelingparen (genetisch identiek) meer op elkaar lijken wat betreft een bepaald kenmerk dan twee-eiige tweelingparen, is dit een indicatie dat erfelijke aanleg een rol speelt bij het kenmerk. Vanuit dit soort onderzoek is gebleken dat middelengebruik deels erfelijk bepaald is. Gemiddeld verklaren genetische factoren zo'n 50% van de verschillen tussen mensen met betrekking tot middelengebruik, al lopen de schattingen uiteen voor specifieke gedragingen en bepaalde sub groepen. De volgende stap is om te onderzoeken welke genetische varianten precies een bijdrage leveren aan welke gedraging. In kandidaat-gen onderzoek wordt een genetische variant geselecteerd waarvan op basis van eerder onderzoek voorspeld wordt dat die een rol speelt. Van zo'n variant is bijvoorbeeld gebleken dat deze effect heeft op een specifiek biologisch proces wat te maken heeft met middelengebruik, zoals het neurale beloningssysteem. Vervolgens wordt gekeken welke versie (allel) van zo'n variant samenhangt met middelengebruik. In de afgelopen twintig jaar is het steeds duidelijker geworden dat deze methode belangrijke tekortkomingen heeft. Omdat het effect van een enkele variant erg

klein is, zijn de steekproeven van honderden tot enkele duizenden deelnemers die werden gebruik vaak ontoereikend om zo'n associatie op een betrouwbare manier te testen. In **hoofdstuk 2** heb ik daarom naar meerdere varianten tegelijk gekeken, en heb ik mijn testen uitgevoerd in twee grote steekproeven. De geselecteerde kandidaat-genen bleken niet geassocieerd te zijn met middelengebruik.

Dit vloeide mogelijk voort uit een andere, meer fundamentele tekortkoming van de kandidaat-gen methode: het selecteren van enkele kandidaten op basis van beperkte kennis. De literatuur over de functie van bepaalde gen-varianten en hun invloed op gedrag is nog verre van compleet, en vaak is de empirische basis voor bepaalde associaties nog wankel. In de afgelopen twintig jaar heeft zich een ware revolutie voltrokken in het veld van de gedragsgenetica. Het complete menselijke genoom werd in 2003 voor het eerst in kaart gebracht. Samen met het inzicht dat de kandidaat-gen methode haar beperkingen had, leidde dit ertoe dat wetenschappers zich verenigden in enorme internationale consortia om iets nieuws te doen: een genoom-wijde associatie studie (GWAS). In plaats van naar de associatie met één of een paar kandidaten te kijken, scande men nu het complete genoom met miljoenen varianten om te kijken of er één tussen zat die een effect had. Omdat je daarvoor miljoenen tests moet uitvoeren met minuscule effecten heb je immense steekproeven nodig, die alleen te verwezenlijken zijn door internationale samenwerkingen en een verregaande bereidheid tot het delen van datasets. Het zijn deze ontwikkelingen, samen met de snelle opeenvolging van nieuwe, publiek toegankelijke methoden en technieken die een stortvloed aan nieuwe kennis hebben opgeleverd. In samenwerking met een groot internationaal team van wetenschappers en data-instanties heb ik voor **hoofdstuk 3** een genoom-wijde associatie studie uitgevoerd voor cannabisgebruik. We vonden een aantal genetische varianten die de kans vergrootten dat iemand ooit in zijn leven cannabis had gebruikt. Weinig van de varianten die we ontdekten waren eerder beschreven in kandidaat-gen onderzoek. Dit laat zien wat de grote kracht is van de GWAS methode: het ontdekken van nieuwe varianten die leiden tot nieuwe voorspellingen over de biologische basis van gedrag.

De ontwikkelingen zijn dermate snel gegaan dat het inmiddels niet meer volstaat om een enkelvoudige GWAS te publiceren (zonder follow-up analyses); met de publiek beschikbare data en analysetools kan ieder lab met de vereiste infrastructuur een dergelijke analyse uitvoeren. Er zijn talloze interessante verdiepende analysetechnieken ontwikkeld die wetenschappers in staat stellen om hun analyses uit te breiden en nieuwe vragen te beantwoorden. Zo kunnen de resultaten van GWASs gebruikt worden om de overlap in de genetische architectuur van verschillende kenmerken te onderzoeken ('genetische correlatie). In hoofdstuk 3 ontdekten we bijvoorbeeld dat de genetische kwetsbaarheid voor cannabisgebruik overlapt met die voor roken, alcoholisme, en ADHD.

Dat betekent dat als je genetische aanleg hebt voor cannabisgebruik, je een verhoogde kans hebt om ook aanleg te hebben voor die andere kenmerken. Een uitbreiding van het principe van genetische correlatie is Genomic Structural Equation Modeling, waarmee onderzocht kan worden of er latente, gemeenschappelijke genetische factoren zijn die zich in verschillende soorten middelengebruik kunnen uiten. In **hoofdstuk 4** presenteren we een dergelijke overkoepelende analyse op de resultaten van 12 verschillende middelengebruik GWASs, met daarin bijvoorbeeld onze eigen resultaten uit hoofdstuk 3 en verschillende rook- en alcohol-GWASs. We vinden bewijs voor vijf latente factoren, waaronder een factor voor verslaving. De genetische varianten die geassocieerd zijn met die factor dragen bij aan verschillende soorten verslaving, zoals alcohol- en cannabisverslaving.

Deel 2: gebruiken en toepassen van gen-ontdekkingen

Deel 2 en deel 3 van mijn proefschrift zijn in essentie verdere toepassingen en verdiepingen van GWAS resultaten. In hoofdstuk 3 (de cannabis GWAS uit deel 1(ontdekten we bijvoorbeeld een gen dat een belangrijke rol speelde in cannabisgebruik. Dit *CADM2* gen was niet voorgesteld in vroegere kandidaat-gen studies. Omdat we nu een solide empirische basis hadden voor dit gen, besloten we om een 'omgekeerde' kandidaat-gen studie uit te voeren. In **hoofdstuk 5** laten we zien dat dit gen niet alleen geassocieerd is met cannabisgebruik, maar met allerlei verschillende soorten middelengebruik, ander risicogedrag en impulsieve persoonlijkheidskenmerken. Voor deze studie konden we kleinere steekproeven gebruiken dan die nodig zijn voor een GWAS: omdat we maar enkele duizenden varianten testten (i.p.v. miljoenen) was de statistische power groter. Het spectrum van associaties was zo breed dat we besloten om verder te kijken. In **hoofdstuk 6** testten we de associatie met 241 verschillende uitkomsten om te onderzoeken of de rol van *CADM2* specifiek is voor risicogedrag, of dat het geassocieerd is met alle soorten gedrag. Het bleek dat *CADM2* inderdaad met een wijd spectrum van gedragingen was geassocieerd, maar ook weer niet met alle aspecten van gedrag. We vonden bijvoorbeeld sterke associaties voor eetgedrag, fysieke activiteit, en cognitie, maar minder met psychische stoornissen en sociaal gedrag. Ook binnen de uitkomstcategorieën waren verschillen, zodat gerelateerde kenmerken soms wel en soms niet geassocieerd waren met het gen. Dit laat zien dat zowel algemene als meer specifieke genetische effecten van belang zijn, en dat het de moeite waard is om apart naar specifieke kenmerken te kijken en niet alles samen te nemen.

De andere twee studies uit deel 2 laten een volgende interessante toepassing van GWAS resultaten zien. Genetische varianten waarvan is vastgesteld dat ze (robuust) samenhangen met een bepaalde gedraging kunnen worden gebruikt als 'instrument' om die gedraging te meten. Het voordeel daarvan is dat je causale verbanden kunt testen. In

observationeel onderzoek kun je op basis van een gevonden associatie tussen twee kenmerken niet concluderen dat de één de ander veroorzaakt; het kan tenslotte ook andersom zijn, of er kunnen andere factoren zijn die de relatie beïnvloeden. Deze problemen spelen minder als je een genetische variant als instrument gebruikt. Een genetische variant kan namelijk niet beïnvloed worden door externe factoren, het DNA ligt vast vanaf de geboorte. Met behulp van sensitiviteitsanalyses kan worden vastgesteld of de variant inderdaad een goed instrument is voor het kenmerk en of er inderdaad geen sprake is van een omgekeerd causaal verband. Dit geheel van technieken en theorie wordt Mendeliaanse Randomisatie (MR) genoemd. MR werd toegepast in hoofdstuk 3 om het causale verband tussen schizofrenie en cannabisgebruik te testen. Die test leverde overtuigend bewijs op voor een causaal effect van cannabisgebruik op schizofrenie, maar zwak bewijs voor een effect in de omgekeerde richting. In **hoofdstuk 7** vind ik sterk bewijs dat insomnia rookgedrag, alcoholisme, en cannabisgebruik kan veroorzaken. In de andere richting vond ik dat roken kan leiden tot insomnia. Dergelijke vondsten hebben duidelijke implicaties: in preventie en interventie moet bijvoorbeeld mogelijk meer aandacht komen voor cannabisgebruik als gevolg van schizofrenie (waar het zwaartepunt nu ligt op het omgekeerde). En aangezien roken en slapeloosheid in twee richtingen causaal gerelateerd zijn, zouden die mogelijk gezamenlijk aangepakt moeten worden in behandeling. Met MR kunnen ook diepere biologische mechanismes worden getest. Veel literatuur heeft gesuggereerd dat verschillen in de volumes van subcorticale hersenstructuren verband hebben met middelengebruik. Het zou kunnen dat dergelijke biologische verschillen leiden tot een grotere kwetsbaarheid voor middelengebruik, maar andersom is het ook mogelijk dat middelengebruik ervoor zorgt dat de volume van deze structuren afneemt. In **hoofdstuk 8** vinden we vooral bewijs voor die laatste voorspelling. Alcoholisme verkleint het volume van de hippocampus en amygdala, en roken leidt tot een kleinere pallidum en hippocampus. Dergelijke vondsten voegen weer een stukje toe aan de puzzel; gegeven de rol van de hippocampus in geheugenprocessen zouden deze vondsten bijvoorbeeld kunnen verklaren waarom alcoholisme gepaard gaat met geheugenproblemen.

Deel 3: gen-omgeving samenspel

De meest toegepaste manier om de resultaten van GWASs te gebruiken in vervolgonderzoek is met behulp van polygenetische scores. Het idee is om in een onafhankelijke steekproef per persoon een score te berekenen die gebasseerd is op de effectgroottes uit de GWAS. Met deze polygenetische score (PGS) meet men zo het genetisch risicoprofiel gebaseerd op de GWAS in een nieuwe groep mensen. PGSs zijn flexibel en eenvoudig toe te passen en kunnen gebruikt worden om een scala aan nieuwe onderwerpen te onderzoeken. In deel 3 van mijn proefschrift gebruik ik ze om het samenspel tussen genetische factoren en omgevingsinvloeden te onderzoeken.

In **hoofdstuk 9** breng ik onderzoek in kaart dat gen-omgeving interactie heeft onderzocht met behulp van PGS (en zet het af tegen oudere vormen van PGS gebaseerd op kandidaat-genen, zoals in mijn studie uit hoofdstuk 2). Bij gen-omgeving interactie hangt het effect van genetische kwetsbaarheid af van omgevingsomstandigheden. De gedachte is bijvoorbeeld dat mensen die al genetisch gevoelig zijn voor een bepaalde uitkomst een extra grote kans hebben op die uitkomst als er ook nog eens risicofactoren aanwezig zijn in de omgeving. Een voorbeeld zou zijn dat genetische kwetsbaarheid voor verslaving een extra groot effect heeft voor mensen die een traumatische gebeurtenis hebben meegemaakt. Mijn review toonde aan dat dergelijke interactie-effecten mogelijk een rol spelen in middelengebruik. Echter, de kwaliteit van de beschouwde studies was beperkt, de interactie-effecten waren klein, en de verschillen tussen studies waren groot. Naar aanleiding van deze bevindingen doe ik enkele aanbevelingen voor vervolgonderzoek naar GxE in middelengebruik. In mijn volgende studie volg ik deze aanbevelingen door gebruik te maken van de nieuwste PGS methoden, grote 'discovery' GWASs en 'target' steekproeven, en te controleren voor belangrijke (genetische) covariaten. In **hoofdstuk 10** beschrijf ik de resultaten. Net als in de review is het bewijs voor GxE niet overweldigend: één van de 14 geteste interacties is significant. Deze interactie laat zien dat een PGS voor alcoholgebruik een sterkere voorspeller is voor alcoholgebruik voor mensen die in een goede buurt wonen dan voor mensen die in een slechte buurt wonen. Mogelijk komt dat doordat mensen met een hogere sociaaleconomische status sowieso al meer alcohol drinken, en wordt dit effect verder versterkt door genetisch risico. Hierbij moet worden opgemerkt dat de effecten van de buurtvariabelen veel kleiner waren dan verwacht, en ook niet altijd in de voorspelde richting. De conclusie was opnieuw dat GxE mogelijk een rol speelt, maar dat het aandeel van dergelijke effecten in de etiologie van middelengebruik waarschijnlijk klein is.

Een andere beperking die ik had opgemerkt in de review van hoofdstuk 9 was dat er in weinig studies rekening werd gehouden met een andere vorm van gen-omgeving samenspel, namelijk gen-omgeving correlatie (rGE). Hierbij bestaat er een associatie tussen genetische aanleg (bijv. gemeten met een PGS) en een omgevingskenmerk. Er zijn verschillende manieren waardoor zo'n associatie kan ontstaan. Door genetische overlap tussen ouders en kinderen kan er bijvoorbeeld een correlatie ontstaan tussen de ouderomgeving en de genetische opmaak van het kind ('passieve' rGE). Bij actieve of reactieve rGE ontstaat er een verband met de omgeving doordat de genetische opmaak leidt tot een bepaalde gedraging die de omgeving beïnvloedt en vormt. Als er bij het bestuderen van gen-omgeving interactie geen rekening wordt gehouden met mogelijke effecten van gen-omgeving correlatie kan het gebeuren dat de interpretatie van interactie-effecten niet klopt. Het optreden van gen-omgeving correlatie kan zelfs leiden

tot vals positieve resultaten van GxE. Daarom is het belangrijk dat er gecontroleerd of tenminste gecheckt wordt voor rGE bij het onderzoeken van GxE. In hoofdstuk 10 heb ik getest op rGE. Ik vond geen correlaties tussen de middelengebruik-PGSs en buurtfactoren, maar wel opmerkelijke samenhang met covariaten zoals de tijd waarin iemand was opgegroeid. In deze studie kon ik niet statistisch controleren voor deze onverwachte effecten. In **hoofdstuk 11** heb ik een methode toegepast die dat wel mogelijk maakte, iets wat bij mijn weten nog niet eerder was uitgeprobeerd. In Structural Equation Modeling (SEM) kunnen hoofdeffecten, interacties, en correlaties tussen variabelen tegelijkertijd gemodelleerd worden. Zo kan ook getest worden in welke mate rGE effecten de resultaten van GxE tests vertroebelen, door modellen met en zonder rGE met elkaar te vergelijken. De resultaten laten bewijs zien voor rGE tussen oudervariabelen (middelengebruik van ouders, betrokkenheid, en de ouder-kindrelatie) en genetisch risico voor roken. Verschillende verklaringen zijn hiervoor mogelijk. Zo lijkt het aannemelijk dat de associatie tussen het middelengebruik van ouders en dat van kinderen te wijten is aan de genetische overlap tussen ouders en kinderen. De associaties tussen genetisch risico voor roken enerzijds en ouderbetrokkenheid en de ouder-kindrelatie anderzijds zouden vormen kunnen zijn van reactieve rGE, waarbij het rookgedrag van het kind een negatieve reactie uitlokt bij ouders, die leiden tot lagere betrokkenheid en een negatievere relatie. Onafhankelijk van deze gen-omgeving correlaties trad er ook een interactie op tussen het middelengebruik van ouders en genetisch risico op roken. Het effect van genetische aanleg op roken werd extra versterkt door het middelengebruik van ouders. Dit complexe geheel van samenspel tussen genetische en omgevingsfactoren laat zien hoe verschillende componenten elkaar kunnen versterken, en hoe complex rookgedrag eigenlijk is. Voor alcohol- en cannabisgebruik werden dergelijke effecten niet geobserveerd, maar dit was mogelijk te wijten aan lagere statistische power voor deze uitkomsten. Ik heb met deze studie laten zien hoe GxE en rGE effecten tegelijkertijd getest kunnen worden. In dit geval bleek niet dat rGE de GxE effecten vertekenden. Wel toonden de resultaten dat rGE minstens even belangrijk is als GxE in middelengebruik, ook al heeft rGE voorheen veel minder aandacht gekregen in de onderzoeksliteratuur.

In **hoofdstuk 12** neem ik opnieuw een stap om GxE en rGE effecten in middelengebruik te ontwarren. Het nadeel van de PGS-methode in hoofdstuk 11 is dat ik weliswaar de relatieve bijdrage van GxE en rGE kon onderzoeken, maar geen mogelijkheid had om te achterhalen welke genetische varianten bijdroegen aan welk effect. Voor mijn volgende studie richtte ik mij op de bijdrage van rGE en GxE met opleidingsniveau op rookgedrag. Ik toon aan dat de genetische kwetsbaarheid voor roken voor een substantieel deel wordt gemedieerd door rGE met opleidingsniveau. Dat wil zeggen: veel van de varianten die geassocieerd zijn met roken zijn eigenlijk geassocieerd met opleidingsniveau, en door het

verband tussen opleidingsniveau en roken raken ze indirect geassocieerd met roken. De volgende stap was om te kijken of er GxE was tussen genetische aanleg voor een lage opleiding en omgevingsrisico (in dit geval de sociaaleconomische kenmerken van de buurt waarin iemand woont) op roken. Dit heb ik vergeleken met GxE met een PGS gebaseerd op de 'directe' genetische kwetsbaarheid voor roken, dus de varianten die niet gemedieerd werden door opleidingsniveau. Op die manier breng ik in kaart of er GxE optreedt met 'directe' versus 'rGE met opleidingsniveau' varianten in de voorspelling van roken. Ik vind aanwijzingen voor GxE, maar deze volgen geen duidelijk onderscheidbaar patroon voor de verschillende PGSs. Ook volgen de GxE effecten niet allemaal dezelfde richting bekeken over verschillende meetinstrumenten en steekproeven. Concluderend toont deze studie aan dat de genetische kwetsbaarheid voor roken kan worden opgedeeld in directe en indirecte componenten, en dat deze componenten op verschillende manieren samenhangen met andere variabelen. Vervolgonderzoek is nodig om vast te stellen in hoeverre de verschillende componenten zich gedragen in interactie met omgevingsrisico.

Samenvattend toont mijn proefschrift de resultaten van studies die met steeds sterkere methoden genetische varianten identificeren die geassocieerd zijn met middelengebruik. In deel 2 en 3 laat ik zien hoe je de vondsten van dergelijke studies kunt gebruiken om nieuwe, belangwekkende onderzoeksvragen te beantwoorden. Mijn omgekeerde kandidaat-gen studies brengen de brede rol van *CADM2* in (risico)gedrag in kaart, daarbij aanknopingspunten biedend voor vervolgonderzoek naar de biologische mechanismen van dat gedrag. Mijn MR studies laten zien hoe middelengebruik causaal samenhangt met psychiatrische stoornissen, en laten bovendien causale effecten zien van middelengebruik op hersenstructuren. Mijn onderzoek naar samenspel tussen genetische en omgevingsfactoren laat een belangrijke rol zien voor gen-omgeving correlatie (en, in minder mate, interactie) in middelengebruik. Genetische en omgevingsfactoren zijn intrinsiek verbonden en moeten gezamenlijk beschouwd worden.

Sterke en zwakke punten

Een sterk punt van dit proefschrift is hoe er flexibel gebruik is gemaakt van steeds nieuwe technieken en datasets. De drijvende kracht achter dit proefschrift, en in feite alle recente ontwikkelingen in gedragsgenetica, is de bereidheid tot grootschalige samenwerking in deze tak van wetenschap. Het delen van onderzoeksresultaten, analysetechnieken, web tools, en zelfs complete, originele datasets biedt ongekende kansen voor wetenschappers wereldwijd om hun onderzoeksvragen te beantwoorden. Ik heb dankbaar gebruik gemaakt van alle beschikbare materialen en technieken, en ingespeeld op de nieuwste ontwikkelingen. Dit proefschrift staat in de meest ware zin van het woord 'on the shoulders of giants'.

Een andere belangrijke ontwikkeling in de wetenschap in de afgelopen jaren is de verschuiving naar ‘open science’. Niet alleen resultaten en data worden gedeeld, daarnaast wordt steeds meer onderzoek gepre-registreerd en delen steeds meer onderzoekers analysescripts en complete resultatenbestanden. Dit laatste is vooral in het veld van de gedragsgenetica steeds gangbaarder. Pre-registratie houdt in dat wetenschappers van tevoren publiceren wat ze precies gaan onderzoeken en hoe ze dat gaan doen. Het idee is dat op die manier wordt voorkomen dat voorspellingen of methoden (veelal onbewust) worden aangepast als de resultaten op de één of andere manier afwijken. Ook wordt de onderzoeker gestimuleerd om van tevoren goed na te denken over methoden en analysetechnieken, en eventuele alternatieven te ontwikkelen voor het geval dat methoden niet blijken te werken. Met name in de gedragswetenschappen pre-registreren steeds meer wetenschappers hun onderzoek. Voor dit proefschrift heb ik eveneens mijn studieplannen gedeeld en gepubliceerd in het kader van transparantie. Op die manier heb ik bijgedragen aan deze beweging gericht op het terugdringen van publicatie-bias en non-replicatie van studieresultaten.

Vanzelfsprekend zijn er daarnaast tekortkomingen waarmee rekening gehouden moet worden bij het interpreteren van dit proefschrift. Zo werden er geen zeldzame genetische varianten opgenomen in de GWAS die ik heb uitgevoerd of waarvan ik de resultaten heb gebruikt. Zeldzame varianten zijn moeilijker om te onderzoeken omdat er nog grotere steekproeven nodig zijn; niettemin zijn er veel aanwijzingen dat de bijdrage van deze varianten aan gedrag substantieel is. Daarnaast is er voor mijn proefschrift vrijwel uitsluitend gebruik gemaakt van steekproeven met een Europese genetische achtergrond, omdat data voor andere etniciteiten zeer beperkt beschikbaar zijn. Doordat er belangrijke verschillen zijn in de genetische opmaak van verschillende bevolkingsgroepen, kan dat betekenen dat de resultaten van veel GWASs slechts beperkt van toepassing zijn in die groepen. Ten slotte is een belangrijke beperking van veel GWASs dat er veelal gebruik gemaakt wordt van ‘oppervlakkige’ maten. Omdat GWASs gebruik maken van grote databases met honderden verschillende variabelen, is de ruimte om gedrag in detail te meten vaak beperkt. Onprecieze meetinstrumenten kunnen leiden tot ruis in de GWASs en verminderen de statistische power.

Daarnaast zijn er meer fundamentele, theoretische overwegingen die in acht moeten worden genomen bij het interpreteren van gedragsgenetisch onderzoek. Gedrag is per definitie lastig te onderzoeken, omdat er zo veel factoren bijdragen aan iedere uitkomst, dat het aantal mogelijke oorzakelijke processen schier oneindig is. Daarnaast opereert de gedragsgenetica inherent op twee compleet verschillende niveaus, namelijk die van het biologische, van de direct observeerbare genetische variant, tegenover die van gedrag,

van complexe, abstracte constructen die niet direct aanwijsbaar zijn. De vertaalslag tussen deze niveaus is buitengewoon moeilijk te maken; er zijn legio nog onbekende processen die ervoor zorgen dat een variant uiteindelijk impact heeft op gedrag. Daarnaast zijn er op het niveau van gedrag nog talloze interacties en correlaties met andere factoren: gedrag komt tot stand in een eindeloos complex netwerk. Of om het met de woorden uit het motto van dit proefschrift te zeggen: 'Alles ist miteinander verbunden,' (*Dark,* Netflixserie).

Het is goed om deze beperkingen in het kader te plaatsen van de enorme wetenschappelijke en technische vooruitgang die is geboekt. Het is moeilijk voor te stellen dat de GWAS-techniek pas 15 jaar oud is, gegeven de razendsnelle ontwikkelingen die het veld heeft doorgemaakt. Toch staat de techniek feitelijk nog in de kinderschoenen, en zijn er nog belangrijke tekortkomingen. Dat wil echter niet zeggen dat we er geen gebruik van moeten maken. Je zou de analogie kunnen maken naar de auto-industrie. In de loop der jaren zijn er steeds verdere verbeteringen doorgevoerd; we begonnen met veiligheidsgordels, voegden airbags toe, en hebben nu automatische remsystemen. Dat wil niet zeggen dat we pas hadden moeten beginnen met autorijden in de jaren '60 na de introductie van de gordel, of zelfs pas halverwege de jaren '70 na de introductie van de airbag, al waren dat nog zulke essentiële verbeteringen. De GWAS-methode is nog jong; ongeacht de huidige tekortkomingen is groot enthousiasme en optimisme op zijn plaats.

Richtingen voor vervolgonderzoek en implicaties

Uit de hierboven genoemde beperkingen vloeien vanzelfsprekende aanbevelingen voort, zoals het gebruiken van gedetailleerdere meetinstrumenten, het meenemen van zeldzame genetische varianten, en het betrekken van steekproeven met een niet-Europese achtergrond. Meer inhoudelijk is een veelbelovende richting voor vervolgonderzoek om in kaart te brengen hoe genetische varianten uiteindelijk leiden tot middelengebruik. Een mooi beginpunt zou zijn om te kijken naar de biologische functie van het *CADM2* gen en het effect van CADMs in het brein, bijvoorbeeld in dieronderzoek of met 'imaging genetics'. Richtingen die direct voortvloeien uit huidig proefschrift richten zich meer op het complexe netwerk van factoren op het niveau van gedrag. Zo zou ik me graag verder willen verdiepen in 'indirecte genetische effecten', door bijvoorbeeld te kijken of iemands middelengebruik beïnvloed wordt door het gemiddelde genetisch risico op middelengebruik van buurtbewoners. Ook zou ik verder willen onderzoeken hoe gen-omgeving correlatie en gen-omgeving interactie van elkaar losgeweekt kunnen worden. Op die manier kan achterhaald worden of een genetische variant nu geassocieerd is met middelengebruik doordat deze samenhangt of interacteert met omgevingskenmerken, of vanwege een meer directe, biologische link. Dergelijke kennis kan helpen bij het verder uitdiepen van de (biologische) mechanismes die

middelengebruik onderliggen, en kunnen ook aanknopingspunten bieden voor behandeling.

Op dit moment zijn GWASs nog niet krachtig genoeg om polygenetische scores te genereren met klinische bruikbaarheid. Het is echter waarschijnlijk dat dit niveau in de nabije toekomst behaald zal worden. Als polygenetische scores sterker worden (door technische vooruitgang en doordat directe, GxE, en rGE effecten beter gemodelleerd zijn), zouden ze bijvoorbeeld gebruikt kunnen worden om risicopopulaties te identificeren. Het in kaart brengen van DNA wordt steeds goedkoper, en op basis van één DNA-profiel kunnen talloze PGSen berekend worden om te kijken op welke uitkomsten iemand een verhoogd risico heeft. Op basis van dergelijke informatie kan bijvoorbeeld gerichte preventie toegepast worden. Ook zouden PGSs kunnen voorspellen welke behandeling voor wie het beste zou werken. Op dit moment is het zelfs al zo dat er enkele varianten bekend zijn die kunnen voorspellen of iemand beter zal reageren op een farmacologische of psychologische behandeling voor alcohol- en nicotine-afhankelijkheid. De bekende effecten zijn op dit moment nog klein, waardoor het vaak niet rendabel is om dergelijke strategieën in de praktijk toe te passen. Maar als de snelheid van de ontwikkelingen in het genetisch onderzoek ook maar enigszins behouden blijft, kunnen we erop rekenen dat we binnen afzienbare tijd op dat niveau zijn.

Conclusie

Dit proefschrift is een waardevolle toevoeging aan de literatuur over de genetica van middelengebruik. Mijn studies hebben verschillende nieuwe varianten geïdentificeerd en hebben inzicht verschaft in de unieke en overlappende genetische factoren voor middelengebruik. Daarnaast heb ik laten zien hoe de vondsten van gen-identificatie onderzoek kunnen worden gebruikt om overlap en causale verbanden tussen middelengebruik en psychiatrische stoornissen te onderzoeken, en om inzicht te krijgen in het samenspel tussen genetisch risico en omgevingsfactoren. De hoofdconclusie van mijn studies is dat er complexe relaties zijn op alle niveaus van de associatie tussen genen en gedrag, of meer poëtisch gezegd: ‘Alles ist miteinander verbunden’. Ten eerste, genetische kwetsbaarheid voor elk soort middelengebruik overlapt met andere soorten middelengebruik en met andere gedragskenmerken, al zijn er ook varianten met meer unieke effecten op specifieke kenmerken. Ten tweede, genetische aanleg voor middelengebruik is ook causaal gerelateerd aan andere kenmerken. Ten derde, genetische kwetsbaarheid voor middelengebruik overlapt met genetische aanleg voor bepaalde omgevingsomstandigheden. Dergelijke overlapeffecten waren in mijn onderzoek meer uitgesproken aanwezig dan gen-omgeving interactie-effecten.

Mijn proefschrift draagt bij aan het fundament dat wordt gelegd voor toekomstige toepassingen van genetische kennis. Op dit moment heeft genetisch onderzoek al een impact doordat het de publieke opinie beïnvloedt en daarmee het beleid voor middelengebruik en verslaving. Ook nu al wordt genetische kennis in beperkte mate toegepast om risicogroepen te identificeren en te ondersteunen bij behandelingskeuze. In de toekomst zullen dat soort toepassingen steeds haalbaarder worden, en ik verwacht dat deze een grote impact zullen hebben op hoe we naar middelengebruik kijken en hoe we het behandelen. Ik geloof dat ik deze verwachtingen nog tijdens mijn carrière zal zien uitkomen en het is mijn ambitie om bij te blijven dragen aan de kennis die dat mogelijk gaat maken.

PUBLICATION LIST

First author publications

Year	Chapter	Reference
2021	5	Arends, R.M., Pasman, J.A. (shared), Verweij, K.J.H., Derks, E.M., Gordon, S.D., Hickie, I., Thomas, N.S., Aliev, F., Zietsch, B.P., van der Zee, M.D., Mitchell, B.L., Martin, N.G., Dick, D., Gillespie, N.A., de Geus, E.J.C., Boomsma, D.I., Schellekens, A.F.A., Vink, J.M. Associations between the CADM2 gene, substance use, risky sexual behavior, and impulsive personality. *Addiction Biology,* e13015.
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submitted	12	Pasman, J.A., Demange, P.A., Gülöksüz, S., Abdellaoui, A., ten Have, M., van Os, J.J., de Graaf, R., Verweij, K.J.H., Smit, D.J., Nivard, M., & Vink, J.M (in preparation). Genetic risk for smoking: disentangling gene environment interplay with socioeconomic status. *Behavior Genetics.*
in prep	4	Allegrini, A., Pasman, J.A. (shared), Smit, D.J., Nivard, M., Verweij, K.J.H., Vink, J.M. (in preparation). A molecular genetics study mapping the common genetic architecture of substance use traits. *Nature Neuroscience.*

Other publications

Year	Chapter	Reference
2017	NA	Roelofs, K., & **Pasman, J.** (2017). Stress, trauma, and cognitive functions in conversion disorder. In: Hallett, M., Stone, J. & Carson, A. (Eds., under revision), *Functional Neurological Disorders.* San Diego: Academic Press Inc.
2017	NA	Mies, G. W., Treur, J. L., Larsen, J. K., Halberstadt, J., **Pasman, J. A.,** & Vink, J. M. (2017). The prevalence of food addiction in a large sample of adolescents and its association with addictive substances. *Appetite, 118*, 97-105.
2018	NA	Ludwig, L., **Pasman, J. A.,** Nicholson, T., Aybek, S., David, A. S., Tuck, S., ... & Stone, J. (2018). Stressful life events and maltreatment in conversion (functional neurological) disorder: systematic review and meta-analysis of case-control studies. *The Lancet Psychiatry, 5*(4), 307-320.
2019	NA	Gillespie, N. A., **Pasman, J. A.,** Treur, J. L., Derks, E. M., Verweij, K. J. H., & Vink, J. M. (2019). High-potency cannabis and incident psychosis: correcting the causal assumption. *The Lancet Psychiatry, 6*(6), 464.
2020	NA	Chang, L. H., Ong, J. S., An, J., Verweij, K. J., Vink, J. M., **Pasman, J.,** ... & Derks, E. M. (2020). Investigating the genetic and causal relationship between initiation or use of alcohol, caffeine, cannabis and nicotine. *Drug and Alcohol Dependence, 210,* 107966.
2020	NA	Winiger, E.A., Ellingson J.M., Morrison C.L., Corley, R.P., **Pasman, J.A.,** Wall, T.L., Hopfer, C.J., & Hewitt, J.K. Sleep Deficits and Cannabis Use Behaviors: An Analysis of Shared Genetics Using Linkage Disequilibrium Score Regression and Polygenic Risk Prediction. *Sleep,* zsaa188*.*
2020	NA	Vink, J.M., Treur, J.L., **Pasman, J.A.,** & Schellekens, A. (2020). Investigating genetic correlation and causality between nicotine dependence and ADHD in a broader psychiatric context. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 2020, 1-7.

2021	8	Overbeek, M.F., Logtenberg, E. (shared first authors), **Pasman, J.A.,** Abdelaoui, A., Smit, D., Treur, J.L., & Verweij, K.J.H. (2021). Investigating the causal nature of the relationship between subcortical brain volume and substance use. *The British Journal of Psychiatry,* first view, 1-9. doi: 10.1192/bjp.2021.81
under review	NA	Marceau, K., Brick, L., **Pasman, J.A.,** Knopik, V.S., & Reijneveld, S.A. (under review). Interactions between genetic, prenatal, cortisol, and parenting influences adolescent substance use and frequency: A TRAILS study.: A TRAILS study. *European Addiction Research.*
accepted	NA	Linner, R.K., Mallard, T.T., Barr, P.B., Sanchez-Roige, S., Madole, J.W., Driver, M.N., …, **Pasman, J.A.,** ... & Liu, M. (2020). Multivariate genomic analysis of 1.5 million people identifies genes related to addiction, antisocial behavior, and health. *Nature Neuroscience, Pre-print available at bioRxiv.*

WORDS OF THANKS

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The roomies
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Writing acknowledgments like this is actually a wonderful tradition. Generally, the (academic) adventure continues, with continued support, but without the acknowledgments sections. How about we start a new tradition of taking a moment every now and then to thank each other for it all? I'll start. You can start looking forward to more of this after my Swedish post doc adventure.

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