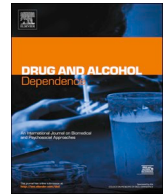




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Illicit drug use and the genetic overlap with Cannabis use

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ABSTRACT

Background: The use of illicit substances is correlated, meaning that individuals who use one illicit substance are more likely to also use another illicit substance. This association could (partly) be explained by overlapping genetic factors. Genetic overlap may indicate a common underlying genetic predisposition, or can be the result of a causal association.

Methods: Polygenic scores for lifetime cannabis use were generated in a sample of Dutch participants (N = 8348). We tested the association of a PGS for cannabis use with ecstasy, stimulants and a broad category of illicit drug use. To explore the nature of the relationship: (1) these analyses were repeated separately in cannabis users and non-users and (2) monozygotic twin pairs discordant for cannabis use were compared on their drug use.

Results: The lifetime prevalence was 24.8 % for cannabis, 6.2 % for ecstasy, 6.5 % for stimulants and 7.1 % for any illicit drug use. Significant, positive associations were found between PGS for cannabis use with ecstasy use, stimulants and any illicit drug use. These associations seemed to be stronger in cannabis users compared to non-users for both ecstasy and stimulant use, but only in people born after 1968 and not significant after correction for multiple testing. The discordant twin pair analyses suggested that cannabis use could play a causal role in drug use.

Conclusions: The genetic liability underlying cannabis use significantly explained variability in ecstasy, stimulant and any illicit drug use. Further research should further explore the underlying mechanism to understand the nature of the association.

1. Introduction

Illicit drugs are substances that either stimulate (e.g. cocaine) or inhibit (e.g. heroin) the central nervous system or cause hallucinogenic effects (e.g. LSD) to the effect that their nonmedical use has been prohibited globally (Hall et al., 2008; Uutela, 2001). For some substances, like cannabis, the prohibition or legalization status varies widely over time and over different countries and states (Wikipedia, 2019). In the present paper we focus on illicit drugs in a broad sense, including cannabis, ecstasy, stimulants, opioids. We do not consider substances that are legal in the Netherlands, such as nicotine and alcohol.

Cannabis is one of the most widely consumed drugs worldwide, with 192.2 million past-year users in 2016, corresponding to 3.9 per cent of the global population aged 15–64 years (Report, 2018). Despite the increasing use of cannabis for medicinal purpose and an ongoing debate about medicalization and decriminalization, associations with

adverse health effects have been reported. These adverse health effects include development of dependence, cardiovascular disease, impaired respiratory function and mental health problems (Hall and Degenhardt, 2009).

Another increasingly popular drug is ecstasy, a psychoactive drug that consists of MDMA. The prevalence in the global population aged 15–64 years is estimated to be 0.4 % (World Drug Report, 2018). In Europe, approximately 1.7 % of young adults (aged 15–34 years) have used ecstasy, with estimates ranging from 0.3%–5.5% between countries (European Drug Report, 2016). Other relatively popular illicit drugs include amphetamine and cocaine (both stimulants), with worldwide past year estimated prevalences of 0.77 %, and 0.35 % respectively (Peacock et al., 2018). The past year prevalence of opioids (including heroin, morphine, codeine, thebaine, oxycodone) was 0.37 % worldwide in 2017 (Peacock et al., 2018). For all illicit drug use together, the overall disease burden was estimated to be 27.8 million

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attributable disability-adjusted life-years (DALYs) in 2017. DALYs reflect the number of years lost due to ill-health, disability or early death. The mortality rate due to illicit drugs was 6.9 deaths per 100,000 people in 2017 (Peacock et al., 2018).

Substance use, including cannabis use, is moderate to highly heritable (Kendler et al. (2012a); Verweij et al., 2010, 2017). The largest genome-wide association (GWA) study for cannabis use to date has successfully identified 35 genes (in 16 regions) associated with lifetime cannabis use (Pasman et al., 2018). Two other genome-wide association studies identified genes for cannabis dependence and cannabis disorder (Agrawal et al., 2018; Demontis et al., 2019). In the current study we have information on use (not abuse or dependence), and will therefore use the GWA for cannabis use (Pasman et al., 2018) as discovery sample.

Epidemiological studies have consistently shown correlations between use of different substances, such that individuals that use one substance are more likely to also use another (Konkoly Thege et al., 2016; McCabe et al., 2015). The phenotypic correlations between substances are partly explained by common genetic influences (Kendler et al., 2012b; Nivard et al., 2016). Many genetic variants, each with a small effect size, contribute to complex behaviors, such as substance use. With methodological advances in molecular genetics and increased sample sizes in GWA studies it has become viable to use many measured genetic variations in individuals to estimate their genetic vulnerability for a certain trait. To do this, polygenic scores (PGS) in individuals from a target dataset can be calculated based on their genome-wide genetic data and the genetic effect sizes estimated in large GWA studies (discovery samples). If the PGS in the target set, for example reflecting the genetic vulnerability for cannabis use, is associated with drug use, for example ecstasy, this would suggest that there is overlap in the genes underlying cannabis and ecstasy use.

In the present study, we used summary-level data from the largest GWA study for lifetime cannabis use to date (Pasman et al., 2018) to generate PGSs in an independent sample of 8348 individuals registered at the Netherlands Twin Register (NTR). We tested the association of the PGS for lifetime cannabis use with ecstasy, stimulants (ecstasy, amphetamines, cocaine) and a broad category of drug use, including stimulants, opioids and hallucinogens. Based on previous literature (using different methods, such as twin models) we expect genetic overlap between cannabis use and drug use. A significant association (genetic overlap) may indicate that there are common underlying genetic predispositions to the use of these substances (*common liability* (Vanyukov et al., 2012)), or can be the result of a *causal association* (Kandel and Kandel, 2015) between the use of the different substances. In that last case, use of cannabis may lead to use of ecstasy or other drugs, and therefore genes associated with cannabis use will also –indirectly– be associated with use of other drugs. The different explanations are not mutually exclusive and are difficult to distinguish. If a significant association is found between the cannabis PGS and use of other drugs, we will explore the nature of this relationship by repeating the same analyses separately in cannabis users and non-users. If the association between the polygenic risk for cannabis and drug use is only significant in cannabis users and not in never users, this might indicate that causal effects play a role (Gage et al., 2016), although other explanations (cannabis users could represent a group with higher risk for substance use, resulting in multiple drug use) are still possible. To further explore the causal role of cannabis in other drug use, we also explored drug use in monozygotic twins discordant for cannabis use.

2. Methods

2.1. Sample

The sample consisted of people registered at the NTR who participated in wave 2 (1993), 3 (1995), 5 (2000), 8 (2009) and/or 10 (2013) of the longitudinal survey study for adult participants and have provided a DNA sample (Boomsma et al., 2006; Treur et al., 2016; Willemsen et al., 2013).

Birth year varied between 1915 and 1996. Median birth year was 1970. The sample consisted for 63.4 % of females.

For the MZ discordant twin analyses we selected MZ twin pairs who both reported on their cannabis use in at least one of the questionnaires. Data were available for 1302 MZ twin pairs. For 802 pairs (62 %), both members of the pair had never used cannabis, for 237 pairs (18 %) both twins had used cannabis and the remaining 263 (20 %) pairs were discordant for cannabis use. The sample of discordant twin pairs consisted of 89 male pairs and 174 female pairs.

2.2. Phenotype data

Cannabis: Data on cannabis use were administered in wave 3, 4, 5, 8 and 10, including a question on lifetime use. All available data were combined into a lifetime use variable (1 = yes, 0 = no).

Ecstasy: In survey 10, participants were asked whether they had ever used ecstasy, lifetime ecstasy use (1 = yes, 0 = no).

Stimulants: We asked about use of stimulant drugs in survey 2, 3, 5, 8 and 10 (see supplemental Table S1¹). We created a new variable for lifetime stimulant use, including ecstasy, amphetamine and cocaine, ‘stimulants’, coded yes (1) if a participant indicated to have used at least one of these substances in survey 2, 3, 5, 8 or 10 and no (0) if a participant indicated to have never used these substances.

Any illicit drugs: Because various questions on drug use were included in other waves as well (in various phrasings, see Supplemental Table 1²) we combined all responses on illicit drug use into the outcome measure: ‘lifetime use of any illicit drug’. This resulted in a variable including use of stimulants, amphetamines, cocaine, speed, ketamine, GHB (gamma-hydroxybutyric acid) and opioids (heroin, morphine, codeine, thebaine). We coded the variable ‘yes’ (1) if a participant reported in at least one wave to have used one of these drugs versus ‘no’ (0) if a participant never used illicit drugs. Please note that cannabis was not included.

Please note that the groups used to examine the three outcome phenotypes are highly overlapping and the results will therefore be redundant to some extent. We have chosen to analyse the three (overlapping) classifications of drug use, because the questionnaire items varied different waves. While a more clearly defined phenotype (ecstasy use only) has the advantage of being more specific, it also led to a smaller sample size. And as ecstasy belongs to the subgroup of stimulants we also decided to combine it with questions on use of other stimulants, resulting in a larger dataset. For the last group we included all available information on drug use.

An overview of the specific questions on illicit drug use per wave can be found in Supplemental Table S1³. The prevalence of drug use per wave can be found in Supplemental Table S2⁴. The total sample of participants with data on (any) illicit drug use and genetic data consist of 8442 subjects.

¹ Supplementary material can be found by accessing the online version of this paper at https://www.mf.surf.net/canit/urlproxy.php?_q=aHR0cDovL2R4LmRvaS5vcmc%3Dand_s=ai52aW5rQGJzaS5ydS5ubA%3D%3Dand_c=fd4512ffand_r=cnU%3D and by entering doi...

² Supplementary material can be found by accessing the online version of this paper at https://www.mf.surf.net/canit/urlproxy.php?_q=aHR0cDovL2R4LmRvaS5vcmc%3Dand_s=ai52aW5rQGJzaS5ydS5ubA%3D%3Dand_c=fd4512ffand_r=cnU%3D and by entering doi...

³ Supplementary material can be found by accessing the online version of this paper at https://www.mf.surf.net/canit/urlproxy.php?_q=aHR0cDovL2R4LmRvaS5vcmc%3Dand_s=ai52aW5rQGJzaS5ydS5ubA%3D%3Dand_c=fd4512ffand_r=cnU%3D and by entering doi...

⁴ Supplementary material can be found by accessing the online version of this paper at https://www.mf.surf.net/canit/urlproxy.php?_q=aHR0cDovL2R4LmRvaS5vcmc%3Dand_s=ai52aW5rQGJzaS5ydS5ubA%3D%3Dand_c=fd4512ffand_r=cnU%3D and by entering doi...

2.3. Polygenic scores (PGS)

Discovery samples: We used SNP effect sizes from the GWA statistics of a genome-wide association meta-analysis of lifetime cannabis use (ever used cannabis yes/no) to generate PGSs (Pasman et al., 2018). The single nucleotide polymorphism (SNP) effects were based on the meta-analytic samples excluding the NTR sample (N = 180,112).

Target sample: SNP data were available from genome-wide SNP arrays, collected at the NTR through several projects between 2004 and 2008 (Willemsen et al., 2013). Genotyping was performed across different platforms, including Perlegen-Affymetrix, Affymetrix 6.0, Illumina 660 and 1 M. After pre-imputation quality control, data were cross-platform imputed against a Dutch reference set. Stringent post-imputation quality thresholds were used. SNPs were removed if imputation quality score below 0.95, minor allele frequency smaller than 0.05 and/or deviation from Hardy-Weinberg equilibrium with p smaller than 0.001. Individuals were removed if their genotype missing rate was higher than 10 %, if they had excess genome-wide homozygosity or if they were of non-Dutch ancestry. We performed Principal Components Analyses (PCA) to exclude individuals with a non-Dutch ancestry and control for Dutch population stratification following procedures described in Abdellaoui et al., 2013. Detailed information on genotyping, genetic QC and imputation is available elsewhere (Abdellaoui et al., 2018).

PGSs were calculated using LDpred (Vilhjálmsón et al., 2015). LDpred computes SNP weights based on their effect size estimates, their linkage disequilibrium with other SNPs and the degree of polygenicity of the trait, quantified as the expected fraction of causal markers contributing to the trait. The reference panel used to determine linkage disequilibrium structure consisted of European populations of the 1000 Genomes project (Delaneau et al., 2014). In the current study we used the 0.3 fraction, representing an expected degree of 30 % polygenicity of the trait, based on estimates of polygenicity of previous studies using LDpred-based polygenic score prediction on cognitive and mental health outcomes (Hugh-Jones et al., 2016; Vilhjálmsón et al., 2015). The computed PGSs were standardized.

2.4. Statistical procedure

Prediction analyses were carried out using generalized estimation equations with a logit link function. To account for familial relatedness, this method uses an exchangeable covariance matrix, allowing for correlated residuals between family members. Analyses were run using robust standard errors for the parameter estimates. Sex, age, and 10 genetic principal components were included as covariates in all analyses. Principal components were included to correct for effects of population stratification. Age was negatively correlated with the outcome measures (the higher the age, the lower the likelihood of having initiated drug use) and males had a higher prevalence of drug use than females. To explore possible sex differences we tested the interaction between the cannabis PGS and sex for ecstasy, stimulants and any illicit drug use (both in total sample as well as in birth cohort > 1968). Estimates of the explained variance (Nagelkerke’s pseudo R²) were obtained from logistic regressions by subtracting the pseudo-R² estimates of the model with only covariates from the model including both the PRSs and covariates. Odds ratios were also obtained through the regression analyses.

2.5. Analyses were performed in SPSS version 24

The prediction analyses were carried out in the total sample as well as in a subsample of participants born after 1968, because the prevalence of illicit drug use among individuals born before 1969 was very low. We corrected for multiple testing with a Bonferroni correction, and considered a p-value smaller than 0.0083 to be significant (0.05/6 tests).

To inspect how drug use varied with increasing cannabis PRS we used quintile plots. The cannabis PRS was divided in quintiles, and we calculated the odds ratio for respectively ecstasy use, stimulants and any illicit drug use within each quintile (compared to the lowest quintile as reference category) (Choi et al., 2018).

For the twin analyses, we compared the prevalence of drug use in the cannabis using twins to that of their non-using co-twins with a McNemar test (a statistical test for paired nominal data). In this design, genetic and common environmental influences are controlled for because MZ twins share all their genetic material and their (early) home environment. If the association between cannabis use and other drug use is solely explained by genes and/or shared environmental factors, then the twins who have used cannabis and their co-twins who have not should be equal in their use of other drugs. In contrast, if the association is to some extent causal or explained by environmental factors for which twin pairs are discordant, we would expect to find significantly higher prevalences in the cannabis users compared to their unaffected MZ co-twins.

3. Results

The lifetime prevalence of cannabis use was 24.8 %, while the lifetime prevalence of ecstasy use was 6.2 % (N = 259/4153). About 6.5 % (N = 448/6877) of the sample reported to have ever used stimulants (ecstasy, amphetamine and/or cocaine) and the overall prevalence of any drug use was 7.1 % (N = 598/8442). Prevalences were significantly higher in the younger birth cohort compared to the older birth cohort, and in men compared to women (Table 1).

As a proof of concept, associations of the PGSs for cannabis use with reported cannabis use was tested. The PGS for lifetime cannabis use was significantly associated with cannabis use in the target sample (B = 0.271 (SE 0.035), p = 4.24 × 10⁻¹⁵, R² = 1.6 %). The PGS for lifetime cannabis use was significantly associated with cannabis use in both birth cohorts (B = 0.237 (SE 0.042), p = 1.29 × 10⁻⁸ in the people born after 1968 and B = 0.355 (SE 0.062), p = 1.08 × 10⁻⁸ in people born before 1969).

Significant, positive associations were found between PGS for cannabis use with ecstasy use, stimulants and any illicit drug use (Table 2), indicating that individuals with a higher genetic predisposition for cannabis use were more likely to have ever tried these drugs. Inspection of quintile plots confirmed that OR for ecstasy use, stimulants or any

Table 1
Prevalence of Ecstasy use, Stimulant use (Ecstasy, Amphetamine, Cocaine) and Any Drug use in the total sample, separately in two birth cohorts (born in or before 1968 versus born after 1968) and separately by sex. The difference in prevalence between the birth cohorts as tested using a Chi-square test.

		Ecstasy	Stimulants	Any illicit drugs
Total sample	N	259	383	598
	N total	4149	7152	8442
	Prevalence	6.2 %	5.4 %	7.1 %
Birth cohort < = 1968	N	19	60	103
	N total	1532	3209	3869
	Prevalence	1.2 %	1.9 %	2.7 %
Birth cohort > 1968	N	240	388	495
	N total	2617	3668	4573
	Prevalence	9.2 %	10.6 %	10.8 %
Difference test	χ ²	103.84	213.13	212.14
	p-value	< .0001	< .0001	< .0001
Men	N	110	207	273
	N total	1363	2433	3089
	Prevalence	8.1 %	8.5 %	8.8 %
Women	N	149	241	325
	N total	2784	4442	5351
	Prevalence	5.4 %	5.4 %	6.1 %
Difference test	χ ²	11.55	24.52	22.73
	p-value	.001	< .0001	< .0001

Table 2

Association of polygenic score for lifetime cannabis use with ecstasy use, stimulants (ecstasy, amphetamine, cocaine), and any illicit drug use in total sample, as well as in a subsample with participants born after 1968.

Polygenic Score	Birth year	N	B	OR	95 % CI	p-value	R ²
Ecstasy	All	4145	0.235	1.27	1.12–1.45	5.41×10^{-4}	0.9%
	> 1968	2615	0.260	1.29	1.13–1.48	1.68×10^{-4}	1.2%
Stimulants	All	7142	0.194	1.22	1.10–1.35	4.14×10^{-4}	0.6%
	> 1968	3666	0.239	1.27	1.13–1.42	4.40×10^{-5}	1.0%
Any illicit drug	All	8438	0.172	1.19	1.09–1.29	1.53×10^{-4}	0.5%
	> 1968	4571	0.196	1.20	1.09–1.32	9.80×10^{-5}	0.7%

illicit drug use increased with increasing polygenetic score for cannabis (see Supplemental Figures 1–3).

To explore possible sex differences we tested the interaction between the cannabis PGS and sex for ecstasy, stimulants and any illicit drug use (both in total sample as well as in birth cohort > 1968) but none of the interactions was significant ($0.10 < p < 0.46$).

The association between cannabis PGS and drug use seemed to be stronger ($p < .05$) in cannabis users compared to non-users for both ecstasy and stimulant use (but only in people born after 1968), although not significant after correction for multiple testing (Table 3).

When comparing the MZ twin pairs discordant for cannabis use on drug use, the prevalence of drug use was higher in the cannabis using twins compared to their co-twins. The difference was not significant for ecstasy use (which was a relatively small sample), but was significant for stimulants and any drug use. This means that cannabis use itself could lead to increased chance of drug use Table 4.

4. Discussion

We showed that the genetic liability underlying cannabis use significantly explained variability in ecstasy, stimulant, and any illicit drug use. When the sample was stratified for lifetime cannabis use, this association seemed to be stronger in cannabis users compared to non-users for ecstasy and stimulants, but not for any drug use. However, this trend was not significant after correction for multiple testing.

The observation that the PGSs for cannabis use were significantly associated with the examined drug use variables (ecstasy use, stimulant use, any drug use), suggests genetic overlap between the traits. The explained variance ranged between 0.5 and 1.2 %, which is quite low but consistent with other PGS studies of addictive phenotypes (Allegrini et al., 2018; Carey et al., 2016; Chang et al., 2019; Vink et al., 2014). As far as we know this is the first study exploring the genetic overlap of the genetic vulnerability for cannabis with other illicit drug use. Only a few studies explored genetic overlap across substances using a PGS method. A previous study showed genetic overlap between PGS for cigarettes per day with glasses of alcohol per week and cannabis initiation as well as between PGS for age at onset of smoking and age at regular drinking. However the PGSs for smoking initiation and smoking cessation did not significantly predict alcohol or cannabis use, possibly due to limited power (Vink et al., 2014). Demontis et al. showed that a PGS-for lifetime smoking was associated with cannabis use disorder (Demontis

Table 3

MZ twins discordant for ever cannabis use. Prevalence (%) of drug use twins who ever used cannabis (affected twins) and their co-twins who never used cannabis (unaffected twins). Differences between affected twins and their unaffected co-twin are tested with the McNemar test for paired samples (p-value in last column).

	N discordant pairs	Cannabis using twins	Non-using co-twins	McNemar test -value
Ecstasy	120	8.3%	2.5 %	0.065
Stimulants	229	9.2 %	2.2 %	0.002
Any drugs	259	15.8%	4.2 %	< .001

et al., 2019). Recently, Chang et al. tested the association between 5 PGSs for licit substances (smoking, alcohol use) with 22 target phenotypes for illicit substance use and substance use disorders. Only 9 of the 110 tested associations were significant. Interestingly, the stimulants (ecstasy, cocaine, amphetamine) showed some significant results, while associations with sedatives or pain killers were not significant. In particular, the PGS for smoking initiation significantly explained variation in the risk of cocaine, amphetamine, hallucinogens, ecstasy and cannabis initiation, as well as DSM-5 alcohol use disorder (0.67–1.54 %). The PGS for drinks per week significantly explained variation in cocaine, amphetamine and ecstasy initiation (0.59 % -0.90 %). Taken together, these results indicate genetic overlap between the use of different substances, although in previous studies not all tested associations were significant.

As explained in the introduction, genetic overlap may indicate that there are common underlying genetic predispositions to the use of these substances (Vanyukov et al., 2012). In case of drug use, this could be genes involved in the vulnerability for reward (associated with drug use), but could also reflect genetic vulnerability for more general personality traits, such as impulsivity, risk-taking behavior or sensation seeking which are also often associated with drug use (Tsavou and Petkari, 2019) or educational attainment (Abdellaoui et al., 2019).

On the other hand, genetic overlap can also be the result of a causal association (Kandel and Kandel, 2015). To explore whether cannabis use itself caused the use of ecstasy, stimulants or any drugs we tested the association between the PGS for cannabis and the outcome variables in cannabis users and never users separately. The association of the cannabis PGS with ecstasy and stimulant use seemed stronger in cannabis users compared to never users which could point to a causal relationship (possibly on top of shared genetic factors). This effect was only observed in people born after 1968, but given the fact that the prevalence is higher in this younger group there is probably more power to detect an association than in the older group. Since the association was not significant after correction for multiple testing we must be cautious with drawing conclusions. In addition, we explored the differences in drug use prevalence in MZ twin pairs discordant for cannabis use. The twins who used cannabis had more often used drugs, compared to their MZ co-twins who never used cannabis. This is in accordance with previous research using the co-twin control methodology (Lynskey et al., 2002, 2006; Vink et al., 2007). This finding suggest that the differences in illicit drug use between twins who used cannabis and their unaffected co-twins cannot solely be explained by genetic influences (or shared family environment) but that individual-specific environmental factors such as cannabis use play a role. Together, this suggested that cannabis use could be a causal factor for other drug use. Future studies should explore causality with more advanced methods such as Mendelian Randomization (a method of using measured genetic variation to examine the causal effect of a modifiable exposure on outcome variables), but larger samples sizes are needed than available in the current study to obtain enough power. In previous studies using two-sample bi-directional Mendelian Randomization analyses, no evidence was found for causal relationships between smoking, alcohol, caffeine, and cannabis (Taylor et al., 2018; Verweij et al., 2018, Chang, L.H. et al., 2020) but these studies did not include

Table 4

Association of Polygenic Score for Cannabis use with Ecstasy use, Stimulants and Any Drug use stratified for cannabis exposure (ever used versus never used). N = number of participants, B = standardized beta, OR = Odds Ratio, P = p-value, R² = explained variance.

					R ²						R ²	
	Birth year	N	B	OR	P		N	B	OR	p		
Ecstasy							Never used cannabis					
PGS Cannabis	All	1174	0.146	1.18	.060	0.6%	2971	-.136	0.87	.414	0.2%	
PGS Cannabis	> 1968	937	0.165	1.20	.039	0.8%	1678	-.104	0.89	.489	0.2%	
Stimulants							Never used cannabis					
PGS Cannabis	All	1576	0.108	1.12	.104	0.3%	5125	-.094	0.91	.476	0.1%	
PGS Cannabis	> 1968	1224	0.152	1.10	.034	0.2%	2551	.004	0.94	.980	< 0.1%	
Any drugs							Never used cannabis					
PGS Cannabis	All	1825	0.057	1.06	.318	0.1%	6165	.012	1.01	.904	< 0.1%	
PGS Cannabis	> 1968	1422	0.087	1.08	.169	0.2%	2896	.076	1.09	.507	0.1%	

other illicit drugs. There might not be a sequential order of use for initiation of smoking, alcohol use or caffeine consumption since these substances are widely available and some people start with smoking while others start with drinking first. A gateway from licit substance use to illicit drug use or from one drug (e.g. cannabis) to other drugs (e.g. ecstasy, stimulants, any drugs) might be more plausible. Ideally, causality should be tested in two directions, because some studies have also found evidence supporting a reverse-gateway hypothesis (i.e. reverse causation). For example, cannabis could influence ethanol (alcohol) levels, although existing findings are inconclusive (Perez-Reyes and Cook, 1993), and a recent MR study did not find evidence for a causal relationship (Chang et al. (2020)). A limitation of the PGS approach is that currently only large GWA studies are available for lifetime cannabis (N = 184,765) (Pasman et al., 2018) and opioid use disorder (N = 82,707) (Zhou et al., 2019), but not for other illicit drugs such as ecstasy. Large genome wide association studies for illicit drugs are needed as input to calculate reliable PGSs.

A strength of the current study is the large discovery sample for cannabis (consisting of 180,112 participants). It is known that a larger discovery sample leads to a more reliable (and powerful) PGS in the target sample. In the present study we showed as a proof of concept that the PGS for lifetime cannabis use was significantly associated with cannabis use in the target sample. A limitation is that the sample size of the target sample was not large enough to carry out more advanced methods such as one-sample Mendelian Randomization to further explore causality between cannabis and other drug use. However, as far as we know this is the first study exploring genetic overlap between cannabis and ecstasy, stimulants and any other drugs and power was sufficient to detect these associations.

In summary, PGS for cannabis use was significantly associated with use of ecstasy, stimulants, and any illicit drugs. An exploratory follow-up analyses indicated that this association was slightly stronger in cannabis users compared to non-users for ecstasy and stimulant use, but only in people born after 1968. The results of the MZ discordant twin analyses were in line with the suggestion that cannabis could be a causal factor for other drug use. Given the exploratory nature of this study, the present findings must be considered as preliminary rather than conclusive. Further unravelling the nature of the co-occurrence between substances will have implications for public health and intervention research. If there is no causal relationship then interventions that target reductions in one drug may not necessarily also lead to any change in use of another drug, and interventions that seek to target both drugs will need to incorporate active ingredients for each substance.

5. Contributors

KJHV and AA created the polygenic scores. JMV, LV and KJHV developed the analyses procedure and LV performed the analyses under supervision of JMV and KJHV. JMV wrote the manuscript, LV contributed to the method section. DIB is PI of the NTR. JJH cleaned and

imputed the genotype data of the NTR. LV, AA, JJH, DIB, KJHV provided critical revision of the manuscript for important intellectual content.

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Declaration of Competing Interest

No conflict declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.drugalcdep.2020.108102>.

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