

2006

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Recommended Citation

Agrawal, A.; Lynskey, M. T.; Bucholz, K. K.; Martin, N. G.; Madden, P. A F; and Heath, A. C., "Contrasting models of genetic co-morbidity for cannabis and other illicit drugs in adult Australian twins." *Psychological Medicine*.37,1. 49-60. (2006).
https://digitalcommons.wustl.edu/open_access_pubs/3887

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Contrasting models of genetic co-morbidity for cannabis and other illicit drugs in adult Australian twins

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ABSTRACT

Background. The use of cannabis and other illicit drugs (OIDs) and their co-morbid misuse are frequently reported in the literature. Correlated vulnerabilities and causal or gateway influences have been implicated in this association. We investigated the source of this co-morbidity between cannabis use (experimentation, early and repeated use, and problems) and OID experimentation and problems using genetic models proposed by Neale and Kendler (*American Journal of Human Genetics* 1995, **57**, 935–953).

Method. In a sample of 4152 same-sex male and female adult Australian twin individuals, we fit 13 genetically informative models of co-morbidity to data on experimentation, early use, repeated use of cannabis and co-morbid OID experimentation, and to abuse/dependence (A/D) problems with cannabis and OIDs.

Results. Model-fitting results suggest that common genetic, shared and unique environmental factors are responsible for the association between cannabis experimentation, early use, repeated use and A/D problems and OID experimentation or problems. The liability causation model, which is a reduced form of the correlated vulnerabilities model, also fit very well. In women, we found evidence for high-risk cannabis experimenters and repeated users to be at increased risk for OID experimentation, despite being below the risk threshold on the liability distribution for OID experimentation (extreme multiformity).

Conclusions. Co-morbid cannabis and OID use and misuse are due partly to a common predisposition to substance use disorders. Putative causal effects could not be ruled out. These models warrant further research, so that features of the correlated vulnerabilities model and the gateway models can be studied jointly in a single series of adaptive nested models.

INTRODUCTION

Results from epidemiological studies suggest that cannabis users are more likely to subsequently use other illicit drugs (OIDs), such as cocaine, hallucinogens, non-prescription sedatives, stimulants and opiates (Kandel, 2002).

Two predominant theories have been proposed to explain this relationship. First, the ‘gateway’ theory posits that (i) cannabis use occurs prior to OID use (*sequence*) and (ii) cannabis users are at increased risk for using OIDs (*association*), to argue that cannabis use may have a causal influence on subsequent use or misuse of OIDs (*causation*) (Kandel, 2003). Second, the correlated vulnerabilities theory suggests that users of cannabis subsequently use OIDs because of a general predisposition to illicit drug

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use, or more globally to deviant behavior, including conduct disorder (MacCoun, 1998; Morral *et al.* 2002*a, b*).

Early family studies found evidence for the clustering of illicit drug abuse amidst family members (Bierut *et al.* 1998; Merikangas *et al.* 1998). Multivariate twin analyses, such as those by Kendler and colleagues, have also shown that a common genetic factor is responsible for the genetic covariation across use and abuse/dependence (A/D) of several classes of illicit drugs, including cannabis (Karkowski *et al.* 2000; Kendler *et al.* 2003). While these genetic analyses supported the correlated vulnerabilities hypothesis, the preceding genetic analyses did not posit or test plausible alternative mechanisms for the association between cannabis and OIDs. Subsequent twin modeling did contrast varying interpretations of a correlated vulnerabilities model with some version of a 'gateway' or 'causal' model (Tsuang *et al.* 1998; Lynskey *et al.* 2003; Agrawal *et al.* 2004*c*; Lessem *et al.* 2006; Lynskey *et al.* 2006) and consistently found overwhelming support for shared genetic risk factors contributing to the co-morbidity between cannabis and OID involvement. However, with the exception of Tsuang *et al.* (1998), who showed that their version of the gateway model provided an inferior fit to data on A/D of cannabis and OIDs when compared to a common liabilities model, in Vietnam era male twins, the remaining genetically informative studies have not been able to unequivocally rule out all gateway-like influences.

Four of the remaining studies used a co-twin control design to test whether an early-onset cannabis-using member of a twin pair was more likely to subsequently use OIDs when compared to their co-twin who was not an early-onset cannabis user. Lynskey *et al.* (2003, 2006), Agrawal *et al.* (2004*b*) and Lessem *et al.* (2006) all found evidence for a relationship between cannabis and OID use that extended beyond their association due to correlated familial (genetic and familial environmental) factors and could be reflective of causal influences or correlated environmental risks. However, none of these studies examined alternative formulations of correlated vulnerabilities and gateway models, which were tested by Agrawal *et al.* (2004*c*), and showed that while the correlated liabilities model fit their data from adult

twin pairs from Virginia very well, a 'gateway' model, which was non-causal, also fit the data well, thus highlighting the need for a more rigorous test of plausible genetic mechanisms contributing to the association between cannabis and OID involvement.

One of the criticisms of epidemiological tests of the 'gateway' theory is that true causality cannot be extricated from covariation due to an unmeasured third correlated variable (Kandel, 2003). This is to say that, if an unmeasured environmental risk factor, such as a traumatic event, is a predictor of both cannabis and OID use and misuse, then even in traditional twin studies, where this unmeasured environmental influence will be unique to each member of the twin pair, but common across traits, this third variable's effects may be confounded with truly causal influences. However, even operating within the restrictive framework of cross-sectional twin data, we can test a series of possible mechanisms by which genetic and environmental risk factors contribute to the association between cannabis and OID use patterns.

Neale & Kendler (1995) proposed a series of 13 genetically informative models (NK models) that address the primary hypothesis of whether two disorders (in our case, cannabis and OID use, or problems) are truly etiologically distinct and, if not, then what are the possible mechanisms that govern the co-occurrence of these two disorders? Based on the co-morbidity models of Klein & Riso (1994), the NK models include seven main models and six submodels (Table 1). The simplest of these models tests the null hypothesis that cannabis and OID experimentation (we use these traits here for illustrative purposes) have distinct non-overlapping vulnerabilities, influenced by uncorrelated genetic and environmental influences. Therefore, according to the *chance* model (Model 1), the observed co-occurrence of cannabis and OID experimentation is due to chance alone. In the *alternate forms* model (Model 2), a single underlying liability governs both cannabis and OID experimentation. Models 3–8 use the concept of multiformity, where a proportion of individuals above a certain threshold for, say cannabis experimentation, are at increased risk for experimenting with OIDs, even if they are below the threshold for OID experimentation itself.

Table 1. *Brief description of each Neale–Kendler co-morbidity model, using lifetime co-morbid cannabis and other illicit drug (OID) experimentation as an example*

Model number	Model	Submodels	Description
1	Chance	—	Co-morbid cannabis and OID experimentation occurs due to stochasticity
2	Alternate forms	—	Single liability: a proportion of individuals experiment with cannabis and a proportion experiment with OIDs
3	Random multiformity	—	Independent liabilities: a proportion of cannabis experimenters are OID experimenters, and vice versa
4		Random multiformity of cannabis	A proportion of cannabis experimenters are OID experimenters, even when below the threshold for OID experimentation
5		Random multiformity of OIDs	A proportion of OID experimenters are cannabis experimenters, even when below the threshold for cannabis experimentation
6	Extreme multiformity	—	Independent liabilities: a proportion of high-risk cannabis experimenters are OID experimenters, and vice versa
7		Extreme multiformity of cannabis	A proportion of high-risk cannabis experimenters are OID experimenters, even when below the threshold for OID experimentation
8		Extreme multiformity of OIDs	A proportion of high-risk OID experimenters are cannabis experimenters, even when below the threshold for cannabis experimentation
9	Three independent disorders	—	Three independent liabilities for cannabis use, OID and cannabis use co-morbid with OID
10	Correlated vulnerabilities	—	Correlated liabilities: correlation between latent genetic and environmental influences on cannabis use and OID experimentation
11	Liability causation	Reciprocal causation	Independent liabilities: liability to experiment with cannabis has causal influence on liability to experimentation with OIDs, and vice versa
12		Unidirectional: cannabis to OIDs	Liability to experiment with cannabis has causal influence on liability to experiment with OIDs
13		Unidirectional: OID to cannabis	Liability to experiment with OIDs has causal influence on liability to cannabis experimentation

This multiformity can be *random*, with one threshold (Models 3–5), or *extreme*, with one low- and one high-risk threshold per trait (Models 6–8). We propose that the multiformity models may be well suited to a test of ‘gateway-like’ mechanisms. In other words, these models allow for an increased risk for OID experimentation in cannabis experimenters who are otherwise not vulnerable to OID experimentation. Hence, this increased risk, which is not due to correlated risk factors, is reflective of the direct influence of cannabis experimentation on OID experimentation. Model 9 models three independent disorders, where co-morbid cannabis and OID experimentation are governed by a unique liability distribution, such that a unique set of risk factors influence the liability to co-morbid experimentation. The *correlated vulnerabilities* hypothesis (Model 10) tests whether shared genetic and environmental risk factors influence co-morbid cannabis and OID experimentation. Finally, the *liability causation* models (Models 11–13), where the liability to experiment with cannabis is regressed on the liability to experiment with OIDs and vice versa, test whether individuals who are at

risk for cannabis experimentation are also more vulnerable to OID experimentation. Although these models appear to test causality, they are more similar to the correlated vulnerabilities model in that they allow for the composite liability to OID experimentation to be influenced by the individual’s liability to experiment with cannabis. Diagrammatic representations may be found in related publications (Neale & Kendler, 1995; Rhee *et al.* 2005).

While these models may not represent the entire gamut of possible sources of covariation between cannabis and OID use patterns, they are a fairly comprehensive series of comparisons of bivariate twin models for co-morbid drug use disorders and represent a highly informative addition to the extant literature on the co-morbidity between cannabis and OID involvement. Therefore, in the present study, using a large sample of adult Australian twins, we tested the 13 NK models of co-morbidity to investigate the co-morbidity between: (i) experimentation with cannabis and OIDs; (ii) early use of cannabis and OID experimentation; (iii) repeated use of cannabis and OID experimentation; and (iii) A/D problems with cannabis and OIDs.

METHOD

Sample

For this study, we used data from 1748 male [487 monozygotic (MZ) and 387 dizygotic (DZ) pairs] and 2404 female (696 MZ and 506 DZ) adult twins from same-sex pairs who were members of the younger cohort of the Australian Twin Registry. As the current formulation of the models require the construction of twin-co-twin frequency tables, only data from these complete pairs of twins were used. We did not find significant differences in rates of cannabis ($\chi^2=0.03$) or OID experimentation ($\chi^2=0.56$) or in cannabis ($\chi^2=2.38$) or OID ($\chi^2=2.30$) problems based on whether a twin was from a complete pair or a singleton. Twin pairs were aged 24–36 years at the time of interviews conducted in 1996–2000. All twins were born between 1964 and 1971 and were initially recruited through the Australian school systems and by mass media appeals. Parents initially registered the adolescent twins in 1980–1982 and the twins themselves were mailed a questionnaire in 1989–1992, when the twins were aged 18–25 years, and subsequently re-interviewed by telephone in 1996–2000, after informed consent, as approved by the Institutional Review Boards of the Washington University in St Louis, USA and the Queensland Institute of Medical Research, Australia, was obtained from all participants. Further details regarding sample ascertainment and collection are presented in related publications (Heath *et al.* 2001; Lynskey *et al.* 2003).

Measures

- (1) Experimentation with cannabis: participant reported having used cannabis at least once in their lifetime.
- (2) OID experimentation: participant reported experimenting with either cocaine or sedatives (e.g. barbiturates) or stimulants (e.g. Ritalin) or opiates (e.g. codeine, heroin) or hallucinogens (e.g. LSD).
- (3) Early-onset cannabis use: participant reported initiation of cannabis use prior to age 17. This cut-off has previously been used in adult samples to denote early use (Lynskey *et al.* 2003; Agrawal *et al.* 2004b).
- (4) Repeated cannabis use: participant endorsed using cannabis 11 or more times in their life.

This cut-off afforded us sufficient cell sizes for twin analyses.

- (5) Cannabis A/D problems: participant endorsed one of the following A/D problems from a non-diagnostic section of the interview:
 - (a) use of drug for more days/greater quantities than intended;
 - (b) needed larger amounts of drug to get same effect or could not get high on amount used first few times;
 - (c) being under influence of drug in hazardous situations (e.g. when driving a car, using machinery);
 - (d) being under the influence or after-effects of drug has interfered with household/work responsibilities;
 - (e) emotional or psychological problems due to use of drug;
 - (f) wanted to cut back on use of drug three or more times in life.
- (6) OID A/D problems: participant reported A/D problems separately for cocaine, sedatives, stimulants, opiates and hallucinogens and A/D problems with any one drug class constituted OID problems.

The prevalence of cannabis experimentation, early use, repeated use and problems, and OID experimentation and problems are shown in Table 2.

Twin models

Data from MZ and DZ twin pairs allowed us to partition the total variance in use (experimentation, early use and repeated use) and A/D problems of cannabis and OID experimentation and problems into three sources: additive genetic (or A), shared environmental (or C) and unique environment (or E) (Neale & Cardon, 1992). Additive genetic influences are shared 100% and 50% between members of an MZ and DZ twin pair respectively while shared environmental influences are shared 100% between members of a twin pair, irrespective of zygosity (under the equal environments assumption). Unique environmental influences also include measurement error and are not shared between members of a twin pair. Heritability refers to the proportion of the total variance

Table 2. Prevalence of cannabis experimentation, early use, repeated use and problems and other illicit drug (OID) experimentation and problems in male and female Australian twins

	Men (n=1748) (%)	Women (n=2404) (%)
Cannabis		
Experimentation	69	53
Early use	28	18
Repeated use	40	22
Problems	28	15
Other illicit drugs (OIDs)		
Experimentation	47	37
Problems	8	5

that is due to genetic factors (Sham, 1998). To fit models to dichotomous twin data, the assumption of an underlying normal distribution is made. It is assumed (as it cannot be tested for dichotomous outcomes) that all individuals in the given population lie along a continuous normally distributed spectrum of vulnerability and that manifesting the trait (or phenotype) occurs when an individual falls above a specific threshold of risk that is imposed upon this underlying distribution.

The model-fitting procedure included fitting all 13 models separately in males and females for (i) co-morbid experimentation with OIDs and cannabis, (ii) co-morbid experimentation with OIDs, with early cannabis use, (iii) co-morbid experimentation with OIDs, with repeated cannabis use, and (iv) co-morbid A/D problems with OIDs and cannabis. To reduce the total number of hypotheses tested, quantitative gender differences were only examined for two best-fitting models. The statistical software package Mx allows for the NK models to be fitted using observed twin-co-twin frequencies (Neale, 2004). Twins were cross-tabulated by their co-twin's drug experimentation, by zygosity, and the observed frequencies were compared with frequencies expected under each of the 13 NK models, to arrive at a χ^2 fit statistic. Models were not nested (although submodels were nested within their main model) and comparison of fit was made using the Akaike Information Criterion (AIC), which is an index of both fit and parsimony (Akaike, 1987; Williams & Holahan, 1994).

RESULTS

The tetrachoric correlations between experimentation with cannabis, early cannabis use and repeated cannabis use, with experimentation with OIDs, were 0.64, 0.53 and 0.75 respectively. The tetrachoric correlation between cannabis A/D problems and A/D problems with OIDs was also high (0.68).

Table 3 shows the AIC (model-fit statistic) for co-occurring experimentation of OID with experimentation, early use and repeated use of cannabis and for cannabis and OID A/D problems. Models were fit separately in male and female same-sex twin pairs for co-occurring use (experimentation, early and repeated use) of and co-occurring problems with cannabis and OIDs. The models are ranked for each set of phenotypes as best-fitting (1) and second-best-fitting (2) model. When comparing nested submodels, only the best-fitting nested model is ranked (e.g. if both the reciprocal causation and unidirectional causation of cannabis model have low AIC values, the submodel with the lower AIC is selected as best-fitting). Table 4 shows the standardized parameter estimates for the best-fitting models, separately for males and females. Constraining variance components across genders did not result in a significant deterioration of fit in the best-fitting and second-best-fitting models, although thresholds could not be equated across male and female twins. However, as in some instances the best-fitting model (or submodel) was different for males and females, we present gender-specific estimates in Table 4.

Overall, cannabis experimentation, early use, repeated use, A/D problems, and also OID experimentation and A/D problems were significantly heritable (Table 4). For cannabis, A/D problems were more strongly influenced by additive genetic factors than experimentation or early use, while the heritability of repeated cannabis use was comparable to that for cannabis A/D problems. Shared environmental factors contributed to the total variance across a majority of the phenotypes, with the exception of repeated cannabis use in both sexes, and OID A/D problems in male twins only.

Table 3 shows that the correlated vulnerabilities model and the related models of liability causation were the best-fitting models for

Table 3. The Akaike Information Criterion (AIC)^a is presented for the 13 co-morbidity models, fitted separately to data on cannabis experimentation, early (before 17 years) use, repeated use (11+ times) and experimentation with other illicit drugs (OIDs) as well as cannabis abuse/dependence (A/D) problems and OID A/D problems in Australian female and male same-sex twin pairs

No.	Model	Experimentation		Early use		Repeated use		A/D problems	
		Female	Male	Female	Male	Female	Male	Female	Male
1	Chance	300.6	177.9	172.7	133.2	506.9	478.6	168.3	126.9
2	Alternate forms	295.1	224.1	107.7	103.6	107.5	114.2	49.4	26.6
3	Random multiformity	58.5	35.3	42.6	36.6	30.7	52.7	10.5	39.0
4	Random multiformity of cannabis	55.9	150.9	41.3	41.1	28.7	45.9	19.5	35.2
5	Random multiformity of OIDs	81.2	32.6	76.4	36.6	82.7	44.1	10.8	54.3
6	Extreme multiformity	-3.9	0.3	9.2	-1.2	8.8 ²	3.8	40.5	3.8
7	Extreme multiformity of cannabis	-6.9 ²	-0.8	15.5	3.9	13.9	0.6	6.9	2.5
8	Extreme multiformity of OIDs	60.9	12.1	30.9	15.6	33.7	19.4	12.0	51.7
9	Three independent disorders	52.4	13.5	33.9	80.0	27.3	59.2	16.2	49.9
10	Correlated vulnerabilities	-5.1	-5.6 ¹	-12.7 ¹	-11.8 ¹	7.4 ¹	-3.1 ²	-3.8 ²	-3.2 ²
11	Reciprocal liability causation	-7.2 ¹	-0.7	-5.2 ²	-7.2 ²	17.2	-2.4	-4.6	-4.8
12	Unidirectional: cannabis causes OID	-5.2	-5.2 ²	1.7	-3.1	15.2	-4.4 ¹	-5.9 ¹	-6.9 ¹
13	Unidirectional: OID causes cannabis	13.7	-4.1	24.0	3.5	36.2	3.2	30.9	-5.8

^a The AIC is an index of both fit and parsimony. The lower (or more negative) the AIC, the better the relative fit of the model.

Numerical superscripts refer to the ranking of models by fit: 1 = best-fitting and 2 = second best-fit. When more than one submodel of a model fit equally well, the submodel with the lower AIC was selected for the relative ranking of AICs. Analyses used 487 monozygotic (MZ) male, 696 MZ female, 387 dizygotic (DZ) male and 506 DZ female complete pairs.

the co-morbidity between cannabis use (experimentation, early and repeated use), cannabis A/D problems, and experimentation and A/D problems with OIDs. Genetic correlations across the cannabis and OID phenotypes were high, as were correlations between shared environmental factors influencing both cannabis and OID experimentation. Likewise, for the liability causation models, the regression parameter was high, showing that common underlying liabilities were largely contributing to co-morbid use and problems with cannabis and OIDs. In women, the extreme multiformity of cannabis model fit better than the correlated vulnerabilities model for co-morbid experimentation. This model also fit well for co-morbid repeated cannabis use and OID experimentation in women.

To illustrate these findings, we describe in detail the results for experimentation with cannabis and OIDs. The best-fitting model in males was the correlated vulnerabilities model (AIC -5.6) followed by the unidirectional causation of cannabis model (AIC -5.2). Genetic influences on OID experimentation were shared ($R_g = 0.57$, $0.57^2 = 33\%$ overlap) with cannabis experimentation, as were the shared ($R_c = 0.98$, $0.98^2 = 96\%$ overlap) and unique environmental ($R_e = 0.40$, $0.40^2 = 16\%$ overlap) influences; however only shared environmental factors

seemed to have less of a specific influence on OID experimentation. The second-best-fitting model was the unidirectional causation model, which suggests that a significant proportion of the total variance in OID experimentation was shared with cannabis experimentation ($\beta = 0.715$). In female twins, the reciprocal causation model (AIC -7.2) and the extreme multiformity of cannabis model (AIC -6.9) fit marginally better than the correlated liabilities model (Table 3). The reciprocal causation model showed a positive association between cannabis experimentation and OID experimentation, as well as a negative reciprocal path. The second-best-fitting model was the extreme multiformity of cannabis model, which was also the third-best-fitting model in men. According to this model (Model 7: third-best-fit in men), 26% of the women who were high-risk cannabis users were at an independent increased risk for using OIDs.

DISCUSSION

We sought to explore the potential mechanisms for the co-morbid use (experimentation, early use and repeated use) of, and problems with, cannabis and OIDs. Although we did not find unequivocal evidence for a single best-fitting

Table 4. Standardized parameter estimates for the best-fitting Neale–Kendler model in female and male adult Australian twins

Variable 1	Variable 2	Model no.	Variable 1			Variable 2			R_g	R_c	R_e	β
			a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)	a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)				
Female twins												
Experimentation with cannabis	OID experimentation	11	0.44 (0.41–0.49)	0.28 (0.26–0.35)	0.28 (0.20–0.29)	0.31 (0.29–0.36)	0.18 (0.13–0.21)	0.51 (0.44–0.55)	—	—	—	$\beta_1 = 0.79$ (0.53–1.00); $\beta_2 = -0.31$ (-0.33 to -0.05)
Early cannabis use	OID experimentation	10	0.44 (0.40–0.47)	0.30 (0.28–0.35)	0.26 (0.23–0.27)	0.39 (0.31–0.41)	0.21 (0.19–0.40)	0.40 (0.36–0.42)	0.66 (0.63–0.68)	0.74 (0.64–0.74)	0.10 (0.07–0.12)	—
Repeated cannabis use	OID experimentation	10	0.58 (0.36–0.60)	0.17 (0.02–0.36)	0.25 (0.20–0.27)	0.30 (0.15–0.55)	0.28 (0.15–0.38)	0.42 (0.36–0.48)	0.86 (0.64–1.00)	0.85 (0.50–0.93)	0.60 (0.48–0.70)	—
Cannabis A/D problems	OID A/D problems	12	0.55 (0.30–0.63)	0.16 (0.11–0.19)	0.29 (0.20–0.31)	0.40 (0.32–0.42)	0.19 (0.17–0.23)	0.41 (0.22–0.43)	—	—	—	0.60 (0.43–0.83)
Male twins												
Experimentation with cannabis	OID experimentation	10	0.48 (0.46–0.50)	0.22 (0.12–0.41)	0.30 (0.28–0.31)	0.35 (0.33–0.55)	0.26 (0.23–0.46)	0.39 (0.28–0.40)	0.57 (0.29–0.51)	0.98 (0.59–1.00)	0.40 (0.29–0.42)	—
Early cannabis use	OID experimentation	10	0.44 (0.40–0.47)	0.30 (0.28–0.35)	0.26 (0.23–0.27)	0.39 (0.31–0.41)	0.21 (0.19–0.40)	0.40 (0.36–0.42)	0.66 (0.63–0.68)	0.74 (0.64–0.74)	0.10 (0.07–0.12)	—
Repeated cannabis use	OID experimentation	12	0.60 (0.30–0.62)	0.16 (0.16–0.43)	0.24 (0.18–0.27)	0.29 (0.24–0.41)	0.35 (0.07–0.56)	0.36 (0.34–0.45)	—	—	—	0.78 (0.72–0.81)
Cannabis A/D problems	OID A/D problems	12	0.68 (0.65–0.69)	0.14 (0.12–0.14)	0.18 (0.16–0.19)	0.67 (0.64–0.70)	0.02 (0.00–0.04)	0.31 (0.22–0.33)	—	—	—	0.35 (0.21–0.52)

CI, confidence interval; OID, other illicit drug; A/D, abuse/dependence.

Model no. refers to number for best-fitting model (see Table 1, which describes each model, and Table 3, which reports the Akaike fit criterion or AIC). R_g , R_c and R_e refer to additive genetic, shared environmental and unique environmental overlap, respectively (shown if best-fitting model was correlated liabilities, Model 10). β refers to the unsquared estimate of the liability causal path (shown if best-fitting model was a liability causation model, Models 11–13).

model, our results suggest that overlapping genetic and environmental factors probably play a significant role in the co-occurrence of cannabis and OID use and misuse. However, strong evidence, across males and females, was also found for a model where the liability to experiment with cannabis, or use of it at an early age or repeatedly, had a causal influence on the liability to experiment with OIDs. In women, we also found evidence for a 'gateway' model, where high-risk cannabis experimenters or repeated users were at increased risk for experimenting with OIDs irrespective of their original liability to do so.

Thus, an accrual of evidence in support of the correlated vulnerabilities model, including evidence from our present study of Australian twins, suggests that cannabis use, including experimentation, early and repeated use, and OID experimentation, as well as A/D problems with cannabis and OIDs, are associated, across gender and cultures (Australian and US samples), due to genetic, shared and unique environmental factors that jointly contribute to the vulnerability to use and misuse these drug classes. These genetic factors may be genes for impulse-disinhibition (e.g. dopaminergic receptors for novelty seeking) (Bardo *et al.* 1996; Miles *et al.* 2001; Kluger *et al.* 2002; Schinka *et al.* 2002; Agrawal *et al.* 2004a), while the environmental factors may refer to negligent parenting (shared environmental) (Burt *et al.* 2003; Walden *et al.* 2004), coercive peer influences (Simons-Morton *et al.* 1999; Kobus, 2003; Lee & Tak, 2005), or childhood sexual or physical abuse (could be shared or unshared across members of twin pairs, i.e. C or E) (Kendler *et al.* 2000). Overall, a correlated vulnerabilities model is congruent with the Problem Behavior Theory (Jessor & Jessor, 1977). According to this theory, deviant behaviors, including illicit drug use and conduct problems, possibly with an early age of onset, cluster in certain individuals due to a general proneness to problem/deviant behaviors (Donovan & Jessor, 1985; Jessor, 1991).

The reciprocal and unidirectional causation models were also supported by our analyses. These models, especially in the absence of multiwave data, do not infer true causality. The total variance in one trait is regressed on the total variance in the other trait and a positive regression path implies that the liability in

OID experimentation is partly shared with the liability to cannabis experimentation. Therefore, the liability causation models are more similar to the correlated vulnerabilities model and may be viewed as a reduced form of the former approach. In the correlated vulnerabilities model, each component of variance, A, C and E, is correlated independently across cannabis and OID experimentation, whereas in the liability causation models, the total variance in cannabis experimentation is regressed on the total variance in OID experimentation and vice versa. Thus, the interpretation of a positive regression path for the unidirectional causation of the cannabis model is that a portion of the total variance in OID experimentation is due to the variance in cannabis experimentation while the remainder of the variance in OID experimentation is shaped by genetic and environmental influences specific to it.

The confounding of the correlated vulnerabilities and liability causal models, as seen in our model-fitting procedure, has been tested previously, using simulated data, by Rhee *et al.* (2004). Using 300 simulated datasets, the authors pointed out that even when the true model was a liability causation model, 26–54 times the correlated liabilities model fit the data best. This finding and our observations, as well as data from the original NK manuscript (Neale & Kendler, 1995), support prior research by Heath *et al.* (1993), which tested the validity of cross-sectional data in discriminating between direction-of-causation models (Duffy & Martin, 1994), especially for two traits with similar magnitudes of genetic, shared and unique environmental influences.

Particularly in female twins, we also found evidence for a model where high-risk cannabis experimenters or repeated users were at increased risk for experimenting with OIDs, despite being below the risk threshold for OID experimentation (extreme multiformity of cannabis). This increase in risk for OID experimentation is not due to correlated risk factors, but is instead due to the individual's cannabis experimentation alone. One possible explanation for this may be exposure opportunity or availability, which is proposed as a contributor to gateway effects (Hall & Lynskey, 2005). It may be that women of this cohort had less frequent exposure to cannabis and were

therefore also less exposed to OIDs, as research has shown exposure opportunity to OIDs to be significantly greater in cannabis users (Wagner & Anthony, 2002). Therefore, co-morbid cannabis and OID experimentation and repeated use may reflect greater drug-seeking or deviancy in this sample of women than in the men. The multiformity models may be viewed as restricted forms of a bivariate moderator model (Martin *et al.* 1987; Dick *et al.* 2001; Purcell, 2002), where the A, C and E influencing cannabis and OID experimentation are uncorrelated and the variance in OID experimentation is estimated freely in individuals who are experimenters (or high-risk users, if extreme multiformity) *versus* those who are not experimenters (including low-risk users for extreme multiformity) of cannabis. If the 'gateway' effect is viewed as an outcome of multiformity, where the liability to experiment with OIDs varies as a function of cannabis experimentation, then using adaptations of the multiformity models, we may, in the future, be able to consolidate features of the competing correlated liabilities and 'gateway' hypotheses.

Overall, our findings, in conjunction with previous results (Agrawal *et al.* 2004c), suggest that cannabis and OID use and misuse co-occur in the population due to common risk factors (correlated vulnerabilities) or a liability that is, in part, shared (liability causation). However, as shown previously with the Virginia sample, a proportion of those who are high-risk cannabis users are also at increased risk for OID experimentation, not due to correlated risk factors but instead due to their cannabis use behavior itself. This second threshold may reflect heterogeneity in the binary construct that we use for cannabis use. In other words, the upper threshold (or high-risk threshold) may reflect a subset of cannabis users with earlier onset, or co-morbid conduct disorder, or negative affect problems, or even susceptibility to subsequent dependence. This is possibly why the extreme multiformity model did not provide as a good a fit when we examined the co-morbidity between early cannabis use and OID experimentation or A/D problems, suggesting that in these models we may have 'captured' the high-risk behavior. A more sophisticated approach, however, would be to modify the NK models to use ordinal data (e.g. abstain *versus* early- and late-onset

cannabis use), with an underlying normal distribution of liability.

The present findings may be viewed with certain limitations in mind. First, the results reported here are based on data from a sample of adult Caucasian twins residing in Australia, and therefore may not be extrapolated to other age cohorts and ethnicities. Second, the models were fitted with certain technical limitations (i.e. usage of dichotomous outcomes, using only bivariate models) and, as a consequence, we may not have explored other potential phenotypic definitions (repeated use as 11+ times *versus* daily use) and mechanisms of association between cannabis and OID use patterns. Third, because of the cross-sectional nature of the data, our definition of causality and our ability to detect it may be somewhat limited. Fourth, we did not have diagnostic assessments of abuse or dependence, nor did we condition A/D for prior use. Fifth, we did not explore the relationship between problems with licit and illicit drugs, where different models may fit well (Rhee *et al.* 2006). Finally, our inability to clearly distinguish the correlated vulnerabilities model from the liability causation and the extreme multiformity model is probably due to limited power, which has previously been observed to contribute to the modest AIC differences across non-nested models. However, it should be noted that a significant number of mechanisms, such as chance, alternate forms, three independent disorders and random multiformity, could be clearly eliminated.

The strengths of this study include the large sample size, extensive model-fitting and use of multiple indicators of cannabis use behavior. Most importantly, the current study, in addition to the correlated vulnerabilities and gateway models, tests multiple alternative mechanisms that may contribute to the relationship between cannabis and OIDs. Therefore, not only do we replicate important findings from previous twin studies but we also add substantively to this growing body of literature by testing alternate phenotypes for cannabis use, including experimentation, early and repeated use, which are both considered to be more accurate markers for subsequent illicit drug involvement than cannabis experimentation alone.

Although there is overwhelming evidence for the correlated vulnerabilities model, we cannot

unequivocally rule out 'gateway' effects of cannabis use on the experimentation with OIDs. This seems to be especially true for the Australian women in our sample. A similar pattern of association has also been seen in the Virginia adult sample. By contrast, the study by Tsuang *et al.* (1998) did not find evidence for a genetic gateway model, which they defined as a reduced correlated liabilities model, where common genetic and environmental influences (or the covariance) across A/D of several classes of illicit drugs was mediated through cannabis abuse. This divergence in findings with Tsuang and colleagues may be due to several reasons, such as their use of a male-only sample, the possibility that their sample of males serving in the military may have had greater access to illicit drugs when compared to their civilian counterparts and their interpretation of the gateway model, which is somewhat different from the ones we test. As pointed out by Morral *et al.* (2002*a, b*), models are required to examine whether the 'gateway' influence persists even after accounting for the shared vulnerability to multiple substance use and misuse. To test this hypothesis within the framework of the comorbidity models, we would need to adapt the extreme multiformity model to include overlapping genetic, shared and unique environmental influences.

Hall & Lynksey (2005) recently reviewed mechanisms by which cannabis use may be associated with patterns of OID use. Broadly defined, these mechanisms may be correlative, causal or both. As discussed previously, a correlative relationship refers to the influence of common risk factors, which may be measured (e.g. novelty-seeking scores) or latent (e.g. genes influencing novelty seeking), that influence experimentation with cannabis and OIDs. Causal influences, however, may be social (e.g. drug availability) or pharmacological (e.g. cross-sensitization of neuroreceptors). Cannabis may be a gateway drug because it introduces users to a provider (peers or black-marketeer) (Pacula *et al.* 2001) who eventually may also become the source for OIDs. Cannabis may also be a gateway drug if neurobiological changes in cannabis users result in increased drug-seeking behavior or sensitivity to OIDs, as supported by some animal studies (Tanda *et al.* 1997). Correlative and causal models are not mutually

exclusive (Agrawal & Lynskey, 2006). Although it is likely that use or misuse of multiple illegal drugs, including cannabis, is in fair part due to common genetic and environmental factors, even after controlling for these correlative influences, some causal influences, which are more likely to be social than pharmacological, plausibly mediate some of the residual influence of cannabis use on OID experimentation and misuse.

What are the public health implications of our findings? If cannabis use has a purely causal impact on experimenting with OIDs, then targeting prevention efforts at reducing cannabis experimentation alone will result in a drastic reduction in subsequent OID experimentation. As this is implausible, prevention efforts that aim to reduce the general level of drug use and problem behavior in adolescents will be more effective than an isolated effort to reduce rates of cannabis use alone. In addition, more research is needed to characterize the high-risk cannabis users who are at risk for OID use and possibly a host of other psychopathology (e.g. childhood psychopathology, early-onset alcohol and tobacco use) and to examine how a disruptive home environment and negative peer affiliations, which inextricably feed back on each other, influence their high-risk status. If there exists a 'gateway' effect, it is unlikely to begin with exposure to cannabis, and the identification of endophenotypes will greatly assist in clarifying the nature of the association between cannabis and OIDs.

ACKNOWLEDGEMENTS

Arpana Agrawal is supported by DA12854 (PI Madden) and AA13321 (PI Heath); research supported by AA07728 (PI Heath), AA11998 (PI Heath), AA10249 (PI Heath); Michael Lynskey is supported by DA18660 and DA18267.

DECLARATION OF INTEREST

None.

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