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Early cannabis use and DSM-IV nicotine dependence: a twin study

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ABSTRACT

Background Evidence suggests that cannabis users are at increased risk for cigarette smoking—if so, this may potentially be the single most alarming public health challenge posed by cannabis use. We examine whether cannabis use prior to age 17 years is associated with an increased likelihood of DSM-IV nicotine dependence and the extent to which genetic and environmental factors contribute to this association. **Methods** A population-based cohort of 24–36-year-old Australian male and female twins ($n = 6257$, 286 and 229 discordant pairs) was used. The co-twin–control method, with twin pairs discordant for early cannabis use, was used to examine whether, after controlling for genetic and familial environmental background, there was evidence for an additional influence of early cannabis use on DSM-IV nicotine dependence. Bivariate genetic models were fitted to the full data set to quantify the genetic correlation between early cannabis use and nicotine dependence. **Results** The early cannabis-using twin was about twice as likely to report nicotine dependence, when compared to their co-twin who had experimented with cigarettes but had never used cannabis. Even when analyses were restricted to cannabis users, earlier age cannabis use onset conferred greater risk (1.7) for nicotine dependence than did later onset. This association was governed largely by common genetic liability to early cannabis use and nicotine dependence, as demonstrated by genetic correlations of 0.41–0.52. **Conclusions** Early-onset cannabis users are at increased risk for nicotine dependence, but this risk is attributable largely to common genetic vulnerability. There is no evidence for a causal relationship between cannabis use and nicotine dependence.

Keywords Cannabis, discordant twins, genetic, Mx, nicotine dependence, twin modeling.

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INTRODUCTION

It is well documented that cannabis use may contribute to an increased likelihood of experimenting with other illicit drugs [1–6]. However, researchers have begun recently to question whether cannabis use is also associated with increased risk for cigarette smoking. In two longitudinal samples of adolescents, those who abstained initially from cigarettes were more likely to report cigarette smoking at a subsequent assessment if they used cannabis in the interim [7,8]. There is also accumulating evidence that cannabis use may impact substantially subsequent important later stages of cigarette smoking, including nicotine dependence (ND) [7–9].

Two epidemiological studies have examined 'reverse gateways'. Patton and colleagues [7], using a longitudinal sample of teens, reported that weekly cannabis use predicted an increase in tobacco initiation. The authors also reported that young adult smokers who used cannabis on a daily basis were at a nearly 3.6 increased odds of developing ND. In a sample of adolescents and young adults, Timberlake and colleagues [9] reported that 23–27-year-olds with a life-time history of cannabis use (>10 times) were twice as likely to develop ND when compared to those young adults who reported no cannabis use at all.

While these studies demonstrate an association between cannabis use and cigarette smoking, they fail to address whether a common genetic predisposition to

cannabis use and cigarette smoking is responsible for this association, or whether this increase in risk is attributable to overlapping environmental influences. Empirical evidence demonstrates the importance of heritable influences on both cannabis use [10] and stages of cigarette smoking [11–16], but we are aware of only one genetically informative study of adult female twins, [17] which showed that the association between cannabis use and cigarette smoking (experimentation and ND) was due to a correlation between the liability to use cannabis and experimentation with cigarettes, both of which are influenced genetically ($h^2_{cannabis} = 0.40$; $h^2_{nicotine} = 0.67$), but not due to any direct influence of cannabis use on ND or vice versa.

Several studies [3–6] show that those who initiate cannabis use earlier in life are at significantly greater risk for experimentation with other illicit drugs, illicit substance use disorders and for alcohol dependence. However, it remains to be seen whether individuals who initiate cannabis use at an early age are more likely to develop ND when matched for genetic background, and possibly for some environmental influences, with individuals who do not use cannabis at all and with individuals who use cannabis but only at a later age. The study of identical and fraternal twin pairs who are matched for familial environment and for genetic background allows us to test this important hypothesis.

In the current study, we use data gathered on young adult Australian twins to explore: (i) whether cannabis use prior to age 17 (early-onset) is associated with an increased risk for onset of DSM-IV ND and (ii) the extent to which this association between early onset cannabis use and DSM-IV ND is explained by genetic and environmental commonality.

MATERIALS AND METHODS

Sample

A sample of 6257 adult Australian male and female twins that included monozygotic (MZ) and dizygotic (DZ) pairs as well as twins from incomplete pairs (although these single-twins were not informative for the discordant twin pair analyses) were used. The full paired sample consisted of 494 MZ and 395 DZ same-sex male pairs and of 698 MZ and 513 DZ same-sex female pairs. Data on 661 DZ opposite sex pairs and 736 twins from pairs where a co-twin did not participate was also available. While opposite-sex pairs and single twins could not be used for the discordant twin pair analyses, data from these twins were utilized for twin analyses. Twins were aged 24–36 [mean 30 (2.5)] years at the time of interviews conducted in 1996–2000, and these data alone were used for the analyses presented here. All twins were born between 1964 and 1971 and were recruited initially through the Australian school systems and via mass media appeals. Parents registered the adolescent twins initially in 1980–82 and the twins themselves were interviewed via 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 [3,18].

Measures

Diagnostic interviews were based on the Semi-Structured Assessment for the Genetics of alcoholism (SSAGA) [19], which was updated for DSM-IV and adapted for telephone use in the Australian sample. ND measures were adapted for inclusion from the CIDI [20].

Early cannabis use (EC)

Self-reports of having used cannabis at least once prior to age 17: a definition shown previously by us to be associated highly with other illicit drug use [3].

Nicotine dependence (ND)

Individuals were coded as meeting criteria for DSM-IV nicotine dependence if they reported experiencing three or more of the seven diagnostic criteria within the same 12-month period.

Individuals who had smoked cigarettes at least once in their life-time, but did not endorse smoking cigarettes 100 or more times [21] during their life-time, were not queried about ND symptoms. These individuals were coded as unaffected for DSM-IV ND.

Covariates

To account for known correlates that may potentially mediate the association between EC and ND, we controlled for (i) DSM-IV major depression: the association between depression and ND has been studied extensively (e.g. [22–24] with evidence for common genetic influence. While the association between depression and cannabis use is controversial (see [25] for a review) genetic studies hint at some overlap [26,27], making this a potential mediating measure. (ii) Two or more DSM-IV conduct problems: if the association between EC and ND represents a general proneness to delinquent behavior [28], including substance use, then controlling for a history of conduct problems is important. (iii) Social

anxiety (defined as experiencing fears, where doing something embarrassing/humiliating caused anxiety, or if experiencing fears caused problems with family, friends, work or other situations): individuals with social anxiety tend to smoke cigarettes more frequently [29]. (iv) Exposure to childhood sexual abuse (self-reported rape or molestation or forced sexual contact with someone within or outside the family, prior to 17 years of age), which has been shown to be a potent risk factor for early onset of substance use and later dependence [30–35]. (v) Life-time use of other illicit psychoactive substances (cocaine, sedatives, stimulants, inhalants, solvents, opiates, hallucinogens or phencyclidine), which has been demonstrated to be an important outcome of EC [3–6], with common genetic underpinnings. (vi) Early drinking (drinking alcohol at least once a month for 6 months or longer when aged 16 years or younger) and early smoking (smoking at least once a week for 3 weeks or longer at age 15 or younger), as EC may simply be a marker for early onset of multiple substances [36].

Sample characteristics

The full sample ($n = 6257$) had a mean age of 30 years and 44.8% were male. Other characteristics are presented elsewhere [18]. Almost 89% of the full sample reported experimenting with cigarettes (3028 male and 2554 female twins) (see previous reports [15,37]. Of these ever-smokers, 56.7% reported smoking 100 or more cigarettes in their life-time and 34.4% met criteria for DSM-IV ND [mean age of onset 21.2 (range 5–34 years)]. In the full sample, 60.2% reported a life-time history of cannabis use with 53.7% of these users (15.6% of the population) reporting initiation prior to 17 years of age [mean age of onset 15.2 (range 9–16 years)]. In ever-smokers, the prevalence of EC was 19% and 12.7% in men and women, respectively. Similarly, in individuals who reported experimentation with cigarettes and a life-time history of cannabis use, the prevalence of ND was 42.8%.

Nearly 83% of all participants reported initiating cigarette smoking prior to 17 years of age. About 55% of the sample reported having smoked 100+ cigarettes prior to 17 years—these individuals are not informative in the discordant twin design and it is for this reason that we chose not to examine the association between EC and this measure. For ND, eight (additionally, six did not report an age of onset) of the EC users reported onset of ND prior to EC and were deleted from those discordant twin analyses.

The mean age of twins constituting the discordant pairs was 30 years [standard deviation (SD) 2.5] and 47.2% of the sample was male; 28.7% of the respondents had 10 or fewer years of education, while 25.7% reported tertiary education (i.e. going to university). About 46% of the sample reported being married (or widowed), while 44% were never married and the remainder were separated or divorced at the time of interview. These estimates were highly comparable to the full sample.

Statistical methods

Discordant twin design

To control for initiation of cigarette smoking, we selected only those twins who reported ever smoking cigarettes. Self-reported age of onset of cannabis use was used to select two subsamples of discordant twin pairs:

1. A total of 286 (129 MZ and 157 DZ) like-sex pairs, where one member reported EC while their co-twin reported cannabis use at a later age or did not report a life-time history of cannabis use (i.e. including life-time non-users);
2. A total of 229 (104 MZ and 125 DZ) like-sex pairs, where one member reported EC while their co-twin reported cannabis use after age 16 (i.e. excluding life-time non-user co-twins).

The discordant twin method is a simple extension of matched-pair conditional logistic regression where, along with matching for age and sex, there is also matching for other latent factors, such as genetic and familial environmental influences, which are shared by members of twin pairs. Kendler and colleagues [22] have presented previously the patterns of odds ratios (OR) that we might expect, depending on whether the association between EC and ND is due to genetic or environmental factors or both:

1. If the association between EC and ND is due exclusively to genetic factors that influence both EC and ND then the OR in MZ twins, who share all their genes (identical-by-descent), would not be significant. However, the OR would be elevated in DZ twins who are matched only for 50% of their genetic background;
2. If the association between EC and ND is due exclusively to shared environmental factors that influence both EC and ND, then we would expect no increase in the OR in either MZ or DZ discordant pairs, as both are assumed to share their familial environment equally. Presumably, when a combination of overlapping genetic and shared environmental influences is at play, we might expect some elevation in the DZ OR.
3. If the association between EC and ND is due neither to genetic nor shared environmental influences, but instead is due entirely to unmeasured individual-specific environmental factors, or represents a truly causal relationship, then both MZ and DZ ORs would be elevated.

4 Finally, if familial (genetic and/or shared environmental) and individual-specific environmental factors contribute jointly to the relationship between EC and ND, then again, both MZ and DZ OR would be elevated; however, the MZ OR would be smaller in magnitude than the DZ OR.

It is important to note here that we are considering only pairs of twins discordant for EC and examining its association with ND in a conditional logistic regression framework (i.e. how likely is the EC member of a discordant pair to be ND and how likely is the non-EC member of a discordant pair likely to not be ND?).

Conditional logistic regression models were fitted in STATA. The within-pair association between EC and ND was examined in the first sample of twins where the unaffected co-twins included life-time cannabis non-users, and the association was then re-examined in the smaller sample where the co-twin population excluded life-time non-users. All models were adjusted for covariates. The significance of interactions between gender and EC and between zygosity and EC was tested. Analyses were re-performed in MZ and DZ pairs discordant for EC use separately to tease apart the role of familial versus causal/non-causal unmeasured environmental influences.

Bivariate twin models

To test the robustness of our results to analytical strategy, we fitted bivariate twin models utilizing data from the entire sample (i.e. not just pairs discordant for cannabis use), to examine the extent to which genetic (A), shared environmental (C) and non-shared environmental (E) factors were correlated across EC and ND. Figure 1 represents, using a path diagram, the within-twin (co-twin not shown) relationship tested in the bivariate model. In the figure, A1, C1 and E1, and A2, C2 and E2 are the genetic, shared and individual-specific influences on EC and ND, respectively. Rg represents the extent to which A1 and A2, the genetic influences on EC and ND, are correlated. Rc and Re are shared and individual-specific environmental correlations, respectively, between EC and ND. Note that A1, the genetic influences on EC, are correlated 1.0 and 0.5 (across-twin within-trait) across members of a pair of MZ and DZ twins, respectively, while Rg represents the within-twin across-trait correlation between EC and ND and may be multiplied suitably by 1.0 or 0.5 (MZ or DZ pair) to explain the across-twin across-trait correlation (i.e. the correlation between EC in twin 1 and ND in twin 2).

Analyses were conducted on both definitions of EC, i.e. where non-user co-twins were included as '0' and where they were set to missing. For primary analyses, ND was set to missing in those who had never smoked even one cigarette—this is comparable to the discordant twin design. In secondary analyses, ND was set to missing in those who had never smoked cigarettes and in those who had smoked <100 cigarettes.

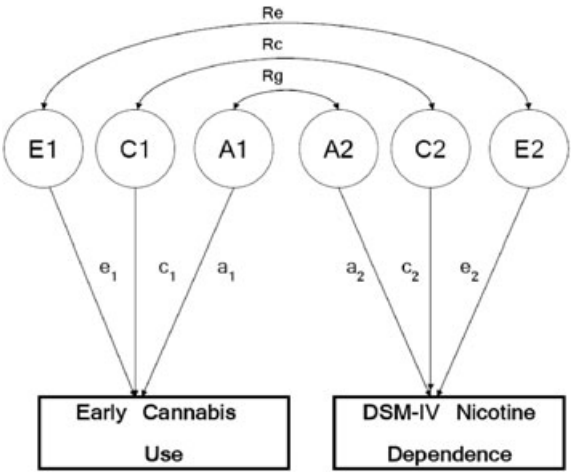

Figure 1 The bivariate twin model, represented as a path diagram (circles = latent variables; A = additive genetic, C = shared environment, E = individual-specific environment; rectangles = observed variable; double-headed arrows = correlations Rg, Rc and Re). Shown here for one twin only

Models were fitted to raw data using a full information maximum-likelihood (FIML) estimation procedure using the software package Mx [38]. A, C and E were allowed to vary, in addition to thresholds, in male and female twins. The correlation across twins was allowed to vary from 0.5 in DZ opposite-sex twin pairs as a test for qualitative sex differences. The fit of submodels was tested using the difference in −2 log-likelihood fit of the models, which follows a χ^2 distribution ($\Delta\chi^2$) for the given degrees of freedom (df).

RESULTS

In this entire sample of twins, EC users were 2.7 [95% confidence interval (CI) 2.3–3.3] times more likely to report ND even after controlling for psychiatric covariates.

Discordant twin analyses

The unadjusted odds of the EC twin meeting criteria for ND were 1.9. This increased likelihood of ND remained even after adjusting for substance use and comorbid psychopathology—EC twins, when compared to their life-time non-user or later-onset user co-twin, were 1.7 times more likely to meet criteria for ND (Table 1). Interactions

Table 1 Conditional odds ratio (OR) between early cannabis use and DSM-IV nicotine dependence in Australian same-sex twin pairs aged 24–36 years.

	Unadjusted ORs (95% CI)	*Adjusted ORs (95% CI)*	*Significant covariates*
Including those who never used cannabis $n = 286$ pairs	1.9 (1.3–2.8)	1.8 (1.1–2.7)	Early smoking, major depression, childhood sexual abuse
Excluding those who never used cannabis $n = 229$ pairs	1.7 (1.1–2.6)	1.5 (0.9–2.7)	Early smoking, major depression, childhood sexual abuse

Covariates tested include DSM-IV major depression, 2+ conduct problems, social anxiety, exposure to childhood sexual abuse, life-time use of other illicit drugs, early drinking and early smoking. CI: confidence interval.

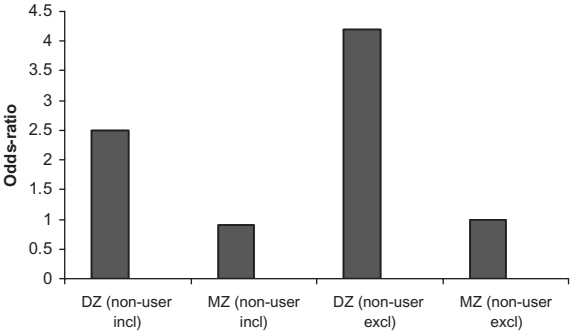

Figure 2 Pattern of monozygotic (MZ) and dizygotic (DZ) odds ratios for the association between early onset cannabis use and DSM-IV nicotine dependence in adult Australian same-sex twins pairs discordant for early cannabis use

between EC and gender or zygosity (being the member of a DZ versus MZ pair) were not significant.

Even upon exclusion of co-twins who had never used cannabis, EC users were still at 1.7 (95% CI 1.1–2.6) greater odds for ND. After adjusting for covariates, this OR was not statistically significant (O.R. 1.5, 95% CI 0.9–2.7, Table 1). Interactions with sex and zygosity were not significant.

Even though interactions with zygosity were not significant in either case, this may have been a consequence of reduced power. When we examined the ORs separately in discordant MZ pairs and DZ pairs, the association between EC (including or excluding cannabis non-user co-twins) and ND was significant only in DZ pairs (Fig. 2). The pattern of MZ–DZ ORs in Fig. 2 is consistent with the conclusion that the association between EC and ND is due to those factors that are correlated fully across MZ pairs (thus, there is no excess risk) but correlated only partially across DZ pairs (thus, the excess risk).

Twin modeling

Genetic (A) and non-shared environmental (E) factors influenced the liability to EC ($a^2 = 72\%$ when non-users included; $a^2 = 66\%$ when non-users excluded) and to ND ($a^2 = 48$–58%) (Table 2). There was no evidence for the DZ opposite-sex correlation being different from the DZ same-sex twin correlation of 0.5 nor for varying magnitudes of A or E in men and women ($\Delta\chi^2 = 10.03$ for 10 df). Shared environmental factors (C) could be dropped without a significant deterioration of fit ($\Delta\chi^2$ ranged from 0 to 2.3 for 1 df). The total correlation between EC and ND was 0.34 when cannabis non-users were included and 0.23 when life-time non-users were excluded. This correlation could be attributed largely to overlapping genetic (Rg = 0.52) factors even when non-users of cannabis were excluded (Rg = 0.41). Consistent with findings from the discordant twin models, there was no evidence for Re ($\Delta\chi^2 = 2.4$ for 1 df), or those individual-specific environmental factors that make one member of an MZ twin pair report EC and progress to ND but does not impact their co-twin. Fitted statistics and the best-fitting model were largely unchanged when excluding non-users of cannabis from the definition of EC (Table 2).

To account for the variance due to smoking 100+ cigarettes, ND was set to missing in those who had smoked less <100 cigarettes in their life-time and models were tested. As expected, this substantially reduced the heritability of ND ($a^2 = 37\%$, 95% CI 25–49%, remainder due to E) as well as the heritability of EC ($a^2 = 52\%$, 95% CI 34-61%, remainder due to E). The genetic correlation between EC and ND was also reduced (Rg = 0.24–0.35); however, the genetic correlations were statistically significant and there was no evidence for shared or individual-specific environmental correlations.

DISCUSSION

In this study of adult male and female twins, onset of cannabis use prior to 17 years of age (EC) increased the odds of DSM-IV ND. However, our analyses also revealed that this excess risk for ND in EC users was due largely to common genetic influences on EC and ND.

Several investigators have now demonstrated the increased risk of problematic cigarette smoking in those with a history of cannabis use [8,9,39–41]. In previous work by us concerning US women aged 18–29 years,

Table 2 Results from best-fitting bivariate model assessing the relationship between early-onset cannabis use and DSM-IV nicotine dependence in adult Australian same- and opposite-sex twins with a life-time history of ever smoking cigarettes.

	h^2 (95% CI)	χ^2 (95% CI)	c^2 (95% CI)	Rg	Rc	Re	Total covariance
Early cannabis use including non-users							
Early cannabis use	0.72 (0.64–0.80)	–	0.28 (0.20–0.36)		–	–	
Nicotine dependence	0.58 (0.50–0.66)	–	0.42 (0.34–0.50)	0.52 (0.44–0.53)	–	–	0.34
Early cannabis use excluding life-time non-users							
Early cannabis use	0.66 (0.56–0.75)	–	0.34 (0.25–0.44)				
Nicotine dependence	0.48 (0.37–0.58)	–	0.52 (0.42–0.63)	0.41 (0.29–0.53)	–	–	0.23

CI: confidence interval; Rg, Rc, Re: correlations.

who had used cannabis, were 2.8 times more likely to transition from smoking 100+ cigarettes to ND [41]. There were, however, several limitations to these previous studies. First, none of these studies explored the possible role of EC, which is a potent predictor of other illicit drug use and psychopathology [3,27]. Secondly, none of them took into account the important etiological contributions of genetic influences and familial environment that may contribute, jointly, to risk for EC and also to progression to more involved stages of cigarette smoking, such as ND. If, indeed, the association between EC and ND extends beyond a shared genetic (and/or environmental) vulnerability, then reducing early exposure to cannabis use may also assist with lower rates of ND. As demonstrated by our study, the risk for ND that is attributable to EC can be attributed largely to the effects of common genetic factors. These common genetic influences overlap only partly with the prior stage of a life-time history of smoking 100+ cigarettes.

This is the first study to document the effects of EC on ND using two complementary genetically informative methods. A feature of the current study is the consistency between the results of these two techniques: the discordant twin design and the bivariate twin model. While a twin model has been fitted previously to data on cannabis and cigarette smoking in adult female Virginia twins, it did not examine EC. These twin models are highly informative as they quantify the magnitude of genetic overlap between cannabis use and stages of cigarette smoking. As expected, these correlations were moderate to high, suggesting that there may exist a cluster of genetic factors that influence an early onset of cannabis use and also influence progression to ND and persistent smoking. This common vulnerability to cannabis and tobacco use has been identified across numerous twin studies [17,42,43] and has been found to be influenced by genetic and shared environmental factors. It is plausible that the genetic influences on this non-specific component of risk may also extend to other aspects of disinhibited and externalizing behaviors [44–48] [for instance, gamma-aminobutyric acid receptor A, subunit 2 (*GABRA2*)]. Identified initially for its role in alcohol dependence [49–52], recent work has implicated polymorphisms in the *GABRA2* gene for their role in cannabis dependence [53], ND [54] and conduct disorder [55].

Genes common to cannabis and tobacco use alone, such as the cannabinoid receptor 1 (*CNR1*) gene may also contribute to the observed genetic correlation. As a target for endogenous cannabinoids [56], its role in mediating the effects of exogenous cannabinoids [57] is supported by animal [58,59] and human research, with some studies reporting association between polymorphisms in *CNR1* and cannabis-related behaviors [60–62] but not others [63]. CB1/CNR1 receptors have also been implicated in ND. Nicotine increases levels of endogenous cannabinoids (e.g. anandamide) and influences reward sensitivity [64]. Rimonabant, a CB1 antagonist, is an emerging drug in tobacco cessation practice [65–70].

Some limitations to this study require discussion. First, our sample is an adult cohort of Caucasian men and women, and these findings may be sample-specific. Secondly, retrospective recall may have affected reports of cannabis use and cigarette smoking. Thirdly, we had fewer than 300 pairs of twins available for analysis, so power may be a concern.

In conclusion, after accounting for genetic commonality, there remains no compelling evidence for causal processes linking EC to ND. This does not imply that reducing rates of EC will have no impact on cigarette smoking behaviors. Instead, it suggests that use of cannabis at an early age may serve as a marker for genetic vulnerability to a host of substance use behaviors.

Providing specialized preventive techniques to such vulnerable adolescents will reduce their general likelihood of progression within their cigarette smoking trajectories and will also probably reduce their levels of general drug involvement.

Declarations of interest

None.

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