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Published in:
Addiction Biology

DOI:
[10.1111/adb.12372](https://doi.org/10.1111/adb.12372)

[Link to publication](#)

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Citation for published version (APA):
Otten, R., Huizink, A. C., Monshouwer, K., Creemers, H. E., & Onrust, S. (2017). Cannabis use and symptoms of anxiety in adolescence and the moderating effect of the serotonin transporter gene. *Addiction Biology*, 22(4), 1081-1089. <https://doi.org/10.1111/adb.12372>

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Cannabis use and symptoms of anxiety in adolescence and the moderating effect of the serotonin transporter gene

Roy Otten^{1,2}, Anja C. Huizink³, Karin Monshouwer⁴, Hanneke E. Creemers⁵ & Simone Onrust⁴

Research & Development, Pluryn, Nijmegen, The Netherlands¹, Behavioural Science Institute, Radboud University Nijmegen, Nijmegen, The Netherlands², Faculty of Psychology and Education, VU University Amsterdam, Amsterdam, The Netherlands³, Netherlands Institute for Mental Health and Addiction, Trimbos Institute, Utrecht, The Netherlands⁴ and Research Institute Child Development and Education, University of Amsterdam, Amsterdam, The Netherlands⁵

ABSTRACT

There is substantial evidence for the assumption that particularly heavy cannabis use is associated with a variety of psychopathologic conditions. Little is known about the relationship between cannabis and anxiety. Prior studies have concluded that cannabis use alone is not sufficient for the development of long-term anxiety, and it has been suggested that cannabis is simply a risk factor that operates in conjunction with other risk factors. One such risk factor may be an individuals' genetic vulnerability. The present study examines the relationship between cannabis use and symptoms of anxiety by taking a developmental molecular-genetic perspective with a focus on a polymorphism involved in the regulation of serotonin. Specifically, we concentrated on changes in cannabis use and symptoms of anxiety over time and differences herein for individuals with and without the short allele of the 5-HTTLPR genotype. Data were from 1424 adolescents over a period of 5 years. We used different statistical analyses to test co-development of cannabis use and symptoms of anxiety throughout adolescence and the possible role of the 5-HTTLPR genotype in this process. Results from different analyses showed that cannabis use is associated with an increase in symptoms of anxiety, but only in carriers of the short allele of the 5-HTTLPR genotype, not in non-carriers. The findings of the present study show first evidence that the links between cannabis use and symptoms of anxiety are conditional on the individuals' genetic make-up.

Keywords Cannabis use, symptoms of anxiety, 5-HTTLPR genotype.

Correspondence to: Roy Otten, Research & Development, Pluryn, PO Box 53 6500 AB Nijmegen, The Netherlands. E-mail: Royotten@pluryn.nl

INTRODUCTION

With between 119 and 224 million users, cannabis is the world's most widely used illicit substance (UNODC 2012). In Europe, in 2011, the lifetime prevalence among 15- to 16-year-olds was 54 percent, whereas use over the last 30 days was 28 percent (Hibell *et al.* 2012). Particularly heavy cannabis use has been associated with a variety of psychopathologic conditions, such as illicit drug use, crime, depression and suicidal thoughts (e.g. Fergusson, Horwood, & Swain-Campbell, 2002; Moore *et al.* 2007; Hall & Degenhardt 2009). Relatively little attention has been paid to the link between cannabis and anxiety. However, a review study based on 15 experimental studies concluded that there

is a positive, dose-dependent link between cannabis use and acute anxiety, and that cannabis use and anxiety symptoms/disorders often co-occur (Crippa *et al.* 2009). Moreover, it was concluded that cannabis use alone would not be sufficient for the development of long-term anxiety and that cannabis is simply a risk factor that operates in conjunction with other risk factors (Crippa *et al.* 2009). One such risk factor is an individuals' genetic make-up. The main objective of the present study was to test the effects of cannabis use on symptoms of anxiety while taking into account the potential moderating effects of a polymorphism involved in the regulation of serotonin, a monoamine neurotransmitter that plays an important role in emotions, among which anxiety (Young 2007).

Cannabis use and anxiety

The potential relationship between cannabis and anxiety is complex, as individuals report reduced levels of anxiety as a motivation to use cannabis, but they also report heightened levels of anxiety and panic as a consequence of use (Fusar-Poli *et al.* 2009). Symptoms of anxiety following cannabis use may be a manifestation of withdrawal symptoms, which, in turn, may motivate people to continue use (Degenhardt *et al.* 2013; Huizink 2013). Furthermore, it has been suggested that repeated intake of cannabis acts on the main stress systems causing a chronic activation and hypervigilant state (Degenhardt *et al.* 2013; Huizink 2013), which is associated with increased anxiety. Although the molecular mechanism underlying the acute and long-term associations between cannabis use and anxiety is still unclear, it is likely that the two main ingredients of cannabis, Δ^9 -tetrahydro-cannabinol (Δ^9 -THC) and cannabidiol (CBD), play a role (Fusar-Poli *et al.* 2009). The main psychoactive ingredient Δ^9 -THC is known to have anxiogenic effects (D'Souza *et al.* 2004), whereas CBD (although not psychoactive) is known to be anxiolytic (e.g. Guimaraes *et al.* 1990; Crippa *et al.* 2009) and capable of reducing the anxiety effects induced by Δ^9 -THC (Zuardi *et al.* 1982). If CBD is less efficient in activating the limbic and paralimbic regions that contribute to the reduction of autonomic arousal and subjective anxiety, the effects induced by Δ^9 -THC on other brain regions may become dominant, causing feelings of anxiety. Hence, following this explanation, symptoms of anxiety following cannabis use may be the result of the interplay between the two main ingredients of cannabis acting in the brain.

Genetic markers

A large number of neurotransmitters, peptides, hormones and other neuromodulators have been implicated in anxiety (Steimer 2002). Serotonin (5-HT), a monoamine neurotransmitter, plays an important role in feelings of wellbeing, happiness and anxiety (Young 2007). Studies have shown that there are genetic markers involved in the regulation of serotonin that predispose individuals to be particularly sensitive to anxiety. A polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) involved in the regulation of 5-HT is such a genetic marker. This polymorphism has been found to affect the transcription rate of the gene that codes for the serotonin transporter (5-HTT), responsible for removing serotonin from the synaptic cleft, returning it to the pre-synaptic neuron (Karg *et al.* 2011), thereby determining the extent, duration and spatial domain of the serotonergic activation. In humans, two common alleles, the short and the long, have been differentially associated with anxiety-related behavioral traits. More specifically,

carriers of the short allele, which is associated with reduced 5-HTT expression and function and increased fear and anxiety-related behaviors, exhibit greater amygdala neuronal activity in response to fearful stimuli (Hariri *et al.* 2002). Hence, carriers of the short allele may be more prone to show symptoms of anxiety in response to fearful stimuli as compared with non-carriers of the short allele. When it comes to cannabis, the short allele of the 5-HTTLPR may be responsible for symptoms of anxiety after frequent use of cannabis. Specifically, as mentioned before, Δ^9 -THC is responsible for the immediate anxiogenic effects of cannabis use. CBD on the other hand functions as a 5-HT receptor agonist, inhibiting the reuptake of 5-HT after its release (Russo *et al.* 2005) and thereby lengthening the serotonergic action (Karg *et al.* 2011). As CBD acts upon the 5-HT receptors, it is likely that in carriers of the short allele of the 5-HTTLPR, the anxiolytic effects of CBD are less effective or pronounced, thereby making the effects of Δ^9 -THC more prominent. The consequences of this process may be more pronounced once cannabis use takes place at more frequent levels, as a consequence of processes of tolerance and withdrawal.

The present study

In the present study, we aim to test the prospective link between cannabis use and symptoms of anxiety while taking into account allelic variation in 5-HTTLPR and by using different analytic strategies. As a consequence of reduced serotonin expression/function in carriers of the short allele of the 5-HTTLPR, we expect that the anxiogenic effects of Δ^9 -THC become dominant over the anxiolytic effects of CBD, which ultimately leads frequent use of cannabis to be prospectively related to higher and increasing levels of anxiety in this group of individuals. By using this developmental molecular-genetic approach, we will gain more information about the process that ultimately leads to the more established link between heavy use of cannabis and clinical levels of anxiety.

MATERIALS AND METHODS

Participants

Data were from the Tracking Adolescents' Individual Lives Survey (TRAILS). The TRAILS target sample involved all 10- to 11-year-old children living in five municipalities in the north of the Netherlands. Seventy-six percent of the target population [$n = 2230$, mean age 10.60, standard deviation (SD) = 0.65, 50.8 percent girls] was enrolled in the study (i.e. both child and parent agreed to participate). As there were hardly any children that used cannabis at the first time of measurement (T1), we only used data from the second (T2), third (T3) and fourth (T4) assessments.

Moreover, we only included those participants who were successfully genotyped ($N=1427$) and had data at least one time of measurement on the main study variables (i.e. cannabis use and anxiety), leading to a study sample of 1424 (i.e. 64 percent of target population). Mean age of the sample that we used at the second time of measurement was 13.02 ($SD=0.60$); at third time of measurement, mean age was 15.73 ($SD=0.74$), and at the fourth time of measurement, it was 18.54 ($SD=0.62$). Fifty-three percent was female. A more detailed description of the sampling procedure and methods can be found in Huisman *et al.* (2008).

Procedures

At each assessment wave, children completed questionnaires either at school (T1–T3), under the supervision of TRAILS assistants or through the Internet (T4). At the third wave, blood or buccal cells were collected for DNA analysis. All procedures were approved by the Central Committee for Research involving Human Subjects. All participants and their parents gave written informed consent.

Cannabis use

Cannabis use was assessed at each measurement by self-report questionnaires. The confidentiality of the study was emphasized, so that adolescents were reassured that their parents or teachers would not have access to the information they provided. Participants were asked to report the frequency of cannabis use in the last 12 months (i.e. current use). More specifically, participants were asked: 'How many times over the last twelve months have you used cannabis?' (0–10 times, 11 = 11–19 times, 12 = 20–39 times, 13 = ≥ 40 times). The average number of times used during the last 12 months at T2–T4 was 0.20 ($SD=1.15$), 1.61 ($SD=3.57$) and 2.27 ($SD=4.11$), respectively.

Anxiety symptoms

An overall score for anxiety symptoms during the past 6 months was assessed at T2, T3 and T4 using the DSM-IV Anxiety Problems Scale of the Youth Self-Report (Achenbach, Dumenci, & Rescorla, 2001; 2003), which consists of six items. Adolescents were requested to respond to each behavior item with (2), (1) or (0), to indicate 'very true or often true', 'somewhat or sometimes true' or 'not true', respectively. An example item stated: 'I am afraid of certain animals, situations, or places, other than school (describe)' The mean item score was calculated at each measurement wave resulting in scores between 0 and 2 (Mean $\alpha=0.62$). Mean item scores in the study sample from T2 to T4 were 0.37 ($SD=0.31$), 0.33 ($SD=0.31$) and 0.39 ($SD=0.35$).

Genotyping of 5-HTTLPR

DNA was extracted from blood samples ($n=1238$) or buccal swabs (cytobrush; $n=361$) using a manual salting out procedure as described by Miller and colleagues (Miller *et al.* 1988). Genotyping the 5-HTTLPR polymorphism in the promoter region of *SLC6A4* (5-HTT, *SERT*) gene was performed by simple sequence length analysis (Nederhof *et al.* 2010). Determination of the length of the 5-HTTLPR alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Call rate was 91.6 percent. The single nucleotide substitution (A_G) present in the HTTLPR long (l) allele (rs25531) was genotyped using a custom-made TaqMan assay (Applied Biosystems). Call rate was 96.5 percent for rs25531. Concordance between DNA replicates showed an accuracy of 100 percent. Because the *lg* polymorphism represents low serotonin expression comparable with the *s* allele, *s* and *lg* alleles were recoded *s*=, and *la* was recoded *l*=(Nakamura *et al.* 2000; Araya *et al.* 2009). The short allele of the 5-HTTLPR genotype was present in 66 percent of the sample, which is in accordance with other studies (e.g. Otten & Engels 2013; Van der Zwaluw *et al.* 2011).

Covariates

In each of the analyses, we controlled for anxiety at the second time of measurement (T2). In addition to the crude models, we also ran the analyses while controlling for sex, age, alcohol use (frequency of use during the week) and tobacco use (frequency of lifetime use). In addition, regarding genetics, we corrected for the possibility of population stratification, as differences in allele frequencies between cases and controls may be due to systematic differences in ancestry rather than association of genes with disease (Purcell *et al.* 2007), by including two principal components resulting from the population stratification analysis.

Statistical analysis

Statistical analyses were conducted with SPSS and MPLUS 6.0 (Muthén & Muthén, 1998–2004). In the first step, we calculated descriptive statistics for anxiety symptoms and cannabis use over the three measurements. In the second step, we conducted a linear regression model to predict anxiety symptoms at T4 by cannabis use at T2, while controlling for anxiety symptoms at T2. Multiple group analyses were conducted to test whether the effects were statistically different for carriers and non-carriers of the short allele of the 5-HTTLPR. To this end, the models were estimated within each group simultaneously (i.e. carriers versus non-carriers of the short allele), and the

parameter specified (i.e. the pathway reflecting the link between cannabis use and anxiety symptoms) was either estimated freely for each group or constrained to be equal across groups. By comparing the model with freely estimated parameters with the nested model with the specific equality constraints, the appropriateness of those constraints was tested using χ^2 -difference tests (cf. Schuck et al. 2012).

In the third step, we conducted a parallel process latent growth model (e.g. Preacher et al. 2008; Barker et al. 2011; Otten & Engels 2013). Latent growth curve modeling permits to capture not only the initial levels of individuals at the beginning of a developmental period but also individual changes over a developmental period (e.g. Muthén & Muthén, 1998–2004). Specifically, intercepts represent initial levels of cannabis use and symptoms of anxiety, and the slopes represent the rates of change in cannabis use and symptoms of anxiety. We tested whether intercepts of cannabis use predicted growth curves of anxiety symptoms and vice versa (to control for the possibility that symptoms of anxiety precede changes in cannabis use) by simultaneously regressing the slope of anxiety symptoms on the intercept of cannabis use and the slope of cannabis use on the intercept of anxiety symptoms in a parallel process latent growth model. In the final step, we examined whether the effects were different for carriers and non-carriers of the short allele of the 5-HTTLPR with multiple group testing. To determine model fit, we used the Comparative Fit Index (CFI), the Tucker-Lewis Index (TLI) (Bentler & Bonett 1980) and the Root Mean Squared Estimate of Approximation (RMSEA) (Browne & Cudeck 1993).

RESULTS

Descriptive analyses

Table 1 shows bivariate correlations between the main study variables and stability rates of cannabis use and symptoms of anxiety over time, separately for short allele carriers and non-carriers. What stands out are the

significant correlations between cannabis use at times 2, 3 and 4 and anxiety symptoms at time 4, but only in carriers and not in non-carriers of the short allele. In non-carriers, at the second time of measurement, higher rates of symptoms of anxiety were associated with less frequent use of cannabis.

Linear regression model

Linear regression models were conducted to predict the level of anxiety symptoms at the last time of measurement by frequency of cannabis use at T2, and controlling for anxiety levels at the first time of measurement in both carriers and non-carriers of the short allele of the 5-HTTLPR (Table 2).

In the overall sample, the first block indicated a small effect of cannabis use on anxiety problems ($B = 0.08$, $P = 0.006$). The second block in which the 5-HTTLPR genotype was added to the model showed no direct effect of the gene ($B = -0.01$, $P = 0.853$). In the third block, the interaction between the 5-HTTLPR and cannabis use was added to the model. The interaction was significant ($B = -0.16$, $P = 0.048$). Multi-group analyses, separating carriers and non-carriers of the short allele, showed that the effect of cannabis use on anxiety was only significant for carriers of the short allele ($B = 0.11$, $P = 0.00$) and not for non-carriers ($B = -0.01$, $P = 0.90$). A χ^2 -difference test showed that this effect was on the border of statistical significance ($\Delta\chi^2 = 3.750(1)$, $P = 0.05$). The results remained significant after controlling for the aforementioned covariates (i.e. sex, age, alcohol use, tobacco use and the possibility of population stratification).

Parallel process latent growth curves

In the first step, separate growth curves were calculated for the total sample. Model fit was good (CFI = 0.95; TLI = 0.88; RMSEA = 0.08). Table 3 shows the means and variances for both the intercepts and the slopes of cannabis use and anxiety of the total sample.

Table 1 Bivariate correlations between main study variables and the different measurements separated for carriers and non-carriers of the short allele of the 5-HTTLPR genotype.

	<i>Cannabis T2</i>	<i>Cannabis T3</i>	<i>Cannabis T4</i>	<i>Anxiety T2</i>	<i>Anxiety T3</i>	<i>Anxiety T4</i>
Cannabis T2	—	0.32**	0.14**	-0.11*	-0.09	-0.04
Cannabis T3	0.21**	—	0.54**	-0.07	-0.03	0.02
Cannabis T4	0.18**	0.51**	—	-0.08	-0.08	-0.01
Anxiety T2	-0.02	-0.06	-0.05	—	0.50**	0.36**
Anxiety T3	0.04	-0.05	-0.04	0.48**	—	0.52**
Anxiety T4	0.10**	0.07*	0.11*	0.38**	0.49**	—

Two-tailed tests. Values below the diagonal refer to the carriers of the short allele. Values above the diagonal refer to non-carriers. Note that mean age at T2 was 13.02, at T3, it was 15.73 and at T4, it was 18.54. * $P < 0.05$; ** $P < 0.01$.

Table 2 Linear regression predicting symptoms of anxiety at T4 by symptoms of anxiety and cannabis use at T2 in the total sample and separated for carriers and non-carriers of the short allele of the 5-HTTLPR genotype.

		Total sample			Carriers of the 5-HTTLPR short allele			Non-carriers of the short allele		
		B	SE	P-value	B	SE	P-value	B	SE	P-value
Block 1	Anxiety T2	0.37	0.02	0.00	0.38	0.03	0.00	0.36	0.04	0.00
	Cannabis use T2	0.08	0.03	0.01	0.11	0.03	0.00	-0.01	0.05	0.90
Block 2	5-HTTLPR	-0.01	0.03	0.85						
Block 3	Interaction ^a	-0.16	0.08	0.05						

^aAfter finding a significant interaction between cannabis use and the 5-HTTLPR was found, the total sample was split, and regression analyses were conducted separated for carriers and non-carriers.

All parameters were significant except for the mean slope of anxiety, which indicates that, overall, mean levels of anxiety did not significantly change over time. In the second step, we tested the actual parallel process latent growth curve models separately for carriers and non-carriers of the short allele of the 5-HTTLPR, by regressing the slope of anxiety problems on the intercept of cannabis use and the slope of cannabis use on the intercept of anxiety problems while controlling for the baseline measure of respectively anxiety problems and cannabis use (Fig. 1). Again, model fit was good (CFI = 0.95; TLI = 0.89; RMSEA = 0.08).

The most important finding is reflected in the link between the intercept of cannabis use and the slope of anxiety symptoms. Specifically, the intercept of cannabis use was positively associated with an increase in anxiety symptoms but only in carriers of the 5-HTTLPR short allele ($B = 0.27$, $P = 0.01$) and not in non-carriers ($B = -0.02$, $P = 0.78$). A χ^2 -difference test established support for this difference between carriers and non-carriers ($\Delta\chi^2 = 5.45(1)$, $P = 0.01$) (Satorra & Bentler 2001). In carriers of the short allele, we also found a significant effect of anxiety on the slope of cannabis use ($B = -0.13$, $P = 0.01$), indicating that individuals with higher levels of anxiety symptoms are less likely to increase their use or reduce their use of cannabis. In non-carriers of the short allele, this effect was similar but just

outside the significance border ($B = -0.12$, $P = 0.07$). A χ^2 -difference test showed that this effect was not statistically different ($\Delta\chi^2 = 0.010(1)$, $P = 0.75$). Finally, all other relationships within the model that appeared different between the two groups were not statistically different after a χ^2 -difference test (results available upon request).

DISCUSSION

The present study, based on a prospectively studied Dutch adolescent cohort, aimed at scrutinizing the link between cannabis use and anxiety by taking a developmental molecular approach. The different analyses point in the direction of a heightened sensitivity to the anxiogenic effects of cannabis in carriers of the short allele of the 5-HTTLPR. Specifically, bivariate correlations, linear regression and parallel growth models showed that in short allele carriers, but not in non-carriers, cannabis use was positively associated with higher and increasing levels of anxiety. As stated in the Introduction section, one explanation for this finding could be (1) that in this specific group, in which serotonin is less effectively regulated, the anxiogenic effects of Δ^9 -THC have more chance of producing anxiety effects or (2) that in this group, CBD (the other main ingredient of cannabis) is less effective in dampening the anxiogenic effects of Δ^9 -THC. In

Table 3 Means and variances for the intercepts and slopes of cannabis use and symptoms of anxiety in the parallel process growth model for the total sample.

	Mean	Mean	Variance	Variance
	Intercept	Slope	Intercept	Slope
Symptoms of anxiety	0.35	0.01	0.05	0.01
	SE = 0.01	SE = 0.01	SE = 0.01	SE = 0.00
	(43.66) ^{***}	(1.16) ^{ns}	(10.58) ^{***}	(3.55) ^{***}
Cannabis use	0.20	1.09	1.29	3.66
	SE = 0.03	SE = 0.06	SE = 0.21	SE = 0.24
	(6.38) ^{***}	(19.37) ^{***}	(6.13) ^{***}	(14.99) ^{***}

T-values are presented in parentheses below their respective associated growth curve parameter. *** $P < 0.001$, two-tailed tests ns = Non significant.

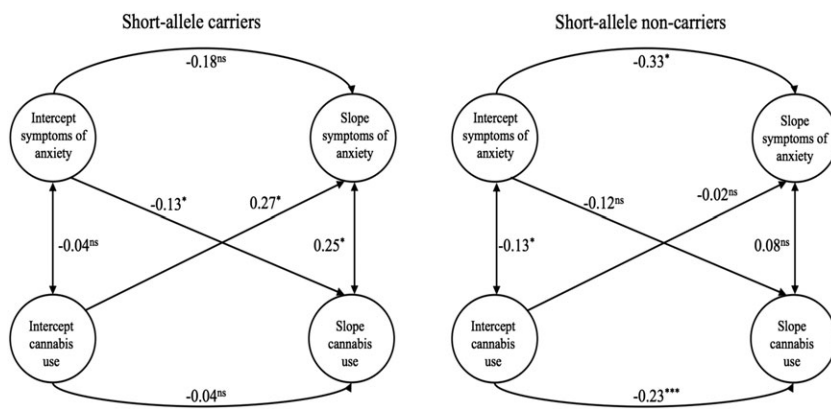


Figure 1 Parallel growth curves of cannabis use and symptoms of anxiety for adolescent carriers and non-carriers of the 5-HTT short allele (standardized estimates), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-tailed tests ns = Non significant

addition to the main findings, within non-carriers of the short allele of the 5-HTTLPR, at the second time of measurement, cannabis use was associated with lower rates of symptoms of anxiety. At this age, frequency of cannabis use is still very low and mostly limited to those who score high on deviant behavior in general. It is likely that this is also the group that scores low on anxiety, and youngsters who score low on anxiety are more likely to experiment with cannabis (Creemers *et al.* 2009).

This study extends the literature in six ways. First, although there is a large body of research concentrating on the link between cannabis use and a variety of psychopathological disorders, little is known about how cannabis use relates to anxiety. In the only review published on this topic, the authors concluded that further research is needed to clarify the mechanisms by which cannabis use may cause acute anxiety and long-lasting anxiety disorders and that ‘... longitudinal studies are helpful in obtaining better understanding of environmental, social, neurobiological and other confounding factors’ (Crippa *et al.* 2009). The current study responds to that need, particularly given its focus on adolescence, the developmental period in which cannabis use usually starts. Second, in addition to the studies that have concentrated on how heavy cannabis use (i.e. cannabis abuse and cannabis dependence) relates to a variety of disorders, there is a need for studies like the present study that concentrate on the process that precipitates this link (Otten *et al.* 2010). This change of perspective will potentially contribute to a better understanding of the process that underlies the ultimate clinical associations between heavy use and psychopathological disorders. In such a way, the link between heavy cannabis use and anxiety disorders may be the result of an iterative and recursive loop in which milder levels of cannabis use and anxiety symptoms interact in a progressive kind of way. Third, this study is one of the first to take a developmental molecular perspective in looking at the link between cannabis use and anxiety. Consistent with prior research on

substance use, this study suggests that the link between cannabis and anxiety is conditional on the presence of a genetic factor (e.g. Otten & Engels 2013). This way, cannabis use could be interpreted as an environmental stimulus that, in interaction with this specific genetic factor, may result in a disease phenotype (i.e. anxiety disorder). Fourth, the longitudinal character of the present study provides first indications that the link between cannabis use and symptoms of anxiety is a causal one. In carriers of the short allele of the 5-HTTLPR, low frequent use of cannabis may have positive immediate effects in the beginning, as in non-carriers of the short allele. However, over time, as a consequence of more frequent use, symptoms of withdrawal may occur, which are possibly stronger in carriers than in non-carriers of the short allele. Prevention of these symptoms may lead to more use and more long-term symptoms of anxiety, suggesting a bidirectional process. Future studies with adequate study designs (e.g. by means of Ecological Momentary Assessments) are needed to elucidate the precise process in which the positive feelings of cannabis use are slowly being replaced by more negative consequences. Fifth, the findings of this study largely fit into models of anxiety that assume that anxiety is a consequence of an interplay between biological factors (e.g. a genetic predisposition to develop symptoms of anxiety) and contextual factors or stimuli (e.g. cannabis use), such as the biopsychosocial model of anxiety. In this study, we found support for the link between cannabis use and anxiety in carriers of the short allele of the 5-HTTLPR genotype; however, this link may even be more complex than presented here. For instance, we did not include information about personality or negative life events. It is likely that the link between cannabis use and anxiety is even stronger in short-allele carriers who score high on neuroticism or in those who experienced more negative life events. Sixth and finally, when it comes to the clinical implications, understanding the link between cannabis use and anxiety is important for paving the way to developing new and effective

treatments and for providing information and knowledge to clinicians. For instance, youngsters who are likely to be genetically at risk should be advised not to use cannabis, which may be in contrast with the public opinion that cannabis reduces symptoms of anxiety.

Limitations

The present findings should be interpreted in the context of five main limitations. First, although our findings were based on longitudinal data covering the most important developmental phase in the adoption of substance use and addictive behaviors, the total number of timepoints was three, which is suboptimal when it comes to the analysis of growth curves. Whereas three timepoints only allow a development to be linear, more timepoints would also allow non-linear development of cannabis use and anxiety (e.g. a quadratic or cubic trend) (Muthén & Muthén, 1998–2004). Second, and related to this issue, these data do not allow testing how the simultaneous development of anxiety problems and cannabis use progresses into adulthood. Although the majority starts using cannabis less frequent as soon as they start working or gain a relationship (White, Labouvie, & Papadaratsakis, 2005), a minority of adults maintain using cannabis. It would be interesting to examine how the association between cannabis use and symptoms of anxiety develops over time within this group. Third, for the measurement of anxiety, we used the YSR-scores (Achenbach *et al.* 2001, 2003), which is a relatively general measure to assess anxiety with relatively low reliability levels (α). To make more specific claims about the link between cannabis and anxiety, the present study needs replication with different measures of anxiety (e.g. social anxiety). Fourth, all measures were based on child reports, which raises the possibility for shared method variance. Moreover, self-report of cannabis use may be subject to social desirability. Therefore, future studies should use biological indices or bogus pipeline procedures to assess cannabis use, in addition to self-report. Fifth, unfortunately, we were not able to control for variation in concentration of CBD through administration via inherent strains and different routes of administration (Bergamaschi *et al.* 2011). Sixth, because the frequency of a single nucleotide polymorphism may differ between ethnic groups, it was decided at the start of this study to exclude subjects with a descent different from Dutch. We corrected for the possibility of population stratification, as differences in allele frequencies between cases and controls may be due to systematic differences in ancestry rather than association of genes with disease (Purcell *et al.* 2007), by including two principal components resulting from the population stratification analysis. Results did not change as a consequence of the

inclusion of these covariates in the analyses. However, in order to test the robustness and generalizability of our findings, future studies should include different ethnic groups. Finally, although the findings of this study confirmed our hypotheses and we made a cautious claim about the potential underlying mechanism caused by a genetic factor, the exact mechanism could not be tested with this study design. More research (e.g. using Functional Magnetic Resonance Imaging) is necessary to elucidate the precise mechanism responsible for this heightened sensitivity for the anxiogenic effects of cannabis. On the basis of our findings, it seems as if carriers of the short allele are more sensitive to the negative effects of Δ^9 -THC. Whether this is exactly what happens or whether this is a simplification of the mechanism at work warrants for more research.

Conclusion

To the best of our knowledge, the present study is the first to test the association between cannabis use and anxiety symptoms with a developmental molecular approach. Using a large longitudinal cohort that covers the adolescent life-span, we highlight that the association between cannabis use and anxiety problems is conditional on the presence of the short allele of the 5-HTTLPR.

Acknowledgements

This research is part of the Tracking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314 and GB-MaGW 452-06-004; and NWO large-sized investment grant 175.010.2003.005); the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006) and the participating universities. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible.

Authors Contribution

RO, AH and SO were responsible for the study concept and design. RO performed the analyses and drafted the manuscript. AH, SO, HC and KM assisted with interpretation of the findings. All authors critically reviewed content and approved final version for publication.

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