Testing bidirectional effects between cannabis use and depressive symptoms: moderation by the serotonin transporter gene

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ABSTRACT

Evidence for the assumption that cannabis use is associated with depression and depressive symptoms is inconsistent and mostly weak. It is likely that the mixed results are due to the fact that prior studies ignored the moderating effects of an individual’s genetic vulnerability. The present study takes a first step in scrutinizing the relationship between cannabis use and depressive symptoms by taking a developmental molecular–genetic perspective. Specifically, we concentrated on changes in cannabis use and depressive symptoms over time in a simultaneous manner and differences herein for individuals with and without the short allele of the 5-hydroxytryptamine (serotonin) transporter gene-linked polymorphic region (5-HTTLPR) genotype. Data were from 310 adolescents over a period of 4 years. We used a parallel-process growth model, which allows co-development of cannabis use and depressive symptoms throughout adolescence, and the possible role of the 5-HTTLPR genotype in this process. We used data from the younger siblings of these adolescents in an attempt to replicate potential findings. The parallel-process growth model shows that cannabis use increases the risk for an increase in depressive symptoms over time but only in the presence of the short allele of the 5-HTTLPR genotype. This effect remained significant after controlling for covariates. We did not find conclusive support for the idea that depressive symptoms affect cannabis use. These findings were replicated in the sample of the younger siblings. The findings of the present study show first evidence that the links between cannabis use and depressive symptoms are conditional on the individual’s genetic makeup.

Keywords 5-HTTLPR genotype, adolescents, cannabis use, depressive symptoms, parallel-process growth model, serotonin.

INTRODUCTION

Cannabis can be considered as the most widely used illicit drug (UNODC, 2010). The consequences of cannabis use over a prolonged period of time are serious and diverse, ranging from a heightened risk for impairment of cognitive functioning to schizophrenia and psychosis (Moore et al. 2007). Evidence for the assumption that cannabis use is associated with depression is inconsistent and mostly weak (Moore et al. 2007). Nevertheless, there may be subgroups particularly at risk (Monshouwer et al. 2006). As recent studies suggest that genetic markers may predispose people to be particularly vulnerable for the effects of drug use on mental health (Degenhardt, Hall & Lynskey 2003), it is likely that the mixed results with respect to the association between cannabis use and depression are due to the fact that prior studies ignored the moderating effects of an individual’s genetic vulnerability. The present study takes a first step in scrutinizing the relationship between cannabis use and depressive symptoms by taking a developmental molecular–genetic perspective.

There are various reasons why to expect a relationship between cannabis use and depression or depressive symptoms (Degenhardt et al. 2003). Instigated by the primary psychoactive ingredient of cannabis [$\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC)], there could be a neurological link between cannabinoid effects and depression. Specifically, $\Delta^8$-THC acts on the cannabinoid system, which is known to be related to emotion regulation. As a consequence, cannabis use could contribute to changes in depressive symptoms. Alternatively, cannabis use may increase the
likelihood for certain contexts or environmental factors (Degenhardt et al. 2003). For instance, cannabis use may instigate a decrease in academic achievement, arguments with parents or peer rejection, which, in turn, makes depressive symptoms more likely to occur.

However, when studying the link between cannabis use and depressive symptoms, there is also the problem of reverse causation. Particularly, in accordance with the self-medication hypothesis, some individuals may use cannabis to cope with depressive symptoms (Grinspoon & Bakalar 1998). Finally, in some cases, the relationship between cannabis use and depressive symptoms may be spurious and disappear after controlling for variables that might be related to both cannabis use and depressive symptoms (e.g. age, sex, personality, tobacco use, alcohol use, socioeconomic status and parenting).

Findings from studies on the link between cannabis use and depression are diverse (Degenhardt et al. 2003). Some studies indeed found that cannabis use precedes depression (e.g. Brook et al. 2002; Fergusson, Horwood & Swain-Campbell 2002), some studies only found a relationship between cannabis use and depression for girls (Patton et al. 2002), while others found no relationship at all (e.g. Fergusson et al. 2002). Support for the self-medication hypothesis is scarce (e.g. Brook, Cohen & Brook 1998). Finally, some studies show a relationship with suicide idealization but not with depression (e.g. Pedersen 2008). It is likely that findings from prior studies are diverse because these studies failed to take into account the role of important moderating mechanisms. Specifically, the link between cannabis use and depressive symptoms is expected to be conditional on certain individual differences that make people more or less susceptible. The role of serotonin (5-hydroxytryptamine, 5-HTT) has been a key aspect in the study of the biochemistry of mental health outcomes (e.g. Kaplan & Sadock 1998). The serotonin transporter gene has been studied as an important candidate gene for its role in depression (for a review, see Levinson 2006). Specifically, this gene encodes the serotonin transporter protein, which is responsible for the reuptake of serotonin into the presynaptic cell after its release into the synaptic cleft to signal the adjacent neuron (Glatt et al. 2003) and terminating its function. One variant of this gene, the 5-HTT (serotonin) transporter gene-linked polymorphic region (5-HTTLPR), is a functional polymorphism in which the short allele is associated with lower 5-HTT transcription and function compared with the long variant (Lesch et al. 1996). It is the efficiency of the serotonin transcription that affects mood tone besides other functions such as sleep, appetite, sexual behavior and motor function (Lesch & Mosnner 1998). It is likely that the link between cannabis use and depressive symptoms is conditional on the presence of the short allele of the 5-HTTLPR polymorphism of the serotonin transporter gene.

In summary, by employing a longitudinal design, the present study will test whether the 5-HTTLPR genotype moderates the link between cannabis use and depressive symptoms.

While most studies that focus on the link between cannabis use and depression concentrate on cannabis use dependence or cannabis abuse and major depressive disorders, we will focus on less frequent levels of cannabis use and depressive symptoms in the general population. Moreover, most studies that concentrate on moderation by a genetic polymorphism use cross-sectional designs or longitudinal designs with only two timepoints over longer periods of time (Van der Zwaluw & Engels 2009). These studies ignore the fact that individuals show different initial levels of use and different individual developmental pathways (e.g. Duncan & Duncan 1996). Hence, instead of using more traditional analyses, we will use a parallel-process latent growth model (e.g. Preacher et al. 2008; Barker et al. 2011), which can provide more fine-grained knowledge of the co-development of cannabis use and depressive symptoms throughout adolescence, and the possible role of the 5-HTTLPR genotype in this process. We expect that individuals with dysfunctioning serotonin reuptake inhibitors are more vulnerable for the effects of cannabis and are more likely to show increasing levels of depressive symptoms over time. In addition, we tested whether the relationship between cannabis use and depressive symptoms is bidirectional.

**METHODS**

**Participants**

Participants were from the Family and Health Study, a prospective study among 428 families (biological parents and two children) that were selected from registers of 22 municipalities in the Netherlands (e.g. Harakeh et al. 2005). Families were visited in their homes by interviewers. To maintain confidentiality, questionnaires were filled in private by each family member. We used data from five waves with 1-year intervals of the oldest child in the family to obtain highest cannabis prevalence rates. Saliva samples were collected for genetic analysis. In total, 310 adolescents could be genotyped after written consent by parents and adolescents. Eventually, genetic data needed for the present study were available for 306 adolescents. At year 1, participants were between 14 and 17 years \([M = 15.23, \text{ standard deviation (SD)} = 0.61]\). The distribution of males and females was almost equal. More than 95% of the family members were of Dutch origin. With respect to education, adolescents were equally divided. Parental consent was obtained for all adolescents.
adolescents who participated and the study was approved by the Central Committee on Research Involving Human Subjects in the Netherlands.

Adolescents who were genotyped did not differ from those who were not genotyped on any of the study variables. Of the dataset including all participants who were genotyped, 98.7% of the cannabis use data were available at T1; 97.7% of the data were available at T2; 95.4% at T3; 71.6% at T4; and 86.6% of the data were available at T5. Participants with a missing on any of the cannabis measures did not differ on any of the study variables, except for age: Older participants had a lower likelihood to have a missing on one of the cannabis measures [odds ratio (OR) = 0.61, confidence interval (CI) = 0.40–0.93]. Data on depressive symptoms were available for all participants at T1; for 99% at T2; 97.7% at T3; 99% at T4; and at T5 87.3% of the data were available. Participants with a missing on any of the depression measures did not differ from those who had no missings on any of the study variables. To make use of all available data, genotyped participants with at least one data point on cannabis use and depressive symptoms were allowed in the latent growth curve analyses.

Cannabis use

Information was collected using self-reports at each assessment point following two items: (1) have you ever used cannabis (0 = Yes, 1 = No); (2) how many times have you used cannabis during the last 4 weeks (1 = Not; 2 = 1–2 times; 3 = 3–4 times; and 4 = 5 times or more). Because some cells consisted of only a small number of respondents, we created a composite score, ranging from 0 to 2, which represented the frequency of cannabis use for each data collection wave (0 = Never used; 1 = Used, but not during the last 4 weeks; and 2 = One time or more during the last 4 weeks) (Monshouwer et al. 2006; Otten et al. 2010). A total of 9.6% reported lifetime use at the first time of measurement, at the fifth time of measurement this was 38.1%. Monthly use increased from 6% at the first time of measurement to 17% at the fifth time of measurement.

Depressive symptoms

To assess the extent to which adolescents experience negative moods, the 6-ite m Depressive Mood List developed by Kandel & Davies (1982, 1986) was used in its Dutch translation (Dékovic, 1996; Otten et al. 2009; 2010). The Depressive Mood List is extensively used in adolescent surveys (Compus, Sydney & Grant 1993) and showed sufficient psychometric properties in terms of internal consistency, reliability and stability over time (Van den Eijnden et al. 2008). On a 5-point scale, respondents were asked to report how often they felt unhappy, sad or depressed, and how often they felt nervous or tensed over the last 12 months. Cronbach’s alphas for the Depressive Mood List used in the present study were between 0.79 and 0.87 [M (SDs): 2.45 (0.65), 2.43 (0.63), 2.43 (0.59), 2.23 (0.68) and 2.13 (0.70)].

Genotyping

5-HTTLPR genotyping of the HTTLPR polymorphism in the SLC6A4 (5-HTT, SERT) gene was performed by simple sequence length analysis. Polymerase chain reaction (PCR) was on 50 ng genomic DNA using 10 pmol of forward primer (50-GGCGTTGCCGCTCTGAATGC-30) and 10 pmol of reverse primer (50-GAGGGACTGAGCTTGACAAACCAC-30), 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Breda, The Netherlands) in a PCR buffer containing 0.3 M Tris-HCl (pH 8.5), 75 mM ammonium sulfate and 7.5 mM MgCl2. The cycling conditions for the PCR started with 5 minutes at 92°C, followed by 35 cycles of 1 minute at 92°C, 1 minute at the optimized annealing temperature (57.5°C), and 1 minute 72°C, then followed by an extra 5 minutes at 72°C. PCR products were analyzed on a 2% agarose gel. The amplification yielded distinct bands at 484 base pairs (bp) (short ‘S’ allele) and 528 bp (long ‘L’ allele). To investigate the random genotyping error rate, the laboratory included five duplicate DNA samples per 96-well plate, which were 100% consistent. In addition, four blanks were included in each plate, which were required to be negative. Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental genotype information using the Markov chain Monte Carlo approximation of the exact test implemented in the GENEPOP package version 3.3 (Laboratoire de Genetique et Environnement, Montpellier, France) (Raymond & Rousset 1995). No deviations from HWE were detected (P = 0.96). To maximize the power of the analyses, 5-HTTLPR genotypes were classified into two groups according to the absence (0) or presence (1) of the short allele (LL = 100 and SL/SS = 206, respectively).

Covariates

Personality

We used the Quick Big Five, a well-validated instrument (Vermulst & Gerris 2005; Otten, Engels, & Van den Eijnden, 2008), to assess the Five-Factor Model of Personality. In a list consisting of 30 traits, respondents were asked to rate on a 7-point scale to what degree they possessed the concerned trait. Openness was measured with items such as creative, artistic and versatile (α = 0.70, M = 4.86, SD = 0.83); conscientiousness was measured with items such as organized, orderly and efficient (α = 0.85, M = 4.19, SD = 1.14); extraversion was...
measured with items such as quiet, withdrawn and shy ($\alpha = 0.84$, $M = 4.75$, $SD = 1.11$); agreeableness with items such as kind, likeable and cooperative ($\alpha = 0.77$, $M = 5.45$, $SD = 0.63$); and emotional stability was assessed with items such as nervous, fearful and sensitive ($\alpha = 0.73$, $M = 4.30$, $SD = 0.93$).

**Alcohol use**

Four questions asked how many glasses of alcohol the respondents had been drinking in the past weeks during weekdays and in weekends, inside and outside the home (Engels, Knibbe & Drop 1999). The sum of these four scores was used to indicate the total amount of alcoholic beverages consumed in the last week, ranging from 0 to 51 ($M = 4.15$, $SD = 6.45$).

**Tobacco use**

Participants were asked to indicate their smoking status on a 9-point ordinal scale (e.g. Kremers, Mudde & de Vries 2001; Otten, Engels, & Van den Eijnden, 2007). We created a composite score ranging from 0 to 2, which represented the frequency of tobacco use for each data collection wave (0 = Never used; 1 = Used, but not during the last 4 weeks; and 2 = One time or more during the last 4 weeks). At the first time of measurement, 162 respondents (53.1%) reported never to have used, 113 respondents (37%) reported used but not during the last month, and 30 respondents (9.8%) reported smoking during the last month.

**Highest attained parental education level**

As a proxy for socioeconomic status, we assessed both maternal and paternal education level (Ensminger & Fothergill, 2003). Parents were asked to indicate their highest attained education level on an 8-point ordinal scale. Most parents [both mothers (34%) and fathers (33%)] reported higher professional education as their highest attained level of education.

**General parenting practices**

Three dimensions were included to represent different aspects of parenting at baseline. The *support* dimension refers to parents giving their adolescents a sense of security and safety (Scholte, Van Lieshout & Van Aken 2001) and consisted of 12 items (e.g. ‘My mother supports me in the things I do’). The response scales ranged from 1 ‘completely not true’ to 5 ‘completely true’ (mother: $\alpha = 0.80$, $M = 4.12$, $SD = 0.43$; father: $\alpha = 0.86$, $M = 3.91$, $SD = 0.54$). *Strict control* refers to the extent to which adolescents perceive their parents to be exerting control on their whereabouts and activities (Engels et al. 2005) and was assessed with four items (e.g. ‘Before you leave on a Saturday evening, does your mother want to know with whom and/or where you are’ (mother: $\alpha = 0.75$, $M = 4.00$, $SD = 0.75$; father: $\alpha = 0.87$, $M = 3.45$, $SD = 0.98$)]. The response scales ranged from 1 ‘never’ to 5 ‘always’. *Psychological control* is the extent to which adolescents perceive their parents to be using psychologically manipulative strategies in order to control the adolescent’s behavior and was assessed by eight items [e.g. ‘When I get a poor grade in school, my mother makes me feel guilty’ (Steinberg, Fletcher & Darling 1994, for the Dutch version: Beyers & Goossens 1999)]. The response scales ranged from 1 ‘completely not true’ to 5 ‘completely true’ (mother: $\alpha = 0.67$, $M = 2.21$, $SD = 0.54$; father: $\alpha = 0.74$, $M = 2.17$, $SD = 0.57$).

**Statistical analysis**

We conducted a parallel-process latent growth model (e.g. Preacher et al. 2008; Barker et al. 2011). First, development was modeled by estimating separate latent growth curves for cannabis use and depressive symptoms. Latent growth curve modeling permits one 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, 1998–2010). Specifically, intercepts represented initial levels of cannabis use and depressive symptoms, and the slopes represented the rates of change in cannabis use and depressive symptoms. Second, we tested whether growth curves of cannabis use predicted growth curves of depressive symptoms and vice versa by regressing the intercept and the slope of depressive symptoms on the intercept of cannabis use and by regressing the intercept and the slope of depressive symptoms on the intercept of cannabis use simultaneously in a parallel-process latent growth model. In the final step, we examined whether the effects were different for adolescents with and without the short allele of the 5-HTTLPR genotype while controlling for the aforementioned set of covariates. The Satorra–Bentler scaled $\chi^2$ difference test was used to test model comparisons (Satorra & Bentler 2001). To determine model fit, we used the comparative fit index (CFI, critical value $\geq 0.90$) (Bentler & Bonett 1980), the Tucker–Lewis index (TLI, critical value $\geq 0.90$) (Bentler 1990) and the root mean squared estimate of approximation (RMSEA, critical value $\leq 0.08$) (Browne & Cudeck 1993).

We used the data of the youngest siblings in an attempt to replicate potential findings ($n = 306$). At year 1, the youngest siblings were between 13 and 15 years ($M = 13.36$, $SD = 0.50$). The distribution of males and females was equal. The short allele of the 5-HTTLPR genotype was present in 66% of the subsample. Lifetime cannabis use increased from 5% at the baseline to 29% at
the fifth time of measurement. Monthly use increased from 3% at the baseline to 12% at the fifth time of measurement. Mean scores on the depressive mood list were 2.45 (0.67), 2.54 (0.71), 2.53 (0.70), 2.31 (0.72) and 2.30 (0.71), respectively.

RESULTS

Descriptive analyses

Table 1 shows the bivariate correlations between the study variables. What stand out are the significant positive relationships between cannabis use and openness, extraversion, alcohol use and tobacco use, and the significant negative relationships between cannabis use and conscientiousness. There were no significant associations between cannabis use and the 5-HTTLPR genotype over the waves. Regarding depressive symptoms, we found consistently significant positive associations with sex (i.e. girls reporting more symptoms than boys) and negative associations with extraversion, emotional stability and support by mother and father. There were no significant associations between depressive symptoms and the 5-HTTLPR genotype over the waves.

Parallel-process latent growth curves

A parallel-process latent growth model was conducted using MPLUS 6.0 (Muthén & Muthén, 1998–2010). First, latent growth curves were estimated for cannabis use and depressive symptoms. Model fit was good (CFI = 0.95; TLI = 0.94; RMSEA = 0.07). Table 2 gives the mean initial values (i.e. intercept) and mean linear rates of changes (i.e. slope) as well as the variability in initial levels and linear rates of change. For both depressive symptoms and cannabis use, the relative fit indices were satisfactory. We also tested whether a quadratic trend would provide a better fit to the data. For each variable, the performed Satorra–Bentler χ² difference tests (Satorra & Bentler 2001) indicated that a quadratic trend did not significantly improve the model fit. More importantly, the analyses revealed significant inter-individual variability with respect to both levels and linear rates of change in both variables.

In the second step, the actual parallel-process latent growth curve model was tested by combining the slope of depressive symptoms with the intercept of cannabis use and the slope of cannabis use with the intercept of depressive symptoms in a simultaneous manner while controlling for the baseline measure of depressive symptoms and cannabis use, respectively, without taking into account the effects of genetic variation (not in the table). In addition to an autoregressive pathway from the intercept of depressive symptoms on the slope of depressive symptoms \((B = -0.46, P = 0.00)\), the crude estimates (without other covariates) showed a significant effect from the intercept of cannabis use on the slope of depressive symptoms \((B = 0.19, P = 0.05)\).

In the third step, we conducted a multi-group analysis to test whether the effects that were found in the total sample were different for adolescents with and without the short allele of the 5-HTTLPR genotype. In order to compare the models for both groups, Fig. 1 depicts the unstandardized crude estimates of dual growth model, separately for adolescents with and without the short allele. The most important finding is reflected in the link between the intercept of cannabis use and the slope of depressive symptoms. Specifically, the intercept of cannabis use was positively associated with an increase in depressive symptoms, but only in the risk group \([B = 0.34 (b = 0.10), P < 0.001]\) and not in the non-risk group \([B = -1.379 (b = -0.14), P = 0.51]\). A χ² difference test established support for this difference \((P < 0.001)\) (Satorra & Bentler 2001). Because the sample size was limited, we conducted the analysis once more using 5000 bootstrap resamples and a bias corrected and accelerated 95% CI. On the basis of the criterion that the null hypothesis of no effect could be rejected if the CI does not contain 0, results showed a significant difference between the group with and without the 5-HTTLPR genotype \((0.10; CI: 0.001, 0.211)\). The intercept of depressive symptoms was marginally positively associated with the slope of cannabis use in the risk group \((B = 0.197, P = 0.06)\) and negatively with the slope of cannabis use in the non-risk group \((B = -0.70, P = 0.06)\). However, with a bootstrap procedure these effects were not significant.

Table 3 shows the results of the parallel-process latent growth curve model controlling for covariates. Specifically, the table shows the results for each growth parameter (intercept and slope) in separate columns. The effect of cannabis use on the slope of depressive symptoms in the group with the short allele of the 5-HTTLPR genotype remains significant. Here, the effect of the intercept of depressive symptoms on the slope of cannabis use was also significant in the group with the short allele of the 5-HTTLPR genotype.

As the aim of the present analysis was to concentrate on a parallel developmental process, we used the intercepts of depressive symptoms and cannabis use to predict the slopes.

One could argue that the prevalence of cannabis use at the first time of measurement was too low to serve as a predictor. However, we were able to replicate the analyses in which we predicted growth parameters of depressive symptoms by the mean of cannabis use over time.

Replication

We ran the exact same model for the youngest siblings. Model fit was good, and similar to the findings for the
Table 1 Bivariate correlations between the study variables.

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<th>Cannabis 3</th>
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Note: *P < 0.05, **P < 0.01; two-tailed tests. 5-HTTLPR genotype = Absence (0) or presence (1) of the short allele of the 5-HTTLPR genotype risk allele.
older siblings, we found a significant effect of the intercept of cannabis use on the slope of depressive symptoms ($B = 0.10, P = 0.04, b = 0.24, P = 0.04$), but only in the presence of the short allele of the 5-HTTLPR genotype, which was supported by a $\chi^2$ difference test ($P < 0.05$).

**DISCUSSION**

The aim of the present study was to use a sophisticated and advanced statistical approach to test whether the prospective association between cannabis use and depressive symptoms could be conditional on the presence of the short allele of the 5-HTTLPR genotype. We expected that individuals with dysfunctioning serotonin reuptake inhibitors would be more vulnerable for the negative emotional consequences of cannabis use and thus more likely to show increasing levels of depressive symptoms over time. The findings indeed show that cannabis use increases the risk for depressive symptoms and the risk of a stronger increase in depressive symptoms, but only in the presence of the short allele of the 5-HTTLPR genotype. As cannabis use does not predict depressive symptoms in all individuals, our results may support a ‘double hit’ hypothesis (Murphy et al. 2008), in which cannabis use functions as a condition that is needed for the susceptibility factor (i.e. the presence of the short allele of the 5-HTTLPR genotype) to become a risk factor. One could argue that the link between cannabis and depressive symptoms is spurious. However, we controlled for a large number of covariates and the effect remained significant. We did not find conclusive evidence for the idea that some individuals use cannabis as a form of self-medication.

With these results, the present study extends the literature in three ways. First, this is the first study that concentrates on the link between cannabis use and depressive symptoms in a population of young adolescents. Most studies that focused on the impact of cannabis use concentrated on more severe forms of psychopathology, such as psychosis and diagnosed depression, mostly in individuals that show cannabis

<table>
<thead>
<tr>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>Slope</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>2.497</td>
</tr>
<tr>
<td>SE</td>
<td>0.035</td>
</tr>
<tr>
<td>(71.987)$^{***}$</td>
<td>(−7.614)$^{***}$</td>
</tr>
<tr>
<td>Cannabis use</td>
<td>0.164</td>
</tr>
<tr>
<td>SE</td>
<td>0.029</td>
</tr>
<tr>
<td>(5.766)$^{***}$</td>
<td>(9.886)$^{***}$</td>
</tr>
</tbody>
</table>

Note: $T$-values are presented in parentheses below their respective associated growth curve parameter. $^{***}P < 0.001$; two-tailed tests. SE = standard error.

**Figure 1** Parallel-process growth models. Parallel growth curves of cannabis use and depressive symptoms separated for adolescents with and without the 5-hydroxytryptamine risk allele (unstandardized estimates); $^{***}P<0.001$; two-tailed tests; † = marginally significant.
dependence or cannabis abuse (Moore et al., 2007). Our findings might indicate that cannabis use, even at relatively low levels, may increase depressive symptoms over time but only in a group with a specific genotype. It is possible that this specific effect reflects the very initial phase of a subtle process that ultimately leads to a bidirectional interplay between cannabis use and depressive symptoms. Specifically, it may be that first symptoms of cannabis dependence develop with an accelerated pace because of the presence of this risk allele. Particularly in individuals with the short allele of the 5-HTTLPR genotype, even low levels of cannabis use may produce feelings of pleasure immediately after using, but also instigate and increase day-to-day unpleasant feelings (reflected in depressive symptoms) as a consequence of withdrawal. In turn, to avoid unpleasant consequences of perceived withdrawal, individuals in the risk group may become more motivated to use cannabis on a more regular level of use, causing more severe levels of depressive symptoms over time (Robinson & Berridge, 2000). Hence, it could still be the case that the link between cannabis use and depressive symptoms is bidirectional.

Second, this is the first study that takes into account genetic variability in establishing the link between cannabis use and depressive symptoms. While previous studies concentrated on the direct link between cannabis and depression (e.g. Degenhardt et al. 2003), this study shows evidence for the idea that the associations may be different depending on individual differences. In our study, we did not find direct effects of the 5-HTTLPR genotype, which may be due to the fact that we did not concentrate on major depression (Van Roekel et al. 2011). Taking a molecular perspective may be an important step necessary to identify other links between substance use and less severe psychopathology (i.e. depressive symptoms rather than depression). Also with respect to other links between substance use and behavior, taking into account an individual’s genetic vulnerability may be necessary to fully understand the underlying mechanisms. For instance, the established link between high levels of alcohol use and violence may be conditional on the presence of certain genetic characteristics, such as the gene that encodes for the neurotransmitter metabolizing enzyme monoamine oxidase (Caspi et al. 2002).

Finally, whereas most studies concentrate on the link between cannabis use and depression or depressive symptoms while looking at two points in time (e.g. Van der Zwaluw & Engels 2009), the present study concentrated on changes in cannabis use and changes in depressive symptoms over time in a simultaneous manner. By using latent growth curves, more subtle effects of predictor variables could be identified, which may be paramount in understanding the underlying mechanisms of development of risk behavior. Specifically, more than traditional statistical techniques, latent growth curves make more efficient use of available data, providing explicit information about the rate of change over time, which may contribute to an increased insight into the development of

### Table 3 Results from the separate latent growth curve parameters for the non-risk and risk individuals.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intercept depressive symptoms</th>
<th>Slope depressive symptoms</th>
<th>Intercept cannabis use</th>
<th>Slope cannabis use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>R</td>
<td>NR</td>
<td>R</td>
</tr>
<tr>
<td>Intercept cannabis</td>
<td>–</td>
<td>–</td>
<td>–0.31</td>
<td>0.17**</td>
</tr>
<tr>
<td>Intercept depressive symptoms</td>
<td>–</td>
<td>–</td>
<td>0.44</td>
<td>–0.07</td>
</tr>
<tr>
<td>Age</td>
<td>0.07</td>
<td>0.03</td>
<td>–0.02</td>
<td>–0.03</td>
</tr>
<tr>
<td>Sex</td>
<td>0.25*</td>
<td>0.32***</td>
<td>–0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Openness</td>
<td>–0.04</td>
<td>0.09</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>–0.01</td>
<td>–0.04</td>
<td>–0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Extraversion</td>
<td>–0.02</td>
<td>–0.07</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Agreableness</td>
<td>–0.05</td>
<td>–0.09</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Emotional stability</td>
<td>–0.21***</td>
<td>0.21***</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.12</td>
<td>0.13*</td>
<td>–0.07</td>
<td>–0.05</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>–0.00</td>
<td>–0.00</td>
<td>0.01</td>
<td>–0.00</td>
</tr>
<tr>
<td>Education mother</td>
<td>0.04</td>
<td>–0.02</td>
<td>–0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Education father</td>
<td>–0.00</td>
<td>–0.01</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Support mother</td>
<td>–0.03</td>
<td>0.19</td>
<td>–0.01</td>
<td>–0.06</td>
</tr>
<tr>
<td>Support father</td>
<td>–0.29*</td>
<td>–0.19</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Behavioral control mother</td>
<td>–0.18*</td>
<td>0.16*</td>
<td>0.01</td>
<td>–0.01</td>
</tr>
<tr>
<td>Behavioral control father</td>
<td>0.16**</td>
<td>–0.11*</td>
<td>–0.07</td>
<td>–0.00</td>
</tr>
<tr>
<td>Psychological control mother</td>
<td>–0.07</td>
<td>0.08</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Psychological control father</td>
<td>–0.10</td>
<td>0.04</td>
<td>0.03</td>
<td>–0.04</td>
</tr>
</tbody>
</table>

Note: *P < 0.05, **P < 0.01, ***P < 0.001; two-tailed tests. I = intercept; S = slope; NR = non-risk; R = risk.

substance dependence. These effects could easily be overlooked in longitudinal studies with only two points in time.

In this study, we concentrated on depressive symptoms in an interplay with cannabis use. It could very well be that important environmental factors play a role in this interplay. For instance, specifically during adolescence, peers may be important in both development of depressive symptoms and cannabis use. Within subgroups of peers, mechanisms of peer contagion could lead to an increase in risk behavior, among which is cannabis use. However, recent studies have also shown support for peer contagion in depressive symptoms (Giletta et al. 2011). In those particular subgroups where adolescents look similar in both cannabis use and depressive symptoms, the effects for the long run could even be more detrimental.

Despite its strengths, the present study has some limitations. First, we acknowledge that the sample size in this study was relatively small to test gene–environment interactions. Even after controlling for covariates and conducting bootstrap analyses, the effect of cannabis use on the slope of depressive symptoms in the group with the 5-HTT risk allele remained significant. In addition, we were able to replicate the findings in the younger sample. Nevertheless, we stress the necessity for replication in larger samples to ensure the stability of such interaction, particularly as recent studies have raised concerns about the replicability of gene–environment interactions involving the serotonin gene (e.g. Fergusson et al. 2011).

Second, the assessment of the key constructs was suboptimal. Cannabis use was measured with two items, indicating the level of use over the last 4 weeks. Because of the low prevalence of frequent cannabis use, we decided to collapse some categories to establish a stronger but less precise measure for monthly use. Otherwise, depressive symptoms were assessed for the last 12 months, illustrating the overall level of depressive symptoms, which could have lead to recall bias. In addition, our study period covered an important but still limited temporal window. By using an extended time frame, we would actually be able to identify not only—as our study shows—how the process starts, but also where it ultimately leads to. Moreover, and related to the assessment issue, while we only concentrated on depressive symptoms and cannabis use in general, it may be that certain links are different for cannabis use disorders and more clinical levels of depression.

Third, all included variables on cannabis use were assessed with self-reported frequency, which may be prone to error. Therefore, a combination of both self-report and more objective measures (Buchan et al. 2002) would have provided more valid measures. Finally, with respect to our sample, participants were all from families with biological parents and two children, which might limit the generalizability of the findings.

In spite of these limitations and the difficulties that often accompany studies focusing on gene–environment studies (Van der Zwaluw & Engels 2009), our findings provide important new insights regarding the link between cannabis use and depressive symptoms. Specifically, for the first time, it is shown that the developmental links between cannabis use and depressive symptoms may be conditional on genetic susceptibility. More insights into the role of genetic variability could potentially help in understanding why some people are more susceptible to the negative consequences of substance use than others.

Authors Contribution

RO designed this present study, conducted the analyses, and wrote the first draft of the manuscript. RE designed the larger study from which the data were drawn and contributed to the writing.

References


