Dopamine receptor (D4) polymorphism is related to comorbidity between marijuana abuse and depression

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HIGHLIGHTS
• Link between DRD4 polymorphism and comorbid marijuana use and depression is examined.
• Those with DRD4 risk allele (≥7R) are at 2.5 times greater risk of comorbidity.
• This study is the first to link DRD4 to comorbid high marijuana use and depression.
• DRD4 may be related to conditions marked by externalizing/internalizing comorbidity.

ABSTRACT
The rates of marijuana abuse are steadily increasing in the U.S. Data suggest that comorbid marijuana abuse and depression is associated with worse outcomes than either diagnosis. Genetic studies independently link the DRD4 gene polymorphism to substance use and to internalizing disorders, but no study has examined whether the DRD4 polymorphism is linked to comorbid marijuana use and depression in a population sample. This study examined associations between the DRD4 gene 48 bp VNTR polymorphism and comorbidity between marijuana use frequency and depression in a diverse, non-clinical adolescent sample (n = 1882; ages 14 to 18) from the National Longitudinal Study of Adolescent Health (Add Health). Multinomial regression analyses indicated that the odds of being comorbid for depressive symptoms and marijuana use are approximately 2.5 times higher for the ≥7R genotype than youths who carry the b7R genotype, controlling for the effects of ethnicity, gender, age, violent victimization, and alcohol related problems. Findings provide genetic clues for psychopathology characterized by prominent externalizing/internalizing comorbidity.

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1. Introduction
Comorbidity between psychological disorders is often the rule rather than the exception (Kessler et al., 2011). Comorbidity is particularly prevalent among “internalizing” psychopathology, such as depression and anxiety disorders; and among disorders in the “externalizing” spectrum, such as antisocial behavior and substance use disorders (Krueger, Caspi, Moffitt, & Silva, 1998, p. 225). Indeed, the degree of association between disorders within each spectrum is so robust that some researchers suggest that the structure of comorbidity among psychological disorders is best accounted by two latent externalizing and internalizing “core psychopathological processes” (Krueger, 1999, p. 921; Kessler et al., 2011).

While there may be separate internalizing and externalizing diatheses, there is a high percentage of comorbidity across externalizing and internalizing psychopathology that remains to be fully explained. Large epidemiological and cross-cultural studies show that substance use disorders (externalizing) and mood disorders (internalizing) co-occur between 30% and 50% of the time (Merikangas et al., 1998). Some theories attempting to explain these high rates of comorbidity have focused on the possibility that some persons with internalizing disorders may use substances to cope with negative emotions (e.g., Buckner et al., 2008; Cooper, Fronc, Russel, & Mudar, 1995), while others suggest that heavy substance use may lead to internalizing disorders such as depression (Steuber & Banner, 2006; van Laar, van Dorsselaer, Monshouwer, & de Graaf, 2007). On the other hand, studies with genetically informative samples indicate that disorders across internalizing and externalizing spectra also share genetic liability factors that may help explain a large part of their comorbidity (e.g., Fu et al., 2002; O’Connor, McGuire, Reiss, Hetherington, & Plomin, 1998). Accordingly, molecular genetics studies have started to determine how genetic polymorphisms that mediate neurotransmitter expression
are linked to internalizing and externalizing disorders as well as their comorbidity (e.g., Gelernter et al., 1997; Nederhof et al., 2010; Noble, 2000).

To date, only a small number of molecular genetics studies have examined the comorbidity between disorders across externalizing and internalizing spectra, and those are only focused on alcohol use disorders in clinical samples (Cerdá, Sagdeo, Johnson, & Galea, 2010). For example, a study of 114 Brazilian patients of European descent with alcohol dependence and 218 controls, found that patients with comorbid major depressive disorder, alcohol abuse, and nicotine dependence, had a higher frequency of the short (S) allele of the serotonin transporter polymorphism (5-HTTLPR) than patients with only a diagnosis of substance abuse, or the control participants (Marques, Hutz, & Bau, 2006). Similarly, a study with a sample of ethnic Han Chinese patients found that the TaqIA polymorphism in the ANKK1 gene, which is proximal to the dopamine D2 receptor gene and has been linked to reduced D2 receptor density (Neville, Johnstone, & Walton, 2004); was associated with comorbid diagnoses of alcohol and anxiety/depression, but not with alcoholism-only, or depression/anxiety-only diagnoses. The effects remained even after controlling for the effects of gender, age, and genetic polymorphisms implicated in the metabolism of alcohol (ADH1B and ALDH2, Huang et al., 2004). A later study by the same research group found higher novelty-seeking scores among patients with the A1 allele of the TaqIA polymorphism in the ANKK1 gene, and higher comorbid alcohol abuse and anxiety/depression than those patients with alcohol-only diagnosis (Lin et al., 2007).

The aforementioned studies suggest that serotonergic and dopaminergic polymorphisms could play a role in explaining the comorbidity between substance use disorders and internalizing syndromes in particular since other substances (e.g., marijuana), appear to activate the same neurobiological reward pathways as alcohol (Filbey, Schacht, Myers, Chavez, & Hutchison, 2009). However, little is known about the relationship between various genetic polymorphisms and comorbid substance abuse (other than alcohol) and internalizing conditions. This gap in the knowledge base is important to address because in the United States, while alcohol and tobacco use is declining, the use of other substances, in particular marijuana, is increasing among adolescents, (Johnston, O’Malley, Bachman, & Schulenberg, 2010). Comorbid marijuana and mood disorders in adolescent populations are important to understand because adolescents appear particularly vulnerable to the co-occurrence of these disorders. Furthermore, comorbid marijuana abuse and depression predict poorer treatment outcomes among adolescents (White et al., 2004), and adolescents are more susceptible to marijuana’s deleterious psychological effects including worsening of depression symptoms (Marmorstein et al., 2010), and increased risk for lifetime psychiatric disorders (Malone, Hill, & Rubino, 2010). Therefore, understanding whether genetic polymorphisms related to the comorbidity of alcohol and depression also are related to other substances, like marijuana, could illuminate future biological early treatments for both types of disorders.

In order to better understand comorbidity between substance abuse and internalizing syndromes like depression, studies examining dopaminergic polymorphisms may be particularly promising. Influential theoretical proposals like the “reward deficiency syndrome” (Blum, Cull, Braverman, Chen, & Comings, 1997) suggest that gene variants of dopamine D2-like receptors make some individuals less susceptible to experience rewards from everyday activities, leading to general dysphoria and higher sensation seeking, each associated with depression and substance misuse. Accordingly, studies have linked dopaminergic polymorphisms to both externalizing and internalizing syndromes (see Alcaro & Panksepp, 2011 for a neuro-affective review and synthesis; Guo, Roettger, & Shih, 2007). One dopaminergic polymorphism in particular, a DRD4 polymorphism, may play individuals at-risk for both substance use disorders and mood disorders.

The DRD4 gene has a 48 bp VNTR polymorphism located in the third exon, and the 48 bp polymorphism can be repeated 2 to 11 times. Research suggests that at the molecular level, the ≥7R allele may correspond to less efficient/unstable transcription and translation (Schoots & Van Tol, 2003), less efficient folding and greater conformational rearrangement of receptor proteins (Van Craenenbroeck et al., 2005), and weaker intracellular dopamine signaling (Asghari et al., 1995). Data show that D4 receptors are densely distributed in areas associated with emotion, reward response and motivation, including the hippocampus and the amygdala (Primus et al., 1997). Based on animal models, some researchers have proposed that poor dopamine functioning in these limbic areas partly underlie depressive symptoms characterized by lack of motivation (e.g., anhedonia and avolition; Nestler & Carlezon, 2006). In humans, amygdaloid D4 function has been linked to depression (Xiang et al., 2008), and activity in the amygdala has also been linked to marijuana craving (Filbey et al., 2008). In addition, individuals with the ≥7R allele report stronger alcohol cravings (Hutchinson, McGarry, Smolen, Bryan, & Swift, 2002), urges to use illicit substances, and greater activity in the striatum in response to substance-related cues (McGeary, 2009). Thus, it is possible that the ≥7R allele of DRD4 may be a common molecular mechanism for motivational symptoms of depression and cravings in marijuana use, contributing to their comorbidity.

Studies have separately linked the ≥7R allele to problematic substance use and depression but data remain somewhat mixed. For example, a study with 184 patients with substance abuse and 122 controls found that individuals with more severe substance use problems had a higher incidence of the “long” version of the polymorphism (i.e., ≥7-11 repeats) relative to controls (Van den Bergh et al., 2000). In a similar way, two recent studies with large cohort studies found higher incidence of marijuana use among adult females (Vaske, 2011), and adolescents (Olsson et al., 2011) who were carriers of the DRD4 7–11 repeat allele. As for depression, a meta-analyses by López León et al. (2005) examining the relationship between the DRD4 polymorphism and mood disorders found an association between the 7–11 repeats of the polymorphism and unipolar depression but not with bipolar disorder. Other studies identify different variants of the DRD4 polymorphism as the genetic “risk allele” for depressive symptoms (Adkins, Daw, McClay, & van den Oord, 2012; Guo & Tillman, 2009). On the other hand, not all studies have found a relationship between DRD4 and heavy substance use (e.g., Creemers et al., 2011; McGarry, Esposito-Smythers, Spirito, & Monti, 2007; van der Zwaluw, Larsen, & Engels, 2012). However, McGarry et al., used a small sample (N = 77), while the study by Creemers et al. was somewhat restricted in range through attrition, and their sample had lower rates of marijuana use than those excluded from the report. For its part, the study by van der Zwaluw et al. only examined alcohol abuse. Importantly, none of these groups examined whether DRD4 was related to comorbidity across internalizing disorders and substance use. Therefore, given the consistent relationship of DRD4 to depression (López León et al., 2005), it may be that mixed findings regarding DRD4 and substance use (e.g., Creemers et al., 2011; Olsson et al., 2011) are accounted for by DRD4’s relationship to the comorbidity between unipolar depression and substance misuse. However, to date no study has examined this possibility.

The current study aimed at helping fill this gap by determining whether the comorbidity of depressive symptoms and marijuana use is linked to a 48 bp VNTR polymorphism in the DRD4 dopamine receptor gene among a large, representative sample (N = 1882) of adolescents. This approach extends current knowledge by using a large, diverse sample free of confounds from long term misuse and also controlling for gender, ethnicity, age, victimization, and alcohol related problems, all variables previously linked to substance use disorders and depression (Kilpatrick et al., 2000; Silver, Arsenault, Langley, Caspi, & Moffitt, 2005). Based on previous findings linking the 7–11 DRD4 polymorphism to substance use and mood disorders.
sive scores in the top 25th percentile but marijuana use scores in the bottom 75% (marijuana abuse group); and (4) youths with both depressive scores and marijuana use scores in the top 25th percentile of the distribution (comorbid group).

In wave I, approximately 65% of youths were in the normative group, while 21.5% were in the depressive group, and 8.5% of youths were in the marijuana abuse group. Approximately 5% of youths were comorbid for high depressive symptoms and marijuana use frequency in wave I. A very similar pattern of results were found in wave II, with 63.4% in the normative group, 20.7% in the depressive group, 10% in the marijuana abuse, and 5.9% of youths in the comorbid group.

2.2.2. Independent variables

The primary independent variable of the analyses was the DRD4 polymorphism but as previously mentioned, gender, ethnicity, age, violent victimization, and alcohol related problems were included as additional control variables due to their link to substance use disorders and depression. The polymorphism was genotyped using PCR methods and a modified assay that have been described elsewhere (Add Health Biomarker Team, 2003). In consideration of research that has found higher mood disorders and substance use is among individuals who have 7–11 repeats on the polymorphism, the current study separated the repeats into the following genotypes: \( 0 \geq 2 \), \( 2 \geq 4 \), \( 4 \geq 7 \), and \( 7 \geq 7 \). The genotypes are in Hardy–Weinberg equilibrium for the full sample (\( \chi^2 = 1.11, p > .05 \)), non-White Hispanics (\( \chi^2 = .16, p > .05 \)), White non-Hispanics (\( \chi^2 = 52, p > .05 \)), and African American non-Hispanics (\( \chi^2 = .83, p > .05 \)) (Rodriguez, Gaunt, & Day, 2009). For analysis purposes, two dummy variables were created from the genotype categories, where the \( 7 \geq 7 \) genotype was the reference category. Therefore, the first variable (one \( 7 \geq 7 \)) compared individuals who have zero \( 7 \) + repeats and who have two \( 7 \) + repeats to individuals with one \( 7 + \) allele (\( 0 \), \( 0 \geq 7 \)). The second variable (two \( 7 \geq 7 \)) compares individuals who are homozygous for the \( 7 \) + allele to individuals with zero and with one \( 7 \) + allele (\( 7 \geq 7 \)).

The control variables were gender, ethnicity, age, violent victimization, and alcohol related problems. Gender was coded as 0 = female (52.3%) and 1 = male. Ethnicity was measured with two dichotomous variables: African American (0 = non-Hispanic Caucasians and Hispanics, 1 = non-Hispanic African American) (17.5%), and Hispanic (0 = non-Hispanic Caucasians and non-Hispanic African Americans, 1 = Hispanics) (14.9%). Ethnicity was included as

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1 The vast majority of authors have categorized the repeats as 7 or more versus less than 7 repeats (as is done in this analyses), while others have included 2 and 4 repeats in the coding method (Guo & Tillman, 2009), and still others have coded it based on whether carriers have at least one 2–11 repeat (Adkins et al., 2012). We have followed the lead of previous research in using the 7R as the cut point, but acknowledge that this is only one way of coding the 48 bp VNTR. Based on the original genotyping data, we identified all genotypes within our data (from 2/2 genotype to 7/10 genotype), and then identified those that had at least 5 cases in each of the four depressive symptoms/marijuana use groups (so that there were no empty cells in the table). The 2/4, 4/4, 4/7, and 7/7 genotypes had at least 5 cases in all of the four groups for waves I and II. A contingency table of these genotypes within the 14–18 age range at wave I (n = 1563) showed that those homozygous for the 7R allele were more likely to be comorbid in wave I (73.3%) and wave II (9.8%) than those in the 2/4 (wave I = 4.5%, wave II = 4.9%), 4/4 (wave I = 4.7%, wave II = 5.2%), and 4/7 (wave I = 5.5%, wave II = 6.8%) genotypes. Thus, the odds of comorbidity during waves I and II are approximately 1.4 to 2.0 times higher for those who are homozygous for the 7R allele compared to the 2/4, 4/4, and 4/7 genotypes.

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(e.g. López León et al., 2005; Vandenbergh et al., 2000) it was hypothesized that individuals with the 7–11 polymorphism would show the highest levels of comorbidity between marijuana heavy use and depression.
control variables in order to address population stratification issues.\(^2\)

\(\text{Age was measured in wave I, with a mean of 15.866 and a standard deviation of 1.300. Violent victimization} \text{ in wave I was assessed with four questions, where respondents were asked whether they had been shot, stabbed, had a gun pulled on them, or were jumped within the past year (0 = never, 1 = at least once). Approximately 19\% of respondents stated that they had been victims of one of these violent acts at least once within the past year, with only 6\% reporting more than one type of violent victimization (\text{alpha} = .56). Alcohol related problems were assessed with nine questions during wave I that asked respondents how often within the past 12 months they had problems resulting from their alcohol consumption (0 = never, 1 = once, 2 = twice, 3 = three to four times, 4 = five or more times). The problems included trouble with parents, problems at school or with school work, problems with friends, problems with someone they were dating, engaging in behavior they later regretted, were hung over, were sick to their stomach, gotten in a sexual situation they regretted, or gotten into a physical fight (\text{alpha} = .82). The average score on the alcohol related problems scale was 1.60 (SD = 3.519), with a range of 0 to 32. This indicates that youths, on average, did not typically experience a large number of alcohol related problems, but that there is a wide amount of variation in their experiences.}\)

\[2\text{Geneticists often agree that controlling for race is one way to deal with issues of population stratification. Other strategies may include using ancestry information markers (AIMS) or examining between-family variance in the association between the polymorphism and the phenotype. AIMS are currently not available in the Add Health, but we did explore whether there was significant between-family variance in the associations between the polymorphism and the phenotype. There was significant variation in the slope for DRD4 and the dichotomous measure of marijuana use for both waves. However, this technique is predicated on the notion that significant variation in the slopes is due to differences in ethnic or genetic lineage, rather than other factors such as family functioning or neighborhood level variables (e.g., social disorganization); thus, ethnicity should explain this variation. In a series of experiments, we controlled for ethnicity related variables—mother's self-reported race and youth's self-reported race—to examine whether the variation in the slopes became non-significant once ethnicity was controlled for in mixed regression models estimated in MPLUS 6.11. The results (available upon request) showed that significant variation in the DRD4-marijuana use slopes remained after controlling for mother's race and youth's race. Furthermore, we re-estimated the mixed regression models with youth's self-reported ancestry at wave III as the between level identifier. This allowed us to examine whether the association between DRD4 and marijuana use varied across ancestries. The results showed that there was not significant variation in the effects of DRD4 on marijuana use across the various ancestries, indicating that population stratification was not operating. Together, these series of results suggest that there may be significant variation in the slopes of marijuana use regressed on DRD4 across families, but that the significant variation may exist for reasons beyond race, ethnicity, or ancestry. Finally, we estimated a multi-level multinomial logistic regression model in MPLUS to examine if the comorbidity variable was related to the DRD4 polymorphism within families—thus holding population stratification issues constant. We found that siblings with one copy of the 7R + allele (\(b = .436, p = .045\)) and siblings with two copies of the 7R + allele (\(b = .366, p = .031\)) were more likely to be in the comorbid group relative to the neither group in wave II, and 7R homozygote siblings were more likely to be in the comorbid class in wave I (\(b = .847, p = .029\)).}\]

\[3\text{Results}\]

\[3.1. Comorbidity analyses}\]

\[3.1.1. Wave I without control variables}\]

\[\text{Table 1 describes the number and percentage of respondents who were classified into the four different categories by DRD4 genotype. Review of the table suggests that youths who carried two copies of the 7R + allele were more likely to be in the comorbidity group for both waves I and II than youths who did not carry a 7R + allele. While informative, the table does not show whether the DRD4 genotypes differentiate between depressive symptoms and marijuana use groups. To address this question, we employ a series of multinomial regression models, where the reference category is alternated between the four groups.}\]

\[\text{The first step in these analyses is to examine whether the DRD4 polymorphism differentiates between the four groups for waves I and II. Multinomial regression models for wave I showed that DRD4 differentiated youths in the comorbid group from those in the normative group (Table 2). The DRD4 polymorphism also differentiated adolescents in the comorbid group from those in the depressive group. Specifically, the results indicated that youths with two copies of the 7R + allele (versus zero) were more likely to be in the comorbid group than in the normative group (\(b = .847, OR = 2.332, p = .029\)) or the depressive group (\(b = .874, OR = 2.396, p = .044\)). Furthermore, DRD4 differentiated adolescents in the marijuana abuse group from youths in the normative group (two \(7R + b = .658, OR = 1.938, p = .042\)). These analyses suggest that DRD4 is associated with comorbid high marijuana use frequency and depressive symptoms, as well as high frequency of marijuana use.}\]

\[3.1.2. Wave I with control variables}\]

\[\text{The next step in the analyses was to examine whether the DRD4 polymorphism was related to marijuana use and depressive symptoms after controlling for the effects of age, ethnicity, gender, violent victimization, and alcohol related problems. As shown in Table 3, the odds of being in the comorbid group (\(b = .978, OR = 2.569, p = .014\)) and in the marijuana abuse (\(b = .698, OR = 2.009, p = .042\)) were significantly higher for youths who carry two copies of the 7R + allele, even after controlling for the effects of demographics, violent victimization, and alcohol related problems.}\]

\[\text{Turning to the control variables, youths with higher levels of alcohol related problems or multiple forms of violent victimization had a higher likelihood of being in the depressive group, the marijuana abuse group, and the comorbid group. In terms of gender, the odds of being comorbid for high depression and marijuana use frequency or having high depressive symptoms were significantly higher for females than for males. Finally, African Americans (\(b = .383, OR = 1.466, p = .013\)) and Hispanics (\(b = .487, OR = 1.627, p = .002\)) were more likely to report high depressive symptoms only, compared to non-Hispanic Caucasians.}\]

\[4\text{We re-estimated the models with youths only ages 14–16 to examine if the findings were subject to nuisance issues. The results were nearly identical to the results garnered from the larger genetic subsample ages 14 to 18.}\]
The main goal of the current study was to examine whether the DRD4 gene polymorphism was associated with comorbidity between depression and marijuana use symptoms in a large, diverse, non-clinical adolescent sample. The study also examined how risk factors previously associated with substance use and depression (ethnicity, age, victimization, and alcohol related problems) were related to the co-occurrence of marijuana use and depression. Consistent with hypothesis, the ≥7R allele may increase the risk of co-occurring recent marijuana use frequency and depressive symptoms. As previously mentioned, a number of studies have linked the ≥7R allele to what may be considered less efficient functioning at the molecular level (e.g., Asghari et al., 1995; Schoots & Van Tol, 2003), and greater urges to use illicit substances and activity in the striatum in response to substance-related cues (McGeary, 2009). Together, these findings could indicate that the ≥7R allele may increase the risk of depression/negative affect and substance use by causing a depletion in D4 receptor activity and overall dopamine activity. Such depletion would be consistent with proposals that dopamine dysfunction is the main underlying factor of a “reward deficiency syndrome” (Blum et al., 1997) which makes some individuals susceptible to negative affect and higher sensation seeking, and thus the development of externalizing and internalizing symptoms (Alcaro & Panksepp, 2011; Zuckerman & Kuhlman, 2000). This hypothesis, while plausible, would need to be empirically evaluated by future research.

These findings complement studies that have examined DRD2 polymorphisms and comorbidity between alcohol abuse diagnoses and internalizing disorders (Huang et al., 2004; Lin et al., 2007). Moreover, given the common mechanisms of action between various substances of abuse, and that dopamine agonists may be a viable therapeutic alternative for depression and other substance use disorders relative to the depressive only group, but this effect did not reach statistical significance (b = .781, OR = 2.183, p = .080). These findings suggested that the polymorphism may differentiate comorbid youths from those with neither disorder, and between those with high comorbid marijuana use and depressive symptoms from those who experience depressive symptoms only. Finally, youths with one copy of the 7R+ allele had a higher risk of being in the marijuana abuse group compared to the normative group (b = .344, OR = .041).

### 3.1.3. Wave II without control variables

Table 1 describes the prevalence of comorbidity across the three DRD4 genotypes. As shown in the table, individuals with two copies of the 7R+ allele were more likely to be in the comorbidity group than with zero or one copy of the 7R+ allele. The table, however, does not allow one to investigate whether DRD4 differentiates between the depressive and marijuana abuse groups.

The wave II analyses for comorbidity parallel the results from the wave I analyses (Table 2). DRD4 differentiates the normative group from the comorbid group and the marijuana abuse group. Youths who carried one copy of the 7R+ allele (b = .435, OR = 1.544, p = .045) and who carried two copies (b = .866, OR = 2.377, p = .032) were more likely to be comorbid for high depressive symptoms and high frequency of marijuana use (relative to the neither disorder) than youths who did not carry a 7R allele. In addition, youths with one copy of the 7R+ allele were more likely to be comorbid relative to youths in the depressive only group (b = .462, OR = 1.587, p = .046). There was a trend for 7R+ homozygotes to be at an increased risk of comorbidity

![Table 1](image)

<table>
<thead>
<tr>
<th>Wave</th>
<th>Normative group</th>
<th>Depression only group</th>
<th>Marijuana use only group</th>
<th>Comorbid group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero 7R+ (n = 1170)</td>
<td>One 7R+ (n = 618)</td>
<td>Two 7R+ (n = 94)</td>
<td></td>
</tr>
<tr>
<td>Wave I</td>
<td>770 (65.8%)</td>
<td>259 (22.1%)</td>
<td>87 (7.4%)</td>
<td>54 (4.6%)</td>
</tr>
<tr>
<td></td>
<td>395 (63.9%)</td>
<td>127 (20.6%)</td>
<td>61 (9.9%)</td>
<td>35 (5.7%)</td>
</tr>
<tr>
<td></td>
<td>55 (58.5%)</td>
<td>18 (19.1%)</td>
<td>12 (12.8%)</td>
<td>9 (9.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wave II</th>
<th>Normative group</th>
<th>Depression only group</th>
<th>Marijuana use only group</th>
<th>Comorbid group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>759 (64.9%)</td>
<td>249 (21.3%)</td>
<td>105 (9.0%)</td>
<td>57 (4.9%)</td>
</tr>
<tr>
<td></td>
<td>379 (61.3%)</td>
<td>121 (19.6%)</td>
<td>74 (12.0%)</td>
<td>44 (7.1%)</td>
</tr>
<tr>
<td></td>
<td>56 (59.6%)</td>
<td>20 (21.3%)</td>
<td>8 (8.5%)</td>
<td>10 (10.6%)</td>
</tr>
</tbody>
</table>

Note: Normative group refers to youths with depressive and marijuana use scores in the bottom 75% of the distribution. The Depression only group refers to youths with depressive scores in the top 25% but marijuana use scores in the bottom 75% of the distribution. The Marijuana use only group refers to youths with marijuana use scores in the top 25% but depressive scores in the bottom 75% of the distribution. The Comorbid group refers to youths with both depressive scores and marijuana use scores in the top 25% of the distribution.

### 3.1.4. Wave II with control variables

The wave II analyses revealed that DRD4 continued to differentiate the normative group from the comorbid group and the marijuana abuse only groups, after controlling for the effects of demographics, violent victimization, and alcohol related problems (Table 3). The odds of comorbidity relative to neither disorder were approximately 2.3 times higher among youths who carried two copies of the 7R+ allele compared to youths who did not carry a 7R+ allele (b = .833, p = .020). The one 7R+ allele was also associated with increased risk of sole marijuana use after controlling for potential confounding variables (b = .393, OR = 1.481, p = .022).

The wave II results for the control variables were also parallel to those from the wave I comorbidity analyses. Again, the odds of sole high depressive symptoms, sole marijuana use, and comorbidity of depressive symptoms and marijuana use were significantly higher among youths reporting higher levels of alcohol related problems and experiencing multiple forms of violent victimization. In addition, females had a higher likelihood of being in the comorbidity group (b = −.528, OR = .949, p = .020) and the high depressive symptoms (b = −.411, OR = .662, p = .001) only group relative to males. Similar to the wave I results, the likelihood of exhibiting high depressive symptoms was greater among African Americans (b = .532, OR = 1.700, p = .001) and Hispanics (b = .539, OR = 1.714, p = .001) relative to non-Hispanic Caucasians.

### 4. Discussion

The main goal of the current study was to examine whether the DRD4 gene polymorphism was associated with comorbidity between depression and marijuana use symptoms in a large, diverse, non-clinical adolescent sample. The study also examined how risk factors previously associated with substance use and depression (ethnicity, age, victimization, and alcohol related problems) were related to the co-occurrence of marijuana use and depression. Consistent with hypothesis, the ≥7R allele may increase the risk of co-occurring recent marijuana use frequency and depressive symptoms. As previously mentioned, a number of studies have linked the ≥7R allele to what may be considered less efficient functioning at the molecular level (e.g., Asghari et al., 1995; Schoots & Van Tol, 2003), and greater urges to use illicit substances and activity in the striatum in response to substance-related cues (McGeary, 2009). Together, these findings could indicate that the ≥7R allele may increase the risk of depression/negative affect and substance use by causing a depletion in D4 receptor activity and overall dopamine activity. Such depletion would be consistent with proposals that dopamine dysfunction is the main underlying factor of a “reward deficiency syndrome” (Blum et al., 1997) which makes some individuals susceptible to negative affect and higher sensation seeking, and thus the development of externalizing and internalizing symptoms (Alcaro & Panksepp, 2011; Zuckerman & Kuhlman, 2000). This hypothesis, while plausible, would need to be empirically evaluated by future research.

These findings complement studies that have examined DRD2 polymorphisms and comorbidity between alcohol abuse diagnoses and internalizing disorders (Huang et al., 2004; Lin et al., 2007). Moreover, given the common mechanisms of action between various substances of abuse, and that dopamine agonists may be a viable therapeutic alternative for depression and other substance use disorders

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Table 2

<table>
<thead>
<tr>
<th>DRD4 polymorphism differentiation of comorbid group from normative, depressive only, and marijuana use only groups in wave I (n = 1882).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wave I</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>One 7R+ allele</td>
</tr>
<tr>
<td>Two 7R+ alleles</td>
</tr>
<tr>
<td><strong>Wave II</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>One 7R+ allele</td>
</tr>
<tr>
<td>Two 7R+ alleles</td>
</tr>
</tbody>
</table>

Note: Wave I coefficients/Wave II coefficients.

* p ≤ .10.
** p ≤ .05.
Neither category is the reference category.

Note.

Table 3

Association of DRD4 polymorphism and comorbidity of depressive symptoms and marijuana use in waves I and II controlling for demographics, violent victimization, and alcohol related problems (n = 1882).

<table>
<thead>
<tr>
<th>Wave I</th>
<th>Depression only (n = 404)</th>
<th>Marijuana use only (n = 160)</th>
<th>Comorbid (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>OR</td>
<td>SE</td>
</tr>
<tr>
<td>One 7R+ allele</td>
<td>-0.110</td>
<td>0.895</td>
<td>0.125</td>
</tr>
<tr>
<td>Two 7R+ alleles</td>
<td>-0.203</td>
<td>0.816</td>
<td>0.291</td>
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<tr>
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<td>-0.419***</td>
<td>0.657</td>
<td>0.122</td>
</tr>
<tr>
<td>African American</td>
<td>0.383*</td>
<td>1.466</td>
<td>0.154</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.487**</td>
<td>1.627</td>
<td>0.159</td>
</tr>
<tr>
<td>Age</td>
<td>0.004</td>
<td>1.004</td>
<td>0.046</td>
</tr>
<tr>
<td>Violent victimization</td>
<td>0.277***</td>
<td>1.319</td>
<td>0.105</td>
</tr>
<tr>
<td>Alcohol related problems</td>
<td>0.101***</td>
<td>1.106</td>
<td>0.024</td>
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</table>

<table>
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<th>Wave II</th>
<th>Depression only (n = 390)</th>
<th>Marijuana use only (n = 187)</th>
<th>Comorbid (n = 111)</th>
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<tbody>
<tr>
<td></td>
<td>b</td>
<td>OR</td>
<td>SE</td>
</tr>
<tr>
<td>One 7R+ allele</td>
<td>-0.093</td>
<td>0.911</td>
<td>0.128</td>
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<tr>
<td>Two 7R+ alleles</td>
<td>-0.030</td>
<td>0.970</td>
<td>0.270</td>
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<tr>
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<td>-0.411***</td>
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<td>0.123</td>
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<tr>
<td>African American</td>
<td>0.532***</td>
<td>1.702</td>
<td>0.153</td>
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<tr>
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<tr>
<td>Age</td>
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<tr>
<td>Alcohol related problems</td>
<td>0.067***</td>
<td>1.069</td>
<td>0.022</td>
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</tbody>
</table>

Note. Neither category is the reference category.

* p ≤ .05.

** p ≤ .01.

*** p ≤ .001.

(e.g., cocaine, Amato et al., 2011; Leventjes, 2011), these findings suggest that polymorphisms on the DRD2 and DRD4 genes that affect dopamine activity may affect the etiology of other substance use disorders, not only marijuana. In short, the current findings sharpen the focus on the dopamine system as a factor in the comorbidity of externalizing and internalizing psychopathology.

These findings could also have direct bearing on efforts aimed at establishing the temporal and dynamic nature of the comorbidity between substance use disorders and internalizing disorders. As previously mentioned, significant debate in the area revolves around whether substance use disorders increase the likelihood of developing internalizing disorders later in life, or rather, people develop substance use problems in an attempt to “cope” with pre-existing internalizing symptoms. A recent structural equation modeling analyses of a large longitudinal birth-cohort indicate that the comorbidity between internalizing disorders and substance use appear to be due to in large part to “common, fixed risk factors” that according to the authors could include genetic factors (Fergusson, Boden, & Horwood, 2011, p. 940).

Our results point to the ≥7R≥7R DRD4 as one such common risk factor that merits further exploration. Notably, the risk conveyed by the ≥7R≥7R DRD4 polymorphism is evident even after accounting for sociodemographic factors that have been linked to internalizing and externalizing psychopathology (Kilpatrick et al., 2000; Silver et al., 2005).

This study has some limitations that need mentioning. First, and consistent with other molecular genetic studies, although the overall study sample was large, a small number of individuals had two copies of the >7R allele (i.e., 5%), which could have exacerbated dimensionality problems in the analyses. Further, the current study is underpowered (approximately 5% power per Quanto and G*Power) to detect the associations given the small number of individuals with the homozygous 7R genotype and the low frequency of the comorbid phenotype (Faul, Erdfelder, Lang, & Buchner, 2007; Gauderman & Morrison, 2006). However, it is noteworthy that the associations were detected across two waves of data and the strength of the associations was similar in both waves, thus slightly lowering the probability of a Type I error. Nonetheless, replication of these findings is necessary preferably in collaborative studies that compile genotypes of interest across multiple heterogeneous samples. Second, although there were efforts to reduce self-report biases on the measures of substance use and depression, these are not completely avoidable and future replication efforts should use third party or objective measures of externalizing and internalizing symptoms. Third, the genetic subsample is not a probability sample. The average level of marijuana use is significantly higher in the full Add Health probability sample relative to the study sample. Although the average level of depressive symptoms and the correlation between marijuana use and depression does not significantly differ between the genetic subsample and the full wave I Add Health sample, nonetheless, some caution should be exercised in generalizing our findings to the larger population.

The study also had strengths, including a heterogeneous representative sample and a robust methodology and analysis. In addition, to our knowledge, this is the first study to implicate the DRD4 48 bp polymorphism as a risk factor for the development of comorbid marijuana and depression symptomatology. The results open research avenues that examine how DRD4 polymorphisms influence psychopathology characterized by “broader” co-occurring internalizing–externalizing psychopathology such as borderline personality disorder. Finally, future studies could examine if DRD4 polymorphisms interact with individual cognitive processes such as expectancies for substance (e.g., belief in anxiety reduction via substance intake), as some recent innovative studies have done with genotypes linked to the metabolism of alcohol (Hendershot et al., 2009). These efforts could prove important in developing effective “tailored” cognitive–behavioral treatment strategies for persons with comorbid diagnoses.

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Contributors

Jamie Vaske and Leonardo Bobadilla conceived the idea for the study and Leonardo Bobadilla researched and wrote the introduction and discussion of the manuscript with literature review and proofing help from Kia Asberg. Jamie Vaske conducted the data analysis. All authors contributed to and have approved the final manuscript.
Conflict of interest
All authors declare that they have no conflicts of interest.

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Add Health Biomarker Team (2003). Biomarkers in wave III of the Add Health Study. Chapel Hill, NC: Population Center, University of North Carolina at Chapel Hill.


