Psychosocial adversity and adolescents' mental health problems
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Chronic stressors and adolescents’ externalizing problems: Genetic moderation by Dopamine Receptor D4. The TRAILS study


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Abstract

The existing literature does not provide consistent evidence that carriers of the Dopamine D4 Receptor 7-repeat allele are more sensitive to adverse environmental influences, resulting in enhanced externalizing problems, compared to noncarriers. One explanation is that the adverse influences examined in prior studies were not severe, chronic, or distressing enough to reveal individual differences in sensitivity reflected by DRD4-7R. This study examined whether the 7-repeat allele moderated the association between chronic stressors capturing multiple stressful aspects of individuals’ lives and externalizing problems in adolescence. We expected that chronic stressor levels would be associated with externalizing levels only in 7-repeat carriers. Using Linear Mixed Models, we analyzed data from 1621 Dutch adolescents, obtained in three measurement waves (mean age approximately 11, 13.5, and 16 years) from the TRacking Adolescents’ Individual Lives Survey population-based birth cohort and the parallel clinic-referred cohort. Across informants, we found that higher levels of chronic stressors were related to higher externalizing levels in 7-repeat carriers but not in noncarriers, as hypothesized. Although previous studies on the 7-repeat allele as a moderator of environmental influences on adolescents’ externalizing problems have not convincingly demonstrated individual differences in sensitivity to adverse environmental influences, our findings suggest that adolescent carriers of the Dopamine D4 Receptor 7-repeat allele are more sensitive to chronic, multi-context stressors than noncarriers.

Keywords: Chronic stressors; Psychosocial adversity; Sensitivity to the environment; Dopamine D4 Receptor 7-repeat allele (DRD4-7R); Externalizing problems; Adolescence.
Dopamine Receptor D4 and chronic stressors

Introduction

Exposure to psychosocial stressors increases adolescents’ risk of psychopathology (for an overview, see Grant, Compas, Thurm, McMahon, & Gipson, 2004), including rule-breaking and aggressive (externalizing) behavior as seen in oppositional defiant disorder (ODD) and conduct disorder (CD). However, individual differences in outcome are large (Jenkins, 2008; Rutter, 2005), suggesting that some individuals are more sensitive to their environment than others. This study aimed to enhance our understanding of these individual differences in adolescence by examining whether the association between chronic stressors and externalizing problems over time is moderated by a genetic variant in the Dopamine D4 Receptor (DRD4) gene, potentially reflecting sensitivity to the environment.

A polymorphism in the third exon of this gene encodes for a variable number of tandem repeats, ranging from 2 to 11 (Bakermans-Kranenburg & van IJzendoorn, 2011; Dmitrieva, Chen, Greenberger, Ogunseitan, & Ding, 2011; Ptacek, Kuzelova, & Stefano, 2011). The 7-repeat (7R) variant results in lower affinity for dopamine (Ptacek et al., 2011), one of the brain’s chemical messengers that is of interest in relation to externalizing problems, through its assumed role in reward mechanisms, motivation, and approach behavior (Dmitrieva et al., 2011).

DRD4-7R has been extensively examined as a moderator of the association between environmental influences and externalizing problems, based on the notion that the 7R allele may reflect sensitivity to the environment, for better and for worse. According to this Differential Susceptibility model (e.g., Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2011), sensitive individuals are likely to be positively affected by beneficial environmental influences and negatively by adverse influences, whereas less sensitive individuals are less affected by both.

Most empirical support for this model comes from studies on laboratory-observed parenting factors in relation to externalizing problems in toddlers and preschoolers. Specifically, 10 month-old 7R carriers exposed to low vs. high laboratory-observed maternal sensitivity showed high vs. low externalizing levels, respectively, approximately 2.5 years later; whereas noncarriers appeared unaffected by maternal sensitivity (Bakermans-Kranenburg & Van IJzendoorn, 2006). In addition, an intervention aimed at reducing toddlers’ externalizing problems by promoting maternal positive discipline proved to be more effective in 7R carriers than in noncarriers at follow-up (mean age 27, 39, and 52 months at pretest, posttest, and follow-up, respectively, Bakermans-Kranenburg, Van IJzendoorn, Pijlman, Mesman, & Juffer, 2008). However, one study showed that in European-Americans, the influence of warm-responsive and negative-intrusive parenting at 6 and 12 months on externalizing levels at 18, 24, and 30 months did not
significantly differ between 7R carriers and noncarriers (Propper, Willoughby, Halpern, Carbone, & Cox, 2007). Another study showed that maternal sensitivity at 14 months, but not at 36 or 48 months, interacted with DRD4-7R in predicting later externalizing problems (Windhorst et al., 2015). Specifically, higher maternal sensitivity at 14 months predicted lower externalizing levels at 18 months (as well as at 60 months, but only via indirect paths across time) in 7R carriers, but did not affect noncarriers. At 36 months, however, 7R carriers showed a similar response, but noncarriers showed the opposite (i.e., higher sensitivity predicted higher externalizing levels), rather than no response. Thus, these prior findings on toddlers and preschoolers do not consistently support the Differential Susceptibility model.

In samples with a broad age range that included middle and late childhood as well as adolescence, findings showed no evidence that DRD4-7R moderated the influence of maternal expressed emotion (i.e., warmth, criticism) on conduct problems (mean age 11 yrs, range 5-17 yrs, Sonuga-Barke et al., 2009) or on prosocial and antisocial behavior (mean age 17 yrs, range 7-28 yrs, Richards et al., 2015). In studies that focused on externalizing problems in (pre)adolescents (between 11-20 years old), the notion that DRD4-7R may reflect sensitivity to the environment, for better and for worse, has received moderate but inconsistent support. One study showed higher sensitivity, for better and for worse, in 7R carriers, compared to noncarriers; to laboratory-observed early maternal stimulation and responsiveness, but not to parent-reported early family adversity, with respect to adolescents’ symptoms of CD/ODD (combined parent-report and self-report) and psychopathy (parent-report, Nikitopoulos et al., 2014). Another study showed relatively high sensitivity, for better and for worse, in 7R carriers to a broad range of intervention-targeted parenting behaviors (reported by parents), with respect to self-reported substance use, but not to parent-reported delinquency (Beach, Brody, Lei, & Philibert, 2010).

Prior studies from our research group TRAILS (“TRacking Adolescents’ Individual Lives Survey”) have, likewise, produced inconsistent results. One of these (Nederhof, Belsky, Ormel, & Oldehinkel, 2012) showed that the 7R allele moderated the association between parental separation and self-reported externalizing problems, although this effect pertained only to boys, not to girls, and only to the absence of parental separation, not to its presence. That is, externalizing levels of 7R-carrying boys compared to noncarriers were relatively low if their families were intact but did not differ if their parents had separated, suggesting sensitivity for better but not for worse. Other studies from our research group showed no evidence that 7R carriers are relatively sensitive to the influence of peers (teacher-reported peer victimization and self-reported social well-being) on self-reported delinquency (Kretschmer, Dijkstra, Ormel, Verhulst, & Veenstra, 2013) or of parenting (rejection, overprotection, and emotional warmth as reported by pre-adolescents) on delinquency and aggression (combined parent-report and self-report,
Marsman, Oldehinkel, Ormel, & Buitelaar, 2013) or substance use (self-report, Creemers et al., 2011).

Taken together, there are clearly many inconsistencies in the literature as to whether DRD4-7R may reflect individual differences in sensitivity to environmental influences. The inconsistencies in prior findings in adolescence, which is also the focus of the present study, do not appear to be driven by an informant effect, nor by differences in operationalization of externalizing problems (e.g., substance use vs. delinquency vs. broader externalizing measures) or environmental influences (e.g., parent vs. peer influence; broad vs. narrow aspects of parenting). Rather, what seems to stand out in these prior findings is the lack of evidence that DRD4-7R reflects sensitivity to the detrimental effects of adverse environmental influences. The few findings that did support high sensitivity not only for better but also for worse in 7R carriers (Beach et al., 2010; Nikitopoulos et al., 2014) were based on the absence of positive (beneficial) environmental influences. For example, while high levels of maternal stimulation and responsivity in the study by Nikitopoulos et al. (2014) were considered to be beneficial, low levels reflect an absence of beneficial influence, rather than presence of adverse influence (e.g., the presence of maternal hostility). In contrast, of the prior findings in adolescence relating to actual adverse influence (i.e., early family adversity, perceived parental rejection or overprotection, peer victimization, parental divorce or separation) none suggested differences in externalizing levels between 7R carriers and noncarriers (Creemers et al., 2011; Kretschmer et al., 2013; Marsman et al., 2013; Nikitopoulos et al., 2014). Thus, the Differential Susceptibility hypothesis, extending the Diathesis-Stress theory (Zuckerman, 1999) that some individuals are more vulnerable to the detrimental effects of adverse influences, has not received much support from the data.

One explanation could be that the adverse environmental influences examined in prior studies were not severe, chronic, or distressing enough to reveal individual differences in sensitivity reflected by DRD4-7R. Adverse environmental influences may become more severe or distressing as they persist over time, taxing individuals’ physical and psychological coping resources. In addition, subtle individual differences in sensitivity may be missed when the adverse environmental influence is rather narrowly operationalized, capturing only one aspect of individuals’ lives. That is, the adverse environmental influences examined in prior studies generally reflected a specific aspect of a single environmental domain (e.g., either family or peer group) while beneficial influences from other domains, if present, will compensate for their impact. Individual differences in sensitivity may thus be easier to detect by assessing environmental influences that are chronic and reflect multiple adverse aspects across multiple environmental domains (e.g., family, peers, school, and neighborhood). We hypothesize that if DRD4-7R truly reflects individual differences in sensitivity to the environment, not only for better, as
some prior findings have shown, but also for worse, this may become evident in the presence of chronic, multi-context, stressors, which may exceed sensitive individuals’ ability to cope. In this study, we have therefore examined whether DRD4-7R is a moderator of the association between chronic stressors, operationalized as number of long-term difficulties, and externalizing (CD and ODD) problems from preadolescence into adolescence. We expected that, only in 7R carriers, chronic stressor levels would be positively associated with externalizing levels, whereas in noncarriers, we expected no influence of chronic stressors on externalizing levels.

Methods and Materials

Sample

We obtained the data used in this study from the first three measurement waves (mean ages about 11, 13.5, and 16 years) of the “TRacking Adolescents’ Individual Lives Survey.” TRAILS aims to contribute to the understanding of the etiology of mental health problems by following 10-12 year-old Dutch children biennially into adulthood.

We pooled data from the TRAILS population-based birth cohort (n = 2230) and the parallel clinic-referred cohort (n = 543), to obtain a large sample with a wide range of problem severity and chronic stress. The sampling procedures, descriptive statistics, and response rates of both cohorts are well-documented (e.g., De Winter et al., 2005; Huisman et al., 2008; Ormel et al., 2012). In brief, TRAILS approached 135 primary schools in five municipalities in the Northern Netherlands to build the population cohort. Of these schools, 90.4 % agreed to participate. TRAILS contacted eligible students and their parents, enrolling 76 % (n = 2230) of those contacted in the study. The three data waves we included in this study ran from March 2001 to July 2002 (T1), September 2003 to December 2004 (T2), and September 2005 to August 2007 (T3); with response rates consistently above 80 %.

The smaller clinic-referred sample (n = 543) consists of pre-adolescents who had been referred to the Groningen University Child and Adolescent Psychiatric Outpatient Clinic at any point in their life (20.8 % ≤5 years; 66.1 % 6-9 years; 13.1 % 10-12 years) for consultation or treatment. The first three data waves in the clinic-referred cohort ran two years behind those of the population cohort: From September 2004 to December 2005 (T1), September 2006 to November 2007 (T2), and September 2009 to February 2011 (T3). The measurement instruments and design for the clinic-referred cohort were the same as those of the population cohort. Of the 1264 eligible pre-adolescents, 543 (65.9 % boys; mean age 11.11; SD 0.50; range 10.13-12.40) enrolled in the study and finished baseline
measurements (T1). Of these 543 baseline participants, 85.1 % (n = 462) participated in the second wave (T2). Of the T2 participants, 83.5 % (n = 386) also participated in the third wave (T3). Another 30 T2 dropouts agreed to participate in the third wave, resulting in a total T3 response rate of 76.6 % (n = 416) of the original sample. Selective attrition analyses have been described elsewhere (De Winter et al., 2005; Huisman et al., 2008; Nederhof et al., 2012; Ormel et al., 2012). Importantly, baseline participants did not differ from non-participants with respect to externalizing problems.

Parents gave written informed consent prior to each assessment wave. Adolescents gave written informed assent at the second and third wave. TRAILS was approved by the National Dutch Medical Ethics Committee, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Measures**

*Externalizing problems*

TRAILS used the Achenbach System of Emperically Based Assessment (ASEBA) family of measures of mental health problems (Achenbach & Rescorla, 2001; Verhulst & van der Ende, 2013) at each time point. The Child Behavior Checklist (CBCL) and the Youth Self-Report (YSR) contain 120 items assessing behavioral and emotional problems in children over the past 6 months. These items can be rated as 0 _not true_, 1 _somewhat or sometimes true_, or 2 _very or often true_. We used DSM-IV-oriented subscales to define externalizing problems as the sum of the average scores of oppositional defiant problems (k = 5; Cronbach’s α = .81 and α = .64 for parent-report and self-report, respectively) and conduct problems (k = 17, α = .82 for parent-report; k = 15, α = .75 for self-report). We chose to use average scale scores and sum these in order to balance the influence of subscales with different numbers of items, then standardized the sum scores.

Externalizing problems correlated significantly (p < .001) with internalizing problems, at both T2 (r = .52, parent-report, and r = .38, self-report) and T3 (r = .54, parent-report, and r = .32, self-report). Therefore, we focused on externalizing problems adjusted for co-occurring internalizing problems (EXTadj). To that end, we computed the summed weighted average of anxiety (k = 6; α = .73 and α = .61 for parent-report and self-report, respectively) and affective problems (k = 13; α = .72 and α = .71 for parent-report and self-report, respectively), after which we computed residual externalizing scores (M = 0; SD = 1).

*Chronic stressors preceding T2 and T3*

We operationalized chronic stressor levels at T2 and T3 as the number of parent-report ed long-term difficulties since the previous measurement. One of the parents, typically
the mother, filled out a TRAILS questionnaire that listed long-term difficulties to which the adolescent might have been exposed since the previous interview (e.g., Oldehinkel, Verhulst, & Ormel, 2008; Zandstra et al., 2015). The stressors included: (1) chronic illnesses or physical handicaps of the child or (2) a family member; (3) high work pressure at school; (4) housing problems; (5) neighborhood problems, such as violence or discrimination; (6) financial problems; (7) lack of friends; (8) being bullied; (9) long-lasting conflicts with family members or (10) others; and (11) long-lasting conflicts between family members. On an open item, parents could also disclose additional long-term difficulties. We coded these additional problems either as a long-term difficulty or dismissed them according to well-defined rules—in particular whether the described situation is typically considered stressful and enduring. For example, we coded a turbulent home environment, such as moving frequently from house to house or parents having an on/off relationship, as long-term difficulties. Situations that we rejected as long-term difficulty included normative or non-enduring situations such as the transition to middle school, puberty, and quarrels with siblings. The number of reported difficulties ranged from 0 to 10. To reduce the influence of extreme and rare scores, we grouped subjects into 4 categories: 0, 1, 2, or 3 or more long-term difficulties.

**DRD4 genotyping**

At T1, DNA was extracted from blood samples or buccal swabs (Cytobrush®) using a manual salting out procedure (Miller, Dykes, & Polesky, 1988). The 48 bp direct repeat polymorphism in exon 3 of *DRD4* was genotyped on the Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA, USA), described in detail elsewhere (Nederhof et al., 2012). The genotyping assay was carried out in a CCKL quality-certified laboratory and has been validated in earlier tests. Three percent blanks as well as duplicates between plates were processed as quality controls during genotyping. Determination of the length of the alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk, The Netherlands) using standard conditions. We formed two groups according to the presence of at least one 7R allele (1 = 7R carrier; 0 = noncarrier).

**Data analysis**

**Data preparation and preliminary analyses**

For this study, our statistical analysis method required at least one value for each predictor on T1-T3 and at least T2 or T3 externalizing problems. Thus, we needed T2 and/or T3 parent-reported and/or self-reported externalizing problems, T2 and/or T3 chronic stressors, and T1 *DRD4*. Participants not from Dutch ancestry were excluded. Of
each sibling pair, we excluded one participant at random. We performed independent samples t-tests to check whether included and excluded subjects differed with respect to our study variables.

**Main analyses**

We computed Pearson’s correlation coefficients between the predictors and T2 and T3 externalizing problems. The possible presence of gene-environment correlations (i.e., \( DRD4 \) genotype is associated with exposure to chronic stressors) may drive gene-environment interaction effects and therefore need to be ruled out.

We used Linear Mixed Modeling (LMM) to investigate the effects of chronic stressors, \( DRD4\)-7R, and their hypothesized interaction in predicting subsequent EXTadj. LMM allows for missing data at different measurement waves, which is an important advantage for a longitudinal design (Kwok et al., 2008). Using PASW Statistics 18, we conducted LMM analyses (T2 and T3 in a single analysis), separately for parent-reported and self-reported EXTadj. We included the independent variables of age (time-variant), sex (0 = female; 1 = male), initial EXTadj at T1, chronic stressors (time-variant), and \( DRD4\)-7R, as well as an interaction between chronic stressors and \( DRD4\)-7R. All non-dichotomous variables were centered prior to analysis. For interpretation of interaction effects we plotted EXTadj levels based on the estimated regression coefficients, for different levels of each predictor. We used the Maximum Likelihood estimation procedure and considered a \( p \)-value < .05 to be statistically significant.

For post-hoc probing of statistically significant interaction effects, we computed simple slopes, which reflect the slopes of regression lines in a plot, and regions of significance, indicating the range of values of a predictor at which the interaction effect is statistically significant (Preacher, Curran, & Bauer, 2006). Regions of significance result from separate analyses that may produce values of a predictor that fall outside the true data range. To examine the potential influence of sex on significant interaction effects, we repeated our main analyses (in which we controlled only for a main effect of sex on EXTadj) by adding sex by \( DRD4\)-7R and sex by stressors interaction terms to the model, as has recently been recommended in the literature (Keller, 2014). If findings showed significant sex by predictor interaction effects, we tested for an additional three-way interaction effect of chronic stressors, \( DRD4\)-7R, and sex, in predicting EXTadj. To check the influence of adjusting externalizing problems for co-occurring internalizing problems, we repeated the analysis replacing the outcome variable EXTadj with EXT; that is, externalizing problems unadjusted for co-occurring internalizing problems.
Results

Results of preliminary analyses

Three hundred and nine participants had missing data for both measurements of chronic stressors, and 137 for parent-reported as well as self-reported externalizing problems.

Of the 1861 participants with available $DRD4$ data, those not from Dutch ancestry ($n = 166$) were excluded. Of the sibling pairs in the remaining groups, one of each was excluded ($n = 22$). Altogether, we excluded a total of 1152 participants ($n = 1005$ population cohort; $n = 147$ clinic-referred cohort) from this study, resulting in a final sample of 1621 subjects ($n = 1225$ population cohort; $n = 396$ clinic-referred cohort).

We compared the final study sample (mean age 11.09; SD 0.55; range 10.01-12.58; 52.2 % boys; 75.6 % population cohort) with those who were not included. We found that participants were somewhat younger, $t (2769) = -2.309$, $p = .021$, and had higher T2 chronic stressor levels, $t (1570.583) = 2.248$, $p = .025$. There were no significant differences between the groups with respect to sex, $p = .69$, $DRD4$-7R, $p = .82$, and parent- and self-reported externalizing problems, $p = .54$ and .25, respectively.

$DRD4$-7R and chronic stressors

Table 1 shows descriptive statistics of the final sample and Table 2 correlations between predictors and parent-reported and self-reported EXTadj. There was no indication of gene-environment correlations as $DRD4$-7R was not significantly associated with chronic stressors at T2 ($r = -.03$, $p = .20$) or T3 ($r = .00$, $p = .88$).

As shown in Table 3, parent-reported and self-reported EXTadj problems were significantly predicted by a two-way interaction effect of chronic stressors and $DRD4$-7R ($p = .023$ and $p = .024$, respectively). We plotted the levels of EXTadj for low, average, high, and very high levels of the truncated chronic stressor variable (corresponding to 0, 1, 2, and 3 or more long-term difficulties, respectively), separately for 7R carriers and noncarriers. Figure 1 shows that higher chronic stressor levels were related to higher EXTadj in 7R carriers, while EXTadj of noncarriers was stable across chronic stressor levels.

Post-hoc probing of these interaction effects resulted in simple slopes and regions of significance. The increase in EXTadj with chronic stress level was statistically significant for 7R carriers, $t (1557.022) = 3.85$, $p < .001$ for parent-report, and $t (1572.308) = 3.74$, $p < .001$ for self-report, while the slope of EXTadj across chronic stress groups did not significantly differ from zero for noncarriers, $t (1557.022) = 1.06$, $p = .29$ for parent-report, and $t (1572.308) = 1.41$, $p = .16$ for self-report.
Table 1 Descriptive statistics of the variables used in this study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wave</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
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<th>Max</th>
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</thead>
<tbody>
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<td>T1</td>
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<tr>
<td></td>
<td>T2</td>
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<td>T3</td>
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<td></td>
<td>T3</td>
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<td>1</td>
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</table>

CBCL = Child Behavior Checklist, YSR = Youth Self-Report, EXT = Externalizing problems (DSM-oriented subscales oppositional defiant problems and conduct problems), DRD4-7R = Dopamine D4 Receptor 7-repeat allele.

*Number of long-term difficulties experienced since previous measurement.

*Sum of 22 item scores for parent-report and 20 items for self-report; range per item 0-2.

*Coded as 0 = noncarrier; 1 = carrier.

Table 2 Correlation matrix of predictors and outcome variables, with parent-reported externalizing problems below and self-reported externalizing problems above diagonal

<table>
<thead>
<tr>
<th>Variables</th>
<th>T2Stressors</th>
<th>T3Stressors</th>
<th>DRD4-7R</th>
<th>Sex</th>
<th>T2EXTadj</th>
<th>T3EXTadj</th>
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<td>.05</td>
<td>.07**</td>
<td>.06*</td>
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<td>-.01</td>
<td>.06*</td>
<td>.09***</td>
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<tr>
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<td>.00</td>
<td>1</td>
<td>.07**</td>
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<td>-.02</td>
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<td>-.01</td>
<td>.07**</td>
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<td>.12***</td>
<td>.16***</td>
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</table>

Parent-report

<table>
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<th>T3EXTadj</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2EXTadj</td>
<td>.15***</td>
<td>.39***</td>
</tr>
<tr>
<td>T3EXTadj</td>
<td>.13***</td>
<td>.30***</td>
</tr>
</tbody>
</table>

DRD4-7R = Dopamine D4 Receptor 7-repeat allele, EXTadj = Externalizing problems adjusted for co-occurring internalizing problems. DRD4-7R was coded as 0 = noncarrier; 1 = carrier. Sex was coded as 0 = female; 1 = male. ***p < .001, **p < .01, *p < .05
Regions of significance showed that the interaction effect between chronic stressors and DRD4-7R in predicting EXTadj was statistically significant below -1.34 and -0.32 chronic stressors for parent-report and self-report, respectively, both non-existent values, and above 1.85 and 4.80 chronic stressors for parent-report and self-report, respectively. We conclude that our findings apply to the upper end of the chronic stressor range, not to the lower end, and that the effect is stronger based on parent-report of externalizing problems than self-report.

Controlling for potential interaction effects of sex with chronic stressors or DRD4-7R, parent-reported and self-reported EXTadj problems were still significantly predicted by
a two-way interaction effect of chronic stressors and $\text{DRD4-7R}$, $p = .037$ and $p = .029$, respectively. In these models, sex did not interact with $\text{DRD4-7R}$ in predicting parent-reported or self-reported EXTadj, $p = .52$ and $p = .24$, respectively, nor with chronic stressors in predicting self-reported EXTadj, $p = .15$. However, sex did significantly interact with chronic stressors in predicting parent-reported EXTadj, $p = .017$, which may explain why the interaction effect of chronic stressors and $\text{DRD4-7R}$ was somewhat weaker compared to our main results. Visual inspection showed that the association between chronic stressor levels and EXTadj was stronger in 7R carriers than in noncarriers (both boys and girls), as in Figure 1, and stronger in boys than in girls (both 7R carriers and noncarriers). However, we found no evidence of a three-way interaction effect of chronic stressors, $\text{DRD4-7R}$, and sex, in predicting parent-reported or self-reported EXTadj, $p = .61$ and $p = .25$, respectively. These posthoc findings suggest that the association between chronic stressors level and EXTadj (at least parent-report) is moderated by $\text{DRD4-7R}$ as well as by sex, but independent of each other.

Without adjusting externalizing for co-occurring internalizing problems, a two-way interaction effect of chronic stressors and $\text{DRD4-7R}$ did not hold in predicting parent-reported EXT, $p = .16$, but still significantly predicted self-reported EXT, $p = .045$. Visual inspection showed that the association between chronic stressor levels and parent-reported EXT was strong overall with negligible differences between 7R carriers and noncarriers (albeit in the same direction as our main results). The association between chronic stressors level and self-reported EXT was similarly strong for 7R carriers but was attenuated in noncarriers, as in Figure 1 but less pronounced. This weakening of effects due to co-occurring internalizing problems may suggest that our main findings pertain especially to ‘pure’ externalizing problems and less to internalizing or comorbid externalizing and internalizing problems.

These post-hoc analyses show the robustness and specificity of our main results. Tables and figures from these analyses are available upon request.

**Discussion**

This study aimed to contribute to the literature by examining whether $\text{DRD4-7R}$ moderated the association between chronic stressors and externalizing problems. As hypothesized, higher chronic stressor levels were related to higher externalizing levels in 7R carriers but not in noncarriers, suggesting high vs. low sensitivity, respectively, to adverse environments. These results were consistent across informants and were not driven by adolescents’ gender. Although it has been posited that the 7R allele reflects sensitivity to adverse as well as to beneficial environmental influences on externalizing
problems (e.g., Bakermans-Kranenburg et al., 2008), this theory has received inconsistent support in adolescence (cf. Beach et al., 2010; Creemers et al., 2011; Kretschmer et al., 2013; Marsman et al., 2013; Nederhof et al., 2012; Nikitopoulos et al., 2014; Richards et al., 2015; Sonuga-Barke et al., 2009). In particular, none of these prior studies have convincingly demonstrated sensitivity to environmental influences in the adverse range. The present study thus adds to the literature by showing this sensitivity to adverse circumstances, at least to chronic stressors.

Our results contrast with prior findings that DRD4-7R did not moderate the association between early family adversity (a relatively broad measure of environmental influence, as the one used in the present study), and adolescents’ symptoms of CD/ODD and psychopathy (Nikitopoulos et al., 2014). These findings were based on data from a parent-interview, conducted when participants were 3 months old, assessing which of eleven family adversity factors (e.g., low educational level, marital discord) were present in the year prior to the child’s birth. One obvious explanation for the difference in findings would be the early age at which the environmental influence was assessed and, consequently, the large amount of time and contextual influences that passed between assessments of the environmental predictor and the behavioral outcome (i.e., 15 years), in contrast to our study, which assessed more recent environmental influences. However, given that the same study did show a moderating effect of DRD4-7R on the influence of laboratory-observed early maternal stimulation and responsiveness, also assessed at 3 months, we cannot conclude that DRD4-7R only moderates recent and not early environmental influences. The null finding may be due to prenatal family difficulties that are resolved before birth or that do not have longlasting effects on children. Moderating effects of DRD4-7R may be easier to detect when focusing on ongoing or chronic environmental difficulties, which presumably have a major impact on sensitive individuals, taxing their ability to cope, but not on less sensitive individuals.

Other prior findings that the 7R allele did not moderate adverse environmental influences on adolescents’ externalizing problems came from our own research group (Creemers et al., 2011; Kretschmer et al., 2013; Marsman et al., 2013; Nederhof et al., 2012). These prior TRAILS findings have shown that the influences of parental rejection and overprotection, as perceived by preadolescents at T1 (mean age 11 yrs) on delinquency and aggression at T2 (mean age 13.5 yrs, combined parent-report and self-report, Marsman et al., 2013) and on substance use at T3 (mean age 16 yrs, self-report, Creemers et al., 2011) were not moderated by DRD4-7R (7R carriers vs. noncarriers). Furthermore, the influence of parental separation, assessed at T1 and T3, on self-reported externalizing levels at T3 did not differ between 7R carriers vs. noncarriers (Nederhof et al., 2012). Finally, teacher-reported peer victimization at T2 did not influence self-reported delinquency at T4 (mean age 19 yrs) in 7R carriers, in contrast to 4R carriers (Kretschmer
et al., 2013). These findings have led our colleagues to suggest that moderating effects of DRD4-7R on environmental influences on externalizing problems apply less to adolescence than to childhood (Kretschmer et al., 2013; Marsman et al., 2013), less to peer influence than to other environmental factors (Kretschmer et al., 2013), or may differ according to the operationalization of externalizing problems (Creemers et al., 2011).

Given that samples, age range, and genetic and outcome measures used in these studies partially overlap with ours, it is likely that our findings differ due to the way in which we have operationalized environmental adversity. Whereas some of the previously addressed adversities may be ongoing, as were the difficulties we have assessed, our study appears to stand alone in its measurement of chronic difficulties that collectively capture many different aspects of individuals’ lives (e.g., both family and peer contexts). Thus, our findings suggest that moderating effects of DRD4-7R on the association between adverse environmental influences and externalizing problems do extend to adolescence when focusing on chronic multi-context stressors. However, this finding will need to be replicated by future research.

Our study included a number of limitations. First, we collected parent-reports, not self-reports, of long-term difficulties because we assumed that parents are better and more stable judges of the difficulties that put chronic strain on family life. The stressors we examined included issues such as chronic housing problems and neighborhood problems. Such factors may be less stressful for adolescents than parents assume. On the other hand, parents may not have full insight into the other chronic stressors we measured, such as bullying, that weigh heavily on adolescents’ life. Although shared method variance may strengthen the interaction effect between chronic stressors and the 7-repeat allele in predicting externalizing problems reported by parents, it does not explain our similar findings on self-reported externalizing problems. Second, we focused on chronic adversities and did not study sensitivity to positive chronic conditions. A formal test of Differential Susceptibility includes both beneficial and adverse aspects of the environment, while we have only addressed the latter Diathesis-Stress model. Although sensitivity to beneficial environmental influences has been examined relatively frequently in relation to DRD4-7R, as outlined in the introduction, it would have complemented our findings nonetheless, had we been able to incorporate this.

Strengths of the study include the large sample of longitudinal, multi-informant data from pre-adolescence well into adolescence, large inter-individual differences in levels of externalizing problems and chronic stressors, and the use of Linear Mixed Modeling that allowed for optimal use of all available data from multiple measurements.

In sum, whereas previous studies on DRD4-7R as a moderator of environmental influences on adolescents’ externalizing problems have not convincingly demonstrated sensitivity to environmental influences in the adverse range, we were able to do so by focus-
ing on chronic multi-context stressors. Our finding that higher levels of chronic stressors were associated with higher externalizing levels in 7R carriers but not in noncarriers suggests high vs. low sensitivity, respectively, to adverse environments. We encourage further studies of environmental influences that reflect multiple adverse aspects across multiple environmental domains (e.g., family, peers, school, and neighborhood).
Dopamine Receptor D4 and chronic stressors

References


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