Gene-environment interactions on the course of Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms
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ADHD symptoms in middle adolescence predict exposure to person-related life stressors in late adolescence in 5-HTTLPR S-allele homozygotes

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ABSTRACT

Background
Literature suggests that life stressors predict Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms and that this relationship is moderated by the serotonin transporter polymorphism (5-HTTLPR). It is less clear whether, on reverse, ADHD symptoms may influence the risk of exposure to life stressors. Furthermore, the role of life stressors may vary across development depending on the type of life stressor.

Methods
We used three-wave longitudinal data of 1,306 adolescents from the general population and clinic-referred cohort of the TRacking Adolescents' Individual Lives Survey. The 5-HTTLPR genotype (SS, LS, LL), parent-reported ADHD symptoms at three time points (T1: Mage = 11.2; T2: Mage = 13.5; T3: Mage = 16.2 years), and the number of person-related (‘dependent’) and environment-related (‘independent’) life stressors occurring between measurements (T1-T2, T2-T3) were assessed. Using path analyses, we examined bidirectional relations between exposure to these life stressors and ADHD symptoms between the separate waves moderated by 5-HTTLPR status.

Results
Exposure to life stressors did not predict ADHD symptoms. Rather, we found that in 5-HTTLPR S-allele homozygotes, ADHD symptoms in middle adolescence (T2) predicted exposure to the number of person-related life stressors later in adolescence (T2-T3, p = .001). There was no relation with environment-related life stressors.

Conclusions
Our study suggests that S-allele homozygotes with higher levels of ADHD symptoms in middle adolescence are more vulnerable to becoming exposed to person-related (‘dependent’) life stressors in late adolescence. Findings emphasize the need to be aware of social-emotional adversities that may occur in genetically vulnerable adolescents with ADHD symptoms in the transition into adulthood.
INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) may be viewed as the extreme end of behaviors varying along a continuum within the population rather than as a categorical disorder with discrete determinants (Levy et al., 1997). Adolescents with ADHD symptoms frequently encounter stressors in many areas of daily life, including academic and social-emotional functioning, such as difficulties dealing with school tasks, presence of family and peer conflict, peer rejection, and social isolation (Nijmeijer et al., 2008).

These life stressors may both impact on and result from ADHD symptoms (Combs et al., 2015; Nijmeijer et al., 2008). While exposure to life stressors has indeed been shown to prospectively predict severity of ADHD (Gunther et al., 2007; Sasser et al., 2016) or related externalizing symptoms (Kim et al., 2003), still little is known about prospective bidirectional relationships. Surprisingly few studies have addressed the impact of ADHD symptoms on subsequent exposure to life stressors. The existing studies merely focused on single specific stressors (Lifford et al., 2009; Matthews et al., 2015; Verlinden et al., 2015). For example, in a large prospective cohort, 5-year-olds with ADHD symptoms and problem behaviors were at elevated risk of becoming more socially isolated at age 12 (Matthews et al., 2015). The effects of ADHD symptoms on life stress may be understood in that an individual’s personal characteristics and behaviors may evoke negative responses in the environment leading to stress exposure; also, getting involved in high-risk environments may lead to future stress exposure (Dick et al., 2006; Reiss, 2010).

Interestingly, studies have shown moderation of the relation between life stressors and ADHD symptoms by the serotonin transporter polymorphism (5-HTTLPR; Van der Meer et al., 2014; Müller et al., 2008; Retz et al., 2008). Only S-allele carriers and not L-allele homozygotes of the 5-HTTLPR genotype showed a prospective positive association between life stressors and severity of ADHD symptoms in a large two-wave study of adolescents with (subthreshold) ADHD and controls (Van der Meer et al., 2014). This gene-environment (GxE) interaction is consistent with the extensive literature on internalizing disorders (Caspi et al., 2010; Nugent et al., 2011), which provides strong evidence for 5-HTTLPR moderating the stress-depression relationship in a comprehensive meta-analysis (Karg et al., 2011). Specifically, the S-allele has consistently been shown to modify an individual’s response to stress and has been linked to increased stress sensitivity and reactivity compared to L-allele homozygotes of the 5-HTTLPR (Caspi et al., 2003, 2010). This may be due to increased amygdala activation in response to emotional stimuli (Hariri et al., 2002; Drabant et al., 2012).

The potential role of 5-HTTLPR genotype in the ADHD-life stressor pathway is less straightforward. While on one hand a meta-analysis showed that it is the 5-HTTLPR L-allele that represents increased risk for ADHD (Gizer et al., 2009), on the other hand it is conceivable that S-allele carriers of the 5-HTTLPR evoke more interpersonal conflict.
as part of their personal characteristics and behaviors (Dick et al., 2006; Shaw, 2015). In these individuals also higher stress sensitivity may play a role (Caspi et al., 2003; Drabant et al., 2012), resulting in increased exposure to life stressors (Kendler et al., 2003; Nijmeijer et al., 2008).

To better understand the role of exposure to life stressors associated with ADHD symptoms, it is important to distinguish between person-related (‘dependent’) and environment-related (‘independent’) life stressors (Johnson et al., 2013). Interestingly, the genetic and environmental origins of person-related and environment-related life stressors have been found to vary over adolescent development (Johnson et al. 2013). This may suggest different developmental risk pathways of stress exposure in relation to psychopathology over time (Johnson et al., 2013). Person-related life stressors are thought to be mainly ‘dependent’ on a person’s behavior and personality traits (Amone-P’Olak et al., 2009; Kendler et al., 1995) and are more genetically influenced than environment-related stressors, with genetic factors increasing in magnitude during the transition to adolescence (Johnson et al., 2013). Environment-related life stressors are thought to occur largely ‘independent’ of the person’s direct involvement and personal traits (Amone-P’Olak et al., 2009; Kendler et al., 1995) and are less genetically influenced than dependent stressors; such shared environmental influences appear to play a greater role in exposure to life stressors in childhood, with decreasing influences during adolescence (Johnson et al., 2013). This distinction is supported by knowledge on adolescents’ social-emotional development towards personality building and growing into independence (Arnett, 1999; Erikson, 1968). Adolescence represents a particularly sensitive period of life, characterized by increasing peer influences and social relations, self-awareness, sensitivity to social environmental cues (particularly social acceptance), inter-personal conflicts, and mental health problems, resulting from personal experiences, maturation, and neurobiological changes (Arnett, 1999; Schriber & Guyer, 2016). This may make the developing adolescent more susceptible to person-related life stressors with increasing age. Indeed, the role of genetic and environmental risk factors for ADHD has been suggested to vary across development (Pingault et al., 2015; Shoval et al., 2014; Thapar et al., 2007).

The current study

The aim of this longitudinal study was to examine bidirectional relations of exposure to different types of life stressors with ADHD symptoms in relation to three serotonin (5-HTTLPR) genotypes (SS, LS, LL) between early (T1; $M_{age} = 11.2$ years), middle (T2; $M_{age} = 13.5$ years), and late adolescence (T3; $M_{age} = 16.2$ years), using data from a large pooled general population and clinic-referred cohort ($n = 1,306$). This allowed us to test both the directionality and specificity (environment versus person dependency) of stress-ADHD relations and to detect possible age-dependent effects. We expected to find that (1) exposure to
life stressors would predict ADHD symptom levels only in S-allele carriers but not in L-allele homozygotes of the 5-HTTLPR genotype; (2) ADHD symptom levels would predict later exposure to life stressors, also only in S-allele carriers; and (3) person-related (‘dependent’) life stressors would play a more prominent role in the relation with ADHD symptoms, particularly during later adolescence in contrast to environment-related (‘independent’) stressors.

METHODS

Participants

Our study included 1,306 participants in the first (T1; \(M_{\text{age}} = 11.2\), range 10.0-12.6 years), second (T2; \(M_{\text{age}} = 13.5\), range 12.6-14.9 years), and third (T3; \(M_{\text{age}} = 16.2\), range 14.9-18.4 years) wave of the Tracking Adolescents’ Individual Lives Survey (TRAILS). TRAILS is an ongoing prospective study of Dutch adolescents with the aim to chart and explain the development of mental health from early adolescence into adulthood. Data were derived from the TRAILS population cohort including both urban and rural areas in the North of the Netherlands, and from the parallel clinic-referred cohort of children who had been referred to the Groningen university child and adolescent psychiatry outpatient clinic at least once in their life. The sampling procedures, descriptive statistics, and response rates of both cohorts have been well-documented elsewhere (Oldehinkel et al., 2015; De Winter et al., 2005).

At baseline, 2,773 adolescents participated in the population-based (\(n = 2,230\)) or clinic-referred cohort (\(n = 543\)), with response rates for both cohorts over 80% at each follow-up assessment. Genotyping data of 5-HTTLPR were available of 1,788 of the 1,922 adolescents who had donated DNA. We further excluded 293 adolescents who missed more than half of the items on the number of life stressors at T1-T2 and/or T2-T3, and another 189 individuals whose age at the subsequent wave was within the range of the age range of the previous measurement wave. Our final sample consisted of 1,306 participants, 85.8% of which stemmed from the general population cohort (90.5% Dutch ancestry; \(n = 105\) with clinical or subclinical levels of ADHD symptoms based on ASEBA cut-off scores [Achenbach 1991]) and 14.2% from the clinic-referred cohort (98.9% Dutch ancestry; \(n = 97\) with clinical or subclinical levels of ADHD symptoms).

The child’s parents or legal guardian and adolescents (\(\geq 12\) years) provided both written informed consent prior to each wave, whereas younger participants provided verbal assent. The TRAILS study was approved by the Central Committee on Research Involving Human Subjects (Dutch CCMO).
Measures

**ADHD symptoms.** At all waves, the DSM-IV-oriented subscale Attention-Deficit/Hyperactivity Problems of the Child Behavioral Checklist (CBCL; Achenbach, 1991; Verhulst & Achenbach, 1995) consisting of 7 items (3 inattention and 4 hyperactivity-impulsivity items) was used to measure parent-reported ADHD symptoms during the previous six months. Items were scored on a 3-point Likert-scale ranging from 0 (‘not true’) to 2 (‘very true or often true’). The DSM-oriented subscale of the CBCL has shown good reliability as well as convergent and discriminative validity in a clinical sample of adolescents (Nakamura et al. 2009).

**Life stressors.** At T2 and T3, the Long-Term Difficulties questionnaire developed by TRAILS (Amone-P’Olak et al., 2009; Oldehinkel et al., 2008) was used to assess the presence of parent-reported person-related (‘dependent’; 6 items) and environment-related (‘independent’; 5 items) life stressors their child may have been exposed to in the past two years (see Supplementary Information 4.1). We categorized adolescents as having been exposed to no (0), one (1), two (2), or three or more (3) life stressors in order to reduce the influence of extreme or rare scores (Zandstra et al., 2015).

**Covariates.** As covariates we assessed past-year ADHD medication use (i.e., methylphenidate, dexamphetamine, and/or atomoxetine) at any time in the preceding year; socioeconomic status (SES) based on five indicators (household income and both parents’ professional occupation and educational attainment) and categorized as low (<P25), intermediate (between P25-P75), or high SES (>P75); two genetic principal component analysis scores to correct for genetic population stratification; and internalizing problems based on the 32-item DSM-oriented broadband internalizing scale of the CBCL (Achenbach, 1991; Verhulst & Achenbach, 1995) with scores from 0-2 over the past six months, measured at all three waves. Aggression problems based on the 18 items of the empirical CBCL aggressive behavior scale (Achenbach, 1991; Verhulst & Achenbach, 1995) were used as an additional covariate as part of a post-hoc analysis (see results section).

**Genotyping.** DNA was extracted from blood samples \(n = 1,211\) or buccal swabs with a Cytobrush \(n = 195\) using a manual salting out procedure as described by Miller, Dykes, and Polesky (1988). Genotyping of the length polymorphism 5-HTTLPR was done by simple sequence length analysis (call rate 91.6%). Furthermore, samples were genotyped for the single-nucleotide polymorphism (SNP) rs25331 (an A to G substitution within the 5-HTTLPR L allele by a custom-made TaqMan assay (Applied Biosystems; call rate 96.5%). Genotyping was done at the Research lab for Multifactorial Diseases within the Human Genetics department of the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. Concordance between DNA replicates showed an accuracy of 100%. As presence of the rs25331 G SNP is known to reduce transcriptional efficiency of the L allele, comparable with the S allele (Hu et al. 2006), \(Ig\) alleles were classified as S, while \(Ia\) alleles were classified as L. Based on these alleles, we refer to the functionality
of the expressed transporter as low (SS), intermediate (LS), and high (LL) expression. The 5-HTTLPR genotype distribution was well within Hardy-Weinberg equilibrium in both cohorts \( (p > .99) \).

**Statistical analysis**

Descriptive analyses were conducted using PASW Statistics 19 whereas Structural Equation Modeling (SEM) with robust Maximum Likelihood estimation (MLR) was done using Mplus Version 6.12 (Muthén & Muthén, 1998-2012). The following fit indices were evaluated to test model fit: the Chi-square \( (\chi^2) \) goodness-of-fit test, the Comparative Fit Index (CFI), and the Root-Mean-Square of Approximation (RMSEA). Values for the CFI should preferably be larger than .95 (Hu & Bentler, 1999) and RMSEA should be below .08 and preferably below .05 (Kline, 2005). Missing data were handled using the Full Information Maximum Likelihood (FIML) method. Chi-square goodness of fit tests were used for testing differences in model fit between nested models (Satorra & Bentler, 2001).

We carried out multigroup analyses with regard to the 5-HTTLPR genotypes (SS, LS, and LL) on the longitudinal bidirectional associations between ADHD symptoms and both person-related and environment-related life stressors between T1-T2 and T2-T3. We specified a multigroup model with stability paths for respectively ADHD symptoms and life stressors between available time points, as well as cross-lagged paths between ADHD symptoms and life stressors across subsequent time points. In this model we included the covariates gender (0 = female, 1 = male), age, SES (0 = high, 1 = intermediate, 2 = low), past-year ADHD medication use (0 = no use, 1 = use), and internalizing problems. Adjustment for internalizing symptoms was done given their importance in previous work related to stress \( \times 5-HTTLPR \) interactions (Caspi et al., 2010). Of note, a priori analyses of quadratic growth did not point to non-linear functions of life stressors \((p\text{-values} > .10)\). A \( p < 0.05 \) was used to indicate statistical significance.

Model fitting analyses were conducted in two steps. First, we checked the equivalence and model fit of the stability paths and relations with covariates across the three 5-HTTLPR genotypes. To the extent that initial model fit of this baseline model was not acceptable we added additional parameters based on the modification indices. Second, having established adequate model fit of this baseline model, we tested whether the strength of the paths between the respective variables differed significantly between the SS, LS, and LL genotypes using the \( \Delta\chi^2 \) difference test (Satorra & Bentler, 2001). When the \( \Delta\chi^2 \) difference test was significant, we used subsequent Wald tests to determine whether the strength of the individual parameters differed between the SS, LS, and LL genotypes.

We additionally conducted a number of sensitivity analyses to check whether (1) results differed when stratified for boys and girls, (2) for subdimensions of ADHD symp-
RESULTS

Descriptive statistics
The characteristics of the study sample are shown in Table 1. Correlations between person-related and environment-related life stressors were \( r = 0.22 \) (\( p < .001 \)) at T1-T2 and \( r = 0.33 \) (\( p < .001 \)) at T2-T3. Correlations between ADHD symptoms and internalizing behavior at the same time points ranged from .37 to .45 (all \( p \)-values < .001) for variations of the 5-HTTLPR genotype. There were no significant gene-environment correlations (\( p \)-values ≥ .05).

Path Analyses

Person-related life stressors. The initial baseline model did not meet the recommended thresholds for acceptable model fit (\( \chi^2(223) = 515.73, p < .001, \text{CFI} = .923, \text{RMSEA} = .055 \)). Based on modification indices we added an additional stability path from ADHD symptoms at T1 to ADHD symptoms at T3. An invariant path across the three 5-HTTLPR genotypes was preferred above the variant path (\( \Delta \chi^2(2) = 1.95, p = .38 \)). Since the model fit of the baseline model with one additional path was still not satisfactory (\( \chi^2(222) = 471.30, p < .001, \text{CFI} = .934, \text{RMSEA} = .051 \)), a second additional path from ADHD medication at T1 to ADHD symptoms at T2 had to be included. Again, the invariant path across the three groups was preferred above the variant path (\( \Delta \chi^2(2) = 0.58, p = .75 \)). This final model, presenting good model fit (\( \chi^2(221) = 414.58, p < .001, \text{CFI} = .949, \text{RMSEA} = .047 \)) is displayed in Figure 1. To simplify graphical presentation, we do not show the second additional path from ADHD medication use at T1 to ADHD symptoms at T2 (\( \beta = .14, p < .001 \)), pointing to slightly higher ADHD symptom levels at T2 for ADHD medication users at T1.

The stability paths of ADHD symptoms between T1 and T2 (\( \Delta \chi^2(2) = 1.48, p = .48 \)) and between T2 and T3 (\( \Delta \chi^2(2) = 3.96, p = .14 \)) were not moderated by 5-HTTLPR status. Overall, we found moderate-to-strong positive associations of ADHD symptoms between T1-T2 and between T2-T3, and small-to-moderate associations between T1-T3. Similarly, the stability paths of the number of person-related life stressors between T1-T2 and T2-T3 were positive with a moderate effect size, and were not moderated by 5-HTTLPR status (\( \Delta \chi^2(2) = 2.34, p = .31 \)).

The number of person-related life stressors did not predict subsequent ADHD symptoms according to 5-HTTLPR status (from T1-T2 to T2: \( \Delta \chi^2(2) = 1.48, p = .48 \); from T2-T3 to T3: \( \Delta \chi^2(2) = 1.83 p = .40 \)). However, we found that ADHD symptoms at T2 predicted
Table 1 Sample characteristics per 5-HTTLPR status (total n = 1,306)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SS allele carriers</th>
<th>LS allele carriers</th>
<th>LL allele carriers</th>
<th>Test statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 323</td>
<td>n = 659</td>
<td>n = 324</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population-based cohort, N (%)</td>
<td>259 (80.2%)</td>
<td>571 (86.6%)</td>
<td>291 (89.8%)</td>
<td>$\chi^2(2) = 9.77$</td>
<td>.001</td>
</tr>
<tr>
<td>Age in years, M (SD)</td>
<td>T1-T3 13.64 (0.44)</td>
<td>13.65 (0.45)</td>
<td>13.61 (0.45)</td>
<td>$F(2) = 0.29$</td>
<td>.75</td>
</tr>
<tr>
<td>Male gender, N (%)</td>
<td>168 (52.0%)</td>
<td>322 (48.9%)</td>
<td>168 (51.9%)</td>
<td>$\chi^2(2) = 1.23$</td>
<td>.54</td>
</tr>
<tr>
<td>Dutch ancestrya, N (%)</td>
<td>289 (89.5%)</td>
<td>609 (92.4%)</td>
<td>298 (92.3%)</td>
<td>$\chi^2(2) = 2.66$</td>
<td>.27</td>
</tr>
<tr>
<td>Socio-economic statusb, N (%)</td>
<td>low 100 (7.7%)</td>
<td>220 (17.0%)</td>
<td>107 (8.2%)</td>
<td>$\chi^2(4) = 3.41$</td>
<td>.49</td>
</tr>
<tr>
<td></td>
<td>medium 156 (12.0%)</td>
<td>330 (25.4%)</td>
<td>166 (12.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>high 64 (4.9%)</td>
<td>106 (8.2%)</td>
<td>48 (3.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD medication usec, N (%)</td>
<td>T1 22 (6.8%)</td>
<td>50 (7.6%)</td>
<td>12 (3.7%)</td>
<td>$\chi^2(2) = 5.55$</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td>T2 34 (10.5%)</td>
<td>64 (9.7%)</td>
<td>37 (11.4%)</td>
<td>$\chi^2(2) = 0.70$</td>
<td>.71</td>
</tr>
<tr>
<td></td>
<td>T3 22 (6.8%)</td>
<td>52 (7.9%)</td>
<td>17 (5.2%)</td>
<td>$\chi^2(2) = 2.36$</td>
<td>.31</td>
</tr>
<tr>
<td>Internalizing problemsd, M (SD)</td>
<td>T1 0.26 (0.21)</td>
<td>0.27 (0.22)</td>
<td>0.26 (0.21)</td>
<td>$F(2) = 0.40$</td>
<td>.67</td>
</tr>
<tr>
<td></td>
<td>T2 0.22 (0.20)</td>
<td>0.22 (0.19)</td>
<td>0.21 (0.20)</td>
<td>$F(2) = 0.47$</td>
<td>.62</td>
</tr>
<tr>
<td></td>
<td>T3 0.23 (0.20)</td>
<td>0.20 (0.21)</td>
<td>0.22 (0.22)</td>
<td>$F(2) = 0.52$</td>
<td>.59</td>
</tr>
<tr>
<td>Main variables</td>
<td>ADHD symptoms e, M (SD)</td>
<td>T1 0.67 (0.53)</td>
<td>0.59 (0.51)</td>
<td>0.59 (0.48)</td>
<td>$F(2) = 2.77$</td>
</tr>
<tr>
<td></td>
<td>T2 0.52 (0.48)</td>
<td>0.46 (0.47)</td>
<td>0.46 (0.44)</td>
<td>$F(2) = 2.14$</td>
<td>.12</td>
</tr>
<tr>
<td></td>
<td>T3 0.48 (0.45)</td>
<td>0.44 (0.43)</td>
<td>0.41 (0.42)</td>
<td>$F(2) = 1.97$</td>
<td>.14</td>
</tr>
<tr>
<td>Person-related life stressorsf,g, M (SD)</td>
<td>T1-T2 0.41 (0.65)</td>
<td>0.39 (0.66)</td>
<td>0.40 (0.63)</td>
<td>$F(2) = 0.44$</td>
<td>.96</td>
</tr>
<tr>
<td></td>
<td>T2-T3 0.78 (1.02)</td>
<td>0.66 (0.87)</td>
<td>0.65 (0.87)</td>
<td>$F(2) = 2.07$</td>
<td>.13</td>
</tr>
<tr>
<td>Environment-related life stressorsh, M (SD)</td>
<td>T1-T2 0.32 (0.51)</td>
<td>0.32 (0.57)</td>
<td>0.25 (0.47)</td>
<td>$F(2) = 2.73$</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>T2-T3 0.48 (0.75)</td>
<td>0.44 (0.70)</td>
<td>0.38 (0.67)</td>
<td>$F(2) = 1.75$</td>
<td>.17</td>
</tr>
</tbody>
</table>

Note. ADHD = Attention-Deficit/Hyperactivity Disorder

* Both parents were born in the Netherlands.
* Groups based on sum score of five indicators (household income and both parents’ occupation and education)
* Methylphenidate, dexamphetamine and atomoxetine use at any time during the past year (1) versus non-use (0)
* Mean of 32-item DSM-oriented broadband internalizing scale of the Child Behavior Checklist (CBCL; Achenbach, 1991; score range 0-2)
* Mean of 7-items DSM-IV-oriented ADHD subscale of the CBCL (Achenbach, 1991; score range 0-2)
* T1-T2 < T2-T3 at $p < .001$.
* Mean number of exposure to life stressors in the past two years before T2 and T3 (score range 0-3)
* SS < LS, SS < LL at $p < .05$. 
person-related life stressors at T2-T3 ($\Delta \chi^2(2) = 12.58, p = .002$) depending on 5-HTTLPR status. Subsequent analyses showed a significant difference between the SS and LS (Wald's statistic: $\chi^2(1) = 11.39, p < .001$), and SS and LL (Wald's statistic: $\chi^2(1) = 10.28, p = .001$), but not between the LS and LL (Wald's statistic: $\chi^2(1) = 0.73, p = .39$) genotypes of the 5-HTTLPR. Thus, only for adolescents with the SS genotype higher levels of ADHD symptoms at T2 significantly predicted exposure to a higher number of person-related life stressors at T2-T3 ($\beta = .20, p < .001$), but not for those with the LS ($\beta = .03, p = .48$) or LL genotype ($\beta = -.04, p = .58$) of the 5-HTTLPR. To illustrate this finding, of the S-allele homozygotes with clinical or subclinical levels of ADHD symptoms at T2 50.9% experienced two or more person-related life stressors at T3 as compared to 15.0% in L-allele homozygotes with clinical or subclinical levels of ADHD symptoms. Moreover, although the 5-HTTLPR status did not differentiate associations between ADHD symptoms at T1 and person-related life stressors at T1-T2 ($\Delta \chi^2(2) = .44, p = .80$), there were small significant associations within each of the SS, LS, and LL genotypes (all $\beta = .08, p$-values < .001).

**Figure 1.** Standardized path coefficients for the associations between ADHD symptoms and the number of person-related life stressors between early (T1), middle (T2), and late (T3) adolescence in relation to 5-HTTLPR status.

Note. ADHD = Attention-Deficit/Hyperactivity Disorder symptom severity. Left, middle, and right path coefficients represent the SS ($n = 323$), LS ($n = 659$), and LL genotypes ($n = 324$) of the 5-HTTLPR genotype, respectively.

***p < .001 indicate significant within-group differences for corresponding path coefficients; brackets represent significant between-group differences in relation to the SS, LS and LL genotypes of 5-HTTLPR ($p < .05$).

**Environment-related life stressors.** As with the model examining the number of person-related life stressors, the initial baseline model had no acceptable fit ($\chi^2(223) = 481.21, p < .001$, CFI = .924, RMSEA = .052). Also here two additional invariant paths were added to the model: a stability path from ADHD symptoms at T1 to ADHD symptoms at T3 and one from ADHD medication at T1 which was regressed on ADHD symptoms at T2. This single adjustment to the initial baseline model resulted in an adequate model.
fit ($\chi^2(221) = 379.54, p < .001, \text{CFI} = .953, \text{RMSEA} = .041$). The final model is displayed in Figure 2 without the second additional path from ADHD medication use at T1 to ADHD symptoms at T2 ($\beta = .14, p < .001$), pointing to slightly higher ADHD symptom levels at T2 in ADHD medication users at T1.

None of the $\Delta \chi^2$ difference tests indicated significant differences across the three $5\text{-HTTLP}$R genotypes regarding the bidirectional associations of ADHD symptoms with environment-related life stressors, and vice versa, between any time points ($p$-values between .06 and .83). Thus, environment-related life stressors did not predict ADHD symptoms, nor the other way around.

**Sensitivity analyses.** All sensitivity analyses confirmed the results of the main analyses, leaving the prospective association between ADHD symptoms at T2 and person-related life stressors at T2-T3 moderated by $5\text{-HTTLP}$R intact both for boys ($\Delta \chi^2(2) = 8.30, p = .02$) and girls ($\Delta \chi^2(2) = 6.74, p = .001$); for inattention ($\Delta \chi^2(2) = 11.45, p = .003$) and hyperactivity-impulsivity symptoms ($\Delta \chi^2(2) = 9.21, p = .01$); and when excluding adolescents of non-Dutch ancestry ($\Delta \chi^2(2) = 10.35, p = .006$).

**Post-hoc analyses.** As results gave rise to the question whether the association between ADHD symptoms and person-related stressors might have been accounted for by co-occurring aggression problems (e.g., by initiating fights with peers), we additionally covaried for aggression problems, also confirming the main results ($\Delta \chi^2(2) = 13.36, p = .001$). Thus, results including examination of person-related life stressors and all other subanalyses were similar to the main results, both in terms of significance levels as well
as direction and magnitude of effects. Finally, to verify that the lack of a prospective association between life stressors and ADHD symptoms in our path analysis models was not an artefact deriving from reduced variance in the life stressor variables, we performed separate linear regression analyses for the 5-HTTLPR genotypes (i.e., regressing T2 ADHD symptoms on T1-T2 stressors, and T3 ADHD symptoms on T2-T3 stressors) adjusted for covariates. In line with our path analyses, we did not find significant associations (all p-values above .28).

**DISCUSSION**

This longitudinal three-wave study investigated bidirectional relations between exposure to (in)dependent life stressors and ADHD symptoms moderated by the 5-HTTLPR genotype in a large pooled general population and clinic-referred cohort spanning early to late adolescence. In contrast to our expectations, our results do not support recent prospective evidence for a G×E interaction between the 5-HTTLPR genotype and exposure to life stressors predicting ADHD symptoms (Van der Meer et al., 2014), nor associations with environment-related (‘independent’) life stressors. However, as hypothesized, ADHD symptoms predicted exposure to person-related (‘dependent’) life stressors (e.g., conflicts with parents or peers, lack of friends, being bullied, school pressures) at two-year follow-up. In particular, adolescents homozygous for the S-allele with higher levels of ADHD symptoms during middle adolescence were exposed to more person-related life stressors in late adolescence. Overall, our findings suggest differential developmental risk pathways to stress exposure associated with psychopathology, varying as per type of stressor, adolescent developmental stage, and underlying genetic vulnerability (Johnson et al., 2013).

**ADHD symptoms predict person-related life stressors**

Our findings substantially add to the sparse longitudinal studies which predominantly focused on children reporting unidirectional effects of ADHD symptoms on exposure to specific person-related stressors. For example, Lifford and colleagues (2009) found that boys’ ADHD symptoms prospectively impacted upon hostile mother-son relations, and not vice versa. Further, it has been reported that 5-year-olds with ADHD symptoms were at elevated risk of social isolation at age 12 years, but not the other way around (Matthews et al., 2015). Also, the presence of ADHD symptoms in preschoolers was associated with the risk of becoming a bully-victim in elementary school (Verlinden et al., 2015). We expand this literature to an adolescent population in the transition into adulthood, while investigating a set of stressors, and pointing to the developmental influence of the 5-HTTLPR genotype.
An explanation for the prospective relation between ADHD symptoms and person-related life stressors is that exposure to these life stressors is a consequence of an individual's personal characteristics, for example, through evoking certain responses in others that may lead to social adversities in interrelationships (Caspi et al., 2005; Dick, 2011; Hampson, 2008; Nijmeijer et al., 2008; Reiss, 2010). Beyond early adolescence, conflicts with parents and peers gain in affective importance (Laurens et al., 1998), given the increasing social sensitivity during adolescence (Schriber & Guyer, 2016), strive for independence, and greater significance of and time spent with peers (Arnett, 1999). Moreover, an individual's characteristics may be associated with the likelihood to get into high risk environments that may set off further problems (Dick et al., 2006; Dick, 2011), such as placement in special education school, in which the child may become exposed to other children with problem behaviors and peer conflicts. On another note, increasing cognitive demands throughout middle and high school may place adolescents with ADHD symptoms at risk for experiencing high pressures at school, another frequent person-related stressor.

No significant gene-environment correlations were found between ADHD symptoms and 5-HTTLPR genotype, which implies that our finding represents a true G×E interaction (Dick, 2011). Thus, being S-allele homozygous of the 5-HTTLPR genotype and having high levels of ADHD symptoms appears to increase exposure to more person-related life stressors in later adolescence; this points to genetic influences on becoming exposed to this type of stressors during development, consistent with Johnson and colleagues (2013). One explanation for this finding is that S-allele homozygotes with a higher number of ADHD symptoms might possess specific personal characteristics, such as aggression or hostility, trait impulsivity, less efficient response inhibition, or autistic traits that regulate social communication (Nijmeijer et al., 2008), known to be related to the S-allele of 5-HTTLPR genotype (Brune et al., 2006; Landrø et al., 2015; Lesch & Merschdorf, 2000; Oades et al., 2008), and that may impact on life stress exposure. Another possibility is that, in line with the comprehensive stress literature regarding the role of 5-HTTLPR genotype (Caspi et al., 2010; Karg et al., 2011), adolescent S-allele homozygotes show an increased sensitivity and reactivity to environmental cues, reflecting an underlying vulnerability to encounter life problems on a broader scale. Individuals are not just passive recipients of life stressors, but there is a continuous interplay between person and environment shaping one's course of life (Cox et al., 2010; Hampson, 2008; Lerner, 1991; Shanahan & Boardman, 2009). To illustrate, adolescents with ADHD symptoms who have become bully-victims and who exhibit high stress sensitivity may develop even more withdrawn, unadjusted, or aberrant behavior, placing them at further risk of being bullied, isolated, having poor-quality relationships, or underachieving at school. Eventually, this may result in a vicious cycle with poor long-term outcomes in multiple domains of daily living.
Interestingly, in early adolescence ADHD symptoms significantly predicted person-related life stressors independently from the 5-HTTLPR genotype. This points to developmental serotonin-related changes across adolescence, as suggested by previous research (e.g., Shoval et al., 2014; Thissen et al., 2015). In particular, the 5-HTTLPR genotype mediated vulnerability may operate during sensitive developmental periods such as late adolescence and young adulthood (Uher & McGuffin, 2008). Taken together, findings are in line with the notion that there is a complex and dynamic interplay between genes and environment changing over the life course (Shanahan & Boardman, 2009).

**Environment-related life stressors were not associated with ADHD symptoms**

Environment-related (‘independent’) life stressors, which mainly occur independently from the individual’s own behavior, did not predict ADHD symptoms moderated by the 5-HTTLPR genotype. Although previous literature has indicated associations between independent life stressors and the broader domain of externalizing problems (e.g., Ciu et al., 2007; Rudolph et al., 2000; Schneiders et al., 2003), also negative findings have been reported (Conway et al., 2014). In our study population, stress exposure (e.g., neighborhood violence) may not have been severe enough to impact on ADHD symptoms (Karg et al. 2011). Moreover, as it has been shown that shared environmental influences (e.g., within the family system) decrease in magnitude as children transition into adolescence (Johnson et al., 2013), we suspect that our study population may already have passed the critical susceptible age to show an association between (family-based) environment-related stressors and ADHD symptoms.

**Life stressors did not predict ADHD symptoms**

Our nullfinding of an effect of life stressors on ADHD symptoms moderated by the 5-HTTLPR genotype appeared not to be a consequence of our pathway model or the presence of non-linear relationships. A few factors may have contributed to our nullfinding compared to previous evidence (Van der Meer et al., 2014; Retz et al., 2008). Most notably, we investigated long-term difficulties as stress measure and not classic incidental life events (e.g., abuse, death of a loved one), although the long-term stressors were similar as in Van der Meer and colleagues (2014). Also, there were some differences with the study population of the previous studies (Van der Meer et al., 2014; Retz et al., 2008), we included the full range of ADHD symptoms from a population-based and clinic-referred sample and exclusively focused on adolescence, whereas the other samples also had young adults and only consisted of persons with more severe symptomatology. Perhaps life stressors have a more pronounced influence with increasing age and/or increasing severity of symptoms. Further studies are warranted to confirm the earlier potentially
interesting findings taking the nature, timing, and severity of life stressors and ADHD symptomatology into account.

**Strengths and limitations**

A strength of this study was the use of a large, longitudinal dataset enabling us to examine bidirectional effects between ADHD symptoms and the exposure to (in)dependent life stressors in relation to 5-HTTLPR status spanning early to late adolescence. Findings were independent from co-occurring internalizing and aggression problems. Additional analyses confirmed that the results were not influenced by sex and ADHD symptom subdimensions (i.e., inattention and hyperactivity-impulsivity). While the use of a CBCL subscale to assess ADHD symptoms may be seen as suboptimal as it does not capture all 18 DSM ADHD symptoms, it has been shown to have adequate discriminative validity (Nakamura et al., 2009). Other limitations have been the use of a small number of parent-reported life stressors, assessing long-term stressors rather than severe adverse life events through which important aspects of life stress may not have been captured. Also the retrospective recall of life stressors may be a potential limitation because of under- and overestimations of stress exposures. While one may question the relevance of some of the person-related stressors (e.g., chronic illness/handicap) we kept with the original scale classification; however, confounding of results is unlikely to have happened, as these stressors typically do not arise as new stressors within the time of our study. Further of note, although the prospective design and the involvement of genetic moderation, as well as the theoretical background may suggest causative pathways between ADHD symptoms and exposure to life stressors, we can neither exclude the influence of other unmeasured traits associated with ADHD symptoms, nor specify whether ADHD symptoms in itself are a necessary or sufficient cause for evoking life stressors. Yet, we could exclude the possibility that our findings were driven by aggression problems. It also deserves mentioning that our pathway model with only two time points of stress exposure does not allow for partialling out within- and between individual patterns and therefore limits causal inferences (Hamaker et al., 2015). Moreover, it should be noted that present findings on the full spectrum of ADHD symptoms in adolescents may not generalize to clinical samples with a diagnosis of ADHD, nor to other age groups. Finally, given the novelty of the findings and concerns about replication in candidate gene studies, future replication is warranted.

**Conclusions**

In conclusion, our study emphasizes that genetically vulnerable adolescents homozygous for the S-allele of the 5-HTTLPR genotype with higher ADHD symptom levels in middle adolescence are at increased risk of becoming exposed to person-related life stressors in late adolescence. Findings support the idea that exposure to person-related
life stressors is not merely a matter of ‘fateful coincidence’, but is influenced by an individual’s personal characteristics and genetic predisposition. This vulnerability likely varies over the life course. Importantly, and also of direct clinical relevance, our study highlights the need for assessment and prevention particularly of social-emotional adversities associated with interpersonal and academic problems (i.e., person-related life stressors) that may occur in the transition from adolescence into adult life in individuals with ADHD symptoms. In other words, clinicians, parents, and teachers should also be aware of the future consequences of problem behavior. Future research should identify the most vulnerable individuals investigating long-term outcomes in multiple domains of functioning far into adulthood including the role of the \(5\)-HTTLPR. Future research could also address the potential role of variation in positive environments given evidence that \(5\)-HTTLPR may represent a plasticity gene rather than a risk gene per se (Belsky & Pluess, 2009).
REFERENCES


Chapter 4 | Gene × stress


