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Interactions between Genetic, Prenatal, Cortisol, and Parenting Influences on Adolescent Substance Use and Frequency: A TRAILS Study

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Keywords
Polygenic risk · G×E · Prenatal stress · Warm parenting · Cortisol reactivity · Adolescent substance use · TRAILS

Abstract
Introduction: Dynamic relations between genetic, hormone, and pre- and postnatal environments are theorized as critically important for adolescent substance use but are rarely tested in multifactorial models. This study assessed the impact of interactions of genetic risk and cortisol reactivity with prenatal and parenting influences on both any and frequency of adolescent substance use. ***Methods:*** Data are from the TRacking Adolescents' Individual Lives Survey (TRAILS), a prospective longitudinal, multi-rater study of 2,230 Dutch adolescents. Genetic risk was assessed via 3 substance-specific polygenic scores. Mothers retrospectively reported prenatal risk when adolescents were 11 years old. Adolescents rated their parents' warmth and hostility at age 11. Salivary cortisol reactivity was measured in response to a social stress task at age 16. Adolescents' self-reported cigarette, alcohol, and cannabis use frequency at age 16. ***Results:*** A multivariate hurdle regression model showed that polygenic risk for smoking, alcohol, and cannabis predicted any use of each substance, respectively, but predicted more frequent use only for smoking. Blunted cortisol reactivity predicted any use and more frequent use for all 3 outcomes. There were 2 interactions: blunted cortisol reactivity exacerbated the association of polygenic risk with any smoking and the association of prenatal risk with any alcohol use. ***Conclusion:*** Polygenic risk seems of importance for early use but less so for frequency of use, whereas blunted cortisol reactivity was correlated with both. Blunted cortisol reactivity may also catalyze early risks for substance use, though to a limited degree. Gene-environment interactions play no role in the context of this multifactorial model.

Introduction

About 90% of individuals with substance use disorders began using in adolescence, highlighting the importance of understanding which adolescents are likely to engage in substance use at early ages [1]. Genetic and both prenatal and postnatal environmental influences are important in the development of alcohol, tobacco, and cannabis use and misuse in adolescence [2]. Further, patterns of

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gene-postnatal environment interaction (G×E) have been found to predict a variety of adolescent substance use phenotypes in twin/family studies [3], candidate gene studies [4], and more recently in studies utilizing polygenic scoring methods of assessing genetic risk [5]. Across these methods, studies most commonly focus on the interaction between genetic risk and high-risk environments, with poorer or more restrictive environments hypothesized to exacerbate genetic risk for a variety of substance use outcomes [5]. Findings typically fit with the diathesis-stress model, which posits that an environmental catalyst strengthens the risk associated with inherited predisposition toward substance abuse. Environmental factors in these investigations most frequently include measures of stress, such as childhood adverse events, trauma or maltreatment, peer factors (i.e., peer substance use and relationships), or parenting factors (i.e., monitoring, rejection, education, and substance use) [6].

The substantial work on G×E for substance use has shown the importance of contextualizing genetic risks for substance use, but has focused nearly entirely on postnatal environments [7]. The neglect of prenatal risk is a key oversight because there is evidence that the prenatal environment plays an organizational role setting the stage for future development, including for substance use [8]. The few studies of genetic-by-prenatal environment interactions examine childhood externalizing problems (a phenotype strongly associated with adolescent substance use), and primarily consist of candidate gene studies showing that maternal substance use during pregnancy exacerbates genetic risk for externalizing problems in offspring, consistent with the diathesis-stress model [9]. This diathesis-stress pattern was also found using an adoption design, although only for children experiencing high levels of parental hostility [7]. A handful of studies have also examined prenatal-by-postnatal interactions primarily for childhood externalizing problems and have found that less warm or more hostile parenting can exacerbate the effects of prenatal risk factors on children's behavior problems [7, 10]. Recent work has found that the combination of genetic, prenatal, and postnatal (e.g., marital hostility [11] and parent-child hostility [7]) risk factors yielded the highest levels of childhood externalizing problems (at 4.5 years [11] and 6–8 years for boys [7]). However, evidence regarding substance use phenotypes (i.e., any use and frequency of use) for these interactions is lacking.

Genetic vulnerability and both prenatal and postnatal environmental influences have been linked to cortisol reactivity and substance use [12]. Furthermore, the influence of environmental stressors should, in theory, operate through calibration of the stress response system [13]. However, physiological aspects of stress have not been considered as contextual factors moderating the influence of genetics of addiction. Cortisol reactivity to stress may also interact with more environmental influences to predict substance use outcomes. Some studies have shown that parent-child closeness and conflict can moderate the influence of stress and pubertal hormones on adolescent adjustment [14, 15], though these studies have not been extended to adolescent substance use. Despite theories suggesting that prenatal risk exposures organize the development of the stress response system in a way that has ramifications for the adolescent brain and behavior [16], studies investigating the potential organizing role of prenatal risk exposures on the effect of cortisol reactivity for adolescent substance use via interactions are needed. Therefore, this study assessed the impact on any use and frequency of adolescent substance use of interactions of genetic risk and cortisol reactivity with prenatal and parenting influences using longitudinal data (Fig. 1). We expected that: (1) prenatal risk, less warm parenting, and dysregulated cortisol reactivity would strengthen the influence of genetic risk on substance use; (2) less warm parenting and dysregulated cortisol reactivity would increase the influence of prenatal risk, on substance use; and (3) dysregulated cortisol reactivity would increase the influence of less warm childhood parenting on substance use later in adolescence. These analyses were not preregistered and the results should be considered exploratory.

Materials and Methods

We tested whether polygenic risk, prenatal risk, lower levels of warm parenting at age 11 years, and cortisol reactivity to a social stress challenge at age 16 years predicted adolescent smoking, alcohol, and cannabis use at 16 years independently and in interaction.

Sample

Participants were from TRAILS (TRacking Adolescents' Individual Lives Survey). TRAILS are a longitudinal study designed to track the development of mental and physical health from preadolescence into adulthood, and includes 2,230 Dutch adolescents (51% female, 85% European ancestry, and representative of the Northern part of the Netherlands). Adolescents were followed prospectively beginning at age 11 years (M = 11.11, standard deviation [SD] = 0.56, range = 10–13 years), with follow-up assessments at average ages of 13 and 16 years. Children were recruited from primary schools within 5 municipalities, beginning in March 2001. Exclusion criteria included mental retardation or physical illness preventing participation and unavailability of a Dutch-speaking parent or guardian. Of the eligible children, 76% (N =

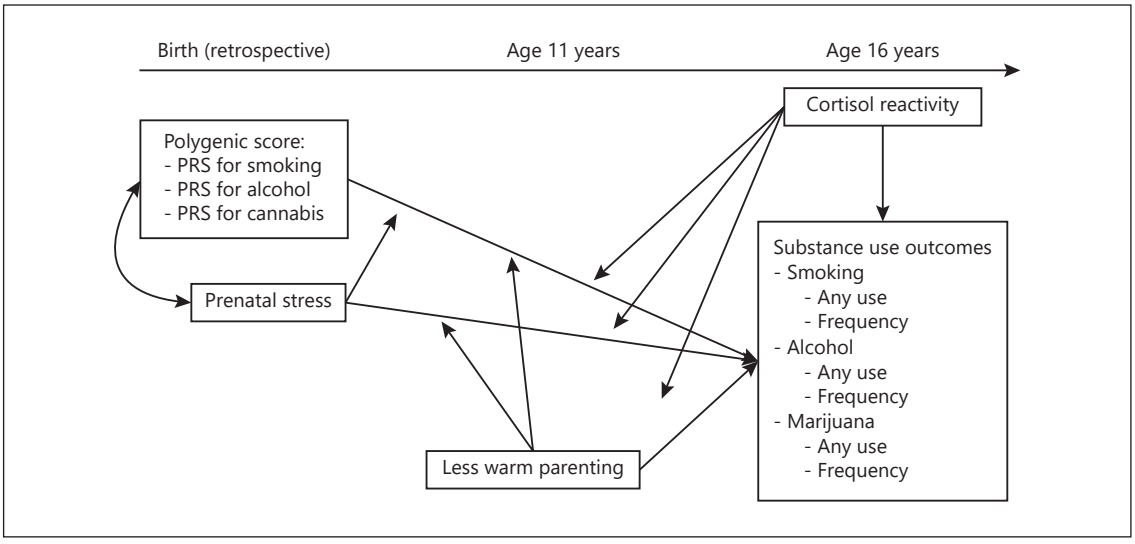

Fig. 1. Conceptual model. All 6 outcomes (any use and frequency of all 3 substances) were modeled, simultaneously. Arrows pointing to other arrows indicate hypothesized moderation of paths (i.e., the construct where the arrow begins is conceptualized as the moderator of the path that it points to). PRS, polygenic risk score.

Table 1. Sample descriptive statistics for survey data

	N	Mean	(SD)	Range
Prenatal stress	2,230	0.78	(0.88)	0.00–5.00
Warm parenting	2,207	0.00	(0.81)	−5.03 to 1.56
Cortisol reactivity	656	−0.03	(0.91)	−6.18 to 3.78
Smoking frequency	1,374	1.58	(2.38)	0.00–7.00
Drinking frequency	1,627	3.61	(3.58)	0.00–13.00
Cannabis use frequency	1,632	0.68	(2.38)	0.00–13.00

Smoking frequency = number of cigarettes consumed in the past month (age 16). Drinking frequency = number of times alcoholic drinks consumed in the past month (age 16). Cannabis use frequency = number of times used in the past month (age 16). Scores for polygenic risk, prenatal stress, warm parenting (age 11), and cortisol reactivity (age 16) reflect descriptive statistics prior to standardizing. SD, standard deviation.

2,230) agreed to participate and were enrolled in the study. Specific sample sizes for each phenotype are in Table 1. The study was approved by the Dutch Central Committee on Research Involving Human Subjects and by Purdue University IRB #1702018756. See [17, 18] for further detail on TRAILS.

Measures

Substance Use

Adolescent self-report of past-month frequency of alcohol, tobacco, and cannabis use were assessed at age 16. Alcohol and cannabis use were reported as counts of units used per day until 10 and thereafter categorized as 11–19 times, 20–39 times, and 40 or more times. Tobacco use was reported as none, <1 cigarette/week, <1 cigarette/day, 1–5 cigarettes/day, 6–10 cigarettes/day, 11–20 cigarettes/day, and >20 cigarettes/day. These variables were zero-inflated, and so were modeled as 2 phenotypes per substance – any use, and if there was any use, frequency of use (see Analytic Strategy).

Genetic Data

Children (N = 1,491) were genotyped for 295,173 single nucleotide polymorphisms (SNPs; all autosomes and × chromosome) using the Illumina Cyto SNP12 v2 array. Genetic data were imputed to the 1,000 Genomes Reference Panel [19] using the Phase 1 global reference set (March 2012), and standard quality control was conducted. We excluded SNPs with call rate <95%, minor allele frequency <5%, missingness >5%, Hardy-Weinberg disequilibrium p value below 1E−06, and individuals with more than 5% missing on SNP data or from non-European ancestry, as described elsewhere [20, 21]. Our main analysis included 3 substance-specific PRS. The largest studies available to date were used to create the substance-specific scores [20]: smoking (having smoked on a reg-

ular basis, and cigarettes per day [22] using multi-trait analysis of GWAS (MTAG), alcohol consumption in glasses per week [22], and lifetime cannabis use (excluding the TRAILS sample [23]). The PRS were created by summing an individual's risk alleles per locus, weighted by the effect size from the relevant source GWAS, after adjusting SNP effect size weights for linkage disequilibrium using the GCTA-SBLUP tool [24]. See [20] for full details on these scores. The first 10 ancestral principal components were included in a baseline regression as predictors of the PRS in order to control for population stratification [25] – the residual was saved and used as a predictor in subsequent hypothesis-testing analyses.

Prenatal Risk

The measures of prenatal risk have been previously described [26, 27]. The TRAILS Family History Interview assessed 12 prenatal experiences retrospectively reported by mothers when children were age 11. Specifically, smoking during pregnancy was assessed by asking mothers whether, and if so, how much, the mother had smoked during pregnancy, categorized into nonsmokers = 0 (67.6%), mild smokers = 1 (<10 cigarettes a day; 23.0%), and heavy smokers = 2 (>10 cigarettes a day; 6.7%; n = 2,169). Alcohol use during pregnancy was assessed by asking mothers whether, and if so, how much, the mother had consumed alcohol during pregnancy, categorized into nondrinkers = 0 (79.0%), mild drinkers = 1 (<1–3 drinks a week; 17.0%), and heavy drinkers = 2 (>4 drinks a week; 1.1%; n = 2,166). These scores, along with the presence of pregnancy complications (i.e., physical, social, or psychological problems during pregnancy), complicated deliveries (i.e., breech presentation and Caesarean section), and hospitalization of the mother (i.e., due to physical problems and postnatal depression) or child (i.e., lack of oxygen, blood transfusion, and jaundice) were summed (as long as individuals had data for 6 or more items, n = 2,187), and winsorized to 4 to correct the distribution (range = 0 [45%]–4, M = 1.87, SD = 2.19) following prior studies [26, 27].

Warm Parenting

TRAILS researchers previously developed a warm parenting at age 11 years [28]. Separately for mothers and fathers, children completed the rejection (α = 0.85-0.88, mother-father r = 0.68), and emotional warmth (α′s = 0.91, mother-father r = 0.71) subscales of the EMBU-C (a Swedish acronym for My Memories of Upbringing; [29]) for children. Rejection and warmth composites were moderately correlated (r = −0.35, p < 0.001). After reverse-coding rejection scores, child reports on warmth and rejection were averaged in order to arrive at a single score that reflects parent-child relationship quality, with higher scores indicating more warmth/support. Lower levels on this score would indicate lower levels of warm parenting (i.e., inverse associations are expected).

Cortisol Reactivity

Cortisol reactivity was assessed in response to the Groningen Social Stress Task (GSST; 30), the Dutch version of the standard Trier Social Stress Test, which elicits a cortisol response through an uncontrollable social evaluative stressor by subjecting participants to a speech task and a mental arithmetic task, judged by 2 research assistants. Cortisol levels were assessed just before the start of the GSST, 20 min later, and 40 min after baseline via Salivette sampling device (Sarstedt, Numbrecht, Germany; see [30] for details on collection, storage, and assays). Cortisol values for 42 participants were set to missing due to medical exclusions (corticosteroids, SSRIs, pain medications, and smoking prior to the GSST). As in prior reports [21, 31], the post-GSST sample was regressed on the pre-task sample, and the standardized residuals saved as the measure of cortisol reactivity. Higher values on this continuous score indicate higher cortisol reactivity.

Analytic Strategy

All main effects and two-way interactions of the predictors (PRS, prenatal risk, less warm parenting, and cortisol reactivity) were included in a negative binomial hurdle regression model. Briefly, hurdle models are 2-stage regression models designed to test count outcomes with a high degree of zero-inflation, and are recommended for analyses of substance use frequency [32]. Thus, these models analyze an outcome with 2 thresholds, creating 2 outcomes that are included simultaneously, first a yes/no outcome, and second a count outcome in case of yes. In this case, adolescents must clear the "use" hurdle (i.e., endorse any substance use) in order to score non-zero values for frequency of use. The zero-inflation part (referred to as "any use") is operationalized in these models as the inability to clear the "use" hurdle; those who do not endorse substance use are a combination of those who did not initiate yet and those who have initiated use at some point but not used in the past month. Any use and frequency of past month use (the count portion) are modeled, simultaneously. A single model was fit that included all 3 outcomes (alcohol, tobacco, and cannabis use frequency) simultaneously, using a structural equation modeling framework. This framework accounts for the correlated nature of the predictors (i.e., rather than artificially splitting the problem into parts), although correlated outcomes in multivariate hurdle models are not currently possible. Age and sex were included as covariates. Analyses were conducted in Mplus [33], using the estimator = MLR option which estimates standard errors that are robust to non-normality and uses Full Information Maximum Likelihood to accommodate missing data. The model was based on all 2,230 adolescents (see online suppl. Appendix A for Mplus output and Table 1 for construct-specific sample sizes; for all online suppl material, see www.karger.com/doi/10.1159/000519864). All variables were standardized to a mean of 0 and SD of 1 prior to analysis. Interactions were probed with simple slopes at the mean, 20th and 80th percentiles of the moderator.

Results

Of the participating adolescents, 34.3% reported any amount of smoking (range = 1–20 + cigarettes/day), 77.2% reported any amount of alcohol use (range = 1–40 + times), and 13.0% reported any amount of cannabis use in the past 4 weeks (range = 1–40 + times). Full model results, including descriptive statistics are provided in Appendix A; there were correlations among the various PRS, as expected, r = 0.17–0.24, and among some interaction terms $|r|$ < 0.32, but otherwise correlations among predictors were small, $|r|$ < 0.12. We provide relevant parameter estimates for smoking in Table 2, alcohol in Table 3, and cannabis in Table 4. Results are graphically summarized in Figure 2.

Table 2. Smoking results from hurdle regression model

Main effects	Smoking							
	any use				frequency			
	β	*B*	(SE)	*p* value	β	*B*	(SE)	*p* value
Polygenic risk (PRS)	**−0.25**	**−0.57***	**(0.08)**	**<0.001**	0.50	0.05*	(0.02)	0.007
Prenatal stress	−0.05	−0.10	(0.07)	0.157	0.29	0.03	(0.02)	0.085
Warmer parenting	0.07	0.15	(0.08)	0.060	−0.10	−0.01	(0.02)	0.605
Cortisol reactivity	0.41	0.87*	(0.17)	<0.001	−0.69	−0.07*	(0.02)	0.001
Interactions								
PRS × prenatal stress	0.00	−0.01	(0.08)	0.918	0.05	0.01	(0.02)	0.776
PRS × warmer parenting	0.02	0.04	(0.08)	0.619	−0.18	−0.02	(0.02)	0.235
PRS × cortisol reactivity	0.13	0.27*	(0.11)	0.016	0.10	0.01	(0.01)	0.372
Prenatal stress × warmer parenting	−0.01	−0.02	(0.08)	0.770	0.25	0.03	(0.02)	0.135
Prenatal stress × cortisol reactivity	−0.18	−0.39	(0.23)	0.087	−0.10	−0.01	(0.02)	0.559
Warmer parenting × cortisol reactivity	−0.18	−0.45	(0.32)	0.155	−0.15	−0.02	(0.02)	0.391
Covariates								
Sex	0.01	0.03	(0.08)	0.681	−0.06	−0.01	(0.02)	0.734
Age	−0.02	−0.04	(0.07)	0.553	0.13	0.01	(0.02)	0.448

β = Standardized betas; *B* = Unstandardized betas with the associated SE and *p* value. PRS for smoking. Bolded effects likely survive adjustment for multiple testing. The any use part of the model predicts the likelihood of having a 0 or not passing the use threshold, whereas the frequency part predicts more frequent use. All effects across Tables 2–4 were estimated in the same model. SE, standard error; PRS, polygenic risk score. * $p < 0.05$.

Table 3. Alcohol results from hurdle regression model

Main effects	Alcohol							
	zero-inflation				frequency			
	β	*B*	(SE)	*p* value	β	*B*	(SE)	*p* value
Polygenic risk (PRS)	−0.13	−0.25*	(0.07)	0.001	0.17	0.04	(0.03)	0.150
Prenatal stress	−0.07	−0.13	(0.07)	0.060	0.07	0.02	(0.03)	0.548
Warmer parenting	−0.02	−0.04	(0.07)	0.604	−0.07	−0.02	(0.03)	0.531
Cortisol reactivity	0.19	0.36*	(0.11)	0.001	−0.73	**−0.16***	**(0.04)**	**<0.001**
Interactions								
PGS × prenatal stress	0.00	0.00	(0.07)	0.973	0.12	0.03	(0.03)	0.363
PGS × warmer parenting	−0.02	−0.04	(0.08)	0.593	−0.19	−0.04	(0.03)	0.143
PGS × cortisol reactivity	0.00	0.00	(0.11)	0.986	−0.10	−0.02	(0.03)	0.528
Prenatal stress × warmer parenting	−0.03	−0.06	(0.07)	0.442	−0.03	−0.01	(0.03)	0.801
Prenatal stress × cortisol reactivity	−0.24	−0.45*	(0.14)	0.001	0.16	0.04	(0.05)	0.490
Warmer parenting × cortisol reactivity	−0.13	−0.28	(0.16)	0.082	0.33	0.08	(0.05)	0.118
Covariates								
Sex	−0.04	−0.09	(0.07)	0.227	0.64	**0.14***	**(0.03)**	**<0.001**
Age	−0.02	−0.03	(0.07)	0.601	0.05	0.01	(0.03)	0.644

β = Standardized betas; *B* = Unstandardized betas with the associated SE and *p* value. PRS. Bolded effects likely survive adjustment for multiple testing. The any use part of the model predicts the likelihood of having a 0 or not passing the use threshold, whereas the frequency part predicts more frequent use. All effects across Tables 2–4 were estimated in the same model. SE, standard error; PRS, polygenic risk score. * $p < 0.05$.

Table 4. Cannabis results from hurdle model

Main effects	Cannabis							
	any use				frequency			
	β	*B*	(SE)	*p* value	β	*B*	(SE)	*p* value
Polygenic risk (PRS)	−0.13	−0.29*	(0.09)	0.001	−0.11	−0.06	(0.09)	0.477
Prenatal stress	−0.12	−0.26*	(0.09)	0.002	−0.31	−0.18*	(0.09)	0.041
Warmer parenting	0.02	0.05	(0.09)	0.554	−0.33	−0.19*	(0.07)	0.004
Cortisol reactivity	**0.40**	**0.84***	**(0.16)**	**<0.001**	−0.49	−0.27*	(0.08)	0.001
Interactions								
PGS × prenatal stress	0.01	0.03	(0.09)	0.757	0.15	0.09	(0.10)	0.345
PGS × warmer parenting	0.05	0.10	(0.08)	0.188	−0.23	−0.13	(0.08)	0.091
PGS × cortisol reactivity	−0.07	−0.15	(0.15)	0.317	0.23	0.13	(0.10)	0.158
Prenatal stress × warmer parenting	−0.05	−0.11	(0.09)	0.182	−0.25	−0.14	(0.08)	0.056
Prenatal stress × cortisol reactivity	−0.17	−0.36	(0.19)	0.056	0.13	0.07	(0.10)	0.493
Warmer parenting × cortisol reactivity	−0.20	−0.50	(0.26)	0.057	−0.11	−0.07	(0.11)	0.541
Covariates								
Sex	**−0.28**	**−0.62***	**(0.10)**	**<0.001**	**0.62**	**0.35***	**(0.09)**	**<0.001**
Age	−0.07	−0.15	(0.08)	0.078	−0.08	−0.04	(0.08)	0.602

β = Standardized betas; *B* = Unstandardized betas with the associated SE and *p* value. PRS. Bolded effects likely survive adjustment for multiple testing. The any use part of the model predicts the likelihood of having a 0 or not passing the use threshold, whereas the frequency part predicts more frequent use. All effects across Tables 2–4 were estimated in the same model. SE, standard error; PRS, polygenic risk score. * $p < 0.05$.

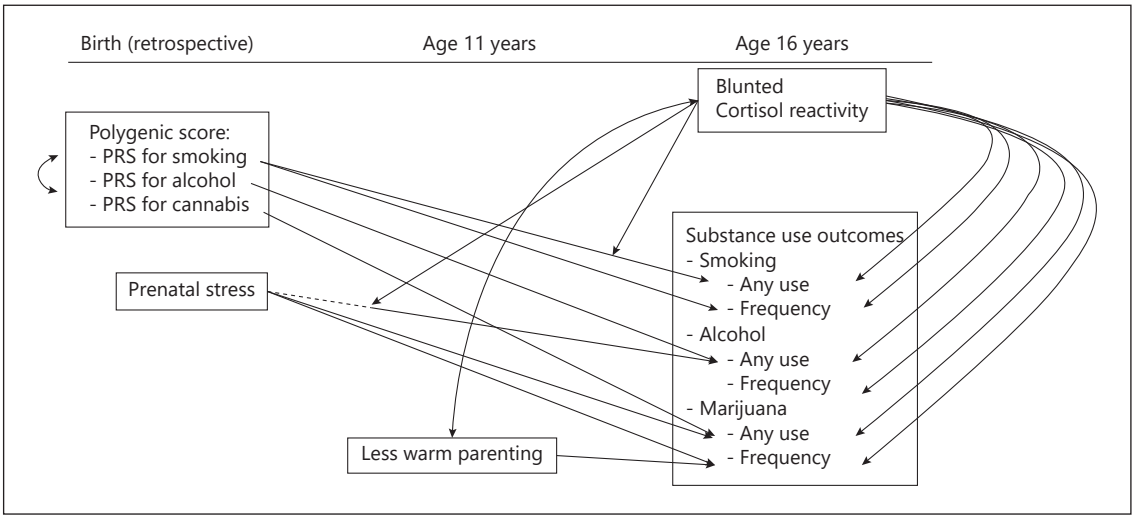

Fig. 2. Graphical summary of results. All 6 outcomes (any use and frequency of all 3 substances) were modeled simultaneously. Age and sex were additionally included as covariates (not depicted for clarity). The dashed line indicates the main effect of prenatal risk on alcohol use had $p > 0.05$. PRS, polygenic risk score.

Any Use

For the zero-inflation part of the hurdle model, as expected, youth with higher PRS for smoking, alcohol, and cannabis use (respectively), and blunted cortisol reactivity were more likely have smoked or used alcohol or cannabis. Specific to cannabis use, females and youth who experienced more prenatal risk used more cannabis, as expected. Two of the 18 tested interactions met the $p <$

0.05 threshold. First, $PRS_{smoking}$ was more strongly associated with any smoking among youth with blunted cortisol reactivity, whereas youth with higher cortisol reactivity evidenced no effect of $PRS_{smoking}$ on any smoking. Second, for average and low levels of cortisol reactivity, youth experiencing more prenatal risks were more likely use alcohol, whereas at high levels of cortisol reactivity prenatal stress was unrelated to alcohol use. Although weaker and statistically uncertain ($p = 0.056$–0.087), we found this same pattern of interaction for cortisol as a moderator of the associations of prenatal risk with cannabis use and smoking, and the associations of less warm parenting with alcohol and cannabis use. In all cases, the risks were increased for youth with blunted cortisol reactivity but not for youth with higher cortisol reactivity.

Frequency of Use

For past month frequency of use (i.e., the count part of the hurdle model), we found that blunted cortisol reactivity was associated with more frequent smoking, alcohol, and cannabis use, as expected. Additionally, higher $PRS_{smoking}$ was related to increased smoking frequency, but PRS did not predict alcohol or cannabis use frequency. Less warm parenting was related to increased cannabis use frequency, as expected, but not for alcohol or smoking frequency. Unexpectedly, less prenatal risk exposure was related to more frequent cannabis use. Being male was related to more frequent alcohol and cannabis use.

Discussion

The present study examined whether interactions between polygenic risk for substance use, prenatal risk, lower levels of warm parenting, and cortisol reactivity predicted smoking, alcohol, and cannabis use and frequency. We hypothesized that the environmental influences would moderate genetic risk, consistent with the diathesis-stress framework, but we did not detect any gene-by-environment interactions. Instead, we found that cortisol reactivity, a physiological response to experienced stress, increased the effect of genetic risk for smoking on mid-adolescent smoking, that is, cortisol reactivity acted as the catalyst in the diathesis-stress model. We also found evidence of a prenatal risk-by-cortisol reactivity interaction for alcohol use, again with blunted cortisol reactivity marking the "stress" that increases the effect of an earlier, in this case prenatal, influence on substance use.

A prior study also found that $PRS_{smoking}$ in particular was implicated in diathesis-stress models of smoking behavior with a different environmental moderator: parental substance use [20]. Taken together, we may conclude that polygenic risk and gene-environment interplay is particularly influential for smoking across development in the TRAILS sample, but not for other substances. The general lack of findings of G×E in our larger model may suggest that G×E findings are weaker than concluded from more specific models that include fewer other variables. Further, our findings in combination with prior evidence in the TRAILS sample suggests that both genetic (i.e., $PRS_{smoking}$) and "environmental catalysts" (in this case prenatal risk and less warm parenting) may operate partially by the blunting of the stress response [21]. There is accumulating evidence that prenatal stress can operate through multiple prenatal biological mechanisms (i.e., mediated by changes in cortisol, serotonin, cytokine, and microbiota in pregnant mothers) that are linked to alteration of several offspring neural phenotypes (e.g., neural circuit formation and pruning processes, brain structural connectivity, and/or epigenetic changes in neural gene expression) that can affect offspring cortisol reactivity [16, 34]. The resulting dysregulation of the stress response system then also may exacerbate these earlier-life influences on substance use. That is, G×E findings may be sparser here because we included a potential mediator of G×E effects – cortisol reactivity. Mediation of G×E effects was not tested here, but these results raise this hypothesis for future work to test.

One key effect was that blunted cortisol reactivity was related to both any and more frequent smoking, alcohol use, and cannabis use. These observations are consistent with past theory and literature of blunted cortisol reactivity and general (i.e., not substance-specific) substance use [35]. These associations could arise either due to allostatic load, that is, chronic stress shifting the set-points for responsivity of cortisol to stressors in a way that confers vulnerability for psychopathology and substance use; [36], or fearlessness and sensation-seeking, whereby youth with blunted cortisol reactivity seek out externalizing behaviors including substance use to increase arousal [37, 38].

However, our findings contrast somewhat from a prior analysis of these data [21], wherein cortisol reactivity was only associated with smoking (not alcohol or cannabis). Analytic choices may explain these differences. First, we used a 2-stage hurdle model to account for the zero-inflated nature of the outcome variables in this study instead of relying only on an estimator with robust standard

errors to adjust for the non-normal outcome data as were done in [21]. Although these models are a better fit for the theory of adolescent substance use uptake and distributions of these phenotypes, a limitation of this approach in comparison to that used in [21] is that it is currently not possible to account for the correlated nature of the outcomes in these models. The associations found for alcohol and cannabis may thus reflect variance shared with and better attributed to smoking. Second, the previous analysis explored developmental pathways among these predictors and showed that cortisol reactivity and substance use codevelop through pathways, including parenting, and middle childhood levels of internalizing problems or changes in externalizing problems. We did not assess earlier behavioral problems in this study. Together, we may conclude that earlier behavior problems may be a "common cause" explaining associations of blunted cortisol reactivity with alcohol and cannabis use that are (a) smaller than associations with smoking, and (b) more specific to initiation of alcohol and cannabis use than for smoking. This discrepancy in findings highlights the need to continue developing better analytical models that can both examine correlated outcomes and predictors together and properly account for the distributions of the outcomes.

A second key finding was the role of PRS for any use, but less support for the role of PRS for frequency of use (i.e., found only for smoking, not alcohol or cannabis use). It is important to bear in mind the context of the GWAS on which the PRS were based. The $PRS_{smoking}$ was based on both initiation and cigarettes per day and predicted both any and frequent use. However, the cannabis PRS was based on a GWAS of initiation, and only predicted any use. Although the alcohol GWAS was based on drinks per week in (middle-aged) adults, in the analysis for which the substance-specific PRS scores were developed [20], there was no evidence of a direct effect of polygenic risk for alcohol on age 22 weekly alcohol use (which may be more normative than in middle age). Here, we found the main effect of polygenic risk for alcohol use but not frequency at age 16. This combination of findings suggests that (a) polygenic risk for smoking is particularly strong in the TRAILS sample, and (b) polygenic risk for alcohol use may better indicate early (and therefore potentially more problematic) alcohol use, at age 16 when the prevalence is lower than at age 22, when alcohol use is more common. These findings further support some specificity of genetic risk on various substance use phenotypes (i.e., initiation vs. use vs. disorder) [39].

Strengths and Limitations

Strengths of this study include that we measured substance use frequency via self-report, in mid-adolescence, prior to the peak of substance use frequency; our phenotypes thus represent earlier onset (potentially more detrimental) frequent use. Early onset of substance use has been linked to greater lifelong problems [1], and thus our data on early adolescence shed light on the processes influencing this earlier, riskier use. Limitations include that we did not exclude girls who used contraceptives from our analytical sample in order to preserve sample size, which could introduce noise in the cortisol reactivity measure. Also, cortisol reactivity was concurrent, and thus is best interpreted as a biomarker, not a potentially causal influence. Second, there may be limited validity of retrospective recall of prenatal risks a decade after birth [27]. Third, the genetic ancestry of this sample was wholly European, and thus we could not conduct analyses in additional ancestrally homogenous subsets and these results may not generalize to other populations. We controlled for population stratification via genetic principal components within the European subsample; however, when data used to derive PRS are not genetically similar to the population of sample data, PRS can be biased and result in false-positives due to differences in allele frequencies across ancestral groups [40]. Finally, we elected not to examine 3- and 4-way interactions or sex differences, given the complexity of models relative to sample size and to limit the multiple testing burden, although there is some preliminary evidence that gene-prenatal-postnatal interactions may be more important for boys than girls [7].

Conclusions

We examined interactions among biological (genetic and cortisol reactivity) and environmental (prenatal risk and parenting) influences on adolescent substance use and frequency of use, in line with current theories that highlight dynamic relations between the child and multiple contextual biological and environmental factors [41]. We found limited evidence of interactions, and only for any use and not for frequency. In general, these findings point to the role of cortisol reactivity as a moderator of early influences for substance use initiation: primarily polygenic risk for smoking initiation and environmental risks for alcohol and cannabis. Polygenic risk was strongest for any use compared to frequency of use across substances (except smoking, for which the PRS did predict

frequency). The strongest correlate of frequency of use was concurrent blunted cortisol reactivity and this was consistent across substances, supporting theoretical models. We add that these correlations are evident across phenotypes (e.g., initiation and frequency of use) and relatively early in development. Taken together, we found that cortisol, directly and as a moderator (for any use), is an important biomarker for adolescent substance use and frequency, whereas gene-environment interaction seems to play less of a role.

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Statement of Ethics

We affirm that this research conforms to the guidelines for human studies and the research which was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the Dutch Central Committee on Research Involving Human Subjects and by Purdue University IRB #1702018756. See [17, 18] for further detail on TRAILS. Parental written informed consent was obtained after the procedures had been fully explained. Adolescents gave written informed consent at the second and third assessment waves.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

K.M. conceptualized the study, completed analysis, and drafted the manuscript. L.B. aided in cleaning and interpretation of genetic data and edited the manuscript. J.P. created the polygenic scores and aided in interpretation of them, as well as edited the manuscript. V.S.K. aided in study conceptualization and interpretation and edited the manuscript. S.A.R. was involved in the overarching study design and data collection, aided in the present study conceptualization and interpretation, and edited the manuscript.

Data Availability Statement

TRAILS data are made available to third parties outside the TRAILS consortium via DANS EASY. The data are not freely accessible, but desired variables can be requested by means of a publication plan. For information on how to access TRAILS data, please see https://www.trails.nl/en/hoofdmenu/data/data-use.

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