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Extending Previous cGxl Findings on 5-HTTLPR's Moderation of Intervention Effects on Adolescent Substance Misuse Initiation

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Abstract

This study addresses replication in cGxE research by investigating if the key findings from Brody, et al., (2009) can be detected using data ($N = 1809$) from the PROSPER substance use preventive intervention delivery system. Parallel to Brody, et al., this study tested the hypotheses that substance misuse initiation would increase faster from age 11 to age 14 and be higher at age 14 among: 1) 5-HTTLPR short carrier adolescents vs. long homozygotes; 2) control vs. intervention adolescents; and 3) 5-HTTLPR short carriers in the control condition vs. all other participants. The hypotheses were generally supported and results were consistent with Brody, et al.'s cGxE finding. Results are discussed in light of replication issues in cGxE research and implications for intervention.

Adolescent substance misuse remains an endemic behavior that places many adolescents at substantial risk. In 2013, a Monitoring the Future report (Johnson, O'Malley, Miech, Bachman, & Schulenberg, 2014) found that 68% of high school seniors reported having consumed alcohol (i.e., more than a few sips) and about half reported having also been drunk. The report also showed rates of any illicit substance misuse (e.g., marijuana, ecstasy, prescription drug misuse) by the end of high school have hovered around 50% since 1992. Adolescent substance misuse is associated with considerable morbidity and mortality in the short term, such as impaired driving and accidents (USDHHS, 2007) and risky sexual behavior (Tapert, Aarons, Sedlar, & Brown, 2001). Adolescent substance misuse also predicts poor adult psychosocial functioning including lower educational and occupational

attainment, greater psychological distress, and mental health problems (e.g., Chassin, Hussong, & Beltran, 2009).

To reduce these harms, substance initiation and subsequent misuse are targeted by several adolescent preventive intervention programs (see Spoth, Trudeau, Gyll, Shin, & Redmond, 2009; Spoth, Greenberg, & Turrise, 2009) and research has demonstrated the success of interventions in delaying initiation and/or reducing adolescent substance misuse. For example, the PROSPER project is a university-school-community partnership system for delivering these types of family- and school-based interventions. Students in PROSPER intervention schools consistently report lower substance initiation and prevalence, including marijuana and prescription drug misuse (Spoth et al., 2007; 2011; 2013) than do control students. Students in PROSPER intervention districts also report less alcohol use compared to control students, although these findings are less consistent (Spoth et al., 2011). Taken together, the PROSPER studies, and those of other interventions (e.g., Guo, Hawkins, Hill, & Abbot, 2001), indicate that preventive intervention during early adolescence can have long-term implications for reducing substance misuse.

Intervention Efficacy and Candidate Gene x Environment Interaction (cGxE) Research

Although research on intervention efficacy suggests that preventive interventions can reduce overall levels of adolescent substance misuse, interventions are not equally effective for all participants. Understanding this heterogeneity is an important direction for enhancing intervention efficacy. Research on individual differences in intervention response include a wide range of putative moderators and many studies focus on program implementation quality and fidelity (see Spoth, et al., 2006). Emergent cGxE literature supplements this earlier work by investigating whether differences in susceptibility to interventions are related to genotype.

To many researchers (see Bakermans-Kranenburg & van IJzendoorn, 2015; Belsky & van IJzendoorn, 2015), cGxE studies have made a strong case that genetics can moderate intervention effects. Other researchers, however, are less convinced, viewing cGxE research in the context of the larger body cGxE work whose replication record render it suspect (e.g., Duncan & Keller, 2012; Duncan, Pollastri, & Smoller, 2014). Much debate has followed these strong critiques, with some researchers arguing against these criticisms on methodological grounds by pointing out differences between initial findings and replication attempts – including variations in study designs and measurement quality (McGuffin, Alsbban, & Uher, 2011; Karg et al., 2009; Rutter, Pickles, & Thapar, 2009). Nonetheless, these critiques persist and have been extended to all cGxE research (Duncan et al., 2014).

Although it is unlikely that this debate will be resolved in the near future, it is important to determine if cGxE findings can be reproduced. Replication of cGxE findings is important but not straightforward. On one hand, replication is the ideal criterion that creates confidence in a set of findings. On the other hand, given that cGxE findings are drawn from studies with different types and perhaps dosages of interventions, different ancestral populations and racial admixtures, as well as differences in study design (e.g., participant age, measurement

specifics), the identical designs, measures, and populations required for direct replications are not available.

With the challenges of this task in mind, the goal of the current study is to contribute to the discussion of replicability of cGxE research by using the PROSPER data for analyses similar to those of one of the first published cGxE studies in the area of behavioral prevention. Specifically, Brody and colleagues (2009) examined the interaction between Strong African American Families (SAAF) program participation and the *5-HTTLPR* polymorphism on *risk behavior initiation*, a composite measure of alcohol and marijuana use and sexual experience, among a sample of early adolescent African Americans (11 to 14 years). Growth modeling results showed that risk behavior initiation increased faster among adolescents with at least one copy of the *5-HTTLPR* short allele (“short carriers”) and slower among SAAF participating youth. Cross-sectional analysis of the age 14 follow-up data showed short carriers in the control condition demonstrated higher risk behavior initiation relative to other adolescents (intervention adolescents of either genotype and long carriers in the control condition).

Since Brody, et al. published these findings, other intervention-by-*5-HTTLPR* interactions have been reported. Brett, et al. (2015) found that children from Romanian institutions who were both homozygous for the short allele and randomly assigned to high-quality foster care at 12 months of age were lowest in externalizing behavior problems at 54 months, compared to *5-HTTLPR* long carrier children and children randomized to care as usual. Similarly, Cleveland and colleagues (2015) found *5-HTTLPR* interacted with intervention status to predict early adolescent alcohol use in a subset of PROSPER participants who completed in-home surveys. However, this effect was limited to adolescents with average or better maternal support/investment. Nonetheless, there are currently few (if any) published attempts at replication or extension within the cGxE literature.

The *5-HTTLPR* Polymorphism

In addition to the need to address replicability, studies about G-E transactions also need to address the biological relevance of the polymorphism(s) examined: Why it should be linked to the outcome being studied and why its impact should change based upon the environment or intervention being studied. The *5-HTTLPR* polymorphism is a well-studied variable number of tandem repeats (VNTR) in the promoter region of the *SLC6A4* gene. This variant exists as two alleles, long and short (16 and 14 repeats; Lesch et al., 1996), and the short allele causes low expression of the serotonin transporter (Bradley et al., 2005). The consequences of altered expression are difficult to assess in a human population and there is no consensus yet on whether the altered expression leads to different concentrations of the serotonin transporter on the cell surface. For example, there is inconsistent evidence from imaging studies (e.g., PET) that *5-HTTLPR* is related to serotonin transporter availability (see Iurescia, Seripa, and Rinaldi, 2015). Nonetheless, the difference in expression makes *5-HTTLPR* a potential causal genetic variant in behavioral analyses and a large body of research documents the relation of *5-HTTLPR* genotype with internalizing behaviors (e.g., Xia & Yao, 2015). Findings from neural physiology fMRI studies converge on the finding that *5-HTTLPR* variation is related to amygdala reactivity in the presence of environmental

threats (e.g., Hariri, et al., 2002) and suggests that short allele carriers are more neurologically susceptible to stressors.

In addition to work on internalizing problems, a growing literature suggests a role for *5-HTTLPR* genotype in substance use. A pair of meta-analyses showed *5-HTTLPR* is associated with alcohol use in both adolescents (Saraceno, Munafó, Heron, Craddock, & van den Bree, 2009) and adults (McHugh, Hofmann, Asnaani, Sawyer, and Otto, 2010). In a third meta-analysis, heroin, cocaine, and methamphetamine use disorders were also associated with *5-HTTLPR* (Cao, Hudziak, and Li, 2013). Pharmacological studies demonstrate the highly interactive nature of serotonin and dopamine neuronal circuits, the latter of which is more commonly thought to be related to addiction. For example, psychostimulant drug effects (e.g., cocaine) are minimal with co-administration of drugs that alter dopamine and serotonin, in comparison to administration of the dopamine-acting drug alone (Baumann, Ayestas, Dersch, Brockington, Rice & Rothman, 2000). In addition, combining serotonin- and dopamine-acting drugs also prevents self-administration in a rodent model (Glatz, et al., 2002). In humans, a drug therapy for cocaine addiction (disulfiram) acts by altering serotonin metabolites, and was shown to be more effective in *5-HTTLPR* short allele carriers (Nielsen, Harding, Hamon, Huang and Kosten, 2012). Taken together, evidence suggests a biological role for *5-HTTLPR* vis-à-vis neurophysiological links to substance use behaviors.

The rs25531 Polymorphism

Subsequent to identifying the *5-HTTLPR* polymorphic region, an A/G Single Nucleotide Polymorphism (SNP; rs25531) was identified within the sixth repeat of *5-HTTLPR* (Hu et al., 2006; Kraft, Slager, McGrath, & Hamilton, 2005; Nakamura, et al., 2000). Work by Hu and colleagues (2006) suggests that this SNP represses transcription by altering a transcription factor binding sight when the rare G allele is in phase with the Long allele (L_G), which leads the L_G allele to function equivalently to a short allele. As with *5-HTTLPR*, there is conflicting evidence regarding the effect rs25531 has on available serotonin (see Iurescia, et al., 2015).

Characterizing Statistical Interactions in cGxE Research

Parallel to an increased emphasis on replication and the biological role of polymorphism(s) has been work on improving the characterization of statistical interaction forms (Reiss, Leve, & Neiderhiser, 2013). Widaman and colleagues (2012) describe a reparametrized regression model that includes the estimated interaction cross-over point (the point where lines cross) as a model parameter. The cross-over point estimate combined with its confidence interval can be used to infer the plausible population interaction form and whether an interaction is ordinal or disordinal (see Figure 1). These interaction forms map onto predictions from a set of three plasticity models, which are often used in cGxE studies. Differential susceptibility (Belsky & Pluess, 2009; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011) resembles a disordinal interaction (Figure 1A) whereas the vantage sensitivity model (Figure 1B; Pluess & Belsky, 2013) and diathesis stress model (Figure 1C) each resemble ordinal interactions. Relevant to the current inquiry,

Brody, et al., (2009) drew from prior work suggesting interventions have their greatest effects on youth who are at greatest risk—representing a diathesis stress interaction form.

The Current Study

We approach the task of partially addressing the replicability issue in cGxE research by extending the primary findings of Brody, et al. in several ways. The Brody, et al. study focused on (1) risk behavior initiation within a (2) sample of African American early adolescents who received a (3) family-based intervention designed to (4) prevent/reduce adolescent substance misuse. Utilizing data from the PROSPER project, we test the proposition that the Brody, et al. findings extend to (1) substance misuse initiation in a (2) primarily rural, Caucasian sample of early adolescents who received (3) PROSPER-delivered family- and school-based interventions designed to (4) prevent/reduce adolescent substance misuse.

Because the purpose of this research was to determine whether the key findings from Brody, et al (2009) could be reproduced using data with similar – though not identical – characteristics, the analyses used in the current inquiry follow those reported in that study. Using growth models, Brody, et al. showed risk behavior initiation tended to increase faster among adolescents with at least one copy of the *5-HTTLPR* short allele and slower among SAAF participating youth (i.e., main effects of *5-HTTLPR* and intervention status, respectively). These findings were not altered when additional covariates were added to the model (i.e., adolescent sex, monthly per capita income, and parent marital status). In addition, Brody, et al. used a planned comparison contrast approach, based on theorizing by Rutter (1985), to test their primary cGxE hypothesis: Adolescents at *5-HTTLPR* genetic risk assigned to the control condition would show greater risk behavior initiation at their age 14 follow-up compared to (a) adolescents at genetic risk assigned to the intervention and (b) adolescents not at genetic risk assigned to either condition. This analytic approach called for an omnibus ANCOVA followed by a non-orthogonal planned contrast in which the hypothesized risk group (control/short carriers) was compared to the remaining groups (intervention/short carriers and homozygous long adolescents in either condition). Because our goal was to replicate and extend Brody, et al., our focal analyses parallel those just described. Following these analyses, we conducted follow-up analyses not conducted by Brody, et al., that test the robustness of the findings as well as formally test the form of the interaction using the Widaman, et al., (2012) technique.

Method

Participants and Procedure

Data for this study came from the PROSPER project, a universal community-university evidence-based prevention intervention delivery system that includes 28 rural and small town communities in Iowa and Pennsylvania randomized into 14 intervention and 14 control units. All 6th grade students in each community were invited to participate, 90% did so at wave 1. Students completed surveys in school initially during fall of 6th grade, they were completed again late in spring of 6th grade, and then annually in schools through 12th grade.

PROSPER intervention communities utilized a partnership-based delivery system to implement a sequence of family-focused and school-based preventative programs during the 6th and 7th grades. Community-university teams selected a universal family-focused program for implementation in 6th grade, from a menu of three choices, for each of the two program types (see Spoth et al., 2004). All 14 communities chose the *Strengthening Families Program: For Parents and Youth 10–14* (SFP 10–14). For the 7th grade in-school program, six teams chose the *All Stars* curriculum (McNeal, et al., 2004) and four each chose *Life Skills Training* (Botvin & Kantor, 2000) and *Project Alert* (Ellickson et al., 2003), respectively. All three programs target personal goal-setting, social norms, peer affiliation, and decision making (see Spoth et al., 2004). Very high implementation levels have been found across programs and cohorts (e.g., Spoth et al., 2007).

DNA was first collected at wave 5 (approximately age 15) from a subset of adolescents who also completed in-home interviews ($N = 537$). During a later young-adult assessment, DNA was collected on an additional 1495 participants, the majority of whom completed in-school assessments only (96.2%) for a total of 2032 DNA samples. In the current study, we utilized data from the first 4 waves to approximate the age range tested in Brody et al. (2009; 11.5 to 14 years). The results reported in this study are based on $N = 1809$ participants whose DNA produced a reliable *5-HTTLPR* genotype and had sufficient across-wave data for longitudinal modeling. The analytic sample was primarily Caucasian ($N = 1624$, 89.8%) and included smaller groups of Hispanic ($N = 86$, 4.8%), African American ($N = 35$, 1.9%), and Asian ($N = 23$, 1.3%) adolescents. A small number of adolescents identified as other ethnicities ($N = 41$, 2.3%). During the initial 6th grade assessment (wave 1), adolescents were $M = 11.77$ years old ($SD = .36$). The second wave was collected 6 months later ($M = 12.24$, $SD = .36$) and subsequent waves were collected annually (wave 3: $M = 13.24$, $SD = .36$; wave 4: $M = 14.25$, $SD = .36$).

Measures

5-HTTLPR Genotyping—DNA collected via buccal swab was genotyped using two slightly different methods. First, samples collected from the in-home participants were genotyped as described in Cleveland, et al. (2015). Second, samples from in-school only students were genotyped using a multiplex PCR of 5 markers developed by Haberstick et al. (2014). The genotypes relevant to this study were produced by *5-HTTLPR* primers (forward NED–ATGCCA GCACCTAACCCCTAATGT and reverse GGACCGCAAGGTGGGCGGGA) and Amelogenin gene primers (forward NED–CCCTGGGCTCTGTAAAGAATAGTG and reverse ATCAGAGC TTAAACTGGGAAGCTG). The Amelogenin gene (*AMEL*) is present on both the X and Y chromosomes (*AMELX* and *AMELY*) with a 6-nucleotide deletion in *AMELX*, which allowed genotyped and self-reported sex comparisons to ensure genotyping accuracy. After the first multiplex, cases that failed to produce a genotype on any of the 5 markers were re-genotyped using multiplex (34%). Re-genotyped failures were excluded from analysis if (1) the sample failed a second time to produce a *5-HTTLPR* genotype ($N = 152$), (2) failed to produce an *AMEL* genotype ($N = 42$), or produced a *5-HTTLPR* genotype but failed on 2 or more other markers within the multiplex ($N = 39$). Among the $N = 1809$ analytic sample, 1228 (67.9%) had at least one copy of the short allele (short carriers) while the remaining

581 (32.1%) were homozygous for the long allele (long homozygotes). *5-HTTLPR* genotype was coded 0 = long homozygote (l/l), 1 = short carrier (s/l or s/s) and was in Hardy-Weinberg Equilibrium ($\chi^2(1) = 1.62, ns$).

rs25531 Genotyping—Genomic DNA (50 ng) was amplified using a Custom TaqMan SNP genotyping assay from Applied Biosystems (Life Technologies, Carlsbad, CA). The 99 individuals who were heterozygous at both rs25531 and *5-HTTLPR* required re-genotyping to determine which allele at rs25531 (G or A) was in phase with the two alleles at *5-HTTLPR* (Long or Short; Kraft et al., 2005; Perroud et al., 2010). PCR amplification of the *5-HTTLPR* site was followed by restriction endonuclease digestion with MspI (New England Biolabs, Beverly, MA). Fragment lengths of the digested DNA were determined by 8% polyacrylamide gel electrophoresis to distinguish between the 4 possible combinations of alleles. Genotypes at rs25531 were in Hardy-Weinberg Equilibrium ($\chi^2(1) = .0002, ns$). This analysis resulted in N = 135 individuals with Long/Long genotypes being included with the individuals classified as short carriers due to the presence of a G allele. Analyses reported below that use *5-HTTLPR* are coded l/l = 0 (N = 446), s/l or s/s or L_G carrier = 1 (N = 1363; also denoted short(L_G) below;).

Substance Misuse Initiation—Brody's *risk behavior initiation* outcome included the following three Yes/No items: (1) Ever drunk beer, wine, wine coolers, whiskey, gin, or other liquor, (2) Ever used marijuana, and (3) Ever had sexual intercourse. For the current study we created a substance misuse initiation outcome defined by averaging three dichotomous items (0 = No, 1 = Yes), each assessed across waves: (1) have you ever been drunk from drinking alcohol? (2) have you ever smoked marijuana?, and (3) have you ever used hard drugs or medications that were prescribed by a doctor to someone else? Because all of the items that Brody, et al used were not available in the PROSPER study (e.g., sex initiation), the outcome measure was created in a manner aimed to parallel the Brody, et al. risk behavior initiation measure and that would reflect risk behavior initiation with regards to substance misuse. Substance misuse initiation values ranged from 0 to 1 and averaged .03 ($SD = .10$, wave 1) to .10 ($SD = .23$, wave 4) across waves. After excluding cases with failed or unreliable genotypes, substance misuse initiation data showed maximum attrition at wave 4 (11.3%, $n = 204$). Participants with and without missing data at wave 4 did not significantly differ on *5-HTTLPR* allele frequencies, intervention status, sex, parent marital status, or free/reduced lunch receipt at wave 1.

Intervention Status—Within the current analytic sample ($N = 1809$), there were 834 control adolescents (46.1%; coded 0) and 975 in the intervention (53.9%; coded 1).

Covariates—Three covariates from the baseline assessment were used in the present analysis. *Parent marital status* (0 = Single Parent ($n = 355, 20.1\%$), 1 = Dual Parent ($n = 1408, 79.9\%$)); *free/reduced school lunch receipt* (0 = No ($n = 1273, 72.4\%$) and 1 = Yes ($n = 485, 27.6\%$)); *adolescent sex* (0 = Male ($n = 704, 38.9\%$) 1 = Female ($N = 1105, 61.1\%$)).

Analysis Plan

Analyses were constructed to approximate those of Brody, et al (2009). Growth curve models were estimated using *Mplus* 6.1 (Muthén & Muthén, 2008). Reflecting the PROSPER data collection spacing, growth model factor loadings were specified as -2.5 , -2 , -1 , 0 across waves, which centers the intercept at wave 4 (age 14). Full information maximum likelihood (FIML) was used to reduce potential bias due to missing data (Schlomer, Bauman, & Card, 2010; results using MLR were the same). First, we present the results of the unconditional growth model. Next, we present results of a main-effects only model that includes Intervention Status and *5-HTTLPR* (homozygous long vs. short(L_G) carrier) effects on wave 4 substance misuse initiation (intercept) and change over time (slope). In addition, we conducted planned comparison analyses similar to Brody, et al (2009). Follow-up analyses centered on testing the robustness of the findings and characterizing the interaction form. In addition to reporting standardized regression coefficients, we include unstandardized coefficients and bootstrapped 95% confidence intervals (1000 draws) that provide information on population parameter reliability (see Salvatore & Dick, 2015; Schlomer, Cleveland, Vandenberg, Fosco, & Feinberg, 2015; similar results were found when we do not include rs25531, results are available in Appendix S1).

Results

Model 1: Unconditional Growth Model

The unconditional growth model fit the data well ($\chi^2(5) = 50.14$, $p < .05$; CFI = .96; TLI = .95; RMSEA = .071). Intercept and slope standardized parameter estimates indicated substance misuse initiation at age 14 was significantly greater than zero ($M = .50$, $p < .05$; unstandardized $M = .090$, $s^2 = .032$, $p < .05$) and that adolescent substance misuse initiation generally increased during early adolescence ($M = .41$, $p < .05$; unstandardized $M = .026$, $s^2 = .004$, $p < .05$).

Model 2: Intervention Status and *5-HTTLPR* Differences

Intervention Status and *5-HTTLPR* were added to the model as predictors of both the slope (denoted β_S) and intercept (denoted β_I). Results showed intervention adolescents' substance misuse initiation increased at a slower rate than control adolescents ($\beta_S = -.087$, $p < .05$; $b = -.011$, 95% CI $[-.020$ to $-.003]$) and was lower at age 14 ($\beta_I = -.088$, $p < .05$; $b = -.032$, 95% CI $[-.052$ to $-.011]$). No main effects were found for *5-HTTLPR* ($\beta_S = .028$, ns ; $b = .004$, 95% CI $[-.006$ to $.014]$; $\beta_I = .037$, ns ; $b = .015$, 95% CI $[-.008$ to $.039]$). This model was estimated a second time using sex, parent marital status, and free/reduced school lunch receipt as exogenous predictors of the intercept and slope. Results showed the intervention effects were robust to these controls ($\beta_S = -.081$, $p < .05$; $\beta_I = -.083$, $p < .05$). *5-HTTLPR* genotype remained non-significant ($\beta_S = .027$, ns ; $\beta_I = .016$, ns).

Model 3: Higher-Risk Adolescents vs. Other Adolescents

Parallel to the planned comparison described by Brody, et al. 2009, a grouping variable was created that distinguished between the higher-risk control adolescents with at least one copy

of the short or L_G allele (coded 1; $N = 648$) and all other adolescents (intervention adolescents of either genotype and long homozygotes; coded 0; $N = 1161$). A multiple group latent growth model was conducted as before except using this grouping variable to compare control/short(L_G) adolescents' substance misuse initiation to the remaining adolescents. Substance misuse initiation was significantly greater than zero at age 14 for both higher-risk adolescents ($M = .54, p < .05$; $b = .118, 95\% \text{ CI } [.098 \text{ to } .138]$) and all other adolescents ($M = .50, p < .05$; $b = .073, 95\% \text{ CI } [.062 \text{ to } .084]$). Similarly, substance misuse initiation significantly increased among control/short(L_G) adolescents ($M = .45, p < .05$; $b = .036, 95\% \text{ CI } [.027 \text{ to } .044]$) and the other adolescents ($M = .39, p < .05$; $b = .020, 95\% \text{ CI } [.016 \text{ to } .025]$). Equality constraints on slope coefficients across groups resulted in significant model misfit ($\chi^2(1) = 9.72, p < .05$) as did constraining substance misuse initiation at age 14 ($\chi^2(1) = 14.46, p < .05$). These results indicate that although both groups showed significant growth in substance misuse initiation and were greater than zero at age 14, control/short(L_G) adolescents showed faster growth and were higher at age 14 compared to other adolescents.

To better approximate the analysis reported by Brody, et al, (2009), a cross-sectional ANCOVA with an accompanying planned comparison was conducted. We created a 4-level variable that represents the 4 possible Intervention Status x *5-HTTLPR* groups: 1 = Control/Short(L_G) Carrier ($n = 573$), 2 = Control/Homozygous Long ($n = 164$), 3 = Intervention/Short(L_G) Carrier ($n = 619$), 4 = Intervention/Homozygous Long ($n = 219$). Note that the total sample size for this analysis ($N = 1575$) is less than that used for the growth model due to attrited cases not picked up by FIML estimation. Similar to Brody, we controlled for substance misuse initiation at baseline (wave 1) and used age 14 substance misuse initiation (wave 4) as the dependent variable. The p -value for the omnibus ANCOVA was statistically significant, suggesting a significant difference between at least two of the four groups ($F(3,1570) = 3.17, p < .05$). The follow-up planned comparison (Helmert contrast) between Control/Short(L_G) Carrier adolescents and other adolescents was significant ($t = 2.69, p < .05$; Cohen's $d = .14$), indicating that, consistent with Brody, et al, (2009), higher-risk adolescents demonstrated greater substance misuse initiation at age 14 compared to other adolescents.

Follow-up Analyses

To test for possible effects due to population stratification, a principal coordinate (PC1) representing variation in non-European ancestry (higher values = more non-European ancestry; see Cleveland, et al., 2015) was added to the unconditional growth model to determine if variability in non-European ancestry was related to model parameters; results for Model 1 showed there were no non-European ancestry associations ($\beta_S = -.032, ns$; $\beta_I = .005, ns$). The unstandardized mean intercept and slope were both .01 larger when including the PC1 covariate, which was likely due to differences in rounding. Variance estimates were identical to those reported in Model 1 above. A similar pattern of results was found for Model 2 wherein intervention status remained associated with the slope and intercept ($\beta_S = -.079, p < .05$; $\beta_I = -.081, p < .05$) and *5-HTTLPR* remained unrelated after including the PC1 covariate ($\beta_S = .025, ns$; $\beta_I = .039, ns$). Differences between control adolescents with at least one copy of the short or L_G allele (higher-risk) and all other adolescents (Model 3) remained significant for both the slope ($\chi^2(1) = 8.66, p < .05$) and

intercept ($\chi^2(1) = 13.25, p < .05$) after adjusting for non-European ancestry; slope and intercept parameters were nearly identical to those reported in Model 3 above (Control/Short(L_G): $M_I = .54, M_S = .44$; Others: $M_I = .50, M_S = .41$). The omnibus ANCOVA also remained statistically significant when adding the PC1 covariate ($F(3, 1532) = 2.69, p < .05$) and the follow-up planned comparison remained statistically significant and similar in effect size ($t = 2.53, p < .05$; Cohen's $d = .13$). Caucasian-only analyses resulted in near identical findings.

To formally test the interaction form found by Brody, et al., and reproduced here, we used SAS (2011) to estimate a non-linear regression model that included the cross-over point as a parameter (Widaman, et al., 2012). Slopes and intercepts from the growth model were exported for use in these analyses. Results for the age 14 intercept showed the cross-over point estimated at 1.00 with a .23 to 1.77 confidence interval (95%). As described by Widaman, et al, (2012) this data pattern suggests an ordinal interaction in the population. In the current case, the combined effect of the intervention and *5-HTTLPR* genotype resembles diathesis stress. Similar results were found for the slope (cross-over = .95, 95% CI: .22 to 1.68). Note that cross-over values for binary variables are descriptive and do not necessarily have intrinsic scale meaning.

Discussion

The purpose of this study was to determine whether the key findings from Brody, et al (2009) could be reproduced using data from a different intervention data set. More specifically, we sought to determine whether the findings reported by Brody, et al., could be extended to (1) substance misuse initiation in (2) a primarily rural, Caucasian sample of early adolescents who received (3) a family- and school-based intervention designed to (4) prevent/reduce substance misuse. As previously shown with the full PROSPER sample using similar measures (e.g., Spoth et al, 2013), results from the genetically informed subsample showed adolescents' substance misuse initiation increased at a slower rate for those in the intervention and was lower at age 14 compared to control adolescents. Unlike Brody, et al. (2009), we found no association between *5-HTTLPR* l/l vs s/l or s/s or L_G genetic variation and substance misuse initiation growth or mean level at age 14. Nonetheless, similar to findings reported by Brody, et al., a significant difference was detected when comparing adolescents at high genetic risk within the control condition to other study adolescents via planned comparison. Our results showed that substance misuse initiation among *5-HTTLPR* short or L_G allele carriers in the control condition increased faster and was higher at age 14 compared to other adolescents. Thus, the PROSPER delivery system had a protective effect on youth at highest risk for substance misuse initiation.

Implications for cGxE Research

We view the results of this paper as generally consistent with Brody and colleagues' findings, with the caveat that we did not find a main effect for *5-HTTLPR*. A possible reason for this difference may be that the *5-HTTLPR* short allele is less frequent, relative to the long, among African ancestry populations, whereas the short is more frequent among European ancestry populations (Gelernter, Kranzler and Cubells, 1997). The differences in

allele frequencies across populations may reflect differences in haplotypes, epistasis patterns, and/or other genomic differences related to *5-HTTLPR* variation and may be important for detecting a *5-HTTLPR* main effect. Furthermore, there may also be systematic environmental or cultural differences among populations (i.e., African Americans vs. European Americans) that are relevant for a *5-HTTLPR* main effect. For example, ethnicity has been shown to moderate *5-HTTLPR* interactions with environmental adversity (van IJzendoorn, Belkys, & Bakermans-Kranenburg, 2012). Our sample is by-and-large ancestrally homogenous and limits our ability to examine possible differences across population groups. Additional studies are needed that examine genetic association differences across ancestral and cultural populations (Causadias, 2013).

Critiques of cGxE and the Current Study

The logic of a direct replication is that if a study is designed and executed in such a way that all the “important” study components (e.g., constructs, measures, sample, etc.) are reproduced with high fidelity, the associations tested in the replication paper should support the same conclusions as the original. However, there is often uncertainty if all the “important” components have been adequately reproduced. Many cGxE studies are derived from data sets that can be quite different, for example, in sample age range, ancestral composition, constructs assessed, measures used, research design, and sampling cohort. Direct replications of this research (or sufficiently close; Munafo, et al., 2009; Rische, et al., 2009) may not be realistic in a field that is largely outside the controlled conditions of a laboratory setting.

Importantly, some of the strongest critiques of the cGxE field are derived from meta-analytic findings that have attempted to distill a varied body of work into direct replications (Munafo, et al. 2009; Rische, 2009). This restrictive strategy would likely exclude the current study as sufficiently incomparable to the Brody, et al. study due to differences in the outcome and intervention. In addition, adopting a more restrictive strategy for grouping studies can reduce effect size variability across studies (see Munafo, et al., 2009; Risch, et al., 2009). Limiting effect size heterogeneity through restrictive inclusion criteria precludes our ability to ask questions about the conditions under which there is evidence for stronger and weaker effects, a key characteristic of meta-analysis. In our view, greater attention to characteristics that vary across studies and how they might relate to effect size heterogeneity is needed in cGxE research (see van IJzendoorn & Bakermans-Kranenburg, 2015 for a recent example). Using the current study as an example, one might conclude that there is no main effect of *5-HTTLPR* on risk behavior/substance misuse initiation in the general population since the sample used in this study is larger than that used in Brody, et al, and is theoretically *more* likely to statistically detect the association. Although increasing sample size, and with it statistical power, is a major focus of genetic association research, it is not the sole approach for improving power in these studies (see Halsey, et al. 2015; Schlomer, et al, 2015). No traits are 100% heritable (Plomin, DeFries, Knopik, & Neiderhiser, *in press*) and heritability estimates differ across time, space, and population (Tenesa & Haley, 2013). It is reasonable to expect that molecular genetic associations will be sensitive to these factors as well—variability which will not be understood (or overcome) by increasing sample sizes only. A

better understanding of cGxE effect size heterogeneity across studies may be particularly enlightening for answering for whom environments (and genotypes) matter.

Implications for Intervention Research

This research also should be evaluated in terms of implications for implementing interventions. Although some may suggest that valuable resources could be saved if we were to identify those adolescents for whom the intervention will be effective and those for whom it will not, we view this approach as problematic for several reasons. First, our findings indicate differences in degree of intervention efficacy in a *universal* prevention-intervention model, which can minimize stigmatization relative to more selective prevention-interventions. Selecting children into programs based on their genotype has consequences that are currently unknown; following this approach has the potential to be socially detrimental, outweighing the positive effects of intervention participation. In addition, phenotypic sensitivity is derived from much more than a single genotype (e.g., Belsky & Beaver, 2011) and extends beyond genetic disposition to conditional phenotypes based on environmental exposures during development (e.g., Del Giudice, Ellis, & Shirtcliff, 2011). Comprehensive information, related to intervention sensitivity (e.g., genetics, personality, mood disorder, peer relations, family history and other influences) would be more defensible and could be implemented as a battery of assessments.

Limitations and Conclusions

Perhaps the most relevant limitation is the ancestrally homogenous sample. We speculated that one possible reason for the difference in the main effect for *5-HTTLPR* between this study and Brody, et al (2009) might be due to differences in genetic ancestry. However, we are unable to empirically test this proposition. Additional studies are needed that have more ancestrally diverse samples to test this hypothesis. Although this study is not a direct replication, it should be viewed as extending previous findings in several ways. The prevailing approach to improving molecular genetic GxE research has focused on increasing sample size, however, the unique characteristics of many intervention studies, such as longitudinal data, well measured phenotypes, and randomization, make cGxE studies uniquely situated for evaluating GxE effects (Bakermans-Kranenberg & van IJzendoorn, 2015).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Group difference forms for differential susceptibility, vantage sensitivity, and diathesis stress models

Note: Figure 1A: differential susceptibility (disordinal); 1B: vantage sensitivity (ordinal); 1C: diathesis stress (ordinal). Dashed and solid lines represent sensitive and non-sensitive phenotypes, respectively.

Figure 2.

Growth model results for *5-HTTLPR* genotype and Intervention Status

Note: Figure 2A: *5-HTTLPR* homozygous long vs. short(L_G) carriers; 2B: control vs. intervention; 2C: higher-risk (control/short(L_G)) vs. other (intervention adolescents of either genotype and I/I); 2D: C/L = Control/Long, I/L = Intervention/Long, I/S = Intervention/Short(L_G), C/S = Control/Short(L_G).