Serotonergic Multilocus Genetic Variation Moderates the Association Between Major Interpersonal Stress and Adolescent Depression: Replication and Candidate Environment Specification

By: Lisa R. Starr, Suzanne Vrshek-Schallhorn, Catherine B. Stroud

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Abstract:

Serotonin-linked genetic risk and stressful life event (SLE) interaction research has been criticized for using single genetic variants with inconsistent replicability. A recent study showed that a multilocus genetic profile score (MGPS) capturing additive risk from five serotonin-linked polymorphisms moderated the association between major interpersonal SLEs and depression, but no subsequent replication attempts have been reported. Moreover, major interpersonal SLEs have been suggested as "candidate environments" for this MGPS, but it has never been demonstrated that gene-environment interactions (G × Es) for major interpersonal SLEs are significantly stronger than for other contexts. Adolescents (N = 241) completed contextual-threat life stress interviews and clinical interviews assessing depressive symptoms, and provided DNA. MGPS intensified the major interpersonal stress-depression association; the interaction accounted for 4% of depressive symptom variance. Genetic moderation was statistically unique to major interpersonal stress versus other environments. Extending previous findings, results support an MGPS approach and underscore the cruciality of the G × E candidate environment.

Keywords: multilocus genetic profile score | serotonin | gene-environment interaction | depression | interpersonal stress

Article:

1. Introduction

Stressful life events (SLEs) are a crucial etiological factor in depression, but there is substantial heterogeneity in the stress-depression association. Genetic variation likely influences who is vulnerable to stress, and who is resilient. Caspi and colleagues' (2003) watershed finding that a

polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) moderates the impact of SLEs on depression inspired a wave of studies examining gene-environment interactions (G × Es), many focusing on 5-HTTLPR. Although supported in some well-conducted studies and two meta-analyses (Bleys et al., 2018, Karg et al., 2011), the flood of 5-HTTLPR G × E studies (which varied in phenotypic measure quality) brought with it concerns about replicability and small effect sizes. After publication of non-supportive (albeit arguably selective) meta-analyses (e.g., Culverhouse et al., 2017, Risch et al., 2009) and critiques (e.g., Dick et al., 2015, Duncan and Keller, 2011), many journals adopted rigid editorial policies and agencies directed funding away from candidate gene research. Within 15 years, 5-HTTLPR G × E research went from inception to crescendo to near-extinction.

Never disputed, however, was the likelihood that genetic variation meaningfully contributes to SLE vulnerability at a conceptual level. However, the nature of genetic risk for depression is likely largely additive, with many genes making small contributions (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013, Sullivan et al., 2012). Single-polymorphism G × E designs may produce unreliable findings because they fail to capture additive risk, as each variant contributes a very small effect. Recently, researchers have begun to devise multilocus genetic profile scores (MGPSs), which comprise summed risk alleles from multiple single nucleotide polymorphisms (SNPs) on genes contributing to biological systems relevant to outcomes of interest. Separate MGPSs have been developed to capture genetic risk for multiple biological systems (Nikolova et al., 2011, Pagliaccio et al., 2014), and have demonstrated G × E effect sizes outsizing those in single-variant studies (e.g., Starr and Huang, in press). For example (Vrshek-Schallhorn et al., 2015b), devised a five-SNP MGPS capturing serotonergic genetic variation, and showed that it interacted with major interpersonal SLEs to predict depression in emerging adults, and with interpersonal SLEs in a replication sample of early adolescents. None of the individual SNPs significantly moderated SLE impact yet all produced individual GxE effect sizes in the predicted direction, suggesting the G × E was indeed driven by additive genetic variance. This study suggested that the moderation of emotional consequences of SLEs by serotonergic genetic variation goes beyond 5-HTTLPR and provided a framework for studying dimensionally distributed serotonin-linked genetic risk.

However, two important questions remain unaddressed. First, Vrshek-Schalhorn et al.'s (2015b) findings have yet to be replicated (although the original manuscript provided an independent replication). Replication is increasingly recognized as paramount, both in genetics and in psychological science as a whole. Therefore, here we sought to replicate the G × E finding that serotonergic MGPS moderates the association between major interpersonal stress and depressive symptoms.

Second, the uniqueness of the "candidate environment" for this G × E needs explicit testing. Although work has focused on identifying specific candidate genes, perhaps equally important is the specification of environmental contexts that interact with genetic risk. Research indicates that severe or major events (those with moderate to major long-term contextual threat) are more etiologically relevant to depression, while minor events (SLEs with lower long-term contextual threat) rarely predict depression (Vrshek-Schallhorn et al., 2015a). Further, theory and evidence implicate interpersonal SLEs in depression over and above non-interpersonal SLEs (Brown et al., 1987, Hammen, 2005, Sheets and Craighead, 2014, Stroud et al., 2011, Vrshek-Schallhorn et al., 2015a), consistent with theoretical models positing an inextricable link between interpersonal functioning and depression (Hames et al., 2013; e.g., Starr and Davila, 2008). Previous research has proposed major interpersonal SLEs as a particularly robust "candidate

environment" for serotonergic GxEs; in one study, 5-HTTLPR genotype predicted depression onset significantly more robustly following major interpersonal SLEs than major non-interpersonal SLEs in months with a major event (Vrshek-Schallhorn et al., 2014). Based on this evidence, the first demonstration of the serotonergic MGPS tested GxE effects with major interpersonal SLEs (Vrshek-Schallhorn et al., 2015b). However, the statistical specificity of the MGPS GxE with major interpersonal SLEs was not tested because GxE effects with major non-interpersonal and minor events were not examined.

The present study sought to replicate and extend findings of a serotonergic MGPS and SLE GxE effect on depressive symptoms in 241 community-dwelling adolescents (an age group at high developmental risk for first onset of depression and increased interpersonal stress; Avenevoli et al., 2015, Rudolph, 2002). We examine depression continuously (incorporating subthreshold episodes) as this arguably maps more closely with genetic risk (Korszun et al., 2004), and because subsyndromal depressive symptoms in adolescents are crucial predictors of later threshold-level episodes and long-term disruptions in functioning (e.g., Klein et al., 2009). We hypothesized that higher MGPSs would strengthen associations between major interpersonal SLEs and depressive symptoms, and that interpersonal SLEs would produce significantly greater GxE effects than other SLEs, including major non-interpersonal, minor interpersonal, and minor non-interpersonal SLEs.

2. Method

2.1. Participants and procedure

Participants were 241 adolescents (Mage = 15.90 years, SD = 1.09; 54% female1) ranging from 14 to 17 years, recruited from a mid-sized metropolitan area using multiple methods (e.g., commercial mailing list, public flyers, online ads; see Starr et al., 2017). Exclusion criteria included prior participation of household members; English impairments; and bipolar, psychotic, or pervasive developmental disorders, or major physical or neurological disorders. Adolescents self-identified as: 73.9% White, 12.2% Black, 4.1% Asian, 7.1% Multiracial, 2.1% other or no race reported, and 0.4% Native American; of these, 9.1% identified as Hispanic/Latino. Participants and a primary caregiver attended a lab session; participants provided consent/assent and adolescents completed interviews and questionnaires, and provided saliva samples for genotyping. Families were paid $160 and entered into raffles. Procedures were approved by the University of Rochester Research Subjects Review Board (#RSRB00053831).

2.2. Measures

SLEs. The UCLA Life Stress Interview (LSI; Hammen, 1991) assessed SLEs over the past year. For each SLE, trained interviewers elicited information regarding the context, duration, prior experiences, and available resources, and prepared narratives (excluding adolescents' subjective responses). Diagnosis-blind trained coding teams rated the long-term contextual threat of each SLE on a 1 (no negative impact) to 5 (extremely severe impact) scale (half-points permitted). Each event was also team-rated as predominantly interpersonal (in nature and/or consequence, including social rejection or disapproval, relationship loss, and/or other disruptions to interpersonal relationships [e.g., peer conflicts, break-ups, parental divorce]), or non-interpersonal (all other events); 65% of events were interpersonal. Ratings were based on specific circumstances; for example, unexpected deaths of close caregivers would be rated as more severe than expected

deaths of distant relatives. Independent coding teams rated a subset of events, with excellent reliability (ICC = 0.87). Events rated ≥3.0 were classified as major (13% of events) and events rated 1.5–2.5 were classified as minor (Stroud et al., 2011). The impact ratings across all major interpersonal events (27% reported ≥1 event), major non-interpersonal (7% reported ≥1 event), minor interpersonal (74% reported ≥1 event), and minor non-interpersonal (56% reported ≥1 event) were summed within category for analyses. The relatively low rates of major non-interpersonal events (although consistent with the literature; Monroe and Harkness, 2005) should be taken under consideration. However, using a more liberal major event severity cut-off of ≥2.5 (i.e., increasing percentage of events classified as major) produced comparable results (both cut-offs have precedence in prior research; Slavich et al., 2014, Stroud et al., 2011).

Depressive Symptoms. The Schedule for Affective Disorders and Schizophrenia for School-Aged Children—Present and Lifetime (KSADS-PL; Kaufman et al., 1997) captured symptoms of major depression and dysthymia. A dimensional scale was used to assess severity: 0 = no clinically significant symptoms, 1 = clinically significant mild symptoms, 2 = moderate, subthreshold symptoms, 3 = DSM-IV criteria met, 4 = DSM-IV criteria met with high severity/impairment (e.g., Rao et al., 2000, Starr et al., 2012a). Ratings for current (past month) dysthymia and major depression were collapsed, forming an overall current depressive symptoms score. Interrater reliability based on blind recoding of 20% of audio-recordings was perfect (ICC = 1.0).

Pubertal Maturation. The mean of the 5-item (two sex-specific) Pubertal Development Scale (PDS; Petersen et al., 1988) was used as an a priori covariate (as puberty appears to moderate both genetic and environmental influences on depression; Silberg et al., 1999).

2.3. Genotyping and MGPS calculation

Adolescents provided saliva samples using DNA Genotek (Ontario, Canada) kits. Samples were genotyped by the University of Wisconsin-Madison Biotechnology Center. DNA concentration was detected and quantified using the Quant-iT™ PicoGreen® dsDNA kit (Life Technologies, Grand Island, NY); extraction followed standard salting-out procedures. Genotyping used KBiosciences' competitive allele specific PCR SNP genotyping assay based on dual FRET (KASPar); assays were amplified with the Eppendorf Mastercycler pro 384 thermal cycler using allele-specific primers. The Synergy 2 (BioTek®) plate reader and Gen 5™ software were used to analyze end-point fluorescence signal.

In the original report (Vrshek-Schallhorn et al., 2015b), construction of the MGPS occurred in two steps: selection based primarily on functionality (i.e., biological impact), and separately, coding of risk direction. First, the 5-SNP MGPS includes four SNPs located on or near four serotonergic genes (HTR1A, HTR2A, HTR2C, and two in TPH2); one TPH2 variant was non-functional and was indicated by a meta-analysis. Second, because there is considerable debate whether low or high serotonergic functioning is linked to depressive mood (e.g., Andrews et al., 2015), risk direction was instead coded based effect sizes of these variants with depression and related outcomes (e.g., suicide, stress-responding), in studies (e.g., Gao et al., 2012, Lemonde et al., 2003, Li et al., 2006). The final MPGS included HTR1A rs6295 G-allele in forward coding direction, HTR2A rs6314 C-allele, HTR2C rs6318 C-allele, TPH2 rs11178997 T-allele and rs4570625 G-allele (the meta-analytic SNP).

In this study, MGPS was coded precisely following Vrshek-Schallhorn et al. (2015b) procedures, with no deviations. SNPs were coded based on the number of risk alleles (0–2), except

for rs6318 which is X-linked (coded as C-allele presence [1] or absence [0]). Scores were summed to create an additive index reflecting number of risk alleles (possible range 0–9, sample range 2–9). We permitted missing data for one SNP (20%), rescaling the MGPS using available data (no participants were missing >1 SNP). All SNPs were in Hardy-Weinberg equilibrium.

2.4. Data analytic approach

All G × Es were tested in separate models using the SPSS PROCESS macro (Hayes, 2013). Models predicted depressive symptoms, and initial "minimal" models included sex, pubertal maturation, age, and White race as covariates; the main effects of stress and MGPS; and the Stress × MGPS interactions. For additional tests for robustness, additional "robust" models were tested that included interactive effects between the environmental variable, MGPS, and each of the four covariates (e.g., MGPS x Sex; Stress x Sex, etc., see Keller, 2014). We then conducted Cook's Distance Tests with robust models to ensure that results were not explained by multivariate outliers (using a critical threshold of 1.0); no problems were identified. Significant interactions were decomposed at M±1 SD (or at the lowest possible value if M-1 SD was out-of-range), and Johnson-Neyman tests (JNT) identified regions of significance. To further test robustness, significant interactions were re-run controlling for quadratic terms for genetic and environmental components (Dick et al., 2015). To reduce Type I error risk, False Discovery Rate (FDR) corrections were applied to interactions following Benjamini and Hochberg's (1995) procedures (4 tests, Benjamini-Hochberg $p < .05$ were considered significant). Deviance tests evaluated whether GxE effect sizes significantly differed from each other, by comparing constrained and unconstrained models with an incremental F test. Significant interactions were also probed with sensitivity tests to gauge direction and magnitude of individual SNP GxE effects and to rule out that one SNP drove effects (Vrshek-Schallhorn et al., 2015b): In individual variant analyses, significant G × E effects were rerun with individual variants in place of the MGPS, and in n-1 analyses, significant G × E effects were rerun with five 4-variant MGPS missing one variant in turn.

3. Results

3.1. Preliminary analyses

Main Effects and Gene-Environment Correlations. See Table 1 for intercorrelations and descriptive statistics. There was no main effect of MGPS on depressive symptoms. Symptoms were significantly correlated with both major and minor interpersonal stress, but not with major or minor non-interpersonal stress. Although major and minor SLEs were significantly correlated within interpersonal and non-interpersonal categories, interpersonal major and minor SLEs were not significantly correlated with non-interpersonal major and minor SLEs, suggesting that interpersonal and non-interpersonal stressors represent orthogonal candidate environments. Interestingly, the gene-environment correlation between MGPS and minor interpersonal stress was significant (but modest).

Table 1. Bivariate correlations among study variables and descriptive statistics.

	1.	2.	3.	4.	5.	6.
1. MGPS	–					
2. Depressive Symptom Rating	.01	–				
3. Interpersonal Major SLE Severity	.07	.33***	–			
4. Non-Interpersonal Major SLE Severity	.00	.06	.03	–		
5. Interpersonal Minor SLE Severity	.13*	.14*	.14*	.04	–	
6. Non-Interpersonal Minor SLE Severity	.09	.07	.10	.13*	.04	–
M	6.45	0.29	0.92	0.29	3.19	1.76
SD	1.34	0.79	1.97	1.20	2.94	2.07
Sample Minimum	2	0	0.0	0.0	0.0	0.0
Sample Maximum	9	4	12.0	11.0	14.5	10.5

*p < .05, ***p < .001.

Notes. N = 241. MGPS = multilocus genetic profile score. SLE = stressful life event.

Tests of Racial Effects. To ensure that results were not influenced by population stratification (i.e., differences in allele distributions across racial groups that coincidentally correlate with racial differences in variables of interest), we examined whether MGPS was associated with race. Adolescents reporting European ancestry (the largest racial subgroup) did not differ from non-Whites on MGPS (t (64.58) = −0.27, p = .789). Moreover, we found no racial differences in the candidate environments, with the exception of non-interpersonal minor stress, which was higher among non-White participants (t (60.45) = 2.45, p = .017). Race did not moderate the association between MPGS and depressive symptoms (p > .05), nor did it moderate the associations between any of the tested candidate environments and depressive symptoms (all ps > .05). Based on these results, we conducted primary analyses in the full sample for maximal power, but then replicated all results in the White subsample.

3.2. Gene-environment interactions

Test of Hypothesized G × E: MGPS × Major Interpersonal Stress. Supporting hypotheses, MGPS significantly moderated the major interpersonal stress-depressive symptoms association (see Table 2), p < .001, FDR-corrected p = .002 (interaction accounted for 4% of variance). Major interpersonal stress was associated with depressive symptoms at high MGPS, b = 0.18, SE = 0.03, p < .001, but not at low MGPS, b = 0.01, SE = 0.04, p = .770 (Fig. 1). JNT revealed that major interpersonal stress was significantly associated with depressive symptoms when MGPS ≥5.87 (76% of sample); moreover, for adolescents with MGPS≤2.16, major interpersonal stress was significantly negatively associated with symptoms, although this result should be interpreted with caution as it applied to <1% of the sample. As an alternative decomposition, the effect of MGPS varied as a function of major interpersonal stress, so that MGPS predicted higher depressive symptoms at higher stress levels (b = 0.10, SE = 0.05, p = .031) but marginally lower symptoms in the absence of such stress (b = −0.08, SE = 0.04, p = .052). In “robust” models with all interactive covariates, results were essentially unchanged, with a significant interaction between MGPS and major interpersonal stressors, β = 0.21, p = .003, and the same decomposition pattern.

Table 2. Full model results for moderation of association between major interpersonal and non-interpersonal stressful life event (SLE) severity and depressive symptoms by serotonergic multilocus genetic profile score (MGPS).

	β	b	SE	p	95% CI
Intercept		.17	.75	.818	[-1.30, 1.65]
MGPS	-.04	-.02	.04	.517	[-.09, .05]
Major Interpersonal SLE Severity	.24	.10	.03	<.001	[.05, .15]
MGPS × Major Interpersonal SLE Severity	**.22**	**.06**	**.02**	**<.001**	**[.03, .10]**

Notes. N = 241. Race, age, pubertal development, and sex were included as covariates.

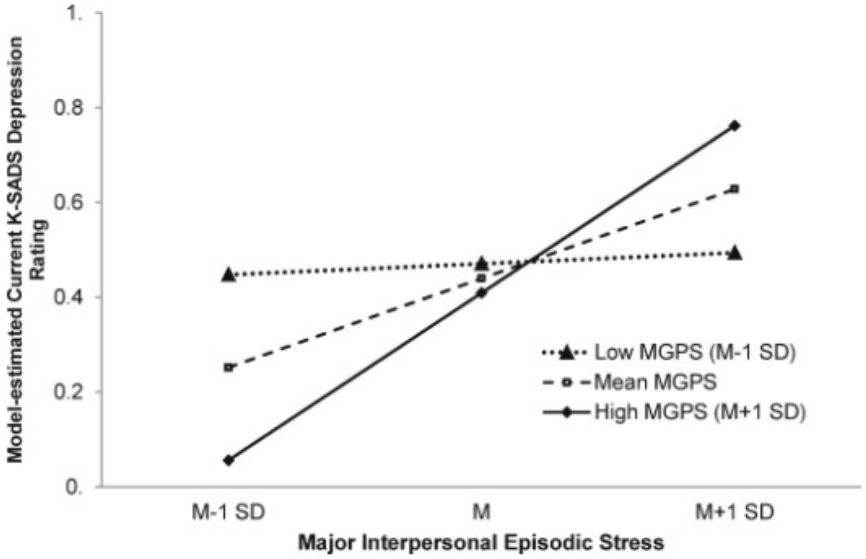

Fig. 1. Depressive symptoms as a function of major interpersonal episodic stress at low, mean, and high levels of serotonergic multilocus genetic profile score (MGPS).

Tests of Non-Hypothesized G × Es. In the initial "minimal" model, the interaction between major non-interpersonal stress and MGPS was significant, but in the complete opposite direction as for major interpersonal stress, β = −0.17, p = .009, FDR-corrected p = .018 (i.e., major non-interpersonal events predicted depressive symptoms at low, but not high, MGPS). However, in the "robust" model that contained interactive covariates, the interaction was no longer significant, β = −0.13, p = .221, suggesting the unanticipated results may have been a consequence of clustering of covariate variables with stress and MGPS variables. Given the non-hypothesized nature of this effect and its non-replication under the robust model, we discourage readers from interpreting the significant of the initial model. To confirm that the major non-interpersonal and interpersonal SLE G × E effects differed significantly, we performed deviance tests, and given the difference in results, to be conservative, we utilized results from both the minimal and robust models. The Major Interpersonal SLE G × E interaction term significantly differed from the Major Non-Interpersonal SLE G × E interaction term in both minimal (F (1, 231) = 15.36, p < .0001) and robust (F (1, 219) = 5.56, p = .019) models.

Interactions between MGPS and minor event severity were non-significant (minor interpersonal β = .01, p = .904, FDR-corrected p = .904; minor non-interpersonal β = .04, p = .501, FDR-corrected p = .523). Results were near-identical when using the robust models. The major

interpersonal GxE effect was significantly larger than the non-significant GxE effects for each minor interpersonal stress ($F(1,231) = 10.39$, $p = .001$) and minor non-interpersonal stress ($F(1,231) = 10.92$, $p = .001$).

Replication in White Sub-Sample. To ensure results were not affected by population stratification, results were replicated in the largest racially homogeneous subsample (White; $n = 192$). MGPS significantly moderated depressive symptom associations with interpersonal major stress in both minimal and robust models ($\beta s = 0.20$ in both models, $ps = .003$ and $.008$, respectively), in the directions reported above. As in the full sample, in the minimal model, MGPS significantly moderated the association between non-interpersonal major stress and depressive symptoms but in the complete opposite direction of effect ($\beta = -0.19$, $p = .012$); however, as in the full sample, in the robust model the non-interpersonal major interaction was non-significant ($\beta = -0.13$, $p = .307$), and thus, not interpreted. MGPS did not moderate either of the minor event indices in the White sample.

Sensitivity Tests. See Table 3. ***Individual variant analyses***. For major interpersonal stress, two SNPs produced significant G × Es (rs6295 and rs4570625), but only rs6295 survived FDR correction; all SNPs showed positive G × E effect sizes. ***N-1 analyses***. All n-1 modified MGPS × Major Interpersonal Stress effects were significant ($ps < .05$), indicating that no single SNP accounted for G × E effects.

Table 3. Interaction Terms for Gene-by-Interpersonal Major SLE Interactions, Predicting Depressive Symptoms, for a) Individual MGPS Constituent SNPs, and b) n-1 Modified MGPSs with SNP Excluded.

SNP	a) Individual SNP				a) n-1 MGPS Excluding SNP			
	b	***SE***	***p***	**FDR**	***b***	***SE***	***p***	**FDR**
rs11178997	.06	.08	.429	.536	.07	.02	<.001	.004
rs4570625	.09	.05	.042	.105	.09	.02	<.001	.003
rs6318	.02	.05	.743	.743	.05	.02	.002	.003
rs6314	.05	.04	.192	.320	.06	.02	.002	.004
rs6295	.10	.03	.002	.010	.04	.02	.049	.049

Notes. MGPS = Multilocus genetic profile score. FDR= False Discovery Rate Correction for 5 tests (Benjamini and Hochberg, 1995).

Exploratory Gender Analyses. As a final step, we examined sex as an exploratory moderator of the MGPS × Major Interpersonal Stress effect. Surprisingly, depressive symptoms were only marginally higher in girls in this sample, $t(235.45) = -1.81$, $p = .072$. A three-way interaction was tested (MGPS × Major Interpersonal Stress × Sex) with White, age, and pubertal maturation as covariates, and it was non-significant (interaction $\beta = 0.02$, $p = .943$).2

4. Discussion

In a community sample of mid-adolescents, we provide a critical replication, showing that a serotonergic MGPS interacts with major interpersonal stress to predict depressive symptoms. We extend this finding with a direct test in support of major interpersonal SLEs as a "candidate environment" for this G × E. Results also offer new evidence that major interpersonal stress represents a particularly potent candidate environment for serotonergic G × Es (e.g., Vrshek-Schallhorn et al., 2014). Although previous tests examining candidate environments have also demonstrated that G × Es are significant with interpersonal stress but non-significant with non-

interpersonal stress (Feurer et al., 2017; e.g., Vrshek-Schallhorn et al., 2014), we provide a more conclusive test by subjecting the differences in magnitude of the interaction terms to significance testing. We show that high MGPS uniquely elevated the association between major interpersonal stress and depressive symptoms, with a significantly larger effect size than for major non-interpersonal events, minor interpersonal events, and minor non-interpersonal events.

Our replication further strengthens the case that this serotonergic MGPS significantly contributes to genetic risk for depression through its interaction with major interpersonal SLEs, here capturing approximately 4% of variance in depressive symptoms, a figure comparable to the initial investigation of this MGPS (unique GxE R-squared = 0.03; see Footnote 5, Vrshek-Schallhorn et al., 2015b), although generalized-R2 is considered to underestimate variance, so actual variance explained may be higher. These findings support claims that the MGPS approach has promise for revealing genetic relationships with psychopathology. The MGPS approach likely enhances power over single variants because dimensional variables reduce Type II error likelihood and because MGPSs capture multiple variants' additive effects, boosting effect sizes over single variants. Effect sizes also may have been enhanced by our use of dimensional depression outcomes (instead of discrete diagnoses), given the inherent power advantages of continuous data. In line with the benefits of the MGPS approach, recent analyses (Starr and Huang, in press) examining a 10-SNP hypothalamic-pituitary-adrenal (HPA)-axis MGPS found a G × E accounting for 8% of continuously-defined depression variance (80 times the presumed effect size of typical "moderate" single-variant GxE [0.1%] that has guided strict sample size requirements; Duncan and Keller, 2011). These larger effect sizes may allow researchers to investigate G × Es in somewhat smaller samples which increases the feasibility of using high-quality, labor-intensive stress assessments (which are themselves associated with higher GxE effect sizes; Karg et al., 2011).

In addition to the power benefits over single-SNP approaches, the MGPS approach offers some advantages over GWAS-derived polygenic risk scores (PRSs; Musliner et al., 2015). PRSs are, without doubt, an important innovation in genomics research with many fascinating research applications (Bogdan et al., 2018). However, PRSs are typically derived from main effect associations with outcomes of interest (e.g., depression), which may mean that a) they are likely less relevant to G × Es (because predicting a main effect does not imply prediction of an interaction effect), and b) individual component SNPs within a depression PRS may predict depression via totally different biological pathways. In contrast, the MGPS approach offers the ability to a) select SNPs related to G × Es, and b) focus on variants related to a single biological system, which are likely to operate mechanistically via shared intermediate phenotypes. This latter feature is especially relevant given the increased incorporation of pharmacogenomics in psychiatry (Reynolds et al., 2014), with potential implications for enhanced personalized medicine.

Next steps could include probing mechanisms by which this MGPS contributes to depression under major interpersonal stress, considering psychological and biological pathways. Although 5-HTTLPR G × E research has generated a highly controversial body of literature, meta-analytic evidence suggested that this polymorphism might confer dysregulated cortisol reactivity (Miller et al., 2013). Research examining multilocus serotonergic risk in relation to HPA-axis functioning has been limited, but one study suggested that the current MGPS interacts with the cortisol awakening response to prospectively predict depression (Vrshek-Schallhorn et al., in press), suggesting complex interplay between serotonergic genetic risk, HPA-axis functioning, and depression. In addition to HPA-axis functioning, reviews of the human and animal literature on the serotonin system suggest that it modulates behavioral constraint (Spoont, 1992) or top-down

versus bottom-up control (Carver et al., 2008); the serotonergic MGPS could potentially manifest as related psychological constructs.

Our findings indicate that major interpersonal SLEs represent a uniquely potent candidate environment for this MGPS. Why would serotonergic genetic variation have uniquely depressogenic implications in the context of interpersonal versus non-interpersonal major events or minor events? For decades, depression theorists have argued that traits (e.g., sociotropy versus autonomy) govern whether individuals are more vulnerable to interpersonal versus non-interpersonal stressors (Beck, 1983, Blatt, 1974), and it is likely that such individual differences would be at least partially genetic mediated. Indeed, some have argued that the serotonin system mediates sensitivity to social contexts (e.g., Way and Taylor, 2010). Moreover, serotonin functioning also has downstream effects on HPA axis activity, which is particularly responsive to interpersonal threats (Dickerson and Kemeny, 2004). It may also be that major interpersonal stress is more likely to yield GxE effects because it is a more potent predictor of depression than other forms of stress, and larger main effects of stress on depression portend greater power for identifying GxE effects. These ideas are highly speculative, and should our results be replicated, further work testing them is needed.

What is clear is that it is important for researchers to include valid measures of the environment, including major interpersonal stress, in research examining genetic risk for depression (Monroe and Reid, 2008). In addition to the differential effects of the 5HT MGPS by type of major SLE, post-hoc analyses of the major interpersonal stress GxE effect revealed a protective effect of the genotype that approached significance ($p = .052$) under relatively better conditions (i.e., lower levels of major interpersonal stress). This echoed prior findings that under relatively less stressful conditions, increasing MGPS was significantly protective against depression (Vrshek-Schallhorn et al., 2015b). Thus, based on 3 samples, we conclude that this GxE effect most likely adheres to a differential susceptibility framework (Belsky and Pluess, 2009). Intriguingly, recent analyses in a large sample using a genome wide association study (GWAS)-based polygenic risk score approach also support for a sex-specific differential susceptibility model, where women (but not men) at "high" polygenic risk for depression were protected against depression if they had experienced no recent SLEs (Arnau-Soler et al., 2019). Critically, if such processes occur on a broad scale for genes involved in depression, this pattern may result in the protective and risk enhancing GxE effects cancelling each other out when the proper candidate environment is not isolated, or when no environmental variables are considered. This may have contributed to replication failures in the 5-HTTLPR literature, and may also thwart attempts to identify variants involved in depression via GWAS. These findings thus argue that stress—and indeed the specific candidate environment of major interpersonal events—is fundamental to accurately understanding the genetic architecture of depression.

Limitations. Despite strengths including using diagnostic and life stress interviews and employing a cutting-edge genetic variable, our study has limitations. Sample size was typical for studies employing these diagnostic and life stress interviews, but small for genetic studies. Stress and depressive symptoms were assessed simultaneously; however, even in prospective longitudinal studies of stress and depression, concurrent interview pairs are used to isolate stress that occurred in the months prior to depression onset. That said, it is possible that the GxE effects are amplified by stress generation among those at higher genetic risk (Huang and Starr, in press, Starr et al., 2012b). Further, adolescents were recruited from the community and rates of clinical depression were low; results should be replicated in more severe samples. Future studies should also consider conducting competitive significance testing comparing the current MGPS to

randomly generated profiles (see Di Iorio et al., 2017); we could not conduct this useful test because we did not utilize a GWAS chip for genotyping.

In conclusion, this study replicates and extends evidence that a serotonergic MGPS intensifies the major interpersonal stress-depressive symptom association, and that this effect is dependent on the nature of the SLEs. Future studies—whether genetic in nature or not—should emphasize major interpersonal stress as a crucial candidate environment for depression. Our results support the MGPS approach as a viable path towards resuscitating theory-driven research on the interplay between genes and the environment.

Conflicts of interest

The authors declare no conflicts of interest.

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