Additive genetic risk from five serotonin system polymorphisms interacts with interpersonal stress to predict depression

By: Suzanne Vrshek-Schallhorn, Catherine B. Stroud, Susan Mineka, Richard E. Zinbarg, Emma K. Adam, Eva E. Redei, Constance Hammen, Michelle G. Craske

Vrshek-Schallhorn, S., Stroud, C.B., Mineka, S., Zinbarg, R.E., Adam, E.K., Redei, E.E., Hammen, C., Craske, M.G. (2015). Additive genetic risk from five serotonin system polymorphisms interacts with interpersonal stress to predict depression. Journal of Abnormal Psychology 124(4): 776-790.

Abstract:

Behavioral genetic research supports polygenic models of depression in which many genetic variations each contribute a small amount of risk, and prevailing diathesis-stress models suggest gene– environment interactions (G×E). Multilocus profile scores of additive risk offer an approach that is consistent with polygenic models of depression risk. In a first demonstration of this approach in a G×E predicting depression, we created an additive multilocus profile score from 5 serotonin system polymorphisms (1 each in the genes HTR1A, HTR2A, HTR2C, and 2 in TPH2). Analyses focused on 2 forms of interpersonal stress as environmental risk factors. Using 5 years of longitudinal diagnostic and life stress interviews from 387 emerging young adults in the Youth Emotion Project, survival analyses show that this multilocus profile score interacts with major interpersonal stressful life events to predict major depressive episode onsets (hazard ratio [HR] = 1.815, p = .007). Simultaneously, there was a significant protective effect of the profile score without a recent event (HR = 0.83, p = .030). The G×E effect with interpersonal chronic stress was not significant (HR = 1.15, p = .165). Finally, effect sizes for genetic factors examined ignoring stress suggested such an approach could lead to overlooking or misinterpreting genetic effects. Both the G×E effect and the protective simple main effect were replicated in a sample of early adolescent girls (N = 105). We discuss potential benefits of the multilocus genetic profile score approach and caveats for future research.

Keywords: serotonin | interpersonal | stressful life events | depression | gene-environment interaction

Article:

A key component of ontogenic models of risk for psychopathology is individual differences in vulnerability to environmental adversity (e.g., Beauchaine & McNulty, 2013). Over the past decade, molecular genetic individual differences (i.e., genetic polymorphisms) have received much attention in terms of their interactions with life stress predicting psychopathology, particularly depression. For example, Caspi and colleagues' (2003) watershed finding that serotonin transporter polymorphism (5-HTTLPR) genotype moderated the effects of recent

stressful life events (SLEs) on risk for depression stimulated hundreds of studies. The largest and most recent meta-analysis supports this gene–environment interaction (G×E), particularly when using objective life stress measurement rather than questionnaire measures (Karg, Burmeister, Shedden, & Sen, 2011). However, two earlier, less-inclusive meta-analyses found no significant G×E effects for 5-HTTLPR (Munafò, Durrant, Lewis, & Flint, 2009; Risch et al., 2009), and the G×E area has also come under significant criticism (Duncan & Keller, 2011).

Existing research has typically examined G×Es using individual polymorphisms, with several exceptions for gene–gene epistatic interactions (e.g., Conway, Hammen, Brennan, Lind, & Najman, 2010; Kaufman et al., 2006). However, behavioral genetic research supports polygenic models of risk wherein many genetic variants each contribute a small amount of risk (Fisher, 1918; Wright, 1921). Evidence suggests that risk is substantially additive in nature (i.e., based on a sum of risk alleles), although a smaller amount of risk arises from epistasis (gene–gene interactions) and dominant or recessive alleles at each marker (e.g., Chipuer, Rovine, & Plomin, 1990). Indeed, polygenic and additive assumptions form the basis for estimations of heritability in twin research, which estimates that genetic factors contribute moderately to depression risk (Sullivan, Neale, & Kendler, 2000). Within hypothesis-driven candidate gene research, new multilocus genetic profile scores offer an approach that is consistent with polygenic and additive assumptions of risk inheritance. For example, Nikolova and colleagues (2011) showed that a novel multilocus genetic profile score using five candidate polymorphisms in the dopamine system predicted activation of a certain brain region involved in reward functioning (the ventral striatum) during a reward-related functional MRI task. (Reflecting Nikolova et al.'s, 2011, terminology, throughout, we use the term "multilocus genetic profile score" to indicate a hypothesis-driven and unweighted sum of SNP risk alleles.) To expand upon prior work, we examined whether a novel multilocus genetic profile score using five polymorphisms in the serotonin system (other than the serotonin transporter polymorphism) moderates the effect of interpersonal stress on risk for depression. We first examined risk for major depressive episode (MDE) onsets in a sample of individuals transitioning from older adolescence to emerging adulthood; second, we predicted depression symptom level in a replication sample of early adolescents, two developmental periods important for depression (Rohde, Lewinsohn, Klein, Seeley, & Gau, 2013).

Multilocus Genetic Profile Scores in Psychological Research

Multilocus profile scores have recently emerged as a means of capturing additive risk across multiple polymorphisms. For example, Nikolova and colleagues constructed a profile score from five functional single-nucleotide polymorphisms (or "SNPs" for short) in dopaminergic genes, each coded with higher values reflecting greater putative levels of midbrain dopamine, and then summed.

Of interest, none of the individual SNPs from this multilocus profile score by itself significantly predicted reward-related ventral striatal brain activation in post hoc analyses, supporting the cumulative effect of the five SNPs. This same dopaminergic multilocus profile score was associated with depression symptoms (Pearson- Fuhrhop et al., 2014). A cumulative serotonergic genetic score (5-HTTLPR, HTR1A rs6295, and HTR2A rs6311) was also applied to predict increased bias for looking at dysphoric images and away from positive ones following a sad mood induction (Disner, McGeary, Wells, Ellis, & Beevers, 2014).

By contrast to these hypothesis-driven approaches, research has also used the most significant SNPs in genome-wide association studies (GWAS) to create weighted polygenic risk

scores, an approach developed in GWAS to identify individuals at high risk for complex diseases (e.g., Wray, Goddard, & Visscher, 2007). For example, Belsky et al. (2013) used a polygenic risk score developed from a prior smoking GWAS to predict the development of nicotine dependence over and above family history. In a G×E application, Salvatore et al. (2015) showed that a polygenic risk score derived from an externalizing disorder GWAS interacted with adolescent parental monitoring and peer substance use to predict adolescent externalizing disorders. The application of multilocus profile scores may prove useful for examining G×E interactions in depression.

Candidate Genes in the Serotonin System

The Serotonin System

Although a simplistic monoamine hypothesis in which low neural levels of serotonin (5-hydroxytryptamine, or 5-HT) "cause" depression is unlikely (e.g., Andrews, Bharwani, Lee, Fox, & Thomson, 2015), the serotonin system remains implicated in depression risk from several very different types of research. For example, protocols that deplete brain serotonin temporarily can induce depression-like symptoms in at-risk individuals (for a review, see Booij, Van der Does, & Riedel, 2003). In addition, a meta-analysis indicates that at least one serotonin-specific reuptake inhibitor, paroxetine, outperformed placebo at treating depression particularly in severe cases (Fournier et al., 2010). Moreover, three separate meta-analyses implicate the serotonin transporter polymorphism 5-HTTLPR in depressive processes: (a) it moderates response to stress on risk for depression (Karg et al., 2011), (b) it predicts amount of cortisol reactivity to lab-induced stress (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013), and (c) it predicts response to SSRI medication during depression (Serretti, Kato, De Ronchi, & Kinoshita, 2007). Further, extensive human and animal research documents serotonin's role in processes related to mood regulation (for reviews, see Carver, Johnson, & Joormann, 2008; Spoont, 1992).

Thus, we sought to investigate the potential additive effect of genetic variants in the serotonin system. A literature search yielded four potential functional variants (those that confer biological differences) other than 5-HTTLPR, on which we previously reported (Vrshek-Schallhorn et al., 2014). This search also yielded one nonfunctional SNP for which there is meta-analytic support for a role in affective disorders. We then reviewed prior research on each of these five SNPs to determine the allele most implicated in affective disorders (i.e., the risk allele).

5-HT1A Receptor SNP rs6295

The 5-HT1A negative autoreceptor downregulates serotonergic activity, and is implicated particularly in recurrent depression (Drevets et al., 2007). In the HTR1A gene encoding this receptor, a functional C to G promoter polymorphism, rs6295, located at basepair 1019, is reported to affect 5-HT1A protein levels and binding potential (Lemonde et al., 2003). Several studies have concluded that either G-carriers or G/G homozygotes are at greater risk for depression (Anttila et al., 2007; Lemonde et al., 2003) or comorbid depression with generalized anxiety disorder (Molina et al., 2011), and that they show poorer response to antidepressant treatment (e.g., Hong, Chen, Yu, & Tsai, 2006; Lemonde, Du, Bakish, Hrdina, & Albert, 2004).

5-HT2A Receptor SNP rs6314

The 5-HT2A receptor is implicated in memory processes, and a C to T substitution at basepair 1354 in its gene HTR2A confers a Histidine to Tyrosine amino acid change at codon 452 (Ozaki et al., 1996), a functional SNP (Ozaki et al., 1997). Several studies indicated that the rarer T-allele is associated with poorer memory recall (de Quervain et al., 2003; Wagner, Schuhmacher, Schwab, Zobel, & Maier, 2008). In a meta-analysis of this genetic variant, a subset of suicidal case-control studies examining suicidal ideation and suicide attempt (vs. suicide completion), found that a lower frequency of the rarer T-allele in cases versus controls, $p = .045$; however, the overall meta-analysis was not significant, $p = .21$ (Li, Duan, & He, 2006). Thus, although the T-allele appears deleterious for memory, there are hints that the C-allele may increase risk for depression.

5-HT2C Receptor SNP rs6318

The 5-HT2C receptor is involved in mood dysregulation (Heisler, Zhou, Bajwa, Hsu, & Tecott, 2007). In the HTR2C gene encoding this receptor, a G to C mutation at basepair 68 leads to a Serine for Cysteine amino acid substitution at codon 23 (Lappalainen et al., 1995), and the C-allele has been shown to confer greater receptor activity (Okada et al., 2004). The HTR2C gene is located on the X-chromosome, of which males have only one copy. Females carry two copies of the gene, one of which is inactivated at random by a well-characterized epigenetic process on a cell-by-cell basis. Female C/C homozygotes, but not male C hemizygotes, experienced greater depression symptoms in the context of higher levels of questionnaire-reported life stress (Brummett, Babyak, Williams, et al., 2014). In addition, C-carriers had greater cortisol and mood reactivity to lab-induced stress in two studies (Brummett, Babyak, Kuhn, Siegler, & Williams, 2014; Brummett et al., 2012).

Two SNPs in the Gene Encoding Tryptophan Hydroxylase-2: rs11178997 and rs4570625

Tryptophan hydroxylase-2 (encoded by the gene TPH2) catalyzes the rate-limiting step in the production of serotonin, and is the predominantly active form of this enzyme in the brain (for a review, see Invernizzi, 2007). We focused on two SNPs in the TPH2 promoter region: rs11178997, a T to A substitution, and rs4570625, a G to T substitution. The A-allele of rs11178997 was shown to confer reduced transcriptional activity of TPH2 because of reduced binding of a key transcription factor; this pattern ought to lead to slower serotonin production for A-carriers (Scheuch et al., 2007). In this same study, rs4570625 was not associated with functional alterations. However, in a meta-analysis examining these and 10 other TPH2 SNPs on risk for major depression, the G-allele of rs4570625 was the only SNP to produce significant results in conservative random-effect models (Gao et al., 2012).1 Similarly, in this same meta-analysis, the rs11178997 T-allele increased risk for major depression in an initial fixed effects meta-analysis of four studies ($p = .0136$), but was nonsignificant in a more stringent random-effects model ($p = .0853$). Notably, these two SNPs are located near one another and are in linkage disequilibrium, indicating they are sometimes inherited together (Zhou et al., 2005).

Interpersonal Major Stressful Life Events and Chronic Stress: The Candidate Environment

Thoughtfully conceptualized and measured environmental measures are integral to the advancement of G×E research (e.g., Monroe & Reid, 2008). For instance, in a 5-HTTLPR G×E metaanalysis, studies using objective or interview-based measures of life stress yielded more robust G×E effects than did studies using questionnaires measures known to lack validity, such as event checklists (Karg et al., 2011). This supports the notion that using higher quality measures provides greater benefit for statistical power than does adding more participants, as suggested in a simulation study (Wong, Day, Luan, Chan, & Wareham, 2003).

Similarly, selecting the most theoretically and empirically "potent" forms of stress ought also to enhance power. Briefly, multiple different forms of stress are associated with depression, but also correlate with one another, necessitating multivariate models to isolate the stressors contributing significant unique variance to depression. Using data from the Youth Emotion Project (YEP) and a second study of emerging adults, we showed that two forms of stress consistently contributed significant unique variance over and above other forms of stress: major interpersonal SLEs and interpersonal chronic stress (Vrshek-Schallhorn et al., in press).2 Major SLEs refer to acute occurrences that carry at least moderate or greater impact or threat, while chronic stress refers to quality of ongoing conditions in various roles, typically measured dimensionally. Interpersonal stressors are those that impact the quality and quantity of relationships with others such as intimate relationships, friendships, social life, and family life (e.g., Vrshek-Schallhorn et al., in press). The stress-depression relationship is robust (e.g., Cole & Dendukuri, 2003; McLeod, Weisz, & Wood, 2007) and interpersonal stress is central to theoretical models of depression (e.g., Hammen, 2003; Joiner & Metalsky, 1995).

The interpersonal distinction may aid GE depression research. Indeed, we showed that an interaction of 5-HTTLPR genotype and major events was driven by interpersonal major events and not noninterpersonal major events (Vrshek-Schallhorn et al., 2014). This highlights the utility for GE research of both carefully conceptualizing and measuring the "candidate environment." Here, we conduct GE analyses using major interpersonal SLEs and interpersonal chronic stress because past research and theory have suggested these are the most potent and uniquely predictive forms of life stress for depression.

Modeling the Main Effects of Genetic Variants

Although one recent GWAS was able to replicate two significant variants (CONVERGE Consortium, in press), GWAS for depression have largely struggled to identify replicable variants reaching genome-wide levels of significance (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). However, recent mega-GWAS for other disorders including schizophrenia have been remarkably successful realizing the potential to reveal never-before-hypothesized genes involved in these diagnoses (e.g., Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014); similarly large GWAS investigations are underway for depression. In contrast to schizophrenia, behavioral genetic studies indicate that depression has a substantially greater environmental basis (an estimated 63% for depression vs. 19% for schizophrenia; Sullivan, Kendler, & Neale, 2003; Sullivan et al., 2000). Thus, if a significant GE can be shown with an additive multilocus profile score, it may also be useful to estimate the main effects of the multilocus profile score and individual SNPs. Difficulty identifying significant

variants in depression GWAS may partly arise from inadequate samples sizes to-date, but may also arise from not accounting for interactions with stress. If this is the case, the main effects of the multilocus profile score and individual SNPs ought to produce effect sizes that are unlikely to be detected by GWAS.

The Present Study

We hypothesized that a serotonergic multilocus profile scoreusing additive genetic effects from five SNPs located in or near four genes (HTR1A, HTR2A, HTR2C, and TPH2) would significantly interact with recent interpersonal major SLEs and separately with interpersonal chronic stress to predict MDEs (i.e., whether or not they were initial episodes). Based on polygenic, additive models, the G×E effects of the individual SNPs should be substantially smaller, leading to insufficient power to detect any at a significant level. In the primary sample, data came from the YEP, a longitudinal study of risk for emotional disorders that oversampled for high levels of neuroticism and began when participants were high school juniors. Oversampling for high levels of neuroticism, leading to larger numbers of prospective episodes of depression, should enhance power to detect significant predictors of episode onset in proportional hazards modeling (Hsieh & Lavori, 2000). Five years of diagnostic and life stress interview data from the YEP were included in analyses. In the replication sample, data came from a 1-year longitudinal study of early adolescent girls and their mothers, who enrolled when the girls were between 6th and 8th grades. Thus, participants were (a) older adolescents to emerging adults and (b) early adolescents, both important developmental periods for understanding the etiology of depression (Rohde et al., 2013).

Primary Sample Method

Participants

Participants (final N = 387) were a subset of the YEP sample that provided DNA (see Zinbarg, Mineka, et al., 2010 for a description of the full sample). Briefly, high school juniors were screened for neuroticism level using the Revised Eysenck Personality Questionnaire (Eysenck, Eysenck, & Barrett, 1985). Those scoring in the top tertile on the Revised Eysenck Personality Questionnaire were oversampled for invitation to the longitudinal study to increase the number of prospective onsets of internalizing disorders. Participants (N = 627) provided informed consent for the longitudinal study and completed the baseline diagnostic and life stress interviews. They were asked to repeat these interviews annually; data from the baseline interview plus four annual interviews (5 years total) are reported. Participants still in contact with the study were invited to provide a DNA sample beginning in the 6th year of the larger YEP study; 410 participants consented and provided samples. Of these, 23 additional participants were excluded for one or more of the following reasons: missing >1 genotype included in the multilocus profile score (n = 5), lacking the baseline socioeconomic status (SES) measurement (n = 7), lifetime diagnosis of bipolar disorder I or II (n = 8) or psychotic symptoms (n = 3), or major depression lasting all months assessed (n = 1). The final sample comprised 387 individuals (268 females, 69.3%; M age at baseline = 16.91 years, SD = 0.37). They had an average of 4.96 (SD = 0.21, range 4–5) out of five genotypes available, and contributed an average of 53.60 person-months (SD = 11.10, range 13–70) to analyses after month-specific exclusions described below. Participants ranged from lower to upper class in SES, and were on average upper-middle class (Hollingshead SES; range

13–66, M = 48.41, SD = 12.51). Self-reported ethnicity was Black, 13.2%; Asian, 4.4%; White, 48.3%; Hispanic, 14.5%; Pacific Islander, 0.8%; multiple races/ethnicities, 13.2%; and other, 5.7%. Participants experienced 145 MDEs during the study (no episodes, n = 290; one episode, n = 60; two episodes, n = 29; three episodes, n = 5; four episodes, n = 3).

Materials and Procedures

Socioeconomic status. Baseline SES scores used Hollingshead's(1975) index of parental education and occupation.

Diagnostic interview. A baseline Structured Clinical Interview for Diagnostic and Statistical Manual for Mental Disorder- Fourth Edition (DSM-IV), nonpatient edition (SCID; First, Spitzer, Gibbon, & Williams, 2001) assessed lifetime diagnoses of mental disorders including MDEs. Four subsequent annual follow-up SCIDs assessed MDE diagnoses since the prior interview. Interviewers possessed at least a bachelor's degree and completed an intensive SCID administration and scoring training program, including matching diagnoses with a set of "gold standard" case ratings. Interviewers were blind to previous assessments and presented all cases to a doctoral level supervisor. Interrater reliability for MDE diagnoses (k values adjusted because of departure from equiprobable distributions) assessed for approximately 10% of SCIDs in the larger study ranged from .82 to .94 (M = .89, SD = .05) across the five interview periods.

Life stress assessment. Chronic and episodic stress were assessed with the University of California–Los Angeles (UCLA) Life Stress Interview (LSI; Hammen et al., 1987; Hammen, Marks, Mayol, & DeMayo, 1985). The baseline interview assessed life stress occurring in the past year, and annual follow-up interviews assessed life stress since the last interview.

Chronic interpersonal stress. The chronic LSI examined the consistent aspect of ongoing objective stress in four interpersonal domains (best friend relationship, peer social circle, romantic relationships, and family relationships) and six noninterpersonal domains not examined in the present report. Interviewers rated participants' chronic stress in each domain on a scale from 1 (excellent or optimal circumstances) to 5 (very negative circumstances) in half-point increments using behavioral descriptions for each scale point. Across the five annual assessments, interrater reliability given by interclass correlation coefficients (ICCs) for a chronic interpersonal stress composite score (the mean of the four domains) ranged from .71 to .91 within site and .76 to .88 cross-site.

Episodic stressful life events. Throughout the LSI, interviewers queried participants whether events related to each domain (and at the end of the LSI, additional unrelated events) had occurred (e.g., Hammen et al., 1985). When events were endorsed, interviewers asked questions to assess the context (such as circumstances and resources to cope with it, expectedness, and prior experience with similar events) and objective impact of each event. Later, interviewers presented narratives of each event, including its duration, consequences, and context, to a team of two or more raters who were blind to participants' diagnoses and emotional responses to events. The team assigned objective impact ratings and a numeric code describing each event (e.g., romantic break-up) from a modified list of events (Paykel & Mangen, 1980).

Severity scores ranged from 1 (*nonevent, or no significant threat or negative impact*) to 5 (*a very severe event, maximal negative impact or threat*) in half-point increments, reflecting the impact of the event for a typical individual given the context. Based on a decision applied to all LSI analyses in this sample, events with a severity score >2.5 were designated as major SLEs,

encompassing moderate to severe events. Interrater reliability (ICCs) for severity over the five annual interview periods ranged from .69 to .76 (M = .72, SD = .03).

To classify each event as interpersonal or noninterpersonal, two raters with LSI experience assigned a category to each of the numeric codes used to describe events (k = .92). Interpersonal SLEs were those that affected primarily the quality or quantity of the participant's relationships. To ensure the temporal precedence of events to MDEs, when an MDE and an SLE were dated to the same person-month, staff examined interview records to determine the order of occurrence. To be conservative in estimating the effects of stress on depression, when the order of occurrence was unclear, the event was excluded from analysis.

DNA collection and genotyping. Participants provided saliva samples in their homes using Oragene collection kits (DNA Genotek, Ontario, Canada) and mailed them to study offices using prepaid postage. DNA extraction and genotyping was performed by Kbioscience (United Kingdom), now LGC Genomics. Genotyping for all five SNPs relied on KASP assays, which use competitive allele-specific PCR permitting biallelic scoring of SNPs at specific loci. The assay-specific KASP Primer mix and a universal KASP master mix were added to DNA samples before thermal cycling, followed by end-point fluorescent detection of genotypes. More specifically, allelic discrimination was accomplished with competitive binding of two allele-specific forward primers, each with a unique tail sequence that corresponds to two universal FRET (fluorescence resonant energy transfer) cassettes, one labeled with fluorescent FAM dye and the other with HEX dye. Results from each SNP assay passed a series of quality control measures.

Analytic Approach

Construction of the serotonergic multilocus profile score. We coded each SNP variable such that increasing values of number of alleles corresponded to increasing risk based on the literature. To model an additive effect, all SNPs were coded in terms of number of risk alleles (0, 1, or 2), with the exception of rs6318 located in HTR2C, which is X-linked, and which we coded as 0 or 1 because males have only one copy. Variants represent the forward coding strand. Following this, we summed all five SNP variables, and calculated each participant's proportion of total possible risk alleles. We permitted up to one missing genotype per person (20%), using a prorated number of possible risk alleles (i.e., if a person was missing a genotype, their proportion of risk alleles was calculated based on the maximum number without the missing genotype). The resulting proportion of risk alleles was standardized for analyses.

Person-month datasets. Consistent with several of our prior reports (Vrshek-Schallhorn et al., 2014, in press), survival analyses were conducted using months as the unit of time. Dates of MDE onset and offset, and dates of SLEs, were coded to the nearest month, with the beginning of the study period corresponding to the first month for which LSI data was available (i.e., 1 year before the baseline interview). In keeping with other studies using this approach (e.g., Kendler, Kuhn, Vittum, Prescott, & Riley, 2005), participants in an ongoing MDE when the study period started were excluded from analyses until recovery. Further, beginning in the month after an individual experienced a new MDE onset, he or she was excluded from analyses until the episode remitted, when he or she was reincluded in analyses. Nearly contiguous MDEs with fewer than 2 months of recovery separating them were coded as a combined single episode following the suggestion of DSM– IV–TR (American Psychiatric Association, 2000). The MDE onset variable was coded as either absent or present in each month (0/1).

Similarly, the occurrence of major interpersonal events was coded as absent or present (0/1) for each month. This time-specific and dichotomous approach is consistent with previous research on SLEs and depression (e.g., Kendler, Karkowski, & Prescott, 1998; Kendler et al., 1995; Kendler, Thornton, & Gardner, 2000, 2001). Because evidence supports that events can significantly increase risk for depression for longer than 1 month (e.g., Kendler et al., 1998; Surtees & Wainwright, 1999), events were lagged 1 additional month beyond the month when they occurred, consistent with previous G×E research using this type of time-specific analysis (Kendler et al., 2005; Vrshek-Schallhorn et al., 2014). For example, if an event occurred in Study Month 7, it was treated as present for both Study Months 7 and 8.

To create the interpersonal chronic stress composite score, ratings on each of the four interpersonal chronic stress domains assessed by the LSI were standardized in the person-month dataset and then averaged to center variables and aid interpretation. This interpersonal chronic stress composite did not vary by month, but was assigned uniformly across all person-months covered by a given annual interview period—typically about 1 year.

Model construction. We covaried several factors known to predict depression or to affect stress-depression associations, including gender (male = 1, female = 0; e.g., Hammen, 2003), SES (e.g., Lorant et al., 2003), and time-varying depression history (coded as 0, 1, or 2+ prior episodes; e.g., Stroud, Davila, & Moyer, 2008). Covarying the main effects of other variables does not statistically remove their influence from interaction effects (e.g., Keller, 2014), but may assist in revealing significant G×E effects by reducing error variance (Zinbarg, Suzuki, Uliaszek, & Lewis, 2010).

In all G×E models, covariates were entered as a first block, main effects of genotype and the life stress variable were entered in a second block, and the G×E interaction was entered in a final block. In secondary models examining the main effects of genotype ignoring stress, covariates were entered in a first block, and genotype was entered in a second block. Reported hazard ratios (HRs) refer to the difference in likelihood of an MDE accompanying a one unit increase in the predictor (Singer & Willett, 2003).

Multiple testing. To reduce the likelihood of a Type I error, we used initial G×E tests of the serotonergic multilocus profile score as omnibus tests (one each for major interpersonal events and interpersonal chronic stress), and only examined the influence of individual SNPs as post hoc analyses for significant profile score result(s).

Sensitivity analyses. To assess the relative importance of each SNP to the multilocus profile score, we followed-up significant profile score G×E tests with a G×E test for each individual SNP. Next, we removed each SNP from the profile score one at a time and reconducted the initially significant model with five separate four-genotype profile scores. Together, these two procedures ought to reveal whether any one SNP contributes substantially more than others to the profile score G×E.

Primary Sample Results

Preliminary Analyses

See Table 1 for correlations and descriptive statistics. There were no significant G-E correlations between the serotonergic multilocus profile score and levels of either form of stress, suggesting that, on average, individuals with more risk alleles have not “selected” significantly riskier

Table 1. *Zero-Order Pearson Correlations and Descriptive Statistics for the Primary Sample*

	Mean	SD	1	2	3	4	5	6
1. Male	.307	.462	—					
2. Socioeconomic Status	48.406	12.510	**.119**	—				
3. Mean of time-varying prior MDEs	.333	.578	-.087	-.072	—			
4. Total number of major interpersonal events for duration of study	1.628	1.813	**-.185**	**-.195**	**.243**	—		
5. Mean of time-varying raw LSI chronic interpersonal stress composite	2.308	.367	-.044	**-.270**	**.343**	**.352**	—	
6. Serotonin multilocus profile score: Proportion of total possible risk alleles	.704	.140	-.017	.058	-.025	-.057	-.088	—
7. Total number of observed MDE onsets for duration of study	.375	.746	**-.102**	-.007	**.594**	**.247**	**.353**	-.043

Note. Significant correlations ($p < .05$) are bolded. Genotype frequencies were HTR1A rs6295 101 CC, 167 CG, 117 GG; HTR2A rs6314 4 TT, 67 CT, 215 CC; HTR2C rs6318 in males, 97 G, 19 C; HTR2C rs6318 in females, 191 GG, 61 CG, 12 CC; TPH2 rs11178997 5 AA, 62 TA, 316 TT; TPH2 rs4570625 26 TT, 168 TG, 190 GG. MDE = major depressive episode; LSI = Life Stress Interview.

Table 2 *Multilocus Profile Score G×E Tests and Post Hoc Constituent GE Tests in the Primary Sample*

				95% Confidence intervals (CIs)		
	B	SE(B)	HR	Lower CI	Upper CI	p value
A. Final step of Primary Model 1: Recent major interpersonal events						
Male	-.329	.206	.719	.481	1.077	.110
Socioeconomic status	.034	.086	1.034	.874	1.224	.694
Prior major depressive episodes	.566	.100	1.762	1.449	2.142	.000
Recent major interpersonal event	.777	.244	2.175	1.349	3.507	.001
Z-scored serotonin multilocus profile score	-.190	.088	.827	.696	.982	.030
Z-Serotonin Multilocus Profile Score × Recent Major Interpersonal Event	.596	.222	1.815	1.174	2.805	.007
B. Final step of post hoc constituent SNP G×E tests for recent major interpersonal events						
Recent major interpersonal event	.779	.243	2.179	1.354	3.507	.001
Z-scored HTR1A rs6295 G-alleles	.051	.092	1.052	.879	1.259	.581
Z-rs6295 G-Alleles × Recent Major Interpersonal Event	.205	.244	1.228	.762	1.979	.400
Recent major interpersonal event	.832	.232	2.298	1.458	3.622	.000
Z-scored HTR2A rs6314 C-alleles	-.043	.085	.958	.811	1.132	.613
Z-rs6314 C-Alleles × Recent Major Interpersonal Event	.321	.250	1.378	.844	2.252	.200
Recent major interpersonal event	.631	.299	1.879	1.045	3.378	.035
HTR2C rs6318 C-carrier	-.139	.216	.870	.570	1.328	.519
rs6318 C-Carrier × Recent Major Interpersonal Event	.519	.472	1.681	.666	4.242	.272
Recent major interpersonal event	.872	.232	2.391	1.519	3.765	.000
Z-scored TPH2 rs11178997 T-alleles	-.182	.079	.833	.713	.974	.022
Z-rs11178997 T-Alleles × Recent Major Interpersonal Event	.241	.217	1.273	.832	1.946	.266
Recent major interpersonal event	.891	.231	2.438	1.551	3.835	.000
Z-scored TPH2 rs4570625 G-alleles	-.235	.092	.790	.660	.946	.010
Z-rs4570625 G-Alleles × Recent Major Interpersonal Event	.411	.220	1.508	.980	2.320	.062

Multilocus Profile Score G×E Tests and Post Hoc Constituent G×E Tests in the Primary Sample

environments. The numbers of risk alleles in individual SNPs were not significantly correlated with each other across the five SNPs (|rs| < .079, ps > .05), except (a) TPH2 SNPs rs11178997 and rs4570625, r = .546, p < .001, consistent with their location near each other in the TPH2 promoter region; and (b) HTR2A rs6314 and HTR1A rs6295, r = -.106, p = .039. SNPs were in Hardy-Weinberg equilibrium (HWE; 2s(1) < 1.89, ps >.05), with the exception of HTR1A rs6295, 2(1) = 6.60, p = .01, and HTR2C rs6318 (tested only in females), x2(1) = 5.52, p = .02. We repeated the primary analyses without these two SNPs in case deviation represented errors in genotyping. Cox regressions include 20,744 person-months.

Primary Models

Major interpersonal stressful life events. A simple main effect for interpersonal major events indicated that, at the mean of the multilocus profile score, the occurrence of such an event significantly predicts MDE onset (HR = 2.175, p = .001; Table 2, Model A). A simple main effect for the multilocus profile score indicated that, in the absence of a recent major interpersonal event, higher-risk alleles were associated with significantly lower risk for MDE onset (HR = 0.827, p = .030), thereby revealing a protective effect in the absence of major interpersonal events. The Multilocus Profile Score = Major Interpersonal Event interaction was also significant (HR = 1.815, p = .007), with increasing multilocus profile score risk alleles associated with greater sensitivity to a major interpersonal event and hence increased likelihood of subsequent MDE onset (see Figure 1).3

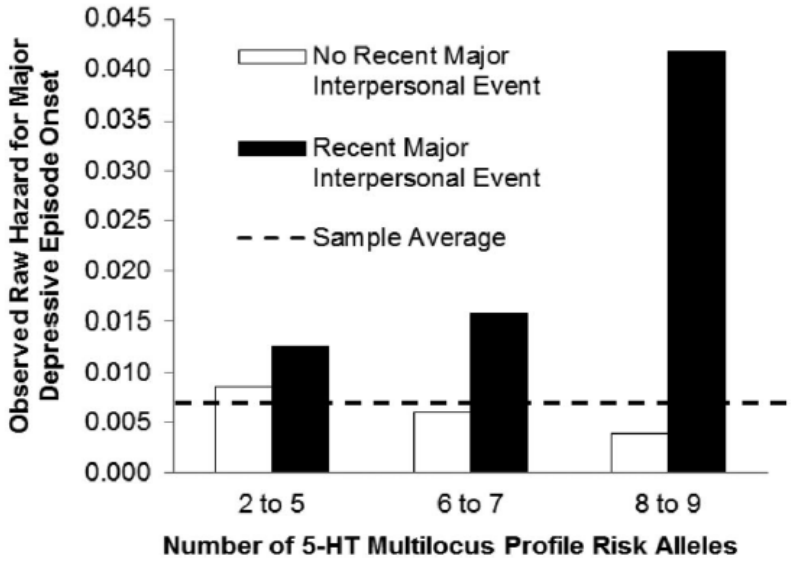

Figure 1. The sample-wide hazard is 0.007. Results represent the primary sample.

Interpersonal chronic stress. Although there was a significant simple main effect of chronic interpersonal stress on depression onset (HR = 2.347, p < .001), the simple main effect of the serotonergic multilocus profile score was nonsignificant (HR = 0.885, p = .197). The G×E interaction between the serotonergic multilocus profile score and interpersonal chronic stress initially approached significance in the predicted direction (HR = 1.187, p = .083). However, when simultaneously examining the G×E interactions of the profile score with major interpersonal

events and with interpersonal chronic stress, the major interpersonal events G×E (HR = 1.809, p = .007) at least partially accounted for the interpersonal chronic stress G×E. The latter effect no longer approached significance, B = .136, SE (B) = .098, HR = 1.146, 95% confidence interval (CI) [.945, 1.389], p = .165.

Population Stratification

To address the possibility of population stratification (i.e., one racial or ethnic subgroup having more risk alleles and coincidentally also a higher rate of MDE onsets under stress than the remaining sample, resulting in a spuriously significant G×E effect) we repeated the significant Multilocus Profile Score × Major Interpersonal Stressful Life Events interaction model in the White only subsample. The G×E effect remained significant, indicating that population stratification cannot explain the observed G×E effect in the full sample, B = 1.498, SE (B) = .524, HR = 4.471, 95% CI [1.600, 12.490], p = .004.

Sensitivity Analysis

To assess the contribution of each SNP to the G×E effect, we repeated this model several ways. First, G×E tests were conducted using the individual constituent SNPs from the multilocus profile score, and second, G×E tests were conducted with five separate four-SNP multilocus profile score variables created by removing one SNP at a time from the original five-SNP multilocus profile score. Results for individual SNP G×Es in Table 2, Section C show that no single SNP significantly interacted with major interpersonal events, although TPH2 rs4570625 approached significance (HR = 1.508, p = .062). In addition, when each of the SNPs were removed one by one, and the four-SNP multilocus profile score variables were examined in five separate G×E tests, each remained significant (HRs > 1.668, ps < .027, not reported in table but available from first author on request). Finally, when both HTR1A rs6295 and HTR2C rs6318 were removed, the G×E effect persisted, B = .494, SE (B) = .224, HR = 1.639, 95% CI [1.056, 2.543], p = .028, indicating that deviation from HWE in these two SNPs did not account for the G×E results. Collectively, these analyses suggest that no individual SNP accounts for the interaction detected with the multilocus profile score, and that the profile score is robust to the removal of any one SNP.

Main Effects of Genetic Factors

To approximate the effect sizes this multilocus profile score and its constituent SNPs might produce in a GWAS, we conducted separate Cox regression models for the multilocus profile score and each individual SNP without including stress variables in the model. Gender and SES were covaried, as these could readily be covaried in GWAS. In interpreting the results, we focused on effect sizes rather than the significance levels. Results indicated that both the multilocus profile score and several individual SNPs may be overlooked or may be interpreted to confer protective effects in GWAS (see Table 3). Specifically, the multilocus profile score produced a modest but nonsignificant protective effect (HR = .914), consistent with its protective simple main effect in the absence of stress in the G×E models. HTR2A rs6314 and HTR2C rs6318 also produced modest effect sizes (respectively, HRs = 0.958 and 1.038) that are unlikely to be detected in case-control designs. One SNP produced modest effects in the expected direction that may be detectable by GWAS (HTR1A rs6295, HR = 1.153). However, in this sample, both TPH2 SNPs (rs11178997 and

rs4570625) produced more robust but protective effect sizes that may be detected in GWAS (respectively, HR = 0.729, p = .071 and HR = 0.707, p = .010).

Replication Sample Method

Participants and Overview

We sought to replicate significant G×E effects in an independent sample. Adolescent girls (N = 126) and their primary female caregivers (here "mothers") were recruited from two New England counties using flyers, word-of-mouth, and local schools between the summer before the girls' 6th grade year and the summer before 8th grade. (Six additional girls were excluded from analyses because they were siblings of existing participants or because their fathers participated.) At baseline (T1), mothers and daughters each completed separate diagnostic and objective stress interviews, and adolescents provided a DNA sample using an Oragene saliva collection kit. One year later, 86% (n = 108) participated in a follow-up (T2) that comprised the same objective and diagnostic stress interviews; three were excluded who either did not provide DNA (n = 2) or provided an insufficient quantity for genotyping (n = 1). Altogether, 105 participants were included in analyses. Participants (M = 12.35, SD = .70) were mostly White (78.1%; Black, 4.8%; Asian, 5.7%; Native American, 1.0%, and multiple or other, 10.5%).

Replication Sample Materials and Procedures

Depressive symptoms. We predicted the level of the worst depressive symptom onset (including no symptoms, mild symptoms, moderate symptoms, and full onsets) and not exclusively MDE onsets because episodes are rare in early adolescence (Rohde, Beevers, Stice, & O'Neil, 2009). Moreover, even early adolescent depressive symptoms are important, predicting the development of later MDEs (Georgiades, Lewinsohn, Monroe, & Seeley, 2006). Interviewers assessed current and past depressive symptoms using the Schedule for Affective Disorders and Schizophrenia for school-age children, present and lifetime version (Kaufman et al., 1997). The T1 interview assessed current (past month) and lifetime history of depressive symptoms and the T2 interview assessed current and past depressive symptoms since T1. For T2 past depressive symptoms, the worst period of symptoms was coded between T1 and T2. Symptom onsets and offsets were dated to establish temporal relationships to life events. Depressive symptoms were rated: 0 = no symptoms; 1 = mild symptoms (e.g., one to two symptoms); 2 = moderate, subthreshold symptoms of depression (e.g., three to four symptoms); 3 = meets DSM–IV criteria for major depression. Interrater reliability (assessed via audio-recordings for 27% of interviews) was good (T1: current: ICC = 1.00; past: ICC = .97; T2: current: ICC = .95; past: ICC = 1.00).

Assessment of acute stress. A version of the UCLA Life Stress Interview (LSI) adapted for adolescents (e.g., Rudolph & Hammen, 1999) was used to assess adolescents' chronic and acute life stress. Only the T2 LSI data, which assessed the interim between T1 and T2, were used. Adolescents and their mothers completed separate interviews with the same interviewer, and LSI interviewers were blind to all other study data (e.g., diagnoses). Although the interview also assessed chronic stress, only events were used in the present analyses. The assessment of SLEs parallels that in the primary sample, except that interviewers created narrative accounts integrating both the mother's and adolescent's report of events (e.g., Rudolph & Flynn, 2007) for presentation to the independent team. The team rated the objective impact and interpersonal nature of each

event on the same scales as in the primary sample, except that the team categorized events as either interpersonal or noninterpersonal (1/0). A second team who was blind to the original ratings, rerated a set of events (n = 132) on objective impact (ICC = .92) and interpersonal status (ICC = .98).

Composite scores were created summing the severity ratings of all interpersonal events (i.e., both major and minor severity events) occurring in the 2 months before the worst depressive symptom onset between T1 and T2, similar to prior work (Davila, Hammen, Burge, Paley, & Daley, 1995). Events included a full spectrum of severity and not only major events because we predicted the spectrum of depressive symptom severity. For participants with no depression symptoms between T1 and T2, a 2-month period was randomly selected and the severity ratings of interpersonal events that occurred during that period were summed. When the temporal precedence of the event to the depressive symptom onset was indeterminate, those events were conservatively excluded from the composite score.

Genotyping. DNA extractions were performed using the Oragene PrepIT L2P DNA Purification Kit and genotyping was carried out using allele-specific PCR with primers designed using PrimerPicker Software (LGC Genomics, Hertfordshire, United Kingdom). Genotypes were ascertained via fluorescent detection with a Synergy II Plate reader (Biotek Instruments, Winooski, VT). Full genotyping details are available on request. The multilocus profile score was calculated as in the primary sample.

Analytic plan. The interpersonal event composite and multilocus profile score were standardized and then entered on the first step of a linear regression predicting the onset of worst depression symptoms between T1 and T2. Symptoms during the T1 to T2 interim, and not the worst lifetime symptoms, were predicted because the stress interview covered only 1 year before T1; a portion of worst lifetime symptoms, therefore, lacked corresponding stress information. The G×E was entered on a second step. We also reran analyses excluding individuals with prior clinically significant depression at or before T1.

Replication Sample Results

SNPs did not deviate from HWE, $\chi 2s(1) \leq 0.92$, ps >.05. The majority of participants experienced no depressive symptoms (n = 80), but others experienced mild symptoms (n = 11), moderate symptoms (n = 8), and full MDEs (n = 6). The multilocus profile score was not significantly correlated with the interpersonal stress composite score, r = .048, p = .624, suggesting that on average, higher multilocus profile scores were not associated with selection into more stressful interpersonal environments. Results of the linear regression indicated no simple main effect of the genetic score at the mean of stress, B = −.014, SE (B) = .081, p = .861, but a significant simple main effect of the interpersonal stress composite score, B = .172, SE (B) = .082, p = .039, indicating that, at the mean of genotype, more stress was associated with higher depression levels. The G×E interaction effect was also significant, B = .213, SE (B) = .068, p = .002. A region of significance analysis (Preacher, Curran, & Bauer, 2006) indicated that the effect of stress on depression level was significant for values of the standardized multilocus genetic profile score greater than −.038 (see Figure 2). Region of significance analyses also showed that the effect of genotype on depression level was significant for values of the standardized stress variable score less than −1.05 (a protective effect of higher profile scores at lower stress levels) or greater than 0.91 (a risk enhancing effect of higher profile scores at higher stress levels).

Discussion

G×E interactions constitute a key area of ontogenic process research. Here we show that a serotonergic multilocus profile score significantly interacts with recent major interpersonal SLEs to predict MDE onsets (including first episodes and recurrent episodes combined), and does so more robustly than any single polymorphism in the profile score. Moreover, we show evidence of conceptual replication in that recent interpersonal acute stress interacted with the profile score to predict depression symptom level (not exclusively MDEs) in a sample of early adolescent girls. This provides the first demonstration of specific molecular genetic variants acting in a polygenic, additive fashion in a G×E interaction with naturalistic life stress to predict depression. In this interaction, higher numbers of risk alleles were associated with greater likelihood of MDE onset following the occurrence of a major interpersonal event. Supporting a truly cumulative interpretation of risk conferred by the five polymorphisms, no single individual polymorphism accounted for the interaction effect emerging with the multilocus profile score. Similarly, the multilocus profile score interaction effect persisted despite removal of any one of the five SNPs. The G×E interaction of this multilocus profile score with interpersonal chronic stress approached significance until we accounted for overlap between it and the G×E with major interpersonal events.

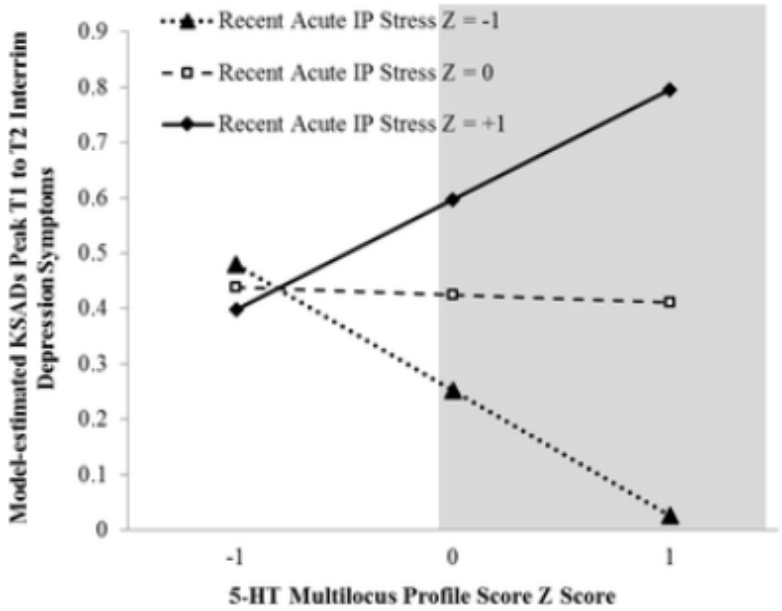

Figure 2. A regions of significance analysis indicated that stress is a significant predictor at the $p \leq .05$ level for values of the multilocus profile score Z-value greater than −0.038. The values of the multilocus genetic profile score for which stress is a significant predictor are depicted by a gray background. Similarly, the effect of genotype on depression level was significant for values of the standardized stress variable score less than −1.05 or greater than 0.91 (regions not depicted). Results represent the replication sample. Genotype frequencies were HTR1A rs6295 21 CC, 56 CG, 28 GG; HTR2A rs6314 0 TT, 18 CT, 87 CC; HTR2C rs6318 78 GG, 23 CG, 3 CC; TPH2 rs11178997 1 AA, 17 TA, 87 TT; TPH2 rs4570625 8 TT, 35 TG, 62 GG. IP = interpersonal; 5-HT = serotonin; Z = standardized score.

Of interest, in addition to the G×E interaction observed with events, we observed a significant but protective simple main effect of the multilocus profile score: In the absence of a recent major interpersonal event, increasing numbers of risk alleles were associated with lower risk of MDE onset. Similarly, a significant protective effect of the profile score at lower levels of interpersonal acute stress emerged in the replication sample. Finally, in the primary sample, main effects of the multilocus profile score and constituent SNPs without accounting for life stress suggest that case-control comparisons will lead to overlooking or misinterpreting some genetic risk factors for depression. These findings emerged from individuals transitioning to adulthood, as well as in a replication sample of early adolescent girls, both key developmental periods for the study of interpersonal stress and depression.

Implications for Genetic Research in Depression

The precise number of genes and genetic variants that contribute to risk for depression is unknown, but is likely to be quite large. Recent estimates for another form of psychopathology, schizophrenia, suggest that 6,300–10,200 common SNPs account for approximately 32% of the total risk for schizophrenia (nearing half of the genetic risk for schizophrenia; Ripke et al., 2013). It must be considered that a similar order of magnitude might also contribute to depression. As such, the present results might best be viewed as a modest proof of the concept that additive risk can act in a diathesis–stress interaction.

However modest these results, they also provide a new framework for interpreting the magnitude of the 5-HTTLPR G×E effect size, and may also adjust expectations for effects sizes of single SNPs in G×E work. Relative to the individual G×E effects of the five SNPs examined here (G×E HRs 1.187–1.681), the G×E effect of 5-HTTLPR might be considered remarkably large for a single SNP. For example, in the primary sample, the G×E effect with this same form of stress yielded an HR = 14.946, 95% CI = [1.933, 115.573] (Vrshek-Schallhorn et al., 2014). This highlights a significant problem facing G×E depression research: Because of the controversy engendered by 5-HTTLPR, some have urged waiting to pursue G×E research in depression until "robust marginal gene associations have been identified" (Risch et al., 2009, p. 2469). However, after no significant variants emerged from the largest-yet GWAS for depression (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013), it may be difficult to identify robust marginal gene associations (i.e., main effects of genetic variants) for single variants. Multilocus profile scores provide an alternative approach to examining single genetic variants—an approach that holds both promise and potential risks.

Multilocus profile scores offer several benefits including a larger cumulative effect size than any one individual SNP, under polygenic and additive assumptions. Further, multilocus profile scores may enhance power because of their dimensional format (vs. the dichotomous format typical of single SNP analyses). These two factors may lead to greater consistency in G×E depression research findings. However, multilocus profile scores have the potential to generate even greater controversy than the field experienced over 5-HTTLPR, particularly under several avoidable conditions. First, we caution against using questionnaire measures of SLEs, for which a robust literature indicates poor validity (for a review, see Monroe & Reid, 2008) and a meta-analysis has demonstrated weaker G×E effects (Karg et al., 2011). Second, it will be challenging to conduct meta-analyses if multilocus profile scores differ in their individual SNPs. Nonetheless, additional SNPs will need to be examined to advance the field. One solution is to report results first with a series of core SNPs and subsequently add other SNPs. Third, multilocus profile scores

may carry greater potential for statistical abuse than single SNP studies. In theory, one could aggregate a large number of nonsignificant small G×E effects to yield an overall significant multilocus profile score G×E effect. Therefore, we urge that multilocus profile scores either use preferably a priori candidate SNPs but at least a priori selected genes, or instead validate a data-driven multilocus profile score in a replication sample. Finally, to aid investigations of the relationships of multilocus profile scores to endophenotypes, we advocate that, initially, hypothesis-driven multilocus profile scores comprise SNPs within individual neurobiological systems rather than across different systems.

Implications of the Main Effects of the Serotonergic Multilocus Profile Score

There are at least two implications of both the simple main effect of the multilocus profile score, and the main effects of this profile score and constituent SNPs examined excluding stress from models. First, in G×E models, the significant simple main effect indicated that individuals with increasing numbers of risk alleles are protected from depression in the absence of major interpersonal events (or, in the replication sample, under lower levels of acute interpersonal stress) compared with their counterparts with fewer risk alleles. This suggests a mechanism by which genetic variants with harmful effects in one context are maintained in the population, instead of being selected out over time: They may provide benefits under less stressful conditions. This interpretation is consistent with the differential susceptibility hypothesis, which states that genetic variants confer greater sensitivity to the environment whether good or bad, and therefore, lead to greater risk but also greater benefits depending on context (J. Belsky & Pluess, 2009), similar to notions of biological sensitivity to context (Boyce & Ellis, 2005). However, we caution that the present results should not lead to the conclusion that this multilocus profile is either beneficial or detrimental overall: Its net effect averaging across months with and without a recent interpersonal event is nonsignificant in the primary sample (see Table 3).

Second, the present results hint at why genetic factors might be missed by most GWAS studies. Main effect sizes for the multilocus profile score and three of the five SNPs suggested they might be overlooked or interpreted in the wrong direction when not accounting for stress. Others have suggested that depression may have a "divergent genetic architecture" from other disorders as one explanation for the missing heritability in depression GWAS (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). This means that for most disorders, genetic factors have small yet detectable main effects, but that for depression, the effects of genes may depend so substantially on the environment, that the effects of genes are not readily detectable without accounting for the environment. The present results provide preliminary support for this notion; thus, the promising potential of GWAS to reveal novel genetic markers for depression may best be realized when accounting for environmental factors, particularly interpersonal ones. However, given the very small sample size of the primary sample relative to GWAS samples, replication and extension of these findings will be essential.

Implications for the Role of Serotonin in Depression

Results provide support for continued examination of the role of serotonin in depression. Instead of a simplistic model focused on serotonin level, recent work suggests that serotonin turnover (breakdown) rates may be critical. For example, in a study examining serotonin functioning in brain-derived (internal jugular venoarterial) blood samples, unmedicated depressed individuals

showed substantially higher serotonin turnover compared with healthy individuals, and depressed 5-HTTLPR S-allele carriers had turnover twice as high as their depressed L/L counterparts. Following treatment with SSRIs for depressed individuals, turnover was reduced to near-normal levels (Barton et al., 2008). Although the present results cannot be interpreted to directly support this pathway, they do support the role of serotonin in depression's etiology, and further suggest that future research should address how the serotonin system might mediate differential sensitivity, conferring benefits under lower levels of stress.

Limitations

Despite several strengths including diagnostic and life stress interviews, a longitudinal design, a relatively novel genetic approach, and evidence of replication, there are limitations of the present study. First, although consistent with one other multilocus profile score (e.g., Nikolova et al., 2011), five SNPs is a relatively small number relative to the number of SNPs that likely to contribute to depression. Second, the sample sizes (Ns = 387 and 105) were quite small compared to GWAS samples, but the primary sample was slightly larger than the median sample size (N = 345) examined in a recent review of candidate gene studies (Duncan & Keller, 2011). The present sample sizes also made it feasible to use fine-grained interviews of life stress. However, given the modest sample sizes, replication of these findings in further independent samples using objective or interview measures of stress is vital.

Third, in the primary sample, two of the SNPs examined here deviated from Hardy-Weinberg equilibrium (HWE), which typically indicates genotyping errors; however, the multilocus profile score G×E remains significant when either or both of these SNPs are excluded from the profile score, and there were no HWE deviations in the replication sample. Fourth, we predicted only depression and not other mental health outcomes. Serotonergic mechanisms likely contribute to other internalizing psychopathologies; however, less is known about the environmental precipitants of these disorders other than depression, presenting challenges in conducting G×E work. For other internalizing disorders, both clarification of the potent forms of stress and examination of G×E interactions remain important future directions. Fifth, the sample is nonrepresentative; however, oversampling (e.g., for high neuroticism) was shown not to bias regression in a latent variable simulation study (Hauner, Zinbarg, & Revelle, 2014) and the replication sample was not oversampled for neuroticism. Sixth, to minimize Type I error, we focused on two forms of interpersonal stress indicated by depression theory and research; we do not report on G×E interactions using noninterpersonal forms of stress. Relatedly, we have not adjusted the significance levels of either the two primary models or the sensitivity analyses for multiple testing. Instead, we adopted a conservative strategy of using limited, planned tests and orthogonal main effects and interactions. Despite these limitations, the present work advances G×E research in depression by providing the first evidence of a multilocus genetic profile score prospectively predicting depression in interaction with acute interpersonal stress in two independent samples.

Conclusions

We provide initial evidence that five serotonergic genetic variants (other than the commonly studied 5-HTTLPR) collectively amplify vulnerability to depression following major interpersonal SLEs in two samples of older and younger adolescents. In both the primary and replication

samples, this occurred in the context of a significant protective effect of these same genetic variants under low interpersonal stress. Finally, when these variants were examined without considering stress, approximating case-control comparisons, the resulting effect sizes suggested that a large percentage of these variants could be missed or misinterpreted.

Footnotes

1 Gao et al. (2012) code analyses of both rs4570625 and rs11178997 in terms of the alternate allele at each marker as we have described. Specifically, in Figure 1 and in Supplemental Materials Table 2, results appear for rs4570625-T. Odds ratios less than 1.0 demonstrate that the T-allele is protective, which indicates that the G-allele increases risk. Similarly, in Supplemental Materials Table 2, results for rs11178997 showed that the A-allele has an odds ration below 1.0, meaning that the A-allele is protective, and T-allele increases risk for major depression, albeit with varying significance across Gao et al.'s different meta-analytic models.

2 Although noninterpersonal chronic stress also contributed significant unique variance in one sample, interpersonal major events and interpersonal chronic stress were the only two that consistently emerged across the two studies.

3 The specific genetic factors that contribute to risk are estimated to heavily, but not perfectly, overlap (r = .57) across sex (Kendler & Prescott, 1999). Thus, as an exploratory analysis in the primary sample, we examined whether the strength of the significant G×E effect differed by sex by examining the three-way G×E × Sex interaction. The interaction was not significant, B = −.307, SE (B) = .684, HR = .736, 95% CI = [.192, 2.815], p = .654. Similarly, heritability is thought to be higher for recurrent cases (e.g., Sullivan et al., 2000), which suggests that individuals who have two or more lifetime episodes by the conclusion of the study period may demonstrate larger G×E effects than individuals with one or zero lifetime episodes, a G×E × Recurrence interaction effect. This interaction was not significant, B = .370, SE (B) = .500, HR = 1.448, 95% CI = [.543, 3.861], p = .459. Separately, the relationship of SLEs to MDE onsets changes with successive MDEs (Stroud, Davila, Hammen, & Vrshek-Schallhorn, 2011). A model examining whether the strength of this G×E effect varied as a function of time-varying episode number—a G×E × History interaction—indicated that there was not a significant difference between the magnitude of the G×E for first onset MDEs versus recurrences, B = .513, SE (B) = .425, HR = 1.671, 95% CI = [.726, 3.843], p = .227. Finally, although heritability calculations in behavior genetics rely on the assumption of additive risk, for some psychological variables, there is evidence for multiplicative effects, that is, epistasis or emergenesis (e.g., Lykken, 2006). Therefore, we created a multiplicative genetic effect variable with all of these five SNPs by standardizing each and multiplying them, to capture variance from all possible lower order gene–gene interactions. This multiplicative genetic score did not significantly predict MDE onsets, B = .057, SE (B) = .170, HR = 1.059, 95% CI = [.758, 1.478], p = .737, or interact with recent major interpersonal events, B = −.237, SE (B) = .402, HR = .789, 95% CI = [.359, 1.733], p = .554.

4 When six individuals with clinically significant major depression at or before T1 were excluded from the analysis, the G×E interaction effect remained significant, B = .193, SE (B) = .063, p = .003.

5 In a single survival model simultaneously testing the 5-HTTLPR G×E effect we previously reported and this multilocus profile score G×E, both interaction effects remained significant predictors of MDE onset, 5-HTTLPR G×E: B = 2.527, SE (B) = 1.044, HR = 12.515, 95% CI = [1.619, 96.751], p = .015; multilocus profile score G×E: B = .631, SE (B) = .222, HR = 1.880, 95% CI = [1.216, 2.907], p = .005. Although the concept of variance is not defined for our categorical outcome, a generalized-R2 value can be calculated for Cox regression that behaves similarly to R2 in linear regression (Allison, 2010, pp. 282–

283). This places effect sizes for both G×Es on the same metric, whereas these HRs are not readily comparable. The 5-HTTLPR G×E contributed a change in generalized R2 of 0.0484 while the multilocus profile score contributed a change in generalized-R2 of 0.0305 (63% of the magnitude of the 5-HTTLPR G×E generalized-R2).

References

Allison, P. D. (2010). Survival analysis using SAS: A practical guide (2nd ed.). Cary, NC: SAS Institute.

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A., Jr. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. Neuroscience and Biobehavioral Reviews, 51, 164–188. 10.1016/j.neubiorev.2015.01.018

Anttila, S., Huuhka, K., Huuhka, M., Rontu, R., Hurme, M., Leinonen, E., & Lehtimäki, T. (2007). Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. Journal of Neural Transmission, 114, 1065–1068. 10.1007/s00702-007-0705-9

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders (Fourth Edition, Text Revision). Washington, DC: American Psychiatric Association.

Barton, D. A., Esler, M. D., Dawood, T., Lambert, E. A., Haikerwal, D., Brenchley, C., . . .Lambert, G. W. (2008). Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. Archives of General Psychiatry, 65, 38–46. 10.1001/archgenpsychiatry.2007.11

Beauchaine, T. P., & McNulty, T. (2013). Comorbidities and continuities as ontogenic processes: Toward a developmental spectrum model of externalizing psychopathology. Development and Psychopathology, 25(4pt2), 1505–1528. 10.1017/S0954579413000746

Belsky, D. W., Moffitt, T. E., Baker, T. B., Biddle, A. K., Evans, J. P., Harrington, H., . . .Caspi, A. (2013). Polygenic risk and the developmental progression to heavy, persistent smoking and nicotine dependence: Evidence from a 4-decade longitudinal study. Journal of the American Medical Association Psychiatry, 70, 534–542. 10.1001/jamapsychiatry.2013.736

Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: Differential susceptibility to environmental influences. Psychological Bulletin, 135, 885–908. 10.1037/a0017376

Booij, L., Van der Does, A. J., & Riedel, W. J. (2003). Monoamine depletion in psychiatric and healthy populations: Review. [Review]. Molecular Psychiatry, 8, 951–973. 10.1038/sj.mp.4001423

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Development and Psychopathology, 17, 271–301. 10.1017/S0954579405050145

Brummett, B. H., Babyak, M. A., Kuhn, C. M., Siegler, I. C., & Williams, R. B. (2014). A functional polymorphism in the HTR2C gene associated with stress responses: A validation study. Biological Psychology, 103, 317–321. 10.1016/j.biopsycho.2014.10.006

Brummett, B. H., Babyak, M. A., Williams, R. B., Harris, K. M., Jiang, R., Kraus, W. E., . . .Siegler, I. C. (2014). A putatively functional polymorphism in the HTR2C gene is associated with depressive symptoms in white females reporting significant life stress. PLoS ONE, 9, e114451. 10.1371/journal.pone.0114451

Brummett, B. H., Kuhn, C. M., Boyle, S. H., Babyak, M. A., Siegler, I. C., & Williams, R. B. (2012). Cortisol responses to emotional stress in men: Association with a functional polymorphism in the 5HTR2C gene. Biological Psychology, 89, 94–98. 10.1016/j.biopsycho.2011.09.013

Carver, C. S., Johnson, S. L., & Joormann, J. (2008). Serotonergic function, two-mode models of self-regulation, and vulnerability to depression: What depression has in common with impulsive aggression. Psychological Bulletin, 134, 912–943. 10.1037/a0013740

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., . . .Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science, 301, 386–389. 10.1126/science.1083968

Chipuer, H. M., Rovine, M. J., & Plomin, R. (1990). LISREL modeling: Genetic and environmental influences on IQ revisited. Intelligence, 14, 11–29. 10.1016/0160-2896(90)90011-H

Cole, M. G., & Dendukuri, N. (2003). Risk factors for depression among elderly community subjects: A systematic review and meta-analysis. The American Journal of Psychiatry, 160, 1147–1156. 10.1176/appi.ajp.160.6.1147

CONVERGE Consortium. (in press). Sparse whole-genome sequencing identifies two loci for major depressive disorder. Nature.

Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium. (2013). A mega-analysis of genome-wide association studies for major depressive disorder. Molecular Psychiatry, 18, 497–511.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. (2014). Biological insights from 108 schizophrenia-associated genetic loci. Nature, 511, 421–427.

Conway, C. C., Hammen, C., Brennan, P. A., Lind, P. A., & Najman, J. M. (2010). Interaction of chronic stress with serotonin transporter and catechol-O-methyltransferase polymorphisms in predicting youth depression. Depression and Anxiety, 27, 737–745.

Davila, J., Hammen, C., Burge, D., Paley, B., & Daley, S. E. (1995). Poor interpersonal problem solving as a mechanism of stress generation in depression among adolescent women. Journal of Abnormal Psychology, 104, 592–600. 10.1037/0021-843X.104.4.592

de Quervain, D. J.-F., Henke, K., Aerni, A., Coluccia, D., Wollmer, M. A., Hock, C., . . .Papassotiropoulos, A. (2003). A functional genetic variation of the 5-HT2a receptor affects human memory. Nature Neuroscience, 6, 1141–1142. 10.1038/nn1146

Disner, S. G., McGeary, J. E., Wells, T. T., Ellis, A. J., & Beevers, C. G. (2014). 5-HTTLPR, HTR1A, and HTR2A cumulative genetic score interacts with mood reactivity to predict mood-congruent gaze bias. Cognitive, Affective & Behavioral Neuroscience, 14, 1259–1270. 10.3758/s13415-014-0267-x

Drevets, W. C., Thase, M. E., Moses-Kolko, E. L., Price, J., Frank, E., Kupfer, D. J., & Mathis, C. (2007). Serotonin-1A receptor imaging in recurrent depression: Replication and literature review. Nuclear Medicine and Biology, 34, 865–877. 10.1016/j.nucmedbio.2007.06.008

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. The American Journal of Psychiatry, 168, 1041–1049. 10.1176/appi.ajp.2011.11020191

Eysenck, S. B. G., Eysenck, H. J., & Barrett, P. (1985). A revised version of the psychoticism scale. Personality and Individual Differences, 6, 21–29. 10.1016/0191-8869(85)90026-1

First, M., Spitzer, R., Gibbon, M., & Williams, J. (2001). Structured Clinical Interview for DSM–IV–TR Axis I Disorders–Non-patient Edition. New York, NY: New York State Psychiatric Institute, Biometrics Research Department.

Fisher, R. (1918). The correlation of relatives on the assumption of Mendelian inheritance. Transactions of the Royal Society of Edinburgh, 52, 399–433. 10.1017/S0080456800012163

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., & Fawcett, J. (2010). Antidepressant drug effects and depression severity: A patient-level meta-analysis. Journal of the American Medical Association, 303, 47–53. 10.1001/jama.2009.1943

Gao, J., Pan, Z., Jiao, Z., Li, F., Zhao, G., Wei, Q., . . .Evangelou, E. (2012). TPH2 gene polymorphisms and major depression—A meta-analysis. PLoS ONE, 7, e36721. 10.1371/journal.pone.0036721

Georgiades, K., Lewinsohn, P. M., Monroe, S. M., & Seeley, J. R. (2006). Major depressive disorder in adolescence: The role of subthreshold symptoms. Journal of the American Academy of Child & Adolescent Psychiatry, 45, 936–944. 10.1097/01.chi.0000223313.25536.47

Hammen, C. (2003). Interpersonal stress and depression in women. Journal of Affective Disorders, 74, 49–57. 10.1016/S0165-0327(02)00430-5

Hammen, C., Adrian, C., Gordon, D., Burge, D., Jaenicke, C., & Hiroto, D. (1987). Children of depressed mothers: Maternal strain and symptom predictors of dysfunction. Journal of Abnormal Psychology, 96, 190–198. 10.1037/0021-843X.96.3.190

Hammen, C., Marks, T., Mayol, A., & deMayo, R. (1985). Depressive self-schemas, life stress, and vulnerability to depression. Journal of Abnormal Psychology, 94, 308–319. 10.1037/0021-843X.94.3.308

Hauner, K. K., Zinbarg, R. E., & Revelle, W. (2014). A latent variable model approach to estimating systematic bias in the oversampling method. Behavior Research Methods, 46, 786–797. 10.3758/s13428-013-0402-6

Heisler, L. K., Zhou, L., Bajwa, P., Hsu, J., & Tecott, L. H. (2007). Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. Genes, Brain & Behavior, 6, 491–496. 10.1111/j.1601-183X.2007.00316.x

Hollingshead, A. (1975). Four factor index of social status. Unpublished manuscript. Yale University, New Haven, CT.

Hong, C. J., Chen, T. J., Yu, Y. W., & Tsai, S. J. (2006). Response to fluoxetine and serotonin 1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder. The Pharmacogenomics Journal, 6, 27–33. 10.1038/sj.tpj.6500340

Hsieh, F. Y., & Lavori, P. W. (2000). Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. Controlled Clinical Trials, 21, 552–560. 10.1016/S0197-2456(00)00104-5

Invernizzi, R. W. (2007). Role of TPH-2 in brain function: News from behavioral and pharmacologic studies. Journal of Neuroscience Research, 85, 3030–3035. 10.1002/jnr.21330

Joiner, T. E., Jr., & Metalsky, G. I. (1995). A prospective test of an integrative interpersonal theory of depression: A naturalistic study of college roommates. Journal of Personality and Social Psychology, 69, 778–788. 10.1037/0022-3514.69.4.778

Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. Archives of General Psychiatry, 68, 444–454. 10.1001/archgenpsychiatry.2010.189

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., . . .Ryan, N. (1997). Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. Journal of the American Academy of Child and Adolescent Psychiatry, 36, 980–988. 10.1097/00004583-199707000-00021

Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., . . .Gelernter, J. (2006). Brain-derived neurotrophic factor-5-HTTLPR gene interactions and

environmental modifiers of depression in children. Biological Psychiatry, 59, 673–680. 10.1016/j.biopsych.2005.10.026

Keller, M. C. (2014). Gene × environment interaction studies have not properly controlled for potential confounders: The problem and the (simple) solution. Biological Psychiatry, 75, 18–24. 10.1016/j.biopsych.2013.09.006

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1998). Stressful life events and major depression: Risk period, long-term contextual threat, and diagnostic specificity. Journal of Nervous and Mental Disease, 186, 661–669. 10.1097/00005053-199811000-00001

Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., & Eaves, L. J. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. The American Journal of Psychiatry, 152, 833–842. 10.1176/ajp.152.6.833

Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. Archives of General Psychiatry, 62, 529–535. 10.1001/archpsyc.62.5.529

Kendler, K. S., & Prescott, C. A. (1999). A population-based twin study of lifetime major depression in men and women. Archives of General Psychiatry, 56, 39–44. 10.1001/archpsyc.56.1.39

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2000). Stressful life events and previous episodes in the etiology of major depression in women: An evaluation of the “kindling” hypothesis. The American Journal of Psychiatry, 157, 1243–1251. 10.1176/appi.ajp.157.8.1243

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. The American Journal of Psychiatry, 158, 582–586. 10.1176/appi.ajp.158.4.582

Lappalainen, J., Zhang, L., Dean, M., Oz, M., Ozaki, N., Yu, D. H., . . .Goldman, D. (1995). Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2c receptor gene (HTR2C). Genomics, 27, 274–279. 10.1006/geno.1995.1042

Lemonde, S., Du, L., Bakish, D., Hrdina, P., & Albert, P. R. (2004). Association of the C(-1019)G 5-HT1A functional promoter polymorphism with antidepressant response. The International Journal of Neuropsychopharmacology, 7, 501–506. 10.1017/S1461145704004699

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., . . .Albert, P. R. (2003). Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. The Journal of Neuroscience, 23, 8788–8799.

Li, D., Duan, Y., & He, L. (2006). Association study of serotonin 2A receptor (5-HT2A) gene with schizophrenia and suicidal behavior using systematic meta-analysis. Biochemical and Biophysical Research Communications, 340, 1006–1015. 10.1016/j.bbrc.2005.12.101

Lorant, V., Deliège, D., Eaton, W., Robert, A., Philippot, P., & Ansseau, M. (2003). Socioeconomic inequalities in depression: A meta-analysis. American Journal of Epidemiology, 157, 98–112. 10.1093/aje/kwf182

Lykken, D. T. (2006). The mechanism of emergenesis. Genes, Brain & Behavior, 5, 306–310. 10.1111/j.1601-183X.2006.00233.x

McLeod, B. D., Weisz, J. R., & Wood, J. J. (2007). Examining the association between parenting and childhood depression: A meta-analysis. Clinical Psychology Review, 27, 986–1003. 10.1016/j.cpr.2007.03.001

Miller, R., Wankerl, M., Stalder, T., Kirschbaum, C., & Alexander, N. (2013). The serotonin transporter gene-linked polymorphic region (5-HTTLPR) and cortisol stress reactivity: A meta-analysis. Molecular Psychiatry, 18, 1018–1024. 10.1038/mp.2012.124

Molina, E., Cervilla, J., Rivera, M., Torres, F., Bellón, J. Á., Moreno, B., . . .Gutiérrez, B. (2011). Polymorphic variation at the serotonin 1-A receptor gene is associated with comorbid depression and generalized anxiety. Psychiatric Genetics, 21, 195–201. 10.1097/YPG.0b013e3283457a48

Monroe, S. M., & Reid, M. W. (2008). Gene-environment interactions in depression research: Genetic polymorphisms and life-stress polyprocedures. Psychological Science, 19, 947–956. 10.1111/j.1467-9280.2008.02181.x

Munafò, M. R., Durrant, C., Lewis, G., & Flint, J. (2009). Gene X environment interactions at the serotonin transporter locus. Biological Psychiatry, 65, 211–219. 10.1016/j.biopsych.2008.06.009

Nikolova, Y. S., Ferrell, R. E., Manuck, S. B., & Hariri, A. R. (2011). Multilocus genetic profile for dopamine signaling predicts ventral striatum reactivity. Neuropsychopharmacology, 36, 1940–1947. 10.1038/npp.2011.82

Okada, M., Northup, J. K., Ozaki, N., Russell, J. T., Linnoila, M., & Goldman, D. (2004). Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant, naturally occurring amino-acid substitution. Molecular Psychiatry, 9, 55–64. 10.1038/sj.mp.4001357

Ozaki, N., Manji, H., Lubierman, V., Lu, S. J., Lappalainen, J., Rosenthal, N. E., & Goldman, D. (1997). A naturally occurring amino acid substitution of the human serotonin 5-HT2A receptor influences amplitude and timing of intracellular calcium mobilization. Journal of Neurochemistry, 68, 2186–2193. 10.1046/j.1471-4159.1997.68052186.x

Ozaki, N., Rosenthal, N. E., Pesonen, U., Lappalainen, J., Feldman-Naim, S., Schwartz, P. J., . . .Goldman, D. (1996). Two naturally occurring amino acid substitutions of the 5-HT2A receptor: Similar prevalence in patients with seasonal affective disorder and controls. Biological Psychiatry, 40, 1267–1272. 10.1016/0006-3223(95)00649-4

Paykel, E., & Mangen, S. (1980). Interview for recent life events. London, United Kingdom: St. George's Hospital Medical School.

Pearson-Fuhrhop, K. M., Dunn, E. C., Mortero, S., Devan, W. J., Falcone, G. J., Lee, P., . . .Cramer, S. C. (2014). Dopamine genetic risk score predicts depressive symptoms in healthy adults and adults with depression. PLoS ONE, 9, e93772. 10.1371/journal.pone.0093772

Preacher, K. J., Curran, P. J., & Bauer, D. J. (2006). Computational tools for probing interactions in multiple linear regression, multilevel modeling, and latent curve analysis. Journal of Educational and Behavioral Statistics, 31, 437–448. 10.3102/10769986031004437

Ripke, S., O'Dushlaine, C., Chambert, K., Moran, J. L., Kähler, A. K., Akterin, S., . . .Sullivan, P. F., & the Multicenter Genetic Studies of Schizophrenia Consortium, & the Psychosis Endophenotypes International Consortium, & the Wellcome Trust Case Control Consortium 2. (2013). Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nature Genetics, 45, 1150–1159. 10.1038/ng.2742

Risch, N., Herrell, R., Lehner, T., Liang, K. Y., Eaves, L., Hoh, J., . . .Merikangas, K. R. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. Journal of the American Medical Association, 301, 2462–2471. 10.1001/jama.2009.878

Rohde, P., Beevers, C. G., Stice, E., & O'Neil, K. (2009). Major and minor depression in female adolescents: Onset, course, symptom presentation, and demographic associations. Journal of Clinical Psychology, 65, 1339–1349. 10.1002/jclp.20629

Rohde, P., Lewinsohn, P. M., Klein, D. N., Seeley, J. R., & Gau, J. M. (2013). Key characteristics of major depressive disorder occurring in childhood, adolescence, emerging adulthood, adulthood. Clinical Psychological Science, 1, 41–53. 10.1177/2167702612457599

Rudolph, K. D., & Flynn, M. (2007). Childhood adversity and youth depression: Influence of gender and pubertal status. Development and Psychopathology, 19, 497–521.

Rudolph, K. D., & Hammen, C. (1999). Age and gender as determinants of stress exposure, generation, and reactions in youngsters: A transactional perspective. Child Development, 70, 660–677. 10.1111/1467-8624.00048

Salvatore, J. E., Aliev, F., Bucholz, K., Agrawal, A., Hesselbrock, V., Hesselbrock, M., . . .Dick, D. M. (2015). Polygenic risk for externalizing disorders: Gene-by-development and gene-by-environment effects in adolescents and young adults. Clinical Psychological Science, 3, 189–201. 10.1177/2167702614534211

Scheuch, K., Lautenschlager, M., Grohmann, M., Stahlberg, S., Kirchheiner, J., Zill, P., . . .Priller, J. (2007). Characterization of a functional promoter polymorphism of the human tryptophan hydroxylase 2 gene in serotonergic raphe neurons. Biological Psychiatry, 62, 1288–1294. 10.1016/j.biopsych.2007.01.015

Serretti, A., Kato, M., De Ronchi, D., & Kinoshita, T. (2007). Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. Molecular Psychiatry, 12, 247–257.

Singer, J., & Willett, J. (2003). Applied longitudinal data analysis: Modeling change and event occurrence. New York, NY: Oxford University Press. 10.1093/acprof:oso/9780195152968.001.0001

Spoont, M. R. (1992). Modulatory role of serotonin in neural information processing: Implications for human psychopathology. Psychological Bulletin, 112, 330–350. 10.1037/0033-2909.112.2.330

Stroud, C. B., Davila, J., Hammen, C., & Vrshek-Schallhorn, S. (2011). Severe and nonsevere events in first onsets versus recurrences of depression: Evidence for stress sensitization. Journal of Abnormal Psychology, 120, 142–154. 10.1037/a0021659

Stroud, C. B., Davila, J., & Moyer, A. (2008). The relationship between stress and depression in first onsets versus recurrences: A meta-analytic review. Journal of Abnormal Psychology, 117, 206–213. 10.1037/0021-843X.117.1.206

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a complex trait: Evidence from a meta-analysis of twin studies. Archives of General Psychiatry, 60, 1187–1192. 10.1001/archpsyc.60.12.1187

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: Review and meta-analysis. The American Journal of Psychiatry, 157, 1552–1562. 10.1176/appi.ajp.157.10.1552

Surtees, P. G., & Wainwright, N. W. (1999). Surviving adversity: Event decay, vulnerability and the onset of anxiety and depressive disorder. European Archives of Psychiatry and Clinical Neuroscience, 249, 86–95. 10.1007/s004060050071

Vrshek-Schallhorn, S., Mineka, S., Zinbarg, R., Craske, M., Griffith, J., Sutton, J., . . .Adam, E. K. (2014). Refining the candidate environment: Interpersonal stress, the serotonin transporter polymorphism, and gene-environment interactions in major depression. Clinical Psychological Science, 2, 235–248. 10.1177/2167702613499329

Vrshek-Schallhorn, S., Stroud, C. B., Mineka, S., Hammen, C., Zinbarg, R. E., Wolitzky-Taylor, K., & Craske, M. G. (in press). Chronic and episodic interpersonal stress as statistically unique predictors of depression in two samples of emerging adults. Journal of Abnormal Psychology.

Wagner, M., Schuhmacher, A., Schwab, S., Zobel, A., & Maier, W. (2008). The His452Tyr variant of the gene encoding the 5-HT2A receptor is specifically associated with consolidation of episodic memory in humans. The International Journal of Neuropsychopharmacology, 11, 1163–1167. 10.1017/S146114570800905X

Wong, M. Y., Day, N. E., Luan, J. A., Chan, K. P., & Wareham, N. J. (2003). The detection of gene-environment interaction for continuous traits: Should we deal with measurement error by bigger studies or better measurement?International Journal of Epidemiology, 32, 51–57. 10.1093/ije/dyg002

Wray, N. R., Goddard, M. E., & Visscher, P. M. (2007). Prediction of individual genetic risk to disease from genome-wide association studies. Genome Research, 17, 1520–1528. 10.1101/gr.6665407

Wright, S. (1921). Systems of mating. Genetics, 6, 111–123.

Zhou, Z., Roy, A., Lipsky, R., Kuchipudi, K., Zhu, G., Taubman, J., . . .Goldman, D. (2005). Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. Archives of General Psychiatry, 62, 1109–1118. 10.1001/archpsyc.62.10.1109

Zinbarg, R. E., Mineka, S., Craske, M. G., Griffith, J. W., Sutton, J., Rose, R. D., . . .Waters, A. M. (2010). The Northwestern-UCLA youth emotion project: Associations of cognitive vulnerabilities, neuroticism and gender with past diagnoses of emotional disorders in adolescents. Behaviour Research and Therapy, 48, 347–358. 10.1016/j.brat.2009.12.008

Zinbarg, R. E., Suzuki, S., Uliaszek, A. A., & Lewis, A. R. (2010). Biased parameter estimates and inflated Type I error rates in analysis of covariance (and analysis of partial variance) arising from unreliability: Alternatives and remedial strategies. Journal of Abnormal Psychology, 119, 307–319. 10.1037/a0017552