

Research Article

GENDER DIFFERENCES IN THE GENETIC AND ENVIRONMENTAL DETERMINANTS OF ADOLESCENT DEPRESSION

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Background: *The well-documented gender differences in the risk for depression may be explained by genetic factors, by different responses to social context, or by a combination of both. We sought to assess whether there were gender differences in the longitudinal associations between serotonin transporter promoter (5-HTTLPR) genotype and depressive symptoms in adolescents, and whether macrosocial context plays a role in explaining any observed differences.* **Methods:** *Using data from a nationally representative survey of adolescents, we applied multilevel mixed models to assess, separately for adolescent males and females (a) the relation between 5-HTTLPR genotype and depressive symptoms and (b) the interaction of county-level deprivation and 5-HTTLPR genotype in models predicting depressive symptoms. All models adjusted for age and other covariates.* **Results:** *Among females (n = 560), main effects models showed an association between the sl genotype and lowered risk of depressive symptoms (b = -.18, P = .03). Among males (n = 524), interaction models showed an association between sl genotype and lowered risk of depressive symptoms in deprived counties only (b = -.32, P = .04).* **Conclusions:** *In adolescent females, the 5-HTTLPR sl genotype confers protection against depressive symptoms independent of county-level social context, whereas in adolescent males, protection by the same genotype is conferred only within the context of county-level deprivation. Future work should aim to understand how genetic and macrosocial factors jointly shape risk for mental illness, and how these factors shape gender differences in mental illness. Depression and Anxiety 27:658–666, 2010.* © 2010 Wiley-Liss, Inc.

Key words: *social epidemiology; serotonin transporter promoter; gene–environment interaction; macrosocial environment*

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INTRODUCTION

Genetic influences on adolescent depression have been confirmed by both twin and adoption studies, with heritability estimates (i.e. the proportion of variation attributable to genetic factors) generally ranging from 30% to 50% (reviewed in^[1]), a finding broadly comparable with estimates of major depressive disorder in adults.^[2] Notably, some studies have reported a greater genetic contribution to depression or depressive symptoms among boys versus girls and a more substantial effect of the common environment among girls versus boys^[3,4]; others, however, have found genetic influences to be more important among adolescent girls.^[5] These conflicting results may be due in part to the fact that genetic influences on depression may only be evident under particular environmental conditions—i.e. that there may be Gene \times Environment (G \times E) interactions, such that individuals of the same genotype may express different phenotypes depending on their surrounding social contexts.^[6]

The gene encoding the serotonin transporter protein (*SLC6A4*) is the best studied in this regard. This protein serves many functions; however, its action has been particularly well-studied in the brain where it transports serotonin at synaptic terminals and other neuronal areas^[7] and serves to regulate emotional aspects of behavior.^[8] Commonly occurring repeat polymorphisms in the promoter (*5-HTTLPR*) region of this gene have previously been associated with differential uptake of serotonin in *in vitro* studies.^[9] In humans, epidemiologic studies have reported G \times E interactions between particular *5-HTTLPR* alleles and depression, with the shorter, less transcriptionally active “s” allele being generally associated with greater risk for depression among individuals who have experienced maltreatment as children.^[10] However, attempts to replicate initial reports of these associations have been mixed and, in some cases, other *5-HTTLPR* alleles and/or genotypes have been implicated in susceptibility to depression (reviewed in^[11]). Most recently, a meta-analysis of *5-HTTLPR* genotype, number of stressful life events (SLE), and potential interactions between the two concluded that only SLE showed evidence for a significant association with depression.^[12]

Findings in adolescent samples have been particularly contradictory and suggest gender difference may be one explanation. The few studies published to date that focus on this age group have found evidence for increased risk for depression and depressive symptoms among carriers of the *ll* genotype when combined with high levels of family adversity^[13,14] and among *ss* carriers when combined with a history of sexual abuse^[15] or high-risk backgrounds.^[16] Notably, a few studies have demonstrated interaction effects only when analyses were conducted stratified by gender,^[16] with some even identifying the same genotype as both a risk and protective factor against depression depending on the gender.^[17] The likelihood that the serotonergic

system—to which the *SLC6A4* locus belongs—operates differently in males and females is substantiated by evidence from animal models showing gender-specific differences in serotonin levels detected in the brain^[18] and blood.^[19] Examples such as these thus suggest that at least some of the heterogeneity in G \times E interactions involving the *5-HTTLPR* locus reported to date may be due to factors that interact differently according to gender and, possibly, developmental period.^[11,20]

An additional consideration in G \times E investigations is the type of environmental exposure being assessed. Although the predominant theme in these studies has been to measure the number of SLE^[12] or presence/absence of maltreatment during childhood,^[10,21,22] evidence from both the non-genetic and genetic literature suggests that the macrosocial environment may also influence the risk for depression or depressive symptoms and that this effect is modified by gender. In the well-known “Moving to Opportunity” study,^[23] adolescent males who moved to less impoverished neighborhoods were significantly less likely to report anxious/depressive problems than adolescent control males who did not move; a similar difference was not detected in adolescent females^[23]; but see^[24] for an alternate analysis). On the genetic side, *5-HTTLPR* \times E interaction effects have been detected for adolescent boys according to their residence in public versus privately owned housing; no such interaction was detected for adolescent females^[17] although other variables, such as traumatic conflicts in the family, did show significant G \times E interactions exclusively in this group. Emerging evidence thus suggests that gender differences in both the genetic and environmental determinants of depression and depressive symptoms exist in adolescent populations.

To test this hypothesis, we investigated the longitudinal association among *5-HTTLPR* genotype, county-level deprivation, and depressive symptom scores in adolescent males and females sampled in the National Longitudinal Study of Adolescent Health (Add Health). Our goals were to test for the effect of *5-HTTLPR* genotype on depressive symptom scores separately in adolescent boys and girls and to test whether any observed *5-HTTLPR* effects were modified by macrosocial conditions in a large, community-based epidemiologic sample.

MATERIALS AND METHODS

SAMPLE

The data for this analysis was drawn from the National Longitudinal Study of Adolescent Health (Add Health), a nationally representative, school-based sample of over 90,000 adolescents in grades 7–12, initially sampled in 1994–1995 in the United States and followed for three subsequent waves. A subsample (~20,745) of participants from the in-school portion of the study was selected to participate in an additional, 90-min in-home interview during Wave I, which provided the primary data source for the analyses reported in this study. In 2002, during Wave III, DNA samples were collected

from a subsample of siblings ($n = 2,574$) who had participated in the in-home interview portion of the study. The in-home and genetic data are part of the restricted use/contractual AddHealth data set^[25] and IRB approval to work with this data set was secured. More detail regarding the design and data availability for the genetic component of AddHealth is available elsewhere.^[26]

The sample for our analysis draws on 1,084 individuals from the sibling subsample who provided DNA, belonged to a same sex sibling cluster of the same sibling type, and for whom there was a complete set of data available for each sibling in the cluster for each of the measures included in our models (described below). The analytic sample did not differ from the excluded sample with respect to genotype, measure of county-level deprivation, or depressive symptom scores, i.e. the main variables in the study.

MEASURES

Individual- and family-level health indicators. *Depressive symptom scores* were obtained using a shortened, 17-item version of the Center for Epidemiological Studies Depression Scale (CES-D),^[27] based on the CES-D questions that were posed in the AddHealth *Feelings Scale* during the in-home interviews conducted during Wave I (April–December 1995) and II (April–August 1996). The internal consistency from Wave I and II were 0.86 and 0.87, respectively. Responses to the 17 questions were ordinal, ranging from 1 (never or rarely) to 4 (most or all of the time) and were summed for use as the outcome variable in all analyses, with higher scores indicative of more depressive symptoms. Respondents were required to answer all 17 questions in Waves I and II in order to be included in our analyzed sample. The final current depression index was standardized to the mean in order to facilitate model interpretation.

Siblings were classified as monozygotic twins (MZ), dizygotic twins (DZ), full siblings (FS), half siblings (HS), or cousins (CO), as indicated in the AddHealth data files.

Genotype: The *5-HTTLPR* locus is characterized by a variable number of tandem repeat polymorphism with two predominant alleles that were assessed in the AddHealth study: the long (*l*) allele with 16 repeats and the short (*s*) allele with 14 repeats, the latter of which corresponds to a ~44-bp deletion in reference to the long allele.^[28] Respondents were assigned one of three possible *5-HTTLPR* genotypes: homozygote long (*ll*; referent category), homozygote short (*ss*), and heterozygote (*sl*).

Age and race/ethnicity: Age was calculated using date of birth and date of interview and left as a continuous variable in the model. Race/ethnicity was self-reported using the following categories: White (reference), African-American, Hispanic, Asian, and other race.

Family structure assessed the number of household resident parent(s) and categorized respondents as belonging to a two-biological parent family (referent category), a one-biological parent family (i.e. single biological parent or one biological parent and a stepparent), or “other family structure.”

Family-level socioeconomic status (SES) was assessed by whether at least one resident parent was receiving public assistance (PA).

Social support was measured by averaging the responses to eight questions that represent respondents’ perceived value and support from family members, friends, and teachers; responses ranged from 1 (not at all) to 5 (very much), with higher scores indicating more social support. If the respondents missed one or more of the eight questions, the average was determined from the remaining answered questions. The internal consistency of the eight social support questions used in this study was 0.78.

County-level environment. Consistent with previous work,^[29] PA was selected as a measure of exposure to poor social

environments, i.e. a proxy for county-level deprivation. The proportion of households receiving PA income in each county for each respondent was assessed using US Census data from 1990, geocoded to respondents’ interview data via the AddHealth contextual database. We calculated the median proportion of PA based on the counties represented by respondents in our data set and dummy variables were then created indicating 1 if the value is greater than the median and 0 otherwise. Individuals who relocated to a different county between Waves I and II were removed from the data set.

STATISTICAL ANALYSIS

A repeated multilevel modeling approach using mixed models was employed in our study, in which level 1 refers to the repeated measurements of individuals’ depressive symptom scores (i.e. the scores obtained from the same individual at Wave I and Wave II), level 2 refers to the individual respondent, and level 3 refers to the family cluster to which the respondent belongs. The following equation describes the basic mixed model used in our analysis:

$$CESD_{j(i)} = \beta_0'X_{ij} + \beta_1'5-HTTLPR_{ij} + \beta_2'family\ structure_{ij} + \beta_3'SES_{ij} + \beta_4'support_{ij} + \beta_5 PA_{ij} + u_{j(i)} + v_{ij} + e_{j(i)}$$

where i, j indicate individual and sibling cluster, respectively. Each beta represents a single coefficient or a vector of coefficients for each predictor component in the model; X represents age and race, *5-HTTLPR* represents the serotonin transporter promoter genotype, family structure represents the variants in resident parents, SES refers to household receipt of PA, support refers to social support, and PA refers to public assistance measured at the county level. The random effect of the family cluster is represented by $u_{j(i)}$, v_{ij} is the random effect of the repeated observations on the same individual, and $e_{j(i)}$ is the error term. This model allows the random effect of family cluster and the error term to vary by sibling type,^[30] denoted by s ($s = MZ, DZ, FS, HS, \text{ and } CO$). All predictors were set at Wave I values and the outcome variable (depressive symptom score) was assessed as a repeated measure across Waves I and II, i.e. across a 1-year interval. Our first model tested the unadjusted (i.e. bivariate) associations between each separate covariate and the outcome. The second model predicted standardized depressive symptom scores adjusting for all the variables included in the model. Our third model again adjusted for all the covariates in the model and included a genotype \times county-level PA interaction term to assess potential $G \times E$ interactions between these two variables (using the *ll* genotype and low PA as the referent categories). All models were stratified by gender, and all analyses were conducted using SAS v. 9.2, SAS Institute, Cary, NC.

RESULTS

Table 1 presents the descriptive statistics and unadjusted associations for the individual-, family- and county-level predictors included in our final model. The average age in both our male ($n = 524$) and female ($n = 560$) samples was approximately 16 years (range in males: 12–19 years; range in females: 12–20 years). The average depressive symptom score was significantly higher in female adolescents (27.8) versus male adolescents (26.5; $t = -3.55, P = .0004$). Although a number of predictor variables also showed gender differences in unadjusted associations (Table 1), notable to this study was the detection of a significant protective effect of the *sl* genotype in females and a significantly increased risk for depressive symptoms among males residing in high PA counties. When one sibling

TABLE 1. Sample descriptives and unadjusted associations predicting standardized depressive symptom score, stratified by gender

	Males (n = 524)		Females (n = 560)		Males (n = 524)			Females (n = 560)				
	n/mean	%/std	n/mean	%/std	b	P	95%CI	b	P	95% CI		
<i>Genotype</i>												
ss	120	22.9	96	17.1	.04	.69	-0.14	0.21	.14	.21	-0.08	0.36
sl	244	46.6	265	47.3	-.13	.07	-0.27	0.01	-.16	.04	-0.32	0.00
ll	160	30.5	199	35.5	.14	.10	-0.02	0.29	.11	.23	-0.07	0.28
<i>Demographics</i>												
Age	16.1	1.6	16.0	1.7	.04	.05	0.00	0.09	.02	.44	-0.03	0.06
White	277	52.9	355	63.4	-.36	<.0001	-0.52	-0.20	-.28	<.01	-0.47	-0.10
Black	82	15.6	76	13.6	.28	.02	0.05	0.50	.10	.46	-0.16	0.35
Hispanic	87	16.6	59	10.5	.07	.53	-0.15	0.30	.02	.88	-0.27	0.32
Asian	44	8.4	28	5.0	.36	.02	0.07	0.65	.58	.01	0.14	1.01
Other	34	6.5	42	7.5	.26	.12	-0.07	0.59	.44	.02	0.09	0.80
<i>Family structure</i>												
Two biological parents	358	68.3	366	65.4	-.26	<.01	-0.43	-0.08	-.33	<.001	-0.52	-0.14
One biological parent	150	28.6	171	30.5	.25	.01	0.07	0.44	.33	<.001	0.14	0.52
Other family structure	16	3.1	23	4.1	.13	.60	-0.35	0.61	.13	.55	-0.30	0.56
<i>SES and social support</i>												
Parent receives public assistance	34	6.5	52	9.3	.32	.06	-0.01	0.66	.37	.01	0.07	0.68
Social support	4.0	0.5	4.0	0.6	-.56	<.0001	-0.68	-0.45	-.75	<.0001	-0.86	-0.63
<i>County-level</i>												
High deprivation	286	54.6	265	47.3	.20	.02	0.04	0.37	.12	.20	-0.06	0.30
<i>17-item CES-D</i>												
Depressive Symptom Score	26.5	5.6	27.8	6.4								

Significant effect estimates at 5% level are bold-faced, *b* is the model parameter estimate, *P* is the *P*-value, and CI is the parameter estimate confidence interval.

TABLE 2. Adjusted main effects model predicting standardized depressive symptom score, stratified by gender

	Male (n = 524)				Female (n = 560)			
	b	P	95% CI		b	P	95% CI	
ss	-.09	.37	-0.28	0.10	-.05	.66	-0.26	0.17
sl	-.12	.13	-0.27	0.03	-.18	.03	-0.34	-0.02
Age	.02	.35	-0.02	0.06	-.01	.63	-0.05	0.03
Black/African-American	.33	<.01	0.10	0.56	.06	.65	-0.19	0.31
Hispanic	.21	.05	0.00	0.41	.13	.32	-0.13	0.38
Asian	.46	<.01	0.18	0.74	.68	<.001	0.31	1.05
Other race	.31	.05	0.00	0.61	.23	.13	-0.07	0.54
One biological parent family	.09	.30	-0.08	0.26	.27	<.01	0.09	0.45
Other family structure	.10	.64	-0.33	0.54	.09	.65	-0.30	0.48
Parent receives public assistance	.25	.10	-0.05	0.55	.18	.23	-0.11	0.46
Social support	-.55	<.0001	-0.66	-0.43	-.74	<.0001	-0.85	-0.62
High deprivation	.06	.49	-0.10	0.21	.02	.84	-0.15	0.18

Significant effect estimates at 5% level are bold-faced, *b* is the model parameter estimate, *P* is the *P*-value, and CI is the parameter estimate confidence interval.

per cluster was sampled randomly from each family, genotype frequencies for the 5-HTTLPR locus were in Hardy-Weinberg Equilibrium ($\chi^2 = 1.87$, $df = 1$, $P = .17$). Consistent with previous work,^[31] the frequency of the ll genotype was higher among black respondents (ll 50.6%; sl 39.2%; and ss 10.1%) compared to whites (ll 31.3%; sl 52.1%; and ss 16.6%). Similarly, the frequency of ss genotypes was higher among Asian (ll 11.1%; sl 58.3%; and ss 30.6%)

and Hispanic (ll 22.2%; sl 50.0%; and ss 27.8%) respondents than among whites. Although these frequencies were statistically significantly different, genotype frequencies for all race/ethnic groups were in Hardy-Weinberg equilibrium.^[32]

Table 2 presents the results of our multivariable, multilevel main effects model. For both males and females, higher social support was associated with significantly lower depressive symptom scores, and

Asian race was associated with significantly higher depressive symptom scores. Among males only, belonging to any of the minority race/ethnic categories was a risk factor for significantly higher depressive symptom scores. Among females only, residing in a family in which there was only one biological parent was associated with significantly higher depressive symptom scores. In contrast, the *sl* genotype showed significant protection against higher depressive symptom scores in this gender: holding all other predictors constant, there was an estimated -0.18 standard deviation change in the predicted mean depressive symptom scores of female *sl* carriers ($b = -.18$, 95% CI: -0.34 , -0.02 ; $P = .03$).

Table 3 presents results from the multivariable models with the interaction term included. Among males, a significant interaction between county-level PA and *5-HTTLPR* genotype was observed, such that males with the *sl* genotype residing in counties with high PA were protected against higher depressive symptom scores ($b = -.32$; 95% CI -0.63 , -0.02 ; $P = .04$). No significant interaction effects were observed among females.

Figure 1 presents the average depressive symptom scores for males and females, respectively, by *5-HTTLPR* genotype and residence in high- versus low-deprivation counties, unadjusted for other covariates. Among males, the protective effect of the *sl* genotype in high deprivation counties can be inferred

TABLE 3. Interaction effects model predicting standardized depressive symptom score, stratified by gender

	Male ($n = 524$)				Female ($n = 560$)			
	<i>b</i>	<i>P</i>	95% CI		<i>b</i>	<i>P</i>	95% CI	
<i>ss</i>	.07	.64	-0.22	0.35	-.08	.63	-0.39	0.23
<i>sl</i>	.05	.63	-0.17	0.28	-.10	.37	-0.32	0.12
Age	.02	.34	-0.02	0.06	-.01	.65	-0.05	0.03
Black/African-American	.31	.01	0.08	0.54	.05	.71	-0.20	0.30
Hispanic	.21	.05	0.00	0.41	.12	.36	-0.14	0.37
Asian	.47	<.001	0.20	0.75	.68	<.001	0.31	1.05
Other race	.30	.05	0.00	0.60	.22	.15	-0.08	0.53
One biological parent family	.09	.29	-0.08	0.26	.28	<.01	0.10	0.45
Other family structure	.09	.69	-0.35	0.52	.08	.69	-0.31	0.47
Parent receives public assistance	.23	.14	-0.07	0.52	.18	.23	-0.11	0.47
Social support	-.55	<.0001	-0.67	-0.44	-.74	<.0001	-0.86	-0.63
High deprivation	.28	.04	0.02	0.54	.10	.46	-0.16	0.35
High deprivation * <i>ss</i>	-.28	.15	-0.66	0.10	.05	.82	-0.38	0.47
High deprivation * <i>sl</i>	-.32	.04	-0.63	-0.02	-.19	.25	-0.50	0.13

Significant effect estimates at 5% level are bold-faced, *b* is the model parameter estimate, *P* is the *P*-value, and CI is the parameter estimate confidence interval.

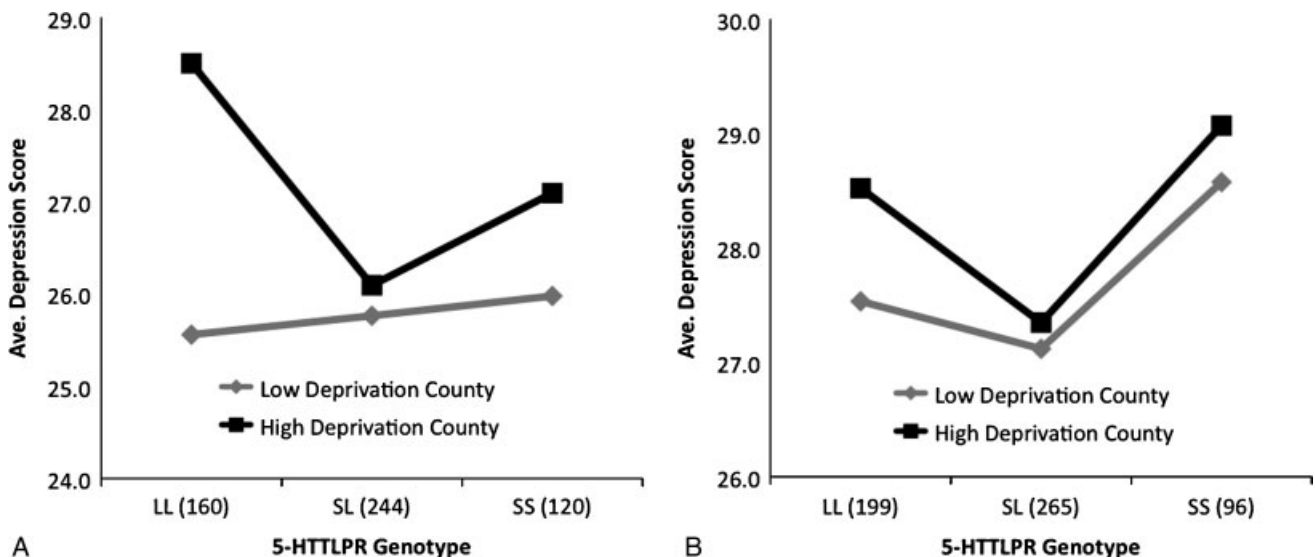


Figure 1. Average depressive symptom scores by *5-HTTLPR* genotype and county-level deprivation, dichotomized as high versus low, among adolescent males (A) and females (B).

from the noticeably lower depressive symptom scores compared to those observed for the *ss* or *ll* genotypes; no similar difference is observed among males residing in counties with low deprivation. Among females, lower depressive symptoms scores are apparent among carriers of the *sl* genotype irrespective of residence in high- or low-deprivation counties, consistent with the results obtained for this predictor in our unadjusted and multivariable main effect models (Tables 1 and 2). Depressive symptom scores were higher in female versus male adolescents for each genotype in each stratum; in low-deprivation counties, these results were statistically significant for the *ll* and *ss* genotypes ($t = -2.04$, $P = .04$ in both tests), and marginally significant for the *sl* genotype ($t = -1.97$, $P = .06$).

DISCUSSION

Our work confirms and extends the existing evidence for gender differences in the genetic and environmental determinants of adolescent depression and depressive symptoms. Using a genetic subsample of the National Longitudinal Study of Adolescent Health, we found that, in males, county-level environment modified the association between *5-HTTLPR* genotype and depressive symptoms across a 1-year interval. No $G \times E$ associations were detected in adolescent females; however, in this group, there was evidence for a protective main effect of the *sl* genotype in multivariable models. Taken together, these results represent the first report of a protective effect of the *sl* genotype against depressive symptoms in adolescents that is differently manifested in males and females, and whose association with depressive symptoms in males is modified by a macro-level feature of the social environment.

Although the detection of the *sl* genotype as protective has, to date, been detected in only one other study of adolescents of which we are aware,^[17] other studies have also presented results that are suggestive of a protective effect against depressive symptoms for this genotype in adults^[33] and children.^[21] In this study, our relatively large sample size may have enabled us to detect associations that other, more underpowered studies may have missed. Interestingly, in the aforementioned study of adolescents that detected a protective *sl* effect and the analyses included what could be considered a measure of the macrosocial environment, i.e. residence in public, multifamily versus privately owned, single family housing; similar to the results presented in this study, depressive symptom scores were lowest only among adolescent males residing in multifamily housing and carrying the *sl* genotype.^[17] When studies have measured “E” via assessments of SLE or psychosocial stress, results have indicated that the *s* allele confers a protective main effect against depressive symptoms in female adolescents^[16] but is associated with increased depressive symptoms in this group when interacted

with family-level environmental risks.^[16,17] Although these latter results have led some to suggest that adolescent boys may be protected from such “pathogenic” $G \times E$ interactions,^[11] it is also possible that current methods may be inadequate for measuring exposures and outcomes pertinent to this group.^[11]

Although our goal in this work was not to address this last point, our working assumption that macro-level environmental features may act as determinants of mental health may indeed have offered a salient lens through which to view $G \times E$ interactions relevant to depressive symptoms that might otherwise be missed among adolescent males. Importantly, our analyses detected an interaction between underlying genetic variability/vulnerability and county-level environment when controlling for potential family-level confounders (i.e. household receipt of PA). That this $G \times E$ effect was detected only among males, in contrast to previous reports demonstrating a preponderance of $G \times E$ interactions among adolescent females when “E” is measured as SLE, suggests that different types of environmental measures may be salient for males and females of this age range; different environmental measures, in turn, may uncover $G \times E$ interactions at work with different alleles even at the same locus. More generally, these results suggest that among adolescents, macrosocial context may have differential effects by gender, such that adolescent males are more susceptible to contextual effects than their female counterparts. This suggestion is consistent with previous evaluations of neighborhood contextual effects on adolescent mental health.^[23] Similar findings have been reported in the twin studies literature, in which shared neighborhood-level environmental influences were found to be more important than genetic effects in determining antisocial behavior among adolescent males, whereas for adolescent girls, genetic factors were more important than shared environmental effects.^[34]

From a population genetic perspective, one scenario that may account in part for the observations reported here is the signal of Tajima’s *D* surrounding the *5-HTTLPR* locus. Tajima’s *D* is a statistical test that aims to distinguish between a DNA sequence evolving randomly (“neutrally”) versus one evolving under a non-random process, such as directional selection or balancing selection.^[35] The University of Santa Cruz genome browser provides a snapshot of Tajima’s *D* values in three populations (in Americans of European, African, and Asian ancestry) estimated from the Perlgen data set.^[36] Tajima’s *D* can adopt values between -2 and $+2$. High values of Tajima’s *D* generally indicate an excess of common variation in a region, which can be consistent with both balancing selection and population contraction.

In the area surrounding the genomic region assessed in this work (rs25531), the value of Tajima’s *D* is estimated to be quite high among individuals of European descent—near the maximum. This group was also

the ethnic group with the greatest representation in our sample (52.9% males and 63.4% females). If we assume that our sampled population is not contracting, then perhaps a scenario of balancing selection can offer insight into our previously unreported observation of a protective effect of the *s/l* genotype. Under this scenario, there would be a heterozygote advantage—a “benefit” of having an *s/l* genotype that would be somewhat akin to the more commonly known sickle cell anemia example. As in the anemia example, the selective advantage of the *s/l* genotype may appear only under certain environmental conditions—in this case, adverse macrosocial conditions. Although this argument is inferential—Tajima’s *D* is assessed using nucleotide-level diversity, which was not directly assessed in the AddHealth genetic sample—and, furthermore, does not account for the protective main effect of the *s/l* genotype among adolescent females, the more general scenario of balancing selection might help to explain the heterogeneity of $G \times E$ results reported thus far for the *5-HTTLPR* locus: maintenance of high levels of diversity at this locus may be advantageous in that different alleles and/or genotypes confer benefits to their bearers in different environmental contexts.

This study has a number of limitations that should be taken into consideration when evaluating its results. First, *5-HTTLPR* genotype was assessed using a bi-allelic system, producing three possible genotypes for analysis in this work; other, relatively low-frequency alleles at this locus have been reported,^[37] including a novel L_G allele thought to behave similarly to the more commonly occurring *s* allele.^[38,39] Due to our reliance on secondary data, however, we were unable to assess their contribution in this work. Lack of consideration of the triallelic genotype, however, would have resulted in misclassification that would have reduced our power to detect genetic effects. Second, we observed significant racial/ethnic differences in *5-HTTLPR* genotype frequencies and depressive symptoms, which raises the possibility that population stratification could have influenced our findings. However, we adjusted for self-reported race/ethnicity in our multivariable models (models 2 and 3), which has been shown to correspond well with ancestral classification using genetic markers.^[40] In addition, we reran all analyses in Whites only and findings were comparable, suggesting that population stratification cannot account for the results presented here.

Finally, there are likely additional environmental and molecular factors, at multiple levels, which contribute to depressive symptoms in adolescents that we were unable to capture in this study. For example, it is possible that including childhood maltreatment as an “E” variable in our models may have enabled us to detect depression-related $G \times E$ interactions among adolescent females consistent with previous reports in the literature^[16]; in addition, evidence is emerging that epigenetic mechanisms may also play a role in

regulating expression of the serotonin transporter gene,^[41,42] with one report indicating a nearly significant ($P = .07$), higher methylation level of *SLC6A4* in those without a lifetime history of major depression when compared to those who have at some point suffered from the disorder.^[41] Investigations focused on $G \times E$ interactions using the *5-HTTLPR* locus are thus inherently complex and will likely require multiple levels of measurement at both the molecular and environmental levels to improve our identification of etiological risk factors for increased depressive symptoms.

Despite these limitations, this study confirms the need to investigate the determinants of depression separately in males and females, particularly in studies involving the *5-HTTLPR* locus in adolescent populations. In addition, this study contributes to emergent literature suggesting that features of the macrosocial environment interact with individual genetic variation in the development of psychiatric disorders.^[43] Our results suggest that adolescent females with the *5-HTTLPR s/l* genotype are conferred protection against depressive symptoms independent of the larger social environment in which they reside; in contrast, among adolescent males, there is an evidence for a $G \times E$ interaction effect at the *5-HTTLPR* locus, such that the *s/l* genotype confers protection against depression in males residing in adverse social environments. Future work should attempt to replicate the results presented in this study in other adolescent populations, and to more thoroughly investigate the distinct causal pathways that may link features of the social environment to risk for/resilience to depressive symptoms separately in males and females.

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