Gene-Environment Interaction in Youth Depression: Replication of the 5-HTTLPR Moderation in a Diverse Setting

Thiago Botter-Maio Rocha, M.D., Mara H. Hutz, Ph.D., Angélica Salatino-Oliveira, B.S., Júlia P. Genro, Ph.D., Guilherme V. Polanczyk, M.D., Ph.D., João Ricardo Sato, Ph.D., Fernando C. Wehrmeister, Ph.D., Fernando C. Barros, M.D., Ph.D., Ana M.B. Menezes, M.D., Ph.D., Luis Augusto Rohde, M.D., Ph.D., Luciana Anselmi, Ph.D., Christian Kieling, M.D., Ph.D.

Objective: Replication of scientific findings is a major challenge in biomedical research. In psychiatry, the identification of measured gene-environment interactions (G×E) has promoted a heated debate over the past decade, with controversial results about its influence on disorders such as major depression. The authors sought to replicate a 2003 study on G×E in youth depression in a large birth cohort from a diverse setting.

Method: Using data from the 1993 Pelotas Birth Cohort Study, and adopting a design as similar as possible to that of the original study, the authors tested whether the relationship between childhood maltreatment and a subsequent depressive episode diagnosis was moderated by 5-HTTLPR genotype. Of 5,249 individuals assessed at birth and followed up to age 18, data on the evaluation for depressive episodes in early adulthood, on childhood maltreatment, and on genotype were available for 3,558 participants, of whom 2,392 remained after conservative screening for previous

depressive symptoms. Associations were investigated with logistic regression analyses and controlling for potential confounders.

Results: The results replicated important findings of the original study, this time in a sample of young adults from a middle-income country: there was a differential dose-response relationship between childhood maltreatment and major depression according to 5-HTTLPR genotype.

Conclusions: After following a research strategy as comparable as possible to that of the original study, the results corroborated the existence of a measured G×E, now in a large sample from a different sociocultural context. These findings provide further evidence that a genetic variant in the 5-HTTLPR moderates the link between childhood maltreatment and youth depression.

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The pursuit of innovative findings has advanced science throughout the years. Studies focusing on replication of initial results, although also recognized as essential in the scientific process (1–4), have received less attention and are frequently regarded as just "another brick in the wall" (5). In recent years, however, the low rate of replicability of results has produced incredulity, doubt, and distrust in the biomedical sciences in general (6–8), as well as in the field of mental health research (9, 10). Pioneer studies that focused on measured gene-environment interactions (G×E) had a tremendous impact in the psychiatric literature (11, 12), instigating many replication efforts. Discrepancies in subsequent results, however, have generated a heated debate over the past decade, casting doubts on whether and to what extent the initial findings are reproducible (13–17).

In the quest to understand why some but not all individuals exposed to stressful life events and maltreatment are at increased risk for major depression, Caspi et al. (12) presented an

innovative framework, providing evidence that this phenomenon was moderated by one's genetic background. In a cohort of individuals followed up to early adulthood, those carrying the short allele of a serotonin-transporter-linked polymorphic region (5-HTTLPR) exhibited an increased risk for depression after experiencing stressful life events or maltreatment compared with those who were homozygotes for the long allele.

Mixed findings among studies trying to replicate the initial report were summarized in meta-analyses that suggested an overall lack of this interaction in the prediction of depression (18) and that the "addition of the 5-HTT genotype did not improve the prediction of depression beyond that associated with negative life events" (13). After criticism regarding selection bias in the included studies (19), a broader meta-analysis was published 2 years later that posited that aggregated results were in accordance with a significant role for

the 5-HTTLPR polymorphism in moderating the relationship between stress and depression (20). Evidence of publication bias of negative studies in the G×E literature and the overinclusiveness of the later meta-analysis (15) have been pointed out as possible reasons for these contrasting conclusions and their failure to elucidate this contradictory issue.

An array of explanations have been proposed for these conflicting findings, among them the heterogeneity of design used in the studies (cross-sectional, retrospective, prospective), the variety of environment assessment tools used (parent/teacher, self, objective assessment), and the nature of the evaluated stressor itself (20). The convergence of all these issues on a single research question has been regarded as a methodological "perfect storm" (21). It has been argued that evidence for the 5-HTTLPR stress sensitivity hypothesis came not only from human but also from animal studies, as well as from research using observational and experimental designs (22).

Given the highly controversial state of current G×E research, unambiguous parameters for statistical replication are required. In line with this idea, recommendations and protocols have been developed to establish common and comparable designs and methodologies (20, 23–25). Culverhouse et al. (26) have developed a sensitive and well-elaborated meta-analysis protocol to address the inconsistencies encountered in previous studies, emphasizing the sources of misinterpretations in G×E research results on depression. An important methodological aspect not prioritized in the protocol, however, is the relevance of the timing of environmental exposure. Multiple lines of evidence corroborate the notion that timing is essential in such investigations (21, 24, 25, 27), as the absence of a clear definition of the temporal order between risk factor and outcome can markedly limit the interpretation of findings. In fact, the centrality of this issue was already demonstrated in the original Caspi et al. study (12), in which the G×E effect was observed only when the reported stressful life events occurred before—but not after—depression.

Up to now, published G×E research originated almost exclusively in high-income countries. In the last three meta-analyses reported, only five of the 54 included studies were from low- and middle-income countries. The underrepresentation of low- and middle-income countries in G×E research contrasts with both the greater frequency of known environmental risks in these low-resource settings and the higher proportion of youths in the population (28). The assessment of G×E in different settings can be useful not only to determine the validity of the G×E construct across multiple contexts but also to identify specific instances when the replication does not occur (22).

Our primary objective in the present study was to test the interaction between maltreatment and 5-HTTLPR in the development of depression in young adults, replicating to the extent possible the design and methods of the original study but in a different sociocultural context—a middle-income country. Given the importance of temporal order in G×E assessment, our analyses also aimed to ascertain the temporality between the stressor and outcome. Using a large and prospectively assessed birth cohort, we sought to employ definitions of variables, a coding strategy, definitions of outcomes, and a statistical approach as similar as possible to those of the original study. As secondary objectives, we also tested whether our model of G×E was specific for major depression and whether our results would stand for the 5-HTTLPR triallelic approach (29).

METHOD

Design and Sample

The data for this study were from the 1993 Pelotas Birth Cohort Study. A detailed description of the cohort characteristics and study design can be found elsewhere (30, 31; see also the data supplement that accompanies the online edition of this article). In brief, all children born in Pelotas, Brazil, in 1993 were eligible for the study. All but 16 mothers agreed to take part, resulting in a total cohort of 5,249 children. Follow-up visits were made at multiple time points, the last in 2011 and 2012. The data used in the present study were collected at the perinatal assessment and the age 11, age 15, and age 18/19 assessments, which had retention rates of 87.5%, 85.7%, and 81.3%, respectively (32).

After initial recruitment, mothers were interviewed on a variety of perinatal health topics. Additional data were obtained on maternal education (number of years of education, categorized into three strata) and monthly family income (measured in number of minimum wages, a standard unit in Brazil valued around US$60 in 1993).

At the age 11 visit (in 2004), data on child mental health were obtained using the Brazilian Portuguese version of the Strengths and Difficulties Questionnaire, with parent and child assessments (33, 34). Data on maternal mental health status were obtained using the Brazilian Portuguese validated version of the Self-Report Questionnaire (35).

For the age 15 visit (in 2008), adolescents filled out a confidential form that included seven dichotomous questions about lifetime emotional, physical, or sexual abuse and child neglect (see Table S3 in the online data supplement for details). We followed the same approach adopted by Caspi et al. in regard to maltreatment classification (11, 36), in which individuals were grouped into three categories: no maltreatment (no positive answer), probable maltreatment (one positive answer), and severe maltreatment (two or more positive answers). Information on mental health problems was collected using the same strategy as in 2004, with the exception of the Strengths and Difficulties Questionnaire, which was available only in the parent version.

Also at age 15, DNA samples were obtained from saliva using the Oragene OG-250 DNA Self-Collection kit, and the 5-HTTLPR polymorphism was amplified as described previously (37–39). The primary focus of interest was the biallelic variant (LL, LS, SS), since it was the genotype system analyzed

TABLE 1. Characteristics of Participants Included in the Primary Analyses[a]

Characteristic	5-HTTLPR Genotype						Childhood Maltreatment						Depressive Episode				Total (N=2,392)	
	LL (N=774)		LS (N=1,144)		SS (N=474)		No (N=1,693)		Probable (N=424)		Severe (N=275)		No (N=2,312)		Yes (N=80)			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Male	362	46.8	524	45.8	207	43.7	838	49.5[b]	169	39.9[c]	86	31.3[c]	1,071	46.3[b]	22	27.5[c]	1,093	45.7
White (self-identified)	474	61.3[b]	828	72.5[c]	362	76.4[c]	1212	71.6[b]	285	67.4[b]	167	60.9[c]	1,614	69.9	50	62.5	1,664	69.7
Maternal education (years)																		
0–4	214	27.6	322	28.2	150	31.6	511	30.2[b]	119	28.1[b]	56	20.4[c]	671	29.0[b]	15	18.8[c]	686	28.7
5–8	365	47.2	554	48.5	217	45.8	813	48.0	202	47.6	121	44.2	1,098	47.5	38	47.5	1,136	47.5
≥9	195	25.2	267	23.4	107	22.6	369	21.8[b]	103	24.3[b]	97	35.4[c]	542	23.5[b]	27	33.8[c]	569	23.8
Maternal psychopathology	185	24.0	266	23.3	102	21.7	353	21.0[b]	113	26.7[c]	87	31.6[c]	528	22.9	25	31.2	553	23.2
5-HTTLPR genotype																		
SS							322	19.0	84	19.8	68	24.7	455	19.7	19	23.8	474	19.8
LS							812	48.0	207	48.8	125	45.5	1,112	48.1	32	40.0	1,144	47.8
LL							559	33.0	133	31.4	82	29.8	745	32.2	29	36.2	774	32.4
Childhood maltreatment																		
No	559	72.2	812	71.0	322	67.9							1,662	71.9[b]	31	38.8[c]	1,693	70.8
Probable	133	17.2	207	18.1	84	17.7							404	17.5	20	25.0	424	17.7
Severe	82	10.6	125	10.9	68	14.3							246	10.6[b]	29	36.2[c]	275	11.5
Depressive episode	29	3.7	32	2.8	19	4.0	31	1.8[b]	20	4.7[c]	29	10.5[d]					80	3.3
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Family income (minimum wages)	4.29	5.51	4.62	6.61	4.90	5.46	4.69	6.15	4.33	5.74	4.22	5.89	4.61	6.10	3.34	4.03	4.57	6.05

[a] Superscripted letters denote column proportions with statistical differences: different letters show significant differences (at $p \leq 0.05$), and equal letters indicate nonsignificant differences for each main variable. Annual family income was measured in number of minimum wages, a standard unit in Brazil valued around US$60 in 1993. 5-HTTLPR=serotonin-transporter-linked polymorphic region; L=long allele; S=short allele.

in the original study as well as in the vast majority of replication attempts (18, 25, 26, 40). However, as a more recently identified single-nucleotide polymorphism (SNP) in the L allele (rs25531, a>g) led to differentiation between the high-functioning La variant versus the Lg variant, more functionally equivalent to the S allele—the so-called triallelic approach (29)—secondary analyses were performed with this latter model, using La, Lg, and S as allele coding.

For the age 18/19 visit (2011–2012), all traceable young adults were assessed by trained psychologists for psychiatric diagnoses, using an instrument derived from the Mini International Neuropsychiatric Interview (41). Depressive episode diagnoses were evaluated with DSM-IV-TR criteria, assessing symptoms in the previous 2 weeks. Other psychiatric diagnoses assessed at this time point were bipolar disorder, generalized anxiety disorder, social anxiety disorder, and attention deficit hyperactivity disorder.

To allow the assessment of timing of events, a sensitive screening index for depressive symptoms was developed with the aim of identifying individuals with a possible major depression diagnosis prior to or at the time of the maltreatment assessment. For this screening index, we used scores from the emotional subscale of the Strengths and Difficulties Questionnaire and the impact supplement administered at the age 11 visit (self-report and parent-rated) and at the age 15 assessment (parent-rated only). To increase sensitivity, an overinclusive index was created using those scores, in which any available data compatible with the presence of emotional problems causing an impact was sufficient for a positive screening. The definition of positive screening followed the borderline cut-point on the emotional and impact subscales, as recommended for the Brazilian Portuguese version of the original instrument. A second strategy was also adopted in order to validate the screening index's findings, this time using the dispersion of emotional scores within the sample. The 80th percentile was used a priori as the cut-point for the construction of the same index above mentioned.

For a flowchart of the study design, see Figure S1 in the online data supplement. The research protocol was approved by the Federal University of Pelotas Ethics Committee. Written informed consent was obtained from primary caregivers or cohort members (in the last visit) prior to each assessment.

Statistical Analysis

Data analyses included a description of the sample characteristics for each variable group and comparison of frequencies between groups, using chi-square tests for categorical variables and t tests and analysis of variance (ANOVA) to compare the group means for quantitative variables. We also tested the independence of childhood maltreatment and 5-HTTLPR genotype variables, using ANOVA for the identification of gene-environment correlation.

To evaluate the association between a depressive episode and 5-HTTLPR genotype, childhood maltreatment

TABLE 2. Logistic Regression Parameters for Each Psychiatric Outcome, Considering All Main Variables and Statistically Significant Covariates[a]

Psychiatric Outcome, Variables, and Covariates	B	SE	Z	p
Major depression				
Gender	0.613	0.259	2.367	0.018
Childhood maltreatment (E)	0.472	0.214	2.206	0.027
5-HTTLPR genotype (G)	−0.531	0.253	−2.099	0.036
G×E interaction	0.466	0.187	2.492	0.013
Bipolar disorder				
Gender	1.091	0.464	2.353	0.019
Childhood maltreatment (E)	0.366	0.358	1.022	0.307
5-HTTLPR genotype (G)	−0.128	0.362	−0.353	0.724
G×E interaction	0.183	0.300	0.610	0.542
Generalized anxiety disorder				
Gender	0.953	0.198	4.813	<0.001
Childhood maltreatment (E)	0.423	0.159	2.660	0.008
5-HTTLPR genotype (G)	−0.279	0.162	−1.722	0.084
G×E interaction	0.097	0.143	0.678	0.496
Social anxiety disorder				
Gender	1.029	0.212	4.853	<0.001
Childhood maltreatment (E)	0.106	0.181	0.585	0.557
5-HTTLPR genotype (G)	−0.269	0.161	−1.670	0.096
G×E interaction	0.080	0.163	0.490	0.622
Attention deficit hyperactivity disorder				
Childhood maltreatment (E)	0.802	0.370	2.167	0.030
5-HTTLPR genotype (G)	−0.436	0.495	0.881	0.378
G×E interaction	0.306	0.345	0.887	0.375

[a] 5-HTTLPR=serotonin-transporter-linked polymorphic region. For each outcome, all potential confounders (see text) were added to the original regression model (5-HTTLPR genotype, childhood maltreatment, and the interaction term). Variables reaching a more flexible statistical threshold ($p<0.2$) were then included in the final model. Additional analyses to assess possible gender moderation for genetic or environmental variables resulted in nonsignificant gender-by-gene and gender-by-maltreatment interaction terms. Reference category for gender: male. For bipolar disorder, gender and family income were included in the final model as statistically significant covariates, but family income did not reach statistical significance in final analysis; for generalized anxiety disorder, gender and skin color were identified as covariates, but skin color did not change the main results; for social anxiety disorder, gender, maternal psychopathology, and maternal education (the latter coded as an ordinal variable) were included in the final model, and all reached statistical significance (for psychopathology, B=0.390, p=0.049; for education, B=0.393, p=0.002); for attention deficit hyperactivity disorder, skin color was identified as a covariate but did not change the main results, and gender was not identified as a statistically significant covariate and was not included in final analysis for that outcome. The 5-HTTLPR genotype was coded as follows: 0=LL, 1=LS, 2=SS.

experience, and their interaction, we performed a binary logistic regression with a first-order interaction model, coding childhood maltreatment exposure by counting the number of maltreatment experiences and coding 5-HTTLPR genotype to reflect the number of short (S) alleles (0=LL, 1=LS, 2=SS). Subsequently, we included all conceptually defined potential confounders in the model, such as gender, maternal education and mental health, family income, and skin color (classified according to self-identification, grouped as white and nonwhite).

Considering our replication focus, we also performed a sensitivity analysis with only participants who self-identified as white. Secondarily, we also assessed the specificity of the logistic regression model, following the analysis strategy described above but with the dependent variable changed to other psychiatric diagnoses, all assessed at the same time point as major depression.

All analyses were carried out with SPSS, version 20 (IBM Corp., Armonk, N.Y.).

RESULTS

Sample Characteristics

From the original sample of 5,249 individuals, 4,052 were evaluated for depressive episode in early adulthood, and within that group, childhood maltreatment and genotype data were available for 3,558 individuals. (See the flowchart in Figure S1 in the online data supplement; see Table S1 for demographic information for the original sample and a comparison of participants who remained in the study and those who were lost to follow-up.)

Using our sensitive screening index for depressive symptoms for the 3,558 retained participants to filter out those likely to have experienced previous depression, a total of 2,392 young adults were selected for our analyses. The final sample characteristics across each group of measures of interest are summarized in Table 1.

Participants who screened positive for early depressive symptoms, excluded from the main analyses (N=1,039), differed significantly from the analyzed sample on several measures, such as gender, skin color, maternal education rate, family income, and exposure to childhood maltreatment (all p values, <0.01; see the online data supplement for further details.)

For those included in the final analyses, the proportion of 5-HTTLPR polymorphism genotypes within the biallelic model was 32.4% for LL, 47.8% for LS, and 19.8% for SS. For the triallelic model, genotype distribution was 40.8% for SLa, 25.0% for LaLa, 19.8% for SS, 7.4% for LaLg, and 7.0% for SLg. Neither distribution deviated from Hardy-Weinberg equilibrium.

Evidence of childhood maltreatment was distributed in the sample as follows: 70.8% were classified as having no maltreatment, 17.7% as having probable maltreatment, and 11.5% as having severe maltreatment. There were gender differences in childhood maltreatment distribution (χ^2=38.74, df=2, p<0.001), with higher levels of maltreatment exposure for females. There was no identifiable evidence of gene-environment correlation, as maltreatment frequency did not differ according to 5-HTTLPR genotype groups.

Gene-Environment Interaction

The assessment of potential confounders indicated that, with the exception of gender, which was included in the final model as a statistically significant covariate, inclusion of other covariates in the model (either individually or as a group) did not change the main results, showing that skin

FIGURE 1. Probability of a Diagnosis of a Depressive Episode at Age 18/19 for Each Childhood Maltreatment Exposure Group, by 5-HTTLPR Genotype, Adjusted for Gender[a]
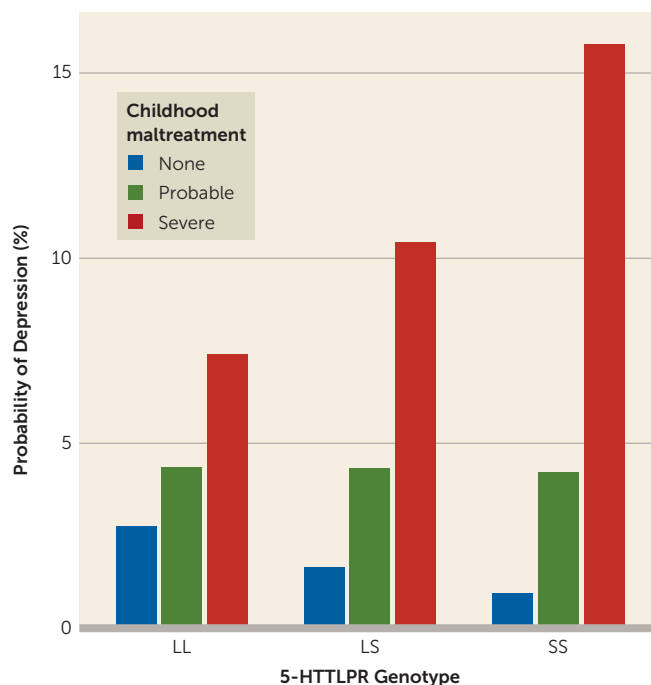
[a] The numbers of individuals for each of the three maltreatment groups (none, probable, severe), by genotype, are as follows: LL: 559, 133, 82; LS: 812, 207, 125; SS: 322, 84, 68. 5-HTTLPR=serotonin-transporter-linked polymorphic region; L=long allele; S=short allele.

color, maternal mental health, maternal education, and family income were not associated with the outcome in our sample.

Our final regression model showed that the association between depression and childhood maltreatment was moderated by 5-HTTLPR genotype (B=0.47, SE=0.19, Z=2.49, p=0.01), predicting depression in our cohort in the same direction as seen in the original study, and with a similar effect size (Table 2). The association between child maltreatment and youth depression was strongest among SS homozygotes (B=1.22, SE=0.30, Z=4.07, p<0.001), followed by SL heterozygotes (B=0.98, SE=0.21, Z=4.60, p<0.001) and LL homozygotes (B=0.50, SE=0.24, Z=2.11, p=0.03).

Figure 1 shows the probability of depression in each 5-HTTLPR genotype group for the three maltreatment exposure strata, adjusted for gender. As identified in the original study, although carriers of the small 5-HTTLPR allele with severe maltreatment exposure represented only 8% of the eligible sample, they accounted for more than 28% of the total cases of depression identified in our study. Among individuals with severe maltreatment, 17.6% of those with the SS genotype became depressed, whereas only 7.3% in the LL group did so.

Sensitivity Analyses

Selecting only individuals who self-identified as white for analysis, using the same final regression model, the interaction term remained associated with depressive episode, in the same direction as in the main analysis (B=0.54, SE=0.24,

Z=2.21, p=0.03). Analysis of all available individuals, regardless of early depression screening status, failed to show an interaction effect on depressive episode occurrence (B=0.21, SE=0.14, Z=1.51, p=0.13), reaffirming the importance of temporal order in G×E studies. Validity analysis using the 80th percentile as a cut-point showed results similar to the original screening index (B=0.35, SE=0.18, Z=1.99, p=0.04 for the interaction term).

Secondary Analyses

Extending the original study, we also assessed the tri-allelic model and the specificity in terms of outcome measure. Regrouping individuals with long 5-HTTLPR alleles according to presumed functionality of the a/g SNP resulted in an association for the interaction term that approached but fell short of significance (B=0.34, SE=0.19, Z=1.86, p=0.07). Examining the specificity of the model by substituting the outcome measure in the moderated regression framework revealed that the G×E was present only when major depression was the outcome, as shown in Table 2.

DISCUSSION

This study replicates the 2003 findings of Caspi et al. (12), reinforcing the role of the interaction between genetic and environmental influences in the etiology of youth major depression. We identified an interaction similar to that observed in the original study, using a categorical outcome within a multiplicative model. The results showed that the association between childhood maltreatment and depression was related to the number of S alleles carried, with progressively higher risk for individuals with more copies of the short variant.

These findings are relevant for several reasons. First, they strengthen and add to the body of evidence that individuals exposed to maltreatment are at an increased risk of developing a depressive disorder. Second, they support the original hypothesis that, given comparable levels of childhood maltreatment, the risk of depression varies according to 5-HTTLPR genotype. The cross-study inconsistency in the measurement of variables has been pointed out as one of the most important factors confounding replication attempts (15, 20, 22, 26, 42). As the quality of meta-analyses is limited by the quality of the studies reviewed, this has also led to difficult-to-interpret statistical summaries.

The low number of standard replications, in comparison to a multiplicity of "approximate replications," has been suggested to be one of the major weaknesses of current psychiatric research (43). In the present study, we adhered as closely as possible to the original design and methods (see Table 3), which is the most recommended strategy for G×E replication (13, 18, 42). Using this approach, we were able to identify a measured G×E, this time in a totally different sociocultural context, giving strength to the validity of the G×E concept in the etiology of depression. Importantly, however, because a previous study that followed a similar

TABLE 3. Comparison of the Original Study by Caspi et al. and the Present Study

Parameter	Caspi et al. 2003	This Study
Sample size	847	2,392
Design	Prospective cohort	Prospective birth cohort
Retention rate	96% at 26 years	81.3% at 18 years
Male	52%	45.7%
Ethnicity	100% Caucasian	69.7% white
Genotype assessment	Biallelic	Biallelic and triallelic
5-HTTLPR genotype distribution[a]		
SS	17%	19.8%
LS	51%	47.8%
LL	31%	32.4%
Environmental assessment	Five items (mother-child interaction observation, parent harsh discipline checklist, disruptive caregiver changes, retrospective assessment for physical and sexual abuse)	Seven retrospective questions (two on child neglect, three on emotional abuse, one each on physical and sexual abuse)
Childhood maltreatment exposure		
None	64.9%	70.8%
Probable	26.4%	17.7%
Severe	8.7%	11.5%
Outcome measurement	Dichotomous and quantitative	Dichotomous
Major depression prevalence	17% (12-month)	3.3% (point)
Temporal order assessment[b]	Yes	Yes
G×E specificity evaluation	No	Yes

[a] 5-HTTLPR=serotonin-transporter-linked polymorphic region; L=long allele; S=short allele.
[b] Between stress exposure and depression diagnosis.

strategy failed to replicate the original findings (17), it is not possible to entirely exclude the nonexistence of a true G×E effect. As the magnitude of the variance attributable to this specific G×E is at most small, literature syntheses aggregating comparable studies are required to further disentangle this issue (44).

The replication of the original finding of G×E in a different culture, especially in a middle-income country, reinforces the G×E construct and provides more robustness to the reproducibility of this evidence. Of note, 91% of the studies included in the above-mentioned meta-analyses were from high-income countries, which account for only 17.8% of world's population (45) and only 10% of people under age 18 (28).

Our methods and data analyses are consistent with the recent recommendations and updated understanding about G×E research. Currently, childhood maltreatment is considered the key risk factor for depression, with stressful life events probably representing a proxy marker of early maltreatment (42, 46). The additive genotypic model for 5-HTTLPR has been the most utilized strategy in G×E studies exploring this gene's moderation (18). We also evaluated the impact of substitution of this biallelic system for the triallelic model, which resulted in a p value of 0.07 for the interaction term.

The importance of timing in G×E research was also evidenced in our results. When the logistic regression including all 3,558 available participants was performed without taking into account the temporal relationship between the stressor and outcome, the model failed to show an effect of the interaction on depression. This result is consistent with the current literature (21, 24, 25, 27), which emphasizes the importance of the assessment of temporal order in G×E studies, suggesting a possible explanation for the mixed results in other replication attempts.

Our study is not without limitations. Given that the 1993 Pelotas Birth Cohort Study was not primarily a mental health study, we had psychiatric diagnoses according to DSM-IV-TR criteria available only in the age 18/19 assessment. On previous visits, mental health problems were evaluated with the Strengths and Difficulties Questionnaire, a screening instrument whose accuracy and predictive capability have undergone extensive evaluation, with good results in different cultures, including the one studied (34). However, for evaluation of temporality between stressor and outcome, we could not rely on DSM-based diagnoses to identify participants who might have had earlier depressive disorders. To reduce this limitation, we used a highly inclusive screening assessment for emotional problems, decreasing the chances of false negatives, despite the risk of a high false positive rate. As our exclusion rate reached 30% of the eligible sample, we decided to run another secondary analysis, using the dispersion of emotional scale scores. After application of these strategies, our exclusion rate dropped to 21.2%, maintaining the same main results previously found. Another potential limitation is the possible modification of the observed interaction of 5-HTTLPR and childhood maltreatment by other, unmeasured, genetic characteristics. Additionally, as our results were based on a limited number of depressed individuals, we cannot rule out an eventual unaddressed admixture in our sample or the instability of our analyses' results. Also, our findings were identified from a majority-white population, and our results may not be generalizable to populations with other ethnic backgrounds. Finally, although results from the city of Pelotas do not indicate population stratification in the region (47), we were not able to rule out this potential bias in our sample.

Our results show not only that S allele carriers are at risk for depression after experiencing childhood maltreatment, but also that early exposure to maltreatment significantly increases the risk for depression regardless of 5-HTTLPR genotype, a finding that differs from the original study. Indeed, a difference of

degree rather than an all-or-nothing phenomenon seems more plausible given the complexity of biological mechanisms. Importantly, the S allele conferred an increased risk for depression at age 18/19 only for those individuals who suffered maltreatment before age 15. For those who did not report any maltreatment, the presence of the S allele was associated with lower rates of depression. The interplay of 5-HTTLPR genotype and childhood maltreatment exhibited specificity for the development of major depression in our study, but not for the other psychiatric diagnoses tested, which is in accordance with previous findings (24, 48).

In conclusion, these results provide further evidence supporting the initial hypothesis of interaction between 5-HTTLPR polymorphisms and childhood maltreatment in the development of youth depression. Despite some differences in study methods, adopting the general principles of the original study proved to be indispensable to adequately assessing replicability—an aspect that was evident especially in regard to the timing of measurements. At a time when high hopes after initial studies are usually followed by discrepant results and skepticism, we believe that groundbreaking advancements in psychiatric knowledge will be contingent on further replication of findings across diverse settings.

AUTHOR AND ARTICLE INFORMATION

From the Department of Psychiatry, Hospital de Clinicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Brazil; the Department of Genetics, Universidade Federal do Rio Grande do Sul, Brazil; the Department of Basic Health Sciences. Universidade Federal de Ciências da Saúde de Porto Alegre; the Department of Psychiatry, Universidade de São Paulo, Brazil; the Center of Mathematics Computation and Cognition, Universidade Federal do ABC, Brazil; the Postgraduate Program in Epidemiology, Universidade Federal de Pelotas, Brazil; and the National Institute for Developmental Psychiatry for Children and Adolescents, Brazil

Address correspondence to Dr. Kieling (ckieling@ufrgs.br).

REFERENCES

1. McNutt M: Reproducibility. Science 2014; 343:229
2. Further confirmation needed. Nat Biotechnol 2012; 30:806
3. Russell JF: If a job is worth doing, it is worth doing twice. Nature 2013; 496:7
4. Wilson K, Atkinson K, Keelan J: The use of preliminary scientific evidence in public health: a case study of XMRV. PLoS Med 2014; 11:e1001623
5. Funder D: NSF gets an earful about replication. Funderstorms [blog], Feb 25, 2014. http://funderstorms.wordpress.com/2014/02/25/nsf-gets-an-earful-about-replication/
6. Begley CG, Ellis LM: Drug development: raise standards for pre-clinical cancer research. Nature 2012; 483:531–533
7. Ioannidis JP: Why most published research findings are false. PLoS Med 2005; 2:e124
8. Must try harder. Nature 2012; 483:509
9. Yong E: Replication studies: bad copy. Nature 2012; 485:298–300
10. Ritchie SJ, Wiseman R, French CC: Failing the future: three unsuccessful attempts to replicate Bem's "retroactive facilitation of recall" effect. PLoS ONE 2012; 7:e33423
11. Caspi A, McClay J, Moffitt TE, et al: Role of genotype in the cycle of violence in maltreated children. Science 2002; 297:851–854
12. Caspi A, Sugden K, Moffitt TE, et al: Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003; 301:386–389
13. Risch N, Herrell R, Lehner T, et al: Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA 2009; 301:2462–2471
14. Munafò MR, Flint J: Replication and heterogeneity in gene x environment interaction studies. Int J Neuropsychopharmacol 2009; 12:727–729
15. Duncan LE, Keller MC: A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Am J Psychiatry 2011; 168:1041–1049
16. Zammit S, Owen MJ, Lewis G: Misconceptions about gene-environment interactions in psychiatry. Evid Based Ment Health 2010; 13:65–68
17. Fergusson DM, Horwood LJ, Miller AL, et al: Life stress, 5-HTTLPR, and mental disorder: findings from a 30-year longitudinal study. Br J Psychiatry 2011; 198:129–135
18. Munafò MR, Durrant C, Lewis G, et al: Gene x environment interactions at the serotonin transporter locus. Biol Psychiatry 2009; 65:211–219
19. Uher R, McGuffin P: The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. Mol Psychiatry 2010; 15:18–22
20. Karg K, Burmeister M, Shedden K, et al: The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Arch Gen Psychiatry 2011; 68:444–454
21. Kraemer HC: Determining gene moderation of environmental risk factors for a mental disorder: a "perfect storm" of methodological problems. Int J Methods Psychiatr Res 2012; 21:185–194
22. Caspi A, Hariri AR, Holmes A, et al: Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am J Psychiatry 2010; 167:509–527
23. Moffitt TE, Caspi A, Rutter M: Strategy for investigating interactions between measured genes and measured environments. Arch Gen Psychiatry 2005; 62:473–481
24. Uher R, McGuffin P: The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Mol Psychiatry 2008; 13:131–146

25. Dunn EC, Uddin M, Subramanian SV, et al: Research review: gene-environment interaction research in youth depression: a systematic review with recommendations for future research. J Child Psychol Psychiatry 2011; 52:1223–1238

26. Culverhouse RC, Bowes L, Breslau N, et al: Protocol for a collaborative meta-analysis of 5-HTTLPR, stress, and depression. BMC Psychiatry 2013; 13:304

27. Moffitt TE, Caspi A: Bias in a protocol for a meta-analysis of 5-HTTLPR, stress, and depression. BMC Psychiatry 2014; 14:179

28. Kieling C, Baker-Henningham H, Belfer M, et al: Child and adolescent mental health worldwide: evidence for action. Lancet 2011; 378:1515–1525

29. Hu XZ, Lipsky RH, Zhu G, et al: Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet 2006; 78:815–826

30. Victora CG, Araújo CL, Menezes AM, et al: Methodological aspects of the 1993 Pelotas (Brazil) Birth Cohort Study. Rev Saude Publica 2006; 40:39–46

31. Victora CG, Hallal PC, Araújo CL, et al: Cohort profile: the 1993 Pelotas (Brazil) Birth Cohort Study. Int J Epidemiol 2008; 37:704–709

32. Gonçalves H, Assunção MC, Wehrmeister FC, et al: Cohort profile update: the 1993 Pelotas (Brazil) Birth Cohort follow-up visits in adolescence. Int J Epidemiol 2014; 43:1082–1088

33. Goodman R: The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry 1997; 38:581–586

34. Fleitlich-Bilyk B, Goodman R: Prevalence of child and adolescent psychiatric disorders in southeast Brazil. J Am Acad Child Adolesc Psychiatry 2004; 43:727–734

35. Mari JJ, Williams P: A validity study of a psychiatric screening questionnaire (SRQ-20) in primary care in the city of Sao Paulo. Br J Psychiatry 1986; 148:23–26

36. Kieling C, Hutz MH, Genro JP, et al: Gene-environment interaction in externalizing problems among adolescents: evidence from the Pelotas 1993 Birth Cohort Study. J Child Psychol Psychiatry 2013; 54:298–304

37. Lahiri DK, Nurnberger JI Jr: A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; 19:5444

38. Ito K, Yoshida K, Sato K, et al: A variable number of tandem repeats in the serotonin transporter gene does not affect the antidepressant response to fluvoxamine. Psychiatry Res 2002; 111:235–239

39. Heils A, Teufel A, Petri S, et al: Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66:2621–2624

40. van Ijzendoorn MH, Belsky J, Bakermans-Kranenburg MJ: Serotonin transporter genotype 5HTTLPR as a marker of differential susceptibility? A meta-analysis of child and adolescent gene-by-environment studies. Transl Psychiatr 2012; 2:e147

41. Amorim P: Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr 2000; 22:106–115

42. Uher R, Caspi A, Houts R, et al: Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: replications and implications for resolving inconsistent results. J Affect Disord 2011; 135:56–65

43. Kapur S, Phillips AG, Insel TR: Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it? Mol Psychiatry 2012; 17:1174–1179

44. Button KS, Ioannidis JP, Mokrysz C, et al: Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013; 14:365–376

45. United Nations, Department of Economic and Social Affairs, Population Division: World Population Prospects: The 2010 Revision, volume II, Demographic Profiles. New York, United Nations, 2011. http://esa.un.org/wpp/Documentation/pdf/WPP2010_Volume-II_Demographic-Profiles.pdf

46. Brown GW, Harris TO: Depression and the serotonin transporter 5-HTTLPR polymorphism: a review and a hypothesis concerning gene-environment interaction. J Affect Disord 2008; 111:1–12

47. Lima-Costa MF, Rodrigues LC, Barreto ML, et al: Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). Sci Rep 2015; 5:9812

48. Kendler KS, Kuhn JW, Vittum J, et al: The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Arch Gen Psychiatry 2005; 62:529–535