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ORIGINAL ARTICLE

Lack of association between the 5-HTTLPR and positive screening for mental disorders among children exposed to urban violence and maltreatment

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Objective: To ascertain whether genetic variations in the serotonin transporter gene (5-HTTLPR 44-bp insertion/deletion polymorphism) influence an increase in depressive and anxiety symptoms in children and adolescents exposed to high levels of violence.
Methods: Saliva samples were collected from a group of children who were working on the streets and from their siblings who did not work on the streets. DNA was extracted from the saliva samples and analyzed for 5-HTTLPR polymorphism genotypes.
Results: One hundred and seventy-seven children between the ages of 7 and 14 years were analyzed (114 child workers and 63 siblings). Data on socioeconomic conditions, mental symptoms, and presence and severity of maltreatment and urban violence were collected using a socio-demographic inventory and clinical instruments. There was no positive correlation between the 5-HTTLPR polymorphism and presence of mental symptoms in our sample, although the children were exposed to high levels of abuse, neglect, and urban violence.
Conclusions: Despite previous studies that associated adult psychiatric disorders with the 5-HTTLPR polymorphism and a history of childhood maltreatment, no such association was found in this sample of children at risk.

Keywords: Serotonin transporter polymorphism; child maltreatment; child labor; street children

Introduction

The serotonin transporter (5-HTT or SERT) and its genetic variations have been associated with depression and other mental disorders.[1] In 2003, Caspi et al.[2] found a significant interaction between the serotonin transporter-linked polymorphic region (5-HTTLPR) and both stressful life events and childhood maltreatment with regard to the development of depression during adulthood. The human 5-HTT gene, *SLC6A4*, is located on chromosome 17q11.1-q12 and presents a 44-bp insertion/deletion polymorphism within the promoter region (5-HTTLPR), with two classical allelic forms, the long (*l*) variant and the short (*s*) variant, which has lower transcriptional activity than the *l* allele.[3] Several studies have confirmed that the short allele variant is a risk factor for developing mood (especially depression) and anxiety disorders.[4-7] Subjects homozygous for the *s* variant of the serotonin transporter gene presented a higher risk of developing depression after exposure to early traumatic events,[2-4,8,9] which suggests a significant gene-environment interaction. A recent meta-analysis of 54 studies reported strong evidence that 5-HTTLPR moderates the relationship between stress and depression and that the *s* allele is associated with an increased risk for developing depression under stress.[10] However, two previous systematic reviews failed to find consistent and replicable gene-environment effects.[11-12]

Child abuse and neglect are risk factors for various psychiatric disorders. A strong link has been identified between childhood trauma and mood and anxiety disorders, including unipolar and bipolar depression.[6,13-15] Preclinical studies found that early stress affects neurodevelopment through long-term alterations in neurochemical systems, including changes in the serotonergic system and increase in circulating glucocorticoids, which is a biological vulnerability for depression development.[16] Clinical data suggest that these alterations, which are associated with early stress, are not irreducible and may be modulated by genetic and epigenetic factors.[17]

Children and adolescents are in their pruning period, in which personality and emotional abilities are developing and the brain is vulnerable to adverse events, such as the presence of violence in their environment.[18-20] Behavioral, cognitive, and emotional problems in children

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and adolescents tend to be multifactorial and encompass genetic and epigenetic features.

The objective of this study was to evaluate the putative relationship between 5-HTTPR and the development of internalizing and externalizing psychopathologic symptoms in a sample of children working on the streets as compared with their siblings who did not work on the streets.

Our hypothesis was that street-working children (children who spend the majority of the day on the street, often selling candy, wiping windshields, and begging, and who return home at the end of the day) would be subjected to a greater number of stressors, which could be considered an environmental risk factor to predict the development of psychopathologic symptoms as compared with their siblings who stayed at home. Moreover, we hypothesized that children bearing the *s/s* genotype of 5-HTTLPR would be more susceptible to an increase of psychopathological symptoms and the interaction gene (bearer of *s* allele) and environment would contribute to the risk of development of psychopathological symptoms.

Methods

Subjects

The sample consisted of children who performed street work in the West Zone of the city of São Paulo, Brazil, who were identified and recruited by social workers from a street work eradication program promoted by the municipal government in partnership with the nongovernmental organization (NGO) RUKHA ("breath of life" in Aramaic).

The NGO selected the West region of the city as the focus of intervention because this area has the highest concentration of street-working children in São Paulo. Although these children work in the West Zone of São Paulo, they live in the South Zone of the city, which is characterized by poverty and extreme violence.

The NGO project aims to remove the children from the streets and encourage them to attend school and recreation centers throughout the day, as well as to educate and teach professional behavior and support the children's caregivers to find paid employment; to evaluate and improve the families' living conditions, hygiene, and nutritional standards; and to provide medical and psychological care for the children and their caregivers.

The present sample is a subset of a larger study that sought to evaluate cortisol responses in children exposed to violence within a psychosocial intervention program.

After initial contact and obtaining informed consent from the children's caregivers and after the study was approved by the Ethics Committee of Universidade Federal de São Paulo (process number 0550/08), the children who were originally selected on the streets and their siblings were included in the study.

The inclusion criteria were as follows: age 7 to 14 years, absence of illnesses or drug use that could interfere with the measurement of salivary cortisol levels, and absence of mental retardation and neurological disorders.

Of 211 children who were approved to participate in the psychosocial intervention program, 191 met the criteria for inclusion in the cortisol response study. Fourteen children were excluded and did not participate in the gene-environment analysis: seven who did not collect saliva samples and seven who did not complete the necessary measures for the cortisol response study. Therefore, a total of 177 children were included in the gene-environment analysis (114 street-working children and 63 siblings).

Clinical data

All of the subjects were evaluated using epidemiological and psychometric scales to collect data concerning socioeconomic conditions, quality of life, presence of emotional symptoms, maltreatment, and urban violence, and presence of mental symptoms.

The interviewers were 11 educators from the NGO staff who were selected and trained to administer the questionnaires. Each interviewer was responsible for collecting the data from a number of residences in which the children who were eligible for the study lived. Except for the WorldSAFE, all instruments were self-reported; the chosen instruments had been previously used and validated for Brazilian samples by other researchers with similar populations.[21-24]

Sociodemographic inventory

A sociodemographic data questionnaire was developed for the present study to collect data on age, sex, educational level, skin color, and religion of the children. Because mixed races comprise a large portion of the Brazilian population, defining people by ethnic group can be difficult. Therefore, the sample was divided into three groups according to participant skin color: white, brown, and black.[25]

Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a self-report instrument completed by the caregivers. However, if the caregiver could not read or write, the interviewers would read the questions to them and precisely record their responses, without any interpretation. This brief questionnaire is a useful measure in psychopathology that is administered to children and young people aged 4 to 16 years. The SDQ is divided into five subscales: emotional symptoms, conduct problems, hyperactivity, relationship problems, and pro-social scale.[21] Scores $\geqslant$ 14 indicate a positive screening, and the interview will be considered valid if at least 12 of the 20 relevant items were completed. In high-risk samples, individuals can be identified as positive even when scores are borderline.[26]

WorldSAFE Core Questionnaire

The WorldSAFE-CQ is a standardized instrument that investigates intrafamilial violence and associated factors; the Brazilian version was based on a consensus among WorldSAFE steering committee members, including behaviors frequently observed in countries such as Brazil, Chile, Egypt, India, the Philippines, and the United States. The original English questionnaire was

developed by the WorldSAFE Steering Committee and copyrighted in 1998.[19] The educators were trained to administer this instrument, and they recorded the answers according to the caregivers' responses; the training for data collection included discussions with two of the investigators (IAB and CSS) to standardize the way in which researchers should collect the information.[22] However, no reliability test was conducted for the present study. The WorldSAFE-CQ includes 31 items that represent different child-rearing behaviors from the child's mother and her husband or partner in the last 12 months. The items were partially derived from the Parent-Child Conflict Tactics Scales with permission from the authors and included parental behaviors usually noted in developing countries. The most important violence measure for this particular inventory is "'severe physical punishment" towards the child, which includes being kicked, choked (putting hand or another object around the neck), smothered (with hand or pillow), burned, beaten repeatedly (with objects or fist), struck on buttocks or other parts of the body (with an object), or threatened with a gun or knife.[22]

Childhood Trauma Questionnaire (CTQ)

The CTQ is a self-report instrument, i.e., it is answered by the subject (child or adolescent); the same procedure as for the SDQ was adopted when respondents needed help to complete the questionnaire. The CTQ was validated for the Brazilian social and cultural context to be applied to children older than 12 years[23]; a second version was adapted for administration to children between the ages of 7 and 11 years.[24] The CTQ has a short-form 28-item questionnaire comprising five subscales of five items each.[27] The cutoff points were developed to split samples between suffering abuse and not suffering abuse for each subscale.[28] The CTQ measures the following: a) emotional abuse; b) physical abuse; c) sexual abuse; d) emotional neglect; and e) physical neglect.

Urban Violence Self-Report Questionnaire

This questionnaire was designed for the present study to collect information about exposure to violence or violent situations on the streets. Based on the 1992 version of the Richters and Martinez instrument, the questionnaire includes 17 items that probe children's exposure to community violence.[29] A pilot study was conducted with 16 children aged 7 to 14 years from the same living area as the children who were included in the present study. However, no reliability test was performed for the present investigation; the data were considered to be a simple description of violent events reported by the children and were not included in the multiple logistic regression model.

DNA samples and extraction

All eligible subjects who agreed to participate in the study had their saliva collected for DNA extraction and analysis. A 2 mL of saliva was passively collected from each subject into an appropriate container (Oragene™, DA Genoteck Inc., Canada) following the manufacturer's instructions. The Oragene™ container allows preservation of saliva for 2 weeks at room temperature, and DNA can be extracted in the same proportions as those obtained in whole blood.

DNA extraction followed the procedures recommended by the Oragene™ kit manufacturer. The samples were collected with the Oragene* DNA self-collection kits and incubated at 50°C according to the instructions of the Oragene* DNA manual purification protocol.

Genotyping

Each sample was diluted to a working concentration of 100 ng/μL. The previously described primer sequences[3-6,8-10,13] HTTLPR-F (5′-tgaatgccagcacctaaccc-3′) and HTTLPR-R (5′-ttctggtgccacctagacgc-3′) were used. The polymerase chain reaction products were amplified in 0.2-mL microtubes containing 1 μL of human genomic DNA, 1 μL of each forward and reverse primer, 1 μ of dNTP Mix (Invitrogen, Brazil), 2.4 μL MgCl, 0.5 μL DMSO, 2.5 μL Taq buffer, and 0.1 μL TaqPolimerase (Applied Biosystems, Brazil) to a final volume of 25 μL for each reaction. The polymerase chain reaction was conducted using a DNA Engine CG 1-96 thermal cycler (Corbett Research, Qiagen). Cycling conditions were as

Table 1 Sociodemographic data from children (n=177)

	n (%)
Sex	
Female	82 (46.3)
Male	95 (53.7)
Total	177 (100.0)
Age	10.5±2.1
Skin color	
White	37 (20.9)
Brown	101 (57.1)
Black	34 (19.2)
Total available	172 (97.2)
Missing	5 (2.8)
Total	177 (100.0)
Religion	
Catholicism	90 (50.8)
Spiritualism	1 (0.6)
Protestant	57 (32.2)
Atheist	3 (1.7)
Others	16 (9.0)
Total available	167 (94.4)
Missing	10 (5.6)
Total	177 (100.0)
Attending school	
No	18 (10.2)
Yes	156 (88.1)
Total available	174 (98.3)
Missing	3 (1.7)
Total	177 (100.0)
Working on the streets	
Yes	114 (60.0)
No	63 (40.0)
Missing	0 (0.0)
Total	177 (100.0)
Income (US$)	194±137

Data presented as n (%) or mean ± standard deviation.

follows: 5 min initial denaturing at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C, with a final extension of 10 minutes at 72°C. HTTLPR long allele (insertion) of 528 base pair (bp) and short allele (deletion) of 484 bp were resolved in 1.5% agarose gels[30-32] and visualized with GelRed pigment (1 : 10.000 – 3 μL).

Genotyping was performed in a blind manner, using proper masked identification, and gels were analyzed by a different examiner blinded to clinical data. In cases where the separation of alleles was not clear, duplicates were performed as recommended in quality control procedure manuals.[30-32]

Statistical analysis

The chi-square test was used to compare the numeric variables, to verify Hardy-Weinberg equilibrium (HWE) between groups, and to compare allele and genotype frequencies. The SDQ scores were used to correlate psychopathologic symptoms with 5-HTTLPR polymorphism variations and the different types of violence and abuse/neglect suffered by the subjects as measured by the scales.

To assess the potential influence of 5-HTTLPR on SDQ scores as well as the effect of the confounders, a multiple logistic regression model with mixed effects was used. The sex, skin color, severe physical punishment reported by the caregivers, emotional abuse, physical abuse, sexual abuse, physical negligence, and emotional negligence variables were included in the model as fixed effects, and dependence between children in the same family was included in the model as a random effect. The variables included in the stepwise regression model were chosen because they are factors previously reported as predictors of the development of mental symptoms in adults with a history of childhood maltreatment with genetic vulnerability.[1,2,4-6,9,10,13,14,16,26,29,33] The stepwise model includes all of the variables that could simultaneously influence the dependent variable, not considering previous single associations. The power of the model was calculated to be 0.98. The result of the stepwise model was reevaluated to avoid multiple corrections. Analyses were conducted using the R statistical program, adopting a significance level of 0.05.[34]

The power of analysis was calculated using the online Genetic Power Calculator software. With the parameter settings used (high risk allele frequency = 0.42; prevalence of X = 0.25; genotype SL relative risk = 0.66; genotype SS relative risk = 0.97; control: case ratio = 0.6), the power of the analysis was 7.7%. SL genotype and SS genotype risk calculated on the basis of the study data bank, using logistic regression.

Results

Sociodemographic data

Sociodemographic data from the children are shown in Table 1. Among the 177 children included in the analysis of interaction between gene and environment, the majority of the children were male and attending school.

Table 2 Sociodemographic data from children working (n=114) and not working (n=63) on the streets (n=177)

	n (%)	p-value
Sex		0.38
Working		
Female	50 (43.9)	
Male	64 (56.1)	
Total	114 (100.0)	
Not working		
Female	32 (50.8)	
Male	31 (49.2)	
Total	63 (100.0)	
Age		< 0.01
Working	10.9±2.1	
Not working	9.8±2.0	
Skin color		0.02
Working		
White	19 (16.7)	
Brown	65 (57.0)	
Black	28 (24.6)	
Total available	112 (98.2)	
Missing	2 (1.8)	
Total	114 (100.0)	
Not working		
White	18 (28.6)	
Brown	36 (57.1)	
Black	6 (9.5)	
Total available	60 (95.2)	
Missing	3 (4.8)	
Total	63 (100.0)	
Religion		0.53
Working		
Catholicism	56 (49.1)	
Spiritualism	0 (0.0)	
Protestant	39 (34.2)	
Atheist	2 (1.8)	
Others	12 (10.5)	
Total available	109 (95.6)	
Missing	5 (4.4)	
Total	114 (100.0)	
Not working		
Catholicism	34 (54.0)	
Spiritualism	1 (1.6)	
Protestant	18 (28.6)	
Atheist	1 (1.6)	
Others	4 (6.3)	
Total available	58 (92.1)	
Missing	5 (7.9)	
Total	63 (100.0)	
Attending school		0.87
Working		
Yes	101 (88.6)	
No	12 (10.5)	
Total available	113 (99.1)	
Missing	1 (0.9)	
Total	114 (100.0)	
Not working		
Yes	55 (87.3)	
No	6 (9.5)	
Total available	61 (96.8)	
Missing	2 (3.2)	
Total	63 (100.0)	
Income (US$)		0.07
Working	205±147	
Not working	197±138	

Data presented as n (%) or mean ± standard deviation.

Table 3 Childhood Trauma Questionnaire (CTQ) scores from all subjects included in the sample

				Valid percent	
	Cutoff*	Yes, n (%)	No, n (%)	% yes	% no
Emotional abuse n=168 (9 missing)	⩾ 8	57 (32.2)	111 (62.7)	33.9	66.1
Physical abuse n=168 (9 missing)	⩾ 7	59 (33.3)	109 (61.6)	35.1	64.9
Sexual abuse n=160 (17 missing)	⩾ 5	27 (15.3)	133 (75.1)	16.9	83.1
Emotional neglect n=166 (11 missing)	⩾ 9	82 (46.3)	84 (47.5)	49.4	50.6
Physical neglect n=165 (12 missing)	⩾ 7	82 (46.3)	83 (46.9)	49.7	50.3

* Based on Paivio & Cramer.[28] Scores range from 5 to 25. The cutoff points discriminate between not suffering and suffering abuse/neglect.

Catholicism is the predominant religion in the country and was reported by 50% of the sample population, followed by Evangelical Christianity. The majority of the children included in the study were brown.

A total of 114 children and adolescents were involved in some form of paid work on the streets, such as selling candy at traffic lights, juggling, or begging. The epidemiologic parameters of the children who worked on the streets in our sample did not differ from their siblings who did not work on the streets, as shown in Table 2. The only significant difference found between the two groups was that more black children were working on the streets.

Exposure to violence

The children included in the project came from the outskirts of the city of São Paulo, with low income, social, and cultural conditions, and lived in settings in which violence is epidemic in the community and the family environment. High levels of abuse and neglect were reported by all of the individuals who completed the CTQ (Table 3). No statistically significant difference in exposure to abuse or neglect was found between the children who were working on the streets and their siblings (Table 4). Boys reported more sexual abuse (28.2% of boys vs. 11.3% of girls; $p = 0.5$).

Results from the WorldSAFE-CQ showed that 105 (63.2%) children had suffered severe physical punishment at home, as reported by their caregivers. The children on the streets had suffered more severe physical punishment (70.5%) than their siblings who remained at home (50.8%) ($\chi^2 = 6.41$; $p = 0.01$) (Table 5).

The results related to urban violence did not show any difference between the children who worked on the streets and those who did not work on the streets. In fact, all of the children in the sample were exposed to a high degree of urban violence.

Psychopathological data

Children working on the streets exhibited more mental symptoms (SDQ+) (67.5%) compared with those who remained at home (52.4%) ($\chi^2 = 3.97$; df = 1; $p < 0.05$). Evaluating the total sample, an extremely high rate of positive screening for mental disorders (66.1%) was found compared with population-based data from Latin America, where rates for mental symptoms range from 8.3 to 35.2%.[19]

Genetic data

There were no between-group differences in allele frequencies for the 5-HTTLPR polymorphism. Both groups were in HWE (Table 6).

To evaluate potential associations between the 5-HTTLPR polymorphism, punishment, and abuse/neglect measures between children who were and were not working on the streets and positive screening for mental symptoms, a mixed-effects multiple logistic regression model was used. SDQ score was considered to be the dependent variable. Genotype, working status, CTQ results, severe physical punishment, sex, and skin color entered the model as independent variables.

Among all of the variables tested, only the presence of severe physical punishment was significantly associated with mental symptoms. Working on the streets and the presence of severe physical punishment correlated significantly with the presence of mental symptoms ($p < 0.01$); however, when analyzed together, the variable working or not working on the streets lost its significance

Table 4 Childhood Trauma Questionnaire (CTQ) values comparing scores between children working or not working on the streets

CTQ subscales	Cutoff point*	Working, n (%)	Not working, n (%)	Total	p-value
Emotional abuse	⩾ 8	35/108 (32.4)	22/38 (28.2)	57/168	0.57
Physical abuse	⩾ 7	38/108 (35.2)	21/60 (35.0)	59/168	0.98
Sexual abuse	⩾ 5	13/100 (13.0)	14/60 (23.3)	27/160	0.09
Emotional neglect	⩾ 9	52/107 (48.5)	30/59 (50.8)	82/166	0.78
Physical neglect	⩾ 7	53/105 (50.4)	26/60 (43.3)	82/165	0.79

* Based on Paivio & Cramer.[28] Scores range from 5 to 25. The cutoff points discriminate between not suffering and suffering abuse/neglect.

Table 5 Results from WorldSAFE Core Questionnaire - Severe Physical Punishment measure for the total sample and stratified by children working or not working on the streets

Severe physical punishment	Yes, n (%)	No, n (%)	Missing, n (%)
Total sample	105 (59.3)	61 (34.5)	11 (6.2)
			p-value
Working on the streets	74/105 (70.5)	31/105 (29.5)	0.01
Not working	31/61 (50.8)	30/61 (49.2)	

(p = 0.29), whereas presence of severe physical punishment remained with the same degree of statistical significance ($p < 0.01$), which indicates that this variable contributes, in large part, toward the outcome.

No association among the different 5-HTTLPR polymorphisms and the presence of mental symptoms was found in this population.

Discussion

The present study could not demonstrate an association between mental symptoms, early-life stressful events, and 5-HTTLPR polymorphisms in the children studied. Although many studies have demonstrated[33,35-37] that adversity in childhood and adulthood is associated with PTSD, major depressive disorder, and other anxiety disorders, some of them failed to show any association with the 5-HTTLPR genotype.[38]

Reinforcing that there is no consensus, several studies rejected a gene-environment interaction between 5-HTTLPR and early life stress, as seen in a 30-year longitudinal study that found no evidence to support the likelihood that the *s* allele increases responsiveness to stress.[39]

A recent meta-analysis that demonstrated conflicting results, published by Munafo et al.,[11] showed that the presence of domestic violence was a strong moderator for increased psychiatric symptoms in this population. Although no relationship with the genetic vulnerability conferred by the presence of the short 5-HTTLPR allele was found in this study, the intensity of the violence factor may have contributed to the lack of association, which indicates that genetic influences may lose importance when environmental factors cause an extremely high burden of trauma to the individual, as observed by Kolassa.[40]

Few studies have evaluated the relationship between early stresses (violence), 5-HTTLPR polymorphism, and the presence of psychopathological symptoms (internalizing and externalizing) in children; the majority of studies have focused on studying the relationship among presence of depression in adulthood, exposure to stressor factors throughout life, and genotype. Kaufman et al.[16,41] are among the few authors who derived their findings from a sample of children and assessed the presence of 5-HTTLPR polymorphism and early stress and the quality and availability of social support as conditioners for an increase in depressive symptoms; they found that the quality and availability of social support could confer resilience to an increase in depression, even in individuals who presented a genotype for vulnerability. Similar data were found in our study, with the intensity and frequency of violence factors correlating more strongly with the risk of psychiatric symptoms than the genotype itself.

We expected that the greatest violence factor for the children would be urban violence, which is typical of the outskirts of the city of São Paulo, where communities are dominated by the drug trade. Additionally, the children who worked on the streets were exposed to several violent situations without any form of protection. However, contrary to our expectations, exposure to violence in the family environment was the major violence factor that was associated with increased risk of psychiatric disorders. The violence perpetrated by their caregivers had a devastating impact on the mental health of these children and adolescents.

Many of the caregivers did not perceive their conduct as violent, but rather as educational. This view of corporal punishment as education seems to recur and be a part of the cultural context of the community. When violence spreads from the home to society, there is a tendency toward perpetuation of this cycle.[22,42,43]

Although poverty and violence are present in many Brazilian communities, the community in which this study was conducted is especially characterized by extreme violence, which may have biased the results of gene-environment interaction.

Table 6 Genetic and allelic distribution for the 5-HTTLPR polymorphism

5HTTLPR	Normal, frequency (%)	Borderline/abnormal, frequency (%)	Total, frequency (%)	p-value
LL	19 (28.4)	39 (35.5)	58 (32.8)	0.40903*
SL	38 (56.7)	51 (46.4)	89 (50.3)	0.71174†
SS	10 (14.9)	20 (18.2)	30 (16.9)	
Total	67 (100.0)	110 (100.0)	177 (100.0)	
Hardy-Weinberg	0.20403	0.64287		

* Chi-square test for genotypes.
† Chi-square test for alleles.

Working on the street was hypothesized to be an environmental risk factor for the development of psychopathologic symptoms (unshared environment). The siblings who stayed at home represented our control group. Most genetic association studies for complex diseases are based on a case-control design; several association studies support the hypothesis that genetic variants can modify the influence of environmental factors on behavioral outcomes, i.e., a gene-environment interaction.[4,37]

The present study design has limitations that may have impacted its negative results. First, the sample size was too small, which may contribute to lack of association. Furthermore, analyzing children during their development at one only point in time does not necessarily consider the influence of the environment prospectively, and analysis of mental symptoms with the SDQ precludes assessment of potential associations between the polymorphism and diagnosed mental disorders, as was reported in the majority of studies published in the field. Moreover, we used a convenience sampling strategy, i.e., the subjects were not randomly selected; they were recruited from a population that was identified to participate in a controlled environmental-enrichment project.

The lack of reliability evaluation of the WORLDSafe instrument is another flaw of the present study, because even with proper training on the use of the instrument, different interviewers may have collected information in an inconsistent manner, and this was not tested.

The choice of sibling-pair design can be controversial, although the use of unrelated controls to assess genetic and gene/environment effects has been called into question because of the potential problem of population stratification.[44,45] The sib-pair design, however, is free of the potential effects of population stratification, because sibling pairs are drawn from the same family and thus from the same genetic stratum.[46] The sib-pair and related small-family study designs have several attractive characteristics for research into complex diseases that result from interactions among loci or from both genetic and environmental effects. Although previous reports[47,48] have shown that use of population-based controls is more efficient in detecting a primary genetic effect than using cousins or sibling controls, with sibling controls being the least efficient, the sibling controls were the most efficient for detecting a gene-environment interaction effect.

As the present sample was not tested regarding genetic ancestry, its genetic structure could be very heterogeneous, even though the children were drawn from the same community and siblings were included. This might jeopardize our results, but the lack of association cannot be attributed to population stratification, as the absence of differences between the groups may be due to a similar genetic background or to between-group similarities in allele frequency regarding the polymorphism studied. On the other hand, if we had found differences in allele frequency, these could be due to unchecked population stratification.

Further studies with high-risk populations and unrelated control groups are required to better understand the gene-environment interaction in these specific groups.

In conclusion, our study failed to confirm the previously reported association between 5-HTTLPR polymorphism and psychiatric disorders in a group of children under severe stress conditions. However, our findings were consistent with those from studies that also failed to find a significant association between this polymorphism and psychiatric conditions.[11,12]

The lack of association between the 5-HTT gene polymorphism and the presence of psychiatric vulnerability under severe stress exposure could be due to factors such as the presence or absence of social support, which strongly influences the phenotype of children exposed to chronic stress conditions despite their genotype, as noted by Kolassa.[40]

We found that early exposure to high levels of violence led to a higher level of psychopathologic symptoms, as expected. Violence perpetrated by caregivers had a high impact in our sample. High exposure to a violent domestic environment appears to be an important risk factor for the emergence of psychopathological symptoms, and could overlap a genetic predisposition possibly conferred by the 5-HTTLPR polymorphism.

In short, we were unable to confirm the hypothesis that 5-HTTLPR polymorphisms are associated with mental disorders. Moreover, methodological factors (i.e., small sample size) may have led to rejection of the true hypothesis (type II error).

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Disclosure

GC is a scientific adviser for Libbs Farmacêutica Ltda. The other authors report no conflicts of interest.

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