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Post-traumatic stress symptoms of children and adolescents exposed to the 2008 Wenchuan Earthquake: A longitudinal study of 5-HTTLPR genotype main effects and gene–environment interactions

Gen Li[1,2], Li Wang[1,2], Chengqi Cao[1,3], Ruojiao Fang[1,2], Brian J. Hall[4,5], Jon D. Elhai[6,7], and Israel Liberzon[8]

[1]Laboratory for Traumatic Stress Studies, CAS Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China
[2]Department of Psychology, University of Chinese Academy of Sciences, Beijing, China
[3]Shenzhen Key Laboratory of Affective and Social Cognitive Science, Shenzhen University, Shenzhen, China
[4]Global and Community Mental Health Research Group, Department of Psychology, Faculty of Social Sciences, University of Macau, Macau SAR, China
[5]Department of Health, Behavior and Society, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[6]Department of Psychology, University of Toledo, Toledo, OH, USA
[7]Department of Psychiatry, University of Toledo, Toledo, OH, USA
[8]Department of Psychiatry, Texas A&M College of Medicine, College Station, TX, USA

Experiencing disasters causes severe mental disorders, among which post-traumatic stress disorder (PTSD) is the most common. We conducted a longitudinal study to examine the effect of 5-hydroxyl tryptamine transporter gene-linked polymorphic region (5-HTTLPR) genotype on child and adolescent PTSD symptom course after the 2008 Wenchuan Earthquake. We genotyped 963 participants who personally experienced the earthquake. PTSD symptoms were measured by University of California, Los Angeles PTSD reaction index at 2.5, 3.5, 4.5 and 5.5 years after the earthquake, respectively. Latent growth model was utilised to examine the main effect and gene–environment interaction effect of 5-HTTLPR on PTSD's symptom course. 5-HTTLPR genotype predicted initial PTSD symptom severity ($\beta = 0.108$, $p = .019$) and rates of symptom recovery ($\beta = -0.120$, $p = .031$) between 2.5 and 5.5 years. Compared with L′ allele carriers, those with S′S′ genotype showed higher initial symptom severity but also faster recovery rate. 5-HTTLPR genotype only predicted symptom severity at 2.5 years after the earthquake, after controlling for sex, age, ethnicity and trauma severity ($\beta = 0.108$, $p = .019$). This is the first evidence of the effect of 5-HTTLPR genotype on child and adolescent PTSD symptoms longitudinally, offering a novel perspective on the effect of 5-HTTLPR on PTSD symptom development following trauma exposure.

Keywords: 5-Hydroxyl tryptamine transporter gene-linked polymorphic region; Post-traumatic stress disorder; Symptom course; Disaster; Children and adolescents.

Correspondence should be addressed to Li Wang, Institute of Psychology, Chinese Academy of Sciences, 16 Lincui Road, Beijing 100101, China. (E-mail: wangli1@psych.ac.cn).

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Disasters have always been a common occurrence. According to the recent World Mental Health Survey, around the world, experiencing man-made disasters has a prevalence rate of 4.0%, while experiencing natural disasters has an even higher prevalence rate of 7.7% (Benjet et al., 2016). China, as a developing country with a large land area and population, has been severely affected by various disasters, especially natural disasters. Thus, further attention is needed on negative consequences of natural disasters. Experiencing natural disasters can cause mental health problems such as anxiety, depression or post-traumatic stress disorders (PTSDs), among children and adolescents. PTSD is the most common type of psychopathology reported in disaster survivors, with the prevalence of diagnosable PTSD in children ranging from 30% to 60% (Yule, 2001).

Genetic factors may shape children's reactions to disasters, including PTSD. Recently, a twin study suggested that the heritability of PTSD in some populations might be as high as 49% (Wolf et al., 2017). In search of genetic vulnerability factors for developing PTSD after trauma exposure, many candidate genes have been suggested, among which, the human serotonin transporter (5- hydroxyl tryptamine transporter, 5-HTT) gene, also known as *SLC6A4*, is the most studied to date (Zhang et al., 2017), with the 5-HTT gene-linked polymorphic region (5-HTTLPR) as a key loci. 5-HTTLPR modifies the promoter activity of the 5-HTT gene, altering expression of the serotonin transporter (Caspi et al., 2003), and thus has been implicated in the development of stress-related disorders. The short allele (S) of 5-HTTLPR is associated with lower serotonin transporter levels than the long allele (L). Moreover, the function of 5-HTTLPR is affected by another A/G single nucleotide polymorphism (SNP), rs25531 in this loci. People with the long allele of 5-HTTLPR and G allele of rs25531 (known as L_G allele) demonstrate similar levels of expression with short allele carriers, while those having the long allele of 5-HTTLPR and A allele of rs25531 (known as L_A allele) show higher expression (Hu et al., 2005). Therefore, the L_G and S alleles are further combined as S′, while L_A is coded as L′. Traumatic events will increase serotonin release at targeted brain regions, and S′ allele carriers would be less efficient than the L' allele at up-regulating the expression level of the serotonin transporter and keeping extracellular serotonin at a normal level, which will increase the risk for developing stress-related mental disorders such as PTSD (Xie et al., 2009). The S′ allele 5-HTTLPR was also found significantly associated with abnormal functional connections between amygdala and medial prefrontal cortex, which is an important endophenotype of PTSD (Madsen et al., 2015). In addition, the association between 5-HTTLPR and PTSD was salient especially in highly trauma-exposed populations, suggesting a potential interaction between trauma exposure and 5-HTTLPR (Gressier et al., 2013). Some studies have reported significant G × E effects for 5-HTTLPR on PTSD among civilian samples, but not in veteran samples (Liu et al., 2015).

According meta-analytic results (Navarro-Mateu, Escámez, Koenen, Alonso, & Sánchez-Meca, 2013), studies on the 5-HTTLPR polymorphism and PTSD yield heterogeneous results. Most studies with significant results indicate that S or S′ allele carriers have a higher risk for developing PTSD (Bryant et al., 2010; Koenen, Amstadter, & Nugent, 2009). However, this result was not replicated in a study of the general population, finding that L_AL_A genotype carriers have the highest risk for PTSD (Grabe et al., 2009). Also, other studies did not find a significant association between PTSD and the 5-HTTLPR genotype (Valente et al., 2011). It is important to note that most of these studies used cross-sectional designs, and PTSD symptoms were measured only once at after the traumatic events, which might account for heterogeneity across these studies and the inconsistent results (Lai, Lewis, Livings, Greca, & Esnard, 2017). Therefore, performing genetic studies at multiple time points after the trauma in a single cohort may help reveal how the associations between 5-HTTLPR and PTSD change over time and provide a possible explanation for the heterogeneity across previous cross-sectional studies.

In addition to PTSD onset, some genes might be also involved in the maintenance, recovery or course of PTSD symptoms (Nugent, Amstadter, & Koenen, 2008). As has been suggested by the Psychiatric Genomics Consortium PTSD (PGC-PTSD) workgroup, PTSD-related genetic variants would also predict the development course of the disorder (Logue et al., 2015). Longitudinal studies focused on the effect of gene to symptom course are needed to help design personalised intervention strategies. However, as a candidate gene showing stable associations with PTSD, no studies have been conducted to investigate the effect of 5-HTTLPR on PTSD's symptom course. Thus little is known about how the 5-HTTLPR genotype affects the symptom course of PTSD after trauma exposure. The current longitudinal study was designed to address this issue.

In the current study, to test the effect of 5-HTTLPR on child and adolescent PTSD symptoms after disaster exposure, we genotyped 963 children and adolescents who personally experienced the 2008 Wenchuan Earthquake in China. PTSD symptoms were assessed at 2.5, 3.5, 4.5 and 5.5 years after the earthquake, respectively. We aimed to examine (a) whether youth with different 5-HTTLPR genotypes had a significant main effect or gene–environment interaction effect on PTSD's symptom course after the disaster; (b) whether the association between 5-HTTLRP and PTSD symptoms differed across different time points after the disaster. Although evidence has accumulated from previous studies on 5-HTTLPR and PTSD, little evidences have been generated from longitudinal studies. Given absence of prior data on

5-HTTLPR's effects on the symptom course of mental disorder, we were cautious to formulate a priori hypotheses regarding directionality of the effects of 5-HTTLPR on PTSD's symptom course.

METHODS

Participants and procedure

Our sample was from Beichuan county of Sichuan Province in China. This county suffered the greatest losses in the 2008 Wenchuan Earthquake, and was almost completely destroyed. In order to investigate effects of the earthquake on mental health of children and adolescents, the local education department conducted four surveys in all primary and secondary schools in the county at 2.5 years (Time 1), 3.5 (Time 2), 4.5 (Time 3) and 5.5 years (Time 4) after the earthquake. The interval between each survey was 1 year. On each survey, participants completed self-reported questionnaires of PTSD symptoms with assistance from trained research assistants and school teachers, after the aim and significance of the survey was introduced. A database was established afterwards based on the clinical data. At 6.5 years after the earthquake, we collected saliva samples and processed them for genotyping from students in the two most affected secondary schools in the county. Their clinical data were paired with the existing data we previously collected. All surveys were conducted by in group classroom settings, and informed consents/assents were obtained from all students and their guardians. The study protocol was approved by the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences, Beijing, China.

At Time 1, 822 participants took part in the survey; Time 2 included 849 participants; Time 3 included 980; and Time 4 included 1104 participants. As recommended by previous longitudinal studies (Su, Supple, Leerkes, & Kuo, 2018), a total of 1039 participants who were surveyed at least twice between Time 1 and Time 4 were included in analyses. Twenty participants were excluded because of missing ethnic information. Six participants were excluded because they did not personally experience the earthquake and 50 participants were not successfully genotyped. Our final sample contained 963 participants, ranging from 7 to 11 years old (mean = 8.3 years, $SD = 0.8$) at the time of the earthquake. There were 439 (45.6%) boys and 524 (54.4%) girls. Self-reported ethnicity for most of our participants was Chinese Qiang (66.9%) with the remaining participants reporting Han ethnicity. Participants who were excluded did not differ on sex, age or earthquake trauma exposure with those included (all $ps > .05$), but ethnicity distribution was different, χ^2 (1) = 3.925, $p = .048$. Among participants included in the final sample, 192 (19.9%) took part in two surveys, 114 (11.8%) took part in three surveys and 657 (68.2%) took part in four surveys between Time 1 and Time 4. The number of completed surveys was associated with age ($p = .001$) and ethnicity ($p = .004$), but not sex or earthquake trauma exposure.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed assent was obtained from all individual child participants included in the study.

Measures

Earthquake-related trauma exposure was assessed by a questionnaire asking five yes (1) or no (0) questions about whether participants experienced (a) traumatic death of a family member; (b) being injured; (c) witnessing injury of someone; (d) witnessing buildings collapse; (e) exposure to a corpse during the earthquake. The five items were chosen as they were most commonly reported in our earlier survey of the earthquake survivors soon after the earthquake. The final score for earthquake-related trauma was calculated by summing item responses. Because the total trauma score was not normally distributed (Kolmogorov–Smirnov test, $p < .001$), as suggested by previous studies (Pietrzak, Galea, Southwick, & Gelernter, 2013), those scoring 0 or 1 were placed in a low exposure group, and those scoring more than 2 were placed in a high exposure group, according to a median split. Results using continuous trauma exposure scores were presented in Tables S1 and S2.

PTSD symptoms in the past month were measured by the University of California, Los Angeles PTSD Reaction Index (PTSD-RI) for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (Child Version) (Steinberg, Brymer, Decker, & Pynoos, 2004) at 2.5, 3.5, 4.5 and 5.5 years after the earthquake. The 20-item PTSD-RI uses a 5-point Likert scale (from 0 = *never* to 4 = *most of the time*) to rate PTSD symptoms individuals experienced during the past month. The PTSD-RI demonstrates good reliability and validity, and is the most commonly used scale in longitudinal studies of child and adolescent PTSD (Lai et al., 2017). In our study, the Chinese version of PTSD-RI was used, and was focused on the earthquake event in particular. The Chinese version of the PTSD-RI was adapted via a two-stage process of translation and back translation, and demonstrated good psychometric properties in Chinese samples (Chen, Lin, Tseng, & Wu, 2002). We used the English version of the PTSD-RI from the official website of Department of Veterans Affairs (VA), USA. Then we translated the English version into a Simplified (modern language) Chinese version according

to the Traditional (more antiquated) Chinese PTSD-RI (Chen et al., 2002). The Chinese version of the PTSD-RI is composed of three subscales measuring re-experiencing (e.g. recurrent thoughts of trauma), avoidance (e.g. avoidance of thoughts of trauma) and hyper-arousal (e.g. sleep difficulty) symptoms. The 17 items with a one-to-one correspondence to DSM-IV PTSD symptom criteria were summed to calculate the final scale score: of the two items assessing restricted affect, the item with a higher score was included; two additional items assessing associated features (i.e. fear of recurrence, trauma-related guilt) were excluded. The PTSD-RI demonstrated good reliability in our sample at all time points. Cronbach's α for the PTSD-RI was 0.858 at Time 1, 0.891 at Time 2, 0.896 at Time 3 and 0.919 at Time 4.

Genotyping

DNA extraction was performed by the OG500 Kit supplied by Oragene (Canada). The genotyping of 5-HTTLPR and rs25531 was conducted by a custom-by-design 2 × 48-Plex SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China). For each participant, 10 ng DNA was amplified by polymerase chain reaction (PCR), and the following genotyping was conducted based on multiplex fluorescence PCR. Blind tests were performed to ensure no genotyping errors. The frequency of the 5-HTTLPR genotype in current sample (S′S′:75%, S′L′:22%, L′L′: 3%) deviated from the Hardy–Weinberg equilibrium (HWE) ($p < .05$). This deviation might have been caused by population stratification in the current study. Our sample contained two different Chinese ethnicities, Han and Qiang. According to previous studies (Gelernter, Cubells, Kidd, Pakstis, & Kidd, 1999; Su et al., 2018), allele frequencies for 5-HTTLPR varied significantly among different ethnicities. Population stratification within an individual study may contribute to deviation from HWE. The frequency of the 5-HTTLPR genotype for the Han ethnicity in the current sample was 73.4% S′S′, 23.2% S′L′ and 3.4% L′L′. The frequency of the 5-HTTLPR genotype for the Qiang ethnicity in the current sample was 75.9% S′S′, 21.3% S′L′ and 2.8% L′L′. To reduce potential bias due to population stratification effects, self-reported ethnicity was controlled as a covariate in all statistical analyses (Su et al., 2018).

Statistical analysis

The effect of 5-HTTLPR on PTSD's symptom course was examined in the whole sample. Latent growth modelling (LGM) was used to model the symptom course of PTSD between 2.5 and 5.5 years after the earthquake. LGM is a structural equation model (SEM) method to describe intra-individual change across time and inter-individual differences in intra-individual change. It is the dominant statistical approach to model change and between-person differences in change, and has been widely used in genetic studies of longitudinal symptom changes (Amstadter et al., 2011). In the current study, in order to test both linear and non-linear change in PTSD symptoms over time, both linear and quadratic growth models were conducted using Mplus 7 software. The models were estimated by full information maximum likelihood estimation with robust standard errors, and the intercept was centred at the first time point, 2.5 years after the earthquake. Full information maximum likelihood estimation is one of the most suggested approaches for processing longitudinal studies with missing data, especially for SEM studies. An acceptable fit is evidenced by the root mean square error of approximation (RMSEA) ≤0.08, standardized root mean square residual (SRMR) ≤0.08, comparative fit index (CFI) ≥0.9 and Tucker–Lewis index (TLI) ≥0.9 as suggested by Hu and Bentler (1999). The main and gene–environment interaction effects with earthquake-related trauma exposure of 5-HTTLPR on the intercept and slope of PTSD symptoms were estimated in the SEM model, with sex, age at the earthquake and self-reported ethnicity controlled as covariates. Furthermore, symptom course of S′ allele homozygotes and L' allele carriers was compared by two-group LGM. LGM analyses were performed in two subsamples divided according to the 5-HTTLPR genotype.

We examined associations between 5-HTTLRP and PTSD symptom severity across different time points within the LGM model by centring the intercept at the four time points (2.5, 3.5, 4.5 and 5.5 years after the earthquake), separately. In order to test the main effect of 5-HTTLPR, the genotype and earthquake-related trauma exposure were set as independent variables and PTSD symptom scores at 2.5, 3.5, 4.5 and 5.5 years after the earthquake were set as dependent variables, respectively. Sex, age at the earthquake and self-reported ethnicity were controlled as covariates. To investigate the gene–environment effect, the interaction between 5-HTTLPR and earthquake trauma exposure (5-HTTLPR×trauma severity) was added into the regression equations as an independent variable. The regression analyses were performed using SPSS 20.

RESULTS

Descriptive analyses

In our sample, the mean score on the PTSD-RI was 17.2 ($SD = 9.6$, range: 0–62) at Time 1, 14.9 ($SD = 10.3$, range: 0–68) at Time 2, 14.9 ($SD = 10.5$, range: 0–58) at Time 3 and 13.8 ($SD = 11.4$, range: 0–68) at Time 4. The mean score of earthquake-related trauma exposure was 1.9 ($SD = 1.2$, range: 0–5). Girls reported more severe PTSD symptoms at all time points ($p < .05$) than

boys. Self-reported ethnicity was related to PTSD-RI scores at Time 1 (β =0.101, p = .032), Time 2 (β =0.092, p = .012) and Time 3 (β =0.076, p = .025). Children reporting Qiang ethnicity had higher PTSD-RI scores than those reporting as Han. Age was not related to PTSD-RI scores at any time.

Latent growth analyses

The linear growth model demonstrated good fit to our data, χ^2 (5, N = 963) = 29.623, RMSEA = 0.072, CFI = 0.958, TLI = 0.950, SRMR = 0.056. The model did not fit well for the quadratic growth model, χ^2 (1, N = 963) = 13.280, RMSEA = 0.113, CFI = 0.979, TLI = 0.875, SRMR = 0.022, suggesting that PTSD symptom course followed a linear pattern. Therefore, the following analyses were all based on the linear growth model. The intercept for PTSD's symptom course, indicating the initial PTSD-RI score at 2.5 years after the earthquake, was 16.6 (p < .001). The slope for the symptom course, indicating changes in PTSD-RI scores between contiguous time points, was −0.9 (p < .001). The intercept and slope were not correlated (r = −0.025, p = .831). The results suggested the PTSD symptoms significantly recovered over time in the whole sample, but the recovery rate was not related to baseline symptom severity.

As for 5-HTTLPR, the genotype significantly affected the intercept (β = 0.108, p = .019) and slope (β = −0.120, p = .031) of PTSD symptom course. The results indicating that compared with L′ allele carriers, those with the S′S′ genotype showed higher initial PTSD symptom severity but faster recovery rates. However, the gene-trauma severity interaction effect was not significant for the intercept (β = −0.101, p = .180) or slope (β = 0.108, p = .267). Sex, ethnicity and trauma exposure only predicted the intercept but not the slope (see in Table 1). The two-group latent growth model demonstrated good fit to the data, χ^2 (10, N = 963) = 31.127, RMSEA = 0.066, CFI = 0.963, TLI = 0.956, SRMR = 0.057. The estimated PTSD symptom course results are presented in Figure 1. According to the two-group LGM, the PTSD symptom course intercept was 16.9 (p < .001) and the slope was −1.1 (p < .001) for S′S′ homozygotes. While for L′ allele carriers, the intercept was 15.6 (p < .001) and slope was −0.5 (p = .067), which indicates that L′ allele carriers did not show a significant decrease in PTSD symptoms over time. The intercept and slope were not correlated in either L′ allele carriers (r = −0.199, p = .173) or S′S′ homozygotes (r = 0.063, p = .697).

The main effect of 5-HTTLPR only predicted PTSD-RI scores at Time 1 (β = 0.108, p = .019) after controlling for covariates (see in Table 2). 5-HTTLPR did not predict PTSD-RI scores at Time 2 (β = 0.060, p = .097), Time 3 (β = 0.016, p = .636) or Time 4 (β = −0.017, p = .644). The S′ allele homozygotes had more severe PTSD symptoms at 2.5 years after the earthquake. Higher earthquake-related trauma exposure led to higher PTSD-RI scores at all time points. The 5-HTTLPR genotype was not found to interact with earthquake-related trauma exposure at any time point (see in Table 2).

TABLE 1
Main effect and gene−environment interaction effect of 5-hydroxyl tryptamine transporter gene-linked polymorphic region (5-HTTLPR) genotype on post-traumatic stress disorder symptom course between 2.5 and 5.5 years after the earthquake

Predictor	B	SE	β	p
Intercept				
Sex	1.450	0.629	0.110	**0.017**
Age	0.043	0.395	0.005	0.914
Ethnicity	1.400	0.644	0.101	**0.032**
Trauma	2.778	0.620	0.208	**<0.001**
5-HTTLPR	1.629	0.696	0.108	**0.019**
5-HTTLPR × trauma	−1.835	1.356	−0.101	0.180
Slope				
Sex	0.562	0.279	0.117	0.053
Age	0.184	0.180	0.060	0.315
Ethnicity	−0.049	0.293	−0.010	0.866
Trauma	0.019	0.277	0.004	0.945
5-HTTLPR	−0.667	0.315	−0.120	**0.031**
5-HTTLPR × trauma	0.713	0.631	0.108	0.267

Note: N = 963. The 5-HTTLPR genotype was coded: S′L′ or L′L′ = 0; S′S′ = 1. Covariates included sex, age at earthquake and self-report ethnicity. In bold values of p < 0.05.

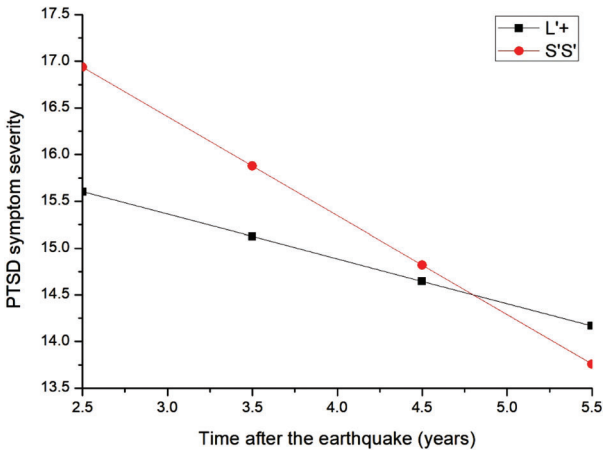

Figure 1. PTSD symptom courses between 2.5 and 5.5 years after the earthquake by 5-HTTLPR genotype. [Colour figure can be viewed at wileyonlinelibrary.com].*Note:* L′+: n = 240, S′S′: n = 723.

DISCUSSION

This study analysed prospective longitudinal data from a child and adolescent sample who experienced the 2008

TABLE 2
Main effect and gene−environment interaction effect of 5-hydroxyl tryptamine transporter gene-linked polymorphic region (5-HTTLPR) genotype on post-traumatic stress disorder symptom at 2.5, 3.5, 4.5 and 5.5 years after the earthquake

Predictor	B	SE	*β*	p
Time 1 (2.5 years)				
Sex	1.450	0.629	0.110	**.017**
Age	0.043	0.395	0.005	.914
Ethnicity	1.400	0.644	0.101	**.032**
Trauma	2.778	0.620	0.208	**<.001**
5-HTTLPR	1.629	0.696	0.108	**.019**
5-HTTLPR×trauma	−1.835	1.356	−0.101	.180
Time 2 (3.5 years)				
Sex	2.012	0.533	0.144	**<.001**
Age	0.227	0.330	0.026	.491
Ethnicity	1.351	0.541	0.092	**.012**
Trauma	2.797	0.526	0.198	**<.001**
5-HTTLPR	0.963	0.583	0.060	.097
5-HTTLPR × trauma	−1.122	1.127	−0.058	.321
Time 3 (4.5 years)				
Sex	2.574	0.573	0.159	**<.001**
Age	0.410	0.355	0.060	.315
Ethnicity	1.302	0.585	0.076	**.025**
Trauma	2.817	0.568	0.171	**<.001**
5-HTTLPR	0.296	0.627	0.016	.636
5-HTTLPR × trauma	−0.409	1.224	−0.018	.738
Time 4 (5.5 years)				
Sex	3.125	0.727	0.162	**<.001**
Age	0.594	0.457	0.048	.195
Ethnicity	1.252	0.750	0.061	.094
Trauma	2.836	0.723	0.144	**<.001**
5-HTTLPR	−0.371	0.803	−0.017	.644
5-HTTLPR × trauma	0.304	1.588	0.011	.848

Note: $N = 963$. The 5-HTTLPR genotype was coded: S′L′ or L′L′ = 0; S′S′ = 1. Covariates included sex, age at earthquake and self-report ethnicity. In bold values of $p < 0.05$.

Wenchuan Earthquake. According to cut-off scores on the PTSD-RI, this sample still suffered from mild PTSD symptoms between 2.5 and 5.5 years after the earthquake (cut-off: 12−23, Steinberg et al., 2004). Although average symptom severity did not reach a cut-off threshold required for diagnosis, it has been reported that sub-syndromal levels of PTSD can still contribute to marked impairment (Stein, Walker, Hazen, & Forde, 1997), and could be clinically meaningful. The PTSD-RI total score at 4.5 years after the earthquake in our sample was still higher than the total score reported in an adolescent sample at 3-months after another earthquake (14.9 vs. 14.1), which implied a strong and prolonged negative effect caused by the earthquake.

The current study intended to examine the effect of 5-HTTLPR on child and adolescent PTSD symptoms longitudinally after the disaster. The 5-HTTLPR genotype significantly predicted PTSD′s symptom course between 2.5 and 5.5 years after the earthquake. Compared with L′ allele carriers, those with the S′S′ genotype showed higher initial PTSD symptom severity but also a faster recovery rate ending with similar levels of severity. 5-HTTLPR predicted PTSD symptom severity at 2.5 years, but not 3.5, 4.5 or 5.5 years, after the earthquake when controlling for covariates. The S′S′ genotype was associated with more severe PTSD symptoms. We did not find a gene-trauma interaction effect for 5-HTTLPR on either symptom severity or course.

Despite the fact that S′ allele homozygotes of 5-HTTLPR showed higher initial PTSD symptoms, they also recovered faster from these symptoms. This interesting pattern of gene effects on PTSD was also reported in a previous longitudinal study that utilised a similar design to that our study (Amstadter et al., 2011). The G allele of *CRHR1* gene was associated with higher initial but greater declines of child PTSD symptoms over time. As we did not find a significant correlation between the intercept and slope of symptom course, this lack of an effect is not due to a negative correlation between the initial symptom levels and recovery rates. Previous cross-sectional studies were based on the a priori hypothesis that individuals with different genetic backgrounds will show different symptom levels. Our longitudinal study suggests that the effect of 5-HTTLPR on PTSD symptoms might also involve changes in symptoms over time. These findings support the notion that genetic factors may also affect the course of PTSD symptoms (Nugent et al., 2008). Clearly, additional genetic studies based on longitudinal datasets are needed.

Consistent with results from cross-sectional studies (Bryant et al., 2010), our study found the S′S′ genotype of 5-HTTLPR conferred risk for greater PTSD symptom severity at 2.5 years after the earthquake. However, this effect was no longer significant at subsequent time points, which might be consistent with results from a prior meta-analysis (Navarro-Mateu et al., 2013), if 5-HTTLPR effects were present only in the earlier period after trauma exposure. The period of PTSD symptom assessment varied between different cross-sectional studies investigating the association between 5-HTTLPR and PTSD, which may account for heterogeneity in the reported results.

Contrary to previous studies, our results did not support a gene−environment interaction effect of 5-HTTLPR. It should be mentioned that early childhood traumatic experience (e.g. childhood abuse) was not measured in the current study. Recent meta-analytic findings in PTSD genetic studies have revealed that the gene-interaction effect of some PTSD risk-related genes of could be developmentally sensitive, and demonstrated more reliable results when interacting with childhood trauma (Wang, Shelton, & Dwivedi, 2018). Another factor accounting for the inconsistent results could be ethnicity. It has been pointed out that G × E interactions of 5-HTTLPR in children and adolescents might be critically dependent on ethnicity, and the G × E effect was usually found in Caucasian samples (Van Ijzendoorn,

Belsky, & Bakermans-Kranenburg, 2012). In fact, a gene–environment effect could be rare, and not necessarily needed to explain the interplay between genes and trauma exposure in PTSD (Stein, 2018). However, it also should be acknowledged that our measure of trauma exposure might not be strong enough to capture all aspects of the event. Therefore, the genetic factor might have no moderation effects on trauma exposure. Moreover, the differential susceptibility of the 5-HTTLPR gene should be mentioned (Van Ijzendoorn et al., 2012), which suggests that the 5-HTTLPR short allele might function as a sensitivity rather than a vulnerability factor for PTSD. It would be interesting to test the interaction effect of 5-HTTLPR with both positive and negative experiences in future studies to address this issue.

The current study was carried out in a unique sample. First, we used individuals of Chinese ancestry, and although there have been a number of studies on 5-HTTLPR and PTSD, most were conducted in western populations. As previously reported (Gelernter et al., 1999), the genotype distribution of 5-HTTLPR differed in eastern and western populations, making the investigation of 5-HTTLPR genotypes and psychopathology in a Chinese population important. We acknowledge that the generalisability of our results in Western samples should be treated with caution considering different genotype distributions of 5-HTTLPR between Chinese and Western countries. A meta-analysis showed that the association between 5-HTTLPR and PTSD could be modified by ethnic distribution of samples (Navarro-Mateu et al., 2013), implying that 5-HTTLPR function might differ across different ethnicities. Our current knowledge on 5-HTTLPR allele functions are mainly based on studies in Caucasian samples. Investigation of 5-HTTLPR allele functions in Chinese sample might help us to integrate current findings, and in turn further our understanding of the relationship between 5-HTTLPR and mental disorders such as PTSD. Moreover, PTSD symptoms were followed-up for a long period after the trauma, which enabled us to focus on chronic post-traumatic responses in children and adolescents. While a number of longitudinal studies on child and adolescent PTSD have been reported, few of them measured PTSD more than 3 years after trauma exposure (Wang et al., 2018).

Several limitations of the current study should be carefully considered. First, our study was conducted between 2.5 and 5.5 years after the earthquake and thus could not capture PTSD symptoms shortly after the disaster. Our results should not be generalised to the whole period after trauma exposure, as PTSD's symptom course might not always follows a linear pattern, especially shortly after the exposure. Second, measurement of earthquake-related trauma exposure was not precise enough to capture more subtle differences in trauma exposure during the earthquake and also does not capture possible stress that occurred in the aftermath of the earthquake (e.g. parents losing their livelihood). The measure of trauma exposure still need to be further validated. Third, the current study only focused on childhood earthquake-related trauma exposure. Results of our study need to be replicated in studies using populations exposed to other trauma types. Finally, a range of covariates before, during and after the earthquake (e.g. socio-economic status, parenting quality, pre-existing PTSD symptoms, whether accepted psychological interventions), which may potentially affect PTSD's symptom course, were not measured or included in analyses.

The current study is the first longitudinal study to investigate the effect of the 5-HTTLPR genotype on child and adolescent PTSD symptoms. By following up on PTSD symptoms between 2.5 and 5.5 years after an earthquake, we found an association between 5-HTTLPR and PTSD symptom levels and course, offering a novel perspective on the effect of 5-HTTLPR on PTSD. Also, our results indicated that the serotonergic system might be a potential treatment target for PTSD symptoms after disaster.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Main effect and gene–environment interaction effect of 5-HTTLPR genotype on PTSD symptom course between 2.5 and 5.5 years after the earthquake using continuous trauma scores

Table S2. Main effect and gene–environment interaction effect of 5-HTTLPR genotype on PTSD symptom at 2.5, 3.5, 4.5 and 5.5 years after the earthquake using continuous trauma scores

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