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Modification of the risk of Post-Traumatic Stress Disorder (PTSD) by the 5-HTTLPR polymorphisms after Lorca's earthquakes (Murcia, Spain).

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Declaration of interest

Dr. Kessler has served on advisory boards for Mensante Corporation, Plus One Health Management, Lake Nona Institute, and U.S. Preventive Medicine, is a co-owner of DataStat, Inc. In the past three years, Dr. Kessler has been a consultant for Hoffman- La Roche, Inc., Johnson & Johnson Wellness and Prevention, and Sonofi-Aventis Groupe. There are no patents, products in development or marketed products to declare. Preliminary results of this study were presented as a poster at the XVI World Congress of the World Psychiatry in Madrid, Spain, in September 2014; at XIX National Congress of Psychiatry in Palma de Mallorca, in October 2016; and at XVII SESPAS Congress ("Ciencia para la Acción") in Barcelona, Septembre 2017.

Data sharing statement

The de-identified minimal dataset needed for only monitoring purposes will be made available on request due to ethical restrictions in the signed agreements with the WHO WMH Survey Initiative.

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Abstract

Information of the modulation effect of the serotonin transporter gene-linked polymorphic region (*5-HTTLPR*) on post-traumatic stress disorder (PTSD) after earthquakes is scarce and contradictory. A cross-sectional face-to-face interview survey of a representative sample of the adults was carried out after the Lorca (Spain) earthquakes (May 11, 2011). Socio-demographic variables, DSM-IV diagnostic assessment and earthquake-related stressors were obtained from the Composite International Diagnostic Interview (CIDI). The triallelic and biallelic classification of the *5-HTTLPR* polymorphism were genotyped from buccal swabs.

Multivariate logistic regression models were used to predict PTSD, including interaction terms to explore gene-environment (G x E) interactions. The vast majority (83%, n=341) of the Lorca survey respondents (*n*=412, 71% response rate) were genotyped. Both classifications of the *5-HTTLPR* genotype were in Hardy-Weinberg equilibrium. Prior lifetime PTSD was the only variable that remained a significant predictor after adjustments. There were no significant main effects of earthquakes-related stressors or *5-HTTLPR*. However, G x E interactions of *5-HTTLPR* with high emotional impact and prior lifetime anxiety disorders were statistically significant. These results provide new evidence of the modulation effect of the *5-HTTLPR* polymorphisms on PTSD risk. This information might characterize people at higher risk of developing PTSD after an earthquake exposure.

Keywords

5-HTTLPR polymorphisms; Post-traumatic Stress Disorder; Gene-Environment Interaction

1. Introduction

Although the vast majority of people in the general population of most countries reports lifetime exposure to one or more traumatic events (Benjet et al., 2015), only a relatively small minority of those exposure to trauma (estimated as averaging 5.6% across studies) develops posttraumatic stress disorder (PTSD) (Koenen et al., 2017). PTSD is a mental disorder characterized by symptoms of re-experiencing, avoidance, dulling of the senses and hyperarousal that occurs following exposure to a potentially traumatic life event (Yehuda et al., 2015). Risk of PTSD is known to vary by type of trauma (Kessler et al., 2017, 2014). Earthquakes and other natural disasters are commonly-occurring types of traumas that have enormous impacts of many sorts (e.g., destruction of infrastructure, personal injury, injury-death of loved ones, geographic displacement, financial adversity) in the general population (Bartholdson and von Schreeb, 2018; Silove and Steel, 2006). PTSD is considered the most frequent psychopathological response to earthquake exposure (Anwar et al., 2013; Cheng et al., 2014; Galea et al., 2005; Neria et al., 2008; Norris et al., 2002a, 2002b). The post-

earthquake risk of PTSD and other mental disorders has been reported to be influenced by experiences that occur within and in the aftermath of the earthquake, pre-disaster characteristics of the exposed individuals and their communities, and interactions between the two broad classes of risk factors (Bromet et al., 2017; Galea et al., 2005; Kessler et al., 2017, 2014).

PTSD is a complex and multifactorial mental disorder (Koenen et al., 2009b). Environmental factors clearly contribute to its development and there is a general consensus on the importance of genetics in its etiology (C.R. Brewin et al., 2000; Dai et al., 2016; Duncan et al., 2018). Several candidate genes related to the current understanding of the neurobiology of the disorder have been studied (Koenen, 2007). One of the most frequently investigated genetic variants is the human serotonin transporter (*5-hydroxytryptamine transporter, 5-HTT*) gene (*SLC6A4*), through polymorphisms in its promoter region (*5-HTTLPR*) (Caspi et al., 2010). The less frequent short allele (*S*) in the *5-HTTLPR* has been associated with a lower transcriptional efficiency compared with the more frequent long (*L*) allele (Lesch et al., 1996). It has been related to different mental outcomes or disorders (Hu et al., 2005; Lesch et al., 1996; Li and He, 2007; Lotrich and Pollock, 2004; Takano et al., 2007). A triallelic model has also been studied since a third functional allele, L_G, was described (Nakamura et al., 2000) with an equivalent expression to the *S* allele (Hu et al., 2005). Thus, *5-HTTLPR* might be considered as a triallelic locus with alleles designated as L_G, L_A, and S, allowing a functional re-arrangement of genotypes (or re-calssification) on the basis of lower and higher levels of expression (Parsey et al., 2006; Zalsman et al., 2006).

Three meta-analyses have analyzed the relation of the *5-HTTLPR* polymorphisms and PTSD. The first of them suggested that current evidence would not support a direct effect of the *5-HTTLPR* polymorphisms on PTSD, with neither the biallelic nor the triallelic approach (Fernando Navarro-Mateu et al., 2013). The second one also found no direct association, but in a sensitivity analysis, being a carrier of the *SS* genotype seemed to represent a risk factor for PTSD among those exposed to higher levels of trauma (Gressier et al., 2013). The possibility of a modulation effect of the *5-HTTLPR* in PTSD through a gene-environment interaction (Koenen et al., 2009b) has been recently explored in the third meta-analyses and its results suggested a modulation effect of the 5-HTTLPR polymorphisms on the association between stress and PTSD (Zhao et al., 2017). Gene-environment (G x E) interactions imply that the effect of a genetic variant is modified by an environmental exposure or vice versa (Koenen et al., 2008, 2009b). Only one of the 14 included studies in the latest meta-analysis focused on earthquakes and it found a significant interaction between the S allele with the exposure on the risk of PTSD after the exposure to 2008 Winchuan earthquake in China (Tian et al., 2015). Since then, only two more studies have analyzed this G x E interaction in survivors of the same Chinese earthquake (Li et al., 2019; Liu et al., 2017). One did not find an association of the biallelic classification of the genotype on total PTSD symptoms (Liu et al., 2017) but the most recent one showed that those child and adolescent carriers with *S'S'* alleles had higher initial PTSD symptoms but also faster recovery rate in a longitudinal study design of 5.5 years of follow-up (Li et al., 2019). Only one of the three latest studies analyzed the triallelic classification of the polymorphism (Li et al., 2019) and none of them controlled for the prior history of mental

disorders, considered as one of the most important factors influencing the risk of mental disorders after a natural disaster (C. R. Brewin et al., 2000; Kessler et al., 2014).

On May 11, 2011 a moderate magnitude earthquake (5.1 Mw) preceded by somewhat smaller one (4.5 Mw) and followed by almost 50 aftershocks of minor magnitude in the following few days took place in Lorca (Murcia, Spain) in the midst of a long European economic crisis at the same time that an epidemiological survey was being carried out in a representative sample of the general population the region of Murcia, the PEGASUS-Murcia ("Psychiatric Enquiry to General Population in Southeast Spain-Murcia") project (Fernando Navarro-Mateu et al., 2013; Navarro-Mateu et al., 2015). Significant differences in the 12-month prevalence of PTSD were found comparing the exposed area of Lorca with the rest of Murcia (3.6% vs 0.5%) (Navarro-Mateu et al., 2017). The aims of the current study were to evaluate *5-HTTLPR* polymorphism as a risk factor for PTSD among people exposed to the earthquakes of Lorca, 2011 (Murcia, Spain) and to explore G x E interactions with earthquake-related stressors and lifetime mental disorders prior to the earthquakes.

2. Methods

2.1. Study design and participants

Details of the PEGASUS-Murcia project protocol, sampling frame, selection and weighting procedures are described elsewhere (F. Navarro-Mateu et al., 2013). Briefly, the study uses a cross-sectional survey design to carry out face-to-face interviews with a representative sample of the adult and non-institutionalized general population of the Region of Murcia, in the southeast of Spain. The eligible population was defined as all people aged 18 or older residing in the household population of Lorca, not living in institutions and registered in PERSAN, a periodically up-dated regional registry containing all residents covered by the public health system, with virtually universal coverage. A stratified, multistage, clustered by health area, probability random sampling design was used. The field work in the area of Lorca was carried out between January and April 2012, which was 8–11 months after the earthquake took place, by certified lay interviewers.

2.2. Diagnostic assessment and socio-demographic variables

The structured interview schedule used in the study was a revised version of the WHO Composite International Diagnostic Interview (CIDI 3.0, hereafter referred to as CIDI) adapted for use in Spain (Navarro-Mateu et al., 2012). The CIDI is a fully structured interview designed by the World Health Organization (WHO) for the purpose of ascertaining diagnoses of mental illnesses for comparative research of the community epidemiology of mental illnesses throughout the world (Kessler and Ustun, 2004). Different mental disorders according to DSM-IV have been considered (i.e. Mood Disorders -including major depression, bipolar and dysthymia-, post-traumatic stress disorder (PTSD), other Anxiety Disorders -generalized anxiety disorder, social phobia, specific phobia, agoraphobia without panic, panic disorder, obsessive compulsive disorder and adult separation anxiety disorder- and Substance Disorders –alcohol and drug abuse and/or dependence-). All diagnoses included organic exclusions and without diagnostic hierarchy rules with the exception of major depressive disorder, dysthymia and general anxiety disorder. For substance use

disorders, abuse was defined with or without dependence in recognition of abuse being a stage in the progression to dependence.

Prevalence estimates of PTSD were determined by whether respondents' symptomatology met the 12-month DSM-IV diagnostic criteria for the mental disorder. Lifetime prevalence of mental disorders prior to the earthquakes was determined by whether respondents had a history of mental disorder with an age-of-onset (AOO) a year prior the interview. Retrospective AOO reports were obtained in the CIDI using a series of questions designed to avoid the implausible response patterns obtained when using the standard CIDI age-of-onset questions (Kessler et al., 2005). Different mental disorders according to DSM-IV have been considered (i.e. Mood Disorders -including major depression, bipolar and dysthymia-, post-traumatic stress disorder (PTSD), other Anxiety Disorders -generalized anxiety disorder, social phobia, specific phobia, agoraphobia without panic, panic disorder, obsessive compulsive disorder and adult separation anxiety disorder- and Substance Disorders – alcohol and drug abuse and/or dependence-). All diagnoses included organic exclusions and without diagnostic hierarchy rules with the exception of major depressive disorder, dysthymia and general anxiety disorder. For substance use disorders, abuse was defined with or without dependence in recognition of abuse being a stage in the progression to dependence.

Socio-demographic variables evaluated in this study were: age at interview, sex, completed years of education (grouped in 2 categories: None, primary or lower: 0–11 years; Secondary or higher: 12 or more years of education); marital status (grouped in 2 categories: living with a partner (married-cohabitating) and not living with a partner (separated-widowed-divorced-never married)). Employment status was categorized in 2 categories (working and not working (student, homemaker, retired/disabled, unemployed and others). Referred ethnicity was grouped in 2 categories (White/Caucasian and Others).

2.3. Earthquake-related Stressors

The questionnaire used in the survey, the WHO Composite International Diagnostic Interview (CIDI 3.0) (Navarro-Mateu et al., 2012) was expanded to include questions to examine respondent involvement and severity of the individual exposure to the catastrophe shortly after the earthquake and the psychopathological effects of this exposure. As described in detail previously (Navarro-Mateu et al., 2017), it included 22 structured questions exploring the exposure to different earthquake-related stressors. Each one of them were categorized into 10 different categories of stressors coded as dichotomous variables (yes/no): (1) Life-threatening experience for you or for close people; (2) death of family members, friends or neighbours; (3) seriously personally injured; (4) seriously family members, friends of close neighbours injured; (5) buried or trapped in rubble; (6) financial loss; (7) property (home) seriously damaged or destroyed; (8) more family or household duties or living with relatives, friends, neighbours or strangers; (9) neighbourhood destroyed or seriously damaged; and (10) job affected or loss. Full text of the interview schedule that includes the complete set of stressor questions is available elsewhere (The World Mental Health Survey Initiative, 2015). Two scores were then calculated to evaluate the impact of the earthquake on individuals: a Global Earthquake Stressor Score (GESS) (range: 0–10), by

adding the individual score of each of the 10 categories, and the Earthquake's Experienced Stress (EES), based on a specific question ("*On a scale between 0 and 10 where 0 means "no stress at all" and 10 means "the most stress you can imagine a person having", what number describes how much stress you experienced as a result of the earthquake?"*). Finally, for this study, these scores were dichotomized into binary variables (yes/no) if the scores were above the sample mean. These new variables were named 'High Earthquake Exposure' (HEE) and 'High Emotional Impact' (HEI), respectively. This particular section, including lifetime and after the earthquakes PTSD evaluation, was asked to all respondents from Lorca, independently of the long-short path itinerary used in other areas of Murcia (F. Navarro-Mateu et al., 2013).

2.4. Genotyping

On completion of the interview, interviewees were asked to provide a biological sample of the buccal mucosal epithelium for genetic analyses. Samples were collected in sterile 1.5 ml tubes and registered and stored at BIOBANC-HCUVA (the biobank for biomedical research network of the Region of Murcia, University Clinical Hospital Virgen de la Arrixaca – TD09/0076/00065) (http://www.imib.es/portal/plataformas/biobanco.jsf?subentrada_actual_web=259&padre=241). Genomic DNA was isolated from buccal swabs of the participants using QIAamp DNA Blood Mini Kit (QIAGEN) and performed automatically in a QIAcube system (QIAGEN) to minimize the variability due to manual handling. The SLC6A4 gene presents a 44 bp variable number of tandem repeats (VNTR) polymorphism in its promoter region, which is located approximately 1 kb upstream from the transcription start site, known as 5-HTTLPR polymorphism. Three variants (alleles) of the 5-HTTLPR polymorphism were genotyped in two steps, involving a polymerase chain reaction (PCR) amplification step, followed by digestion with HpaII. The primers used to perform the PCR were previously described (Mellman et al., 2009): sense-ATCGCTCCTGCATCCCCCATTAT and antisense- GAGGTGCAGGGGGATGCTGGAA. Briefly, 25 μl reaction included 50 ng genomic DNA, 1X amplification buffer, 0.2 mM dNTPs, 1.5 mM MgSO4, 0.2 μM of each primer, 1 unit Platinum Taq PCRx polymerase (Invitrogen) and 1X PCR enhancer owing to the high GC content in the polymorphism region. The reaction was initially heated to 95°C (5 min), followed by 35 cycles of 95°C (35 sec), 60°C (30 sec) and 68°C (30 sec) and a final elongation step of 72°C (5 min). To distinguish between S (103 bp) and L (146 bp) alleles, PCR product reactions were analyzed by size determination on a QIAxcel Advanced System (QIAGEN) by high-resolution capillary electrophoresis. As a result of biallelic genotyping, individuals were genotyped as S/S, S/L or L/L. Afterwards and according to the manufacturer's instructions, fast HpaII restriction enzyme digestion (Thermoscientific) was carried out for SNP rs25531. This SNP consist in the presence of adenine (A) or guanine (G), being digested in the last position. Final digested products were visualized on a QIAexcel, and individuals were genotyped as S/S, S/L_A, S/L_G, L_A/L_A, L_A/L_G and L_G/L_G. The "triallelic" approach (S, L_A, L_G) allow the functional re-classification of S/L_G and L_G/L_G as $S\acute{S}$, L_A/L_G as $S\acute{L}$ and L_A/L_A as $L\acute{L}$. The products' sizes after digestion were: S (103 bp), L_A (146 bp) and L_G (83 bp, 63 bp). All genetic analyses were performed blinded to the diagnostic status of participants by the same geneticist.

2.5. Statistical methods

Calculations for deviation from the Hardy–Weinberg equilibrium were performed using chi-square tests for biallelic and triallelic genotype frequencies. Differences between those participants who did and did not provide genetic samples by socio-demographic variables and diagnostic categories were calculated by chi-square or student's t tests. Initially, bivariate analyses were used to explore differences by the presence (no/yes) of 12-month PTSD and each of the demographic characteristics (female sex, age, marital status, level of education and employment status), prior mental disorders (PTSD, other anxiety, mood and substance disorders), exposure-related variables (HEE and HEI) and the genotype frequencies measured as the number of *L* alleles.

Hierarchical logistic regression models were estimated to predict 12-month PTSD. Logits were exponentiated to create Odds Ratios (OR) and their 95% Confidence Intervals (95%CI). Model 1 (M1) was implemented as a simple logistic regression with each of the previous relevant independent variables. Model 2 (M2) included all sociodemographic variables and all lifetime disorders prior to the exposure. Model 3 (M3) added the *5-HTTLPR* polymorphism to M2. As there is no consensus on the inheritance model of the 5-HTTLPR polymorphisms, three different approaches for each classification (biallelic or triallelic genotypes) were used to explore their relationship with 12-month PTSD: a codominant model (*SS, SL* or *LL* and *S'S', S'L'* or *L'L'*), a *S* or *S'* dominant model (*LL* vs *SS+SL* or *L'L'* vs *S'S'+S'L'*) and a *S* or *S'* recessive model (*LL+SL* vs *SS* or *L'L'+S'L'* vs *S'S'*) were analyzed in model M2. Finally, to test for gene-environment interactions, another hierarchical series of multivariate logistic regression models were performed with the sequentially inclusion of the different previously defined interaction terms formed by the combination of the 5-HTTLPR genotype (both, with the triallelic and biallelic approaches) with earthquake-related measures (HEE and HEI) and the four prior lifetime mental disorders (PTSD, other anxiety, mood and substance disorders). All statistics used two-sided tests with alpha level of 0.05. No correction for multiple testing was performed. In exploratory analyses of a genetically complex trait in which the relationship between genotype and phenotype has not been yet established (Fernando Navarro-Mateu et al., 2013), multiple test adjustment are not strictly required (Bender and Lange, 2001) as it can increase the likelihood that real effects would be missed out (type II error rates) (Rothman, 1990).

The Clinical Research Ethics Committee of the University Hospital Virgen de la Arrixaca of Murcia approved the protocol. Written informed consent was obtained from all participants before starting the interview. The study was carried out in accordance with the STREGA (Strengthening The Reporting of Genetic Association Studies) guidelines, an extension of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines, for candidate gene studies (Little et al., 2009).

3. Results

The vast majority (83.5%, n = 344) of the Lorca survey participants (n= 412, 71.0% response rate) provided a biological sample to facilitate genetic analyses. The remaining survey respondents (16.5%, n=68) declined. The socio-demographic variables for the entire sample comparing both groups are presented in Supplementary table S1. The groups differed

only in mean age and employment status, with those providing biological samples younger and more likely to be employed than refusers. DNA from three biological samples could not be genotyped due to a very low DNA concentration levels. Thus, the final sample used in this study consists of the 341 people interviewed after the Lorca earthquakes for whom we had complete *5-HTTLPR* genotype data.

Description of socio-demographic variables, lifetime mental disorders prior to the exposure, earthquakes exposure description, and the triallelic and the biallelic genotype frequencies are presented in Table 1. The global allele frequencies of the different alleles were: *S*=369 (54.1%), L_A=18 (2.6%) and L_G =295 (43.3%). Both genotype classifications did not deviate from Hardy-Weinberg equilibrium ("triallelic" approach: *S'S'*=113 (33.1%), *S'L'*= 161 (47.2%), *L'L'*= 67 (19.6%), $\chi^2_{,1\ d.f.}$= 0.498, p-value=0.48; "biallelic" approach: *SS*=101(29.6%), *SL*=167 (49.0%), *LL*=73 (21.4%), $\chi^2_{,1\ d.f.}$=0.066, p-value=0.80). Participants with 12-month PTSD were more frequent having other work status different as working (student, homemaker, retired/disabled and unemployed among others), had more lifetime antecedents of PTSD, other anxiety, mood and substance disorders prior to the earthquakes and were more affected by the exposure to the natural catastrophes, both, in terms of the number of related-stressful events and in terms of the emotional impact secondary to the exposure, than people without 12-month PTSD. There were no differences in terms of the distributions of the "triallelic" and the "biallelic" *5-HTTLPR* genotype classification between both groups.

Table 2 summarizes the association between the variables with PTSD status with the "triallelic" approach in a bivariate model (M1) and the multivariate model (M2). The only variable that remained significant after the adjustment for socio-demographic, prior mental disorders and earthquake-related variables was the lifetime history of PTSD prior to the earthquake's exposure. There was no significant main effect for earthquake exposure-related variables and *5-HTTLPR* "triallelic" genotype. A similar pattern was found with the "biallelic" genotype (supplementary table 2).

Different gene-environment interaction models were analyzed with the sequential inclusion of different interaction terms formed with the combination of *5-HTTLPR* genotype with earthquakes exposure-related measures (HEE and HEI) and the four prior lifetime mental disorders (PTSD, other anxiety, mood and substance disorders). Interaction terms were significant only in two models, specifically: the number of *L'* alleles x HEI (p-value= 0.027), and the number of *L'* alleles and other prior anxiety disorders (p-value=0.027) (see table 2). The same pattern of interaction was found with the "biallelic" approach (number of *L* alleles x HEI, p-value= 0.026, and number of *L* alleles and other prior anxiety disorders, p-value=0.027) (see supplementary table 2).

The analysis of the association between the three different models of inheritance of the *5-HTTLPR* genotype in the "triallelic" (per *L'* allele: adjusted OR (95%CI) = 1.81 (0.65; 5.02); *S'* > *L'*: 0.25 (0.06; 1.13) and *L'* > *S'*: 0.94 (0.20; 4.37) and the "biallelic" (per *L* allele: 1.73 (0.62; 4.78); *S* > *L*: 0.28 (0.06; 1.22) and *L* > *S*: 1.00 (0.21; 4.69) approach did not found any significant effect for any of them.

Stratified analyses with a multivariate logistic regression adjusted for variables included in model 2 (M2) showed that the number of *Ĺ* alleles does not influence the risk of PTSD among persons who suffered low emotional impact (adjusted OR =0.29; 95%CI: 0.02, 4.05) but increased the risk among those suffering high emotional impact (adjusted OR = 4.84; 95%CI: 1.26, 18.64) and predicted an increased risk of PTSD among those with no history of any anxiety disorder with the exception of prior lifetime PTSD (3.74, 1.14; 12.07). A similar pattern was found with the number of L alleles. Stratified analyses with a multivariate logistic regression adjusted for variables included in model 2 (M2) showed a similar pattern among persons who suffered low emotional impact (aOR=0.27; 95%CI: 0.02, 3.83) but increased risk of PTSD among those suffering high emotional impact (aOR = 4.55; 95%CI: 1.19, 17.46) and an increased risk of PTSD among those with no history of any anxiety disorder with the exception of prior lifetime PTSD (3.43, 1.07; 11.02). Limitations of the sample size did not allow calculating the risk associated with one or two or more anxiety disorders.

4. Discussion

The aims of this study were to evaluate the association of the *5-HTTLPR* polymorphism and PTSD and to explore the moderation effect of this genotype on the PTSD risk associated to earthquake-related stressors and lifetime mental disorders prior to the exposure in a representative sample of the general population exposed to the earthquakes of Lorca, 2011 (Murcia, Spain). The results suggest that the *5-HTTLPR* polymorphism had no main effect in predicting PTSD is consistent with previous meta-analytic research which no direct association described (Gressier et al., 2013; Fernando Navarro-Mateu et al., 2013). To analyze the impact of the earthquake exposure, two scores approaches were measured: the severity (HEE) and the emotional impact (HEI) of the exposure. Contrary to our expectations and despite finding significant differences between participants who did and those who did not develop 12-month PTSD in terms of high exposure and high emotional impact in the bivariate analyses, none of both scores remained significant in multivariate analyses. Unfortunately, we did not have data on previous emotion regulation skills or cognitive biases of participants that could confound the effect (Woud et al., 2017). A prior history of lifetime PTSD was the most consistent and strongest risk factor predicting PTSD is consistent with a recent cross-national epidemiological analysis of risk factors for PTSD among people exposed to trauma in the context of the WHO World Mental Health (WMH) Surveys (Kessler et al., 2018), where only prior anxiety disorders significantly predicted PTSD in a multivariate model.

The *5-HTTLPR* genotype modulated the effect of the emotional impact of the earthquakes' exposure and of the history of previous anxiety disorders on the risk of PTSD, both with the tri- and biallelic functional classification of the polymorphism. L'L' and LL alleles carriers were at higher risk of PTSD in the context of higher emotional impact and in the absence of prior anxiety disorders. Three papers have analyzed the *5-HTTLPR* x earthquake exposure (Li et al., 2019; Liu et al., 2017; Tian et al., 2015) and only one of them found a statistical significant G x E interaction with bialellic genotype (Tian et al., 2015). These interactions are broadly consistent with previous studies finding the *L* or *L'* alleles to be involved risk of PTSD instead of the *S* or *S'* alleles. A risk-reducing effect of the *SS* genotype has been

previously described in relation to chronic PTSD in victims of motor-vehicle accidents (Thakur et al., 2009) and in the development of re-experiencing and arousal symptoms of PTSD in two independent African American samples exposed to childhood emotional abuse (Walsh et al., 2014). Moreover, two other studies implicated *L'* or *L* alleles in G x E interactions predicting PTSD. The *L'* allele was found to interact with the number of trauma events in PTSD with a stronger effect in homozygous (Grabe et al., 2009). In the second study, the probability of developing lifetime PTSD was dependent on genotype (Kolassa et al., 2010). While *SS* homozygous were at higher risk for developing PTSD independently of the number of traumatic events, *SL* or *LL* carriers showed a S-shaped dose-response relationship with the increased number of traumas. However, only a trend towards an interaction between genotype polymorphisms and number of traumatic events experienced was found. It is possible that participants with those antecedents were more prone to have a higher emotional impact after such an exposure, though this possibility could not be confirmed in an exploratory bivariate analysis in our sample (results not presented). The risk of developing PTSD following an stressful exposure has been previously described to be mediated by preexisting psychopathology, including panic or generalized anxiety disorder (Koenen et al., 2002).

PTSD offers unique opportunities to study G x E in psychiatry (Koenen et al., 2008, 2009b) but also enormous challenges. This mental disorder has been considered a complex and heterogeneous phenotype composed of a combination of symptom dimensions potentially influenced by different G x G, G x E and/or E x E interactions (Smoller, 2016; Yehuda et al., 2015), and by even more complex interaction models (e.g. G x G x E or G x E x E interactions) (Mehta and Binder, 2012). An important limitation of PTSD studies is the great heterogeneity in the index trauma analyzed as it influences the consequent PTSD conditional risk (Kessler et al., 2018, 2014). The study of PTSD after the exposure to an earthquake has, at least, two advantages: i) it limits the variability in the index trauma exposure, and ii) it reduces the possibility of a gene-by-environment correlation (rGE), where the genetic variants influencing the disorder risk may act through the effect of exposure to index traumas (Koenen et al., 2008; Mehta and Binder, 2012). Research on psychological consequences after an earthquake is extremely complicated due to the specific difficulties inherent to the complex logistic organization needed to start a survey after an unpredicted disaster (Kessler et al., 2008). Besides, a high heterogeneity has been found in published literature related to characteristics: i) of the disaster exposure, such as the type of trauma, timing, intensity and/or duration of the trauma; ii) of the exposed population, including the region/country affected and group-level environmental factors, such as social support or rates of crime and unemployment; and iii) different methodological issues, such as study design, power and sample size and diagnostic instruments selected (Bromet and Dew, 1995; Galea et al., 2008; Kilpatrick et al., 2007; Koenen et al., 2009a, 2008).

Several strengths of the study should be highlighted. First, the study was performed in a representative sample of the general population from Lorca (F. Navarro-Mateu et al., 2013; Navarro-Mateu et al., 2017). The target population was selected before the earthquake took place in Lorca with a response rate of 71% of participants, above the 60% conventionally considered as a minimum standard in general population surveys (Johnson and Wislar, 2012). There were only slight differences (mean age and employment status) between those

who provided biological samples (85%) and those who declined (16.5%), and these did not seem to be related to genotype or disease status. Thus, the data seems to have a good external validity. Second, a careful evaluation of the prior history of mental disorders in combination to other sociodemographic variables allowed a comprehensive control for potential confounders. This is more important in the context of shared genetic effects between PTSD and other disorders (Duncan et al., 2018). Finally, both, the "triallelic" and "biallelic" approaches were considered in the analyses. Nevertheless, some limitations deserve careful consideration. First, DSM-IV diagnoses of PTSD and prior lifetime mental disorders were based on a fully structured lay-administered interview, the CIDI 3.0, rather than semi-structured clinical interviews. However, this has been evaluated with a moderate-to-excellent concordance with diagnosis of most mental disorders (Haro et al., 2006; Kessler et al., 2004) and the instrument has been widely used in epidemiologic surveys of general population all over the world (Kessler and Ustun, 2004). Second, some concerns about population stratification could be raised as the method used to evaluate ethnicity was not based on genetic markers but on the declared ethnicity. All statistical analyses were adjusted to control for the differences were found in the declared ethnicity. Third, other environment variables might have obscured other potential interactions, such as the history of childhood adversities (Kessler et al., 2010; Walsh et al., 2014; Zhao et al., 2017), previous stressful life events (Kessler et al., 2018), specific personality traits (e.g. neuroticism and/or resilience) (Aburn et al., 2016; Munafo et al., 2006) or epigenetic mechanisms such as the level of methylation (Koenen et al., 2011). Future studies should explore their modulation effect as this is the first genetic analysis in our sample. Fourth, the cross-sectional design limits the interpretation of the associations found being causal and might explain the heterogeneity of results. More longitudinal designs (Li et al., 2019) are needed. Finally, the relatively small sample size and the investigation of a number of interactions raise the possibility that the observed significant interactions might fail to replicate in future studies, although the broad consistency of these interactions with the results of other studies suggests that they might be stable. Future research would be needed to investigate this issue.

The risk of developing PTSD after the exposure to the earthquakes of Lorca is influenced by the previous history of PTSD and the emotional impact of the experience and the previous history of anxiety disorders are modulated by the *5-HTTLPR* genotype. Therapeutic interventions to prevent mental disorders after the exposure to earthquakes should be focused on people at higher risk and our study shows that this should particularly include those with a higher direct exposure to the natural disaster and those with a prior history of mental disorders. Nowadays, to identify genetic biomarkers that would allow to distinguish between persons at high or low risk of developing PTSD after trauma exposure, more independent replicative studies with larger samples, future up-date or new meta-analysis with the *5-HTTLPR* or other candidate genes, and large genome-wide association (GWAS) studies are warranted to improve our knowledge of the etio-pathogenesis of the PTSD phenotype (Koenen et al., 2008; Logue et al., 2015).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Aburn G, Gott M, Hoare K, 2016 What is resilience? An Integrative Review of the empirical literature. J. Adv. Nurs 72, 980–1000. 10.1111/jan.12888 [PubMed: 26748456]

Anwar J, Mpofu E, Matthews LR, Brock KE, 2013 Risk factors of posttraumatic stress disorder after an earthquake disaster. J.Nerv.Ment.Dis 201, 1045–1052. [PubMed: 24284639]

Bartholdson S, von Schreeb J, 2018 Natural Disasters and Injuries: What Does a Surgeon Need to Know? Curr. Trauma Rep 4, 103–108. 10.1007/s40719-018-0125-3 [PubMed: 29888166]

Bender R, Lange S, 2001 Adjusting for multiple testing--when and how? J. Clin. Epidemiol 54, 343–349. [PubMed: 11297884]

Benjet C, Bromet E, Karam EG, Kessler RC, McLaughlin KA, Ruscio AM, Shahly V, Stein DJ, Petukhova M, Hill E, Alonso J, Atwoli L, Bunting B, Bruffaerts R, Caldas-de-Almeida JM, de Girolamo G, Florescu S, Gureje O, Huang Y, Lepine JP, Kawakami N, Kovess-Masfety V, Medina-Mora ME, Navarro-Mateu F, Piazza M, Posada-Villa J, Scott KM, Shalev A, Slade T, Ten Have M, Torres Y, Viana MC, Zarkov Z, Koenen KC, 2015 The epidemiology of traumatic event exposure worldwide: results from the World Mental Health Survey Consortium. Psychol. Med 1–17. 10.1017/S0033291715001981

Brewin CR, Andrews B, Valentine JD, 2000 Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. JConsult Clin Psychol 68, 748–766. [PubMed: 11068961]

Brewin CR, Andrews B, Valentine JD, 2000 Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. J. Consult. Clin. Psychol 68, 748–766. [PubMed: 11068961]

Bromet E, Dew MA, 1995 Review of psychiatric epidemiologic research on disasters. Epidemiol.Rev 17, 113–119. [PubMed: 8521929]

Bromet EJ, Atwoli L, Kawakami N, Navarro-Mateu F, Piotrowski P, King AJ, Aguilar-Gaxiola S, Alonso J, Bunting B, Demyttenaere K, Florescu S, de Girolamo G, Gluzman S, Haro JM, de Jonge P, Karam EG, Lee S, Kovess-Masfety V, Medina-Mora ME, Mneimneh Z, Pennell B-E, Posada-Villa J, Salmerón D, Takeshima T, Kessler RC, 2017 Post-traumatic stress disorder associated with natural and human-made disasters in the World Mental Health Surveys. Psychol. Med 47, 227–241. 10.1017/S0033291716002026 [PubMed: 27573281]

Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE, 2010 Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am.J.Psychiatry 167, 509–527. 10.1176/appi.ajp.2010.09101452 [PubMed: 20231323]

Cheng Y, Wang F, Wen J, Shi Y, 2014 Risk factors of post-traumatic stress disorder (PTSD) after Wenchuan earthquake: a case control study. PLoS.One 9, e96644. [PubMed: 24800944]

Dai W, Chen L, Lai Z, Li Y, Wang J, Liu A, 2016 The incidence of post-traumatic stress disorder among survivors after earthquakes:a systematic review and meta-analysis. BMC Psychiatry 16, 188 10.1186/s12888-016-0891-9 [PubMed: 27267874]

Duncan LE, Ratanatharathorn A, Aiello AE, Almli LM, Amstadter AB, Ashley-Koch AE, Baker DG, Beckham JC, Bierut LJ, Bisson J, Bradley B, Chen C-Y, Dalvie S, Farrer LA, Galea S, Garrett ME, Gelernter JE, Guffanti G, Hauser MA, Johnson EO, Kessler RC, Kimbrel NA, King A, Koen N, Kranzler HR, Logue MW, Maihofer AX, Martin AR, Miller MW, Morey RA, Nugent NR, Rice JP, Ripke S, Roberts AL, Saccone NL, Smoller JW, Stein DJ, Stein MB, Sumner JA, Uddin M, Ursano RJ, Wildman DE, Yehuda R, Zhao H, Daly MJ, Liberzon I, Ressler KJ, Nievergelt CM, Koenen KC, 2018 Largest GWAS of PTSD (N=20 070) yields genetic overlap with schizophrenia and sex differences in heritability. Mol. Psychiatry 23, 666–673. 10.1038/mp.2017.77 [PubMed: 28439101]

Galea S, Maxwell AR, Norris F, 2008 Sampling and design challenges in studying the mental health consequences of disasters. IntJMethods PsychiatrRes 17 Suppl 2:S21–8. doi: 10.1002/mpr.267., S21–S28.

Galea S, Nandi A, Vlahov D, 2005 The epidemiology of post-traumatic stress disorder after disasters. Epidemiol Rev 27, 78–91. [PubMed: 15958429]

Grabe HJ, Spitzer C, Schwahn C, Marcinek A, Frahnow A, Barnow S, Lucht M, Freyberger HJ, John U, Wallaschofski H, Volzke H, Rosskopf D, 2009 Serotonin transporter gene (SLC6A4) promoter polymorphisms and the susceptibility to posttraumatic stress disorder in the general population. Am.J.Psychiatry 166, 926–933. 10.1176/appi.ajp.2009.08101542 [PubMed: 19487392]

Gressier F, Calati R, Balestri M, Marsano A, Alberti S, Antypa N, Serretti A, 2013 The 5-HTTLPR polymorphism and posttraumatic stress disorder: a meta-analysis. JTrauma Stress 26, 645–653. 10.1002/jts.21855 [PubMed: 24222274]

Haro JM, Arbabzadeh-Bouchez S, Bugha T, De Girolamo G, Guyer M, Jin R, Lepine JP, Mazzi F, Reneses B, Vilagut G, Sampson N, Kessler RC, 2006 Concordance of the Composite International Diagnostic Interview Version 3.0 (CIDI 3.0) with standardized clinical assessments in the WHO World Mental Health Surveys. IntJ Methods PsychiatrRes 15, 167–180.

Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA, 2005 An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol ClinExpRes 29, 8–16.

Johnson TP, Wislar JS, 2012 Response rates and nonresponse errors in surveys. JAMA 307, 1805–1806. 10.1001/jama.2012.3532 [PubMed: 22550194]

Kessler RC, Abelson J, Demler O, Escobar JI, Gibbon M, Guyer ME, Howes MJ, Jin R, Vega WA, Walters EE, Wang P, Zaslavsky A, Zheng H, 2004 Clinical calibration of DSM-IV diagnoses in the World Mental Health (WMH) version of the World Health Organization (WHO) Composite International Diagnostic Interview (WMHCIDI). IntJMethods PsychiatrRes 13, 122–139.

Kessler RC, Aguilar-Gaxiola S, Alonso J, Benjet C, Bromet EJ, Cardoso G, Degenhardt L, de Girolamo G, Dinolova RV, Ferry F, Florescu S, Gureje O, Haro JM, Huang Y, Karam EG, Kawakami N, Lee S, Lepine J-P, Levinson D, Navarro-Mateu F, Pennell B-E, Piazza M, Posada-Villa J, Scott KM, Stein DJ, Ten Have M, Torres Y, Viana MC, Petukhova MV, Sampson NA, Zaslavsky AM, Koenen KC, 2017 Trauma and PTSD in the WHO World Mental Health Surveys. Eur. J. Psychotraumatology 8, 1353383 10.1080/20008198.2017.1353383

Kessler RC, Aguilar-Gaxiola S, Alonso J, Bromet EJ, Gureje O, Karam EG, Koenen KC, Lee S, Liu H, Pennell B-E, Petukhova MV, Sampson NA, Shahly V, Stein DJ, Atwoli L, Borges G, Bunting B, de Girolamo G, Gluzman SF, Haro JM, Hinkov H, Kawakami N, Kovess-Masfety V, Navarro-Mateu F, Posada-Villa J, Scott KM, Shalev AY, ten Have M, Torres Y, Viana MC, Zaslavsky AM, 2018 The associations of earlier trauma exposures and history of mental disorders with PTSD after subsequent traumas. Mol. Psychiatry 23, 1–8. 10.1038/mp.2017.194

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE, 2005 Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch.Gen.Psychiatry 62, 593–602. 10.1001/archpsyc.62.6.593 [PubMed: 15939837]

Kessler RC, Keane TM, Ursano RJ, Mokdad A, Zaslavsky AM, 2008 Sample and design considerations in post-disaster mental health needs assessment tracking surveys. Int J Methods Psychiatr Res 17 Suppl 2:S6–S20., S6–S20. [PubMed: 19035440]

Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Aguilar-Gaxiola S, Alhamzawi AO, Alonso J, Angermeyer M, Benjet C, Bromet E, Chatterji S, Girolamo G. de, Demyttenaere K, Fayyad J, Florescu S, Gal G, Gureje O, Haro JM, Hu C, Karam EG, Kawakami N, Lee S, Lépine J-P, Ormel J, Posada-Villa J, Sagar R, Tsang A, Üstün TB, Vassilev S, Viana MC, Williams DR, 2010 Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. Br. J. Psychiatry 197, 378–385. 10.1192/bjp.bp.110.080499 [PubMed: 21037215]

Kessler RC, Rose S, Koenen KC, Karam EG, Stang PE, Stein DJ, Heeringa SG, Hill ED, Liberzon I, McLaughlin KA, McLean SA, Pennell BE, Petukhova M, Rosellini AJ, Ruscio AM, Shahly V, Shalev AY, Silove D, Zaslavsky AM, Angermeyer MC, Bromet EJ, de Almeida JM, De GG, de JP, Demyttenaere K, Florescu SE, Gureje O, Haro JM, Hinkov H, Kawakami N, Kovess-Masfety V, Lee S, Medina-Mora ME, Murphy SD, Navarro-Mateu F, Piazza M, Posada-Villa J, Scott K, Torres Y, Carmen VM, 2014 How well can post-traumatic stress disorder be predicted from pre-trauma risk factors? An exploratory study in the WHO World Mental Health Surveys. World Psychiatry 13, 265–274. [PubMed: 25273300]

Kessler RC, Ustun TB, 2004 The World Mental Health (WMH) Survey Initiative Version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI). IntJ Methods PsychiatrRes 13, 93–121.

Kilpatrick DG, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Resnick HS, Roitzsch J, Boyle J, Gelernter J, 2007 The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults. Am.J.Psychiatry 164, 1693–1699. 10.1176/appi.ajp.2007.06122007 [PubMed: 17974934]

Koenen KC, 2007 Genetics of posttraumatic stress disorder: Review and recommendations for future studies. JTrauma Stress 20, 737–750. 10.1002/jts.20205 [PubMed: 17955543]

Koenen KC, Aiello AE, Bakshis E, Amstadter AB, Ruggiero KJ, Acierno R, Kilpatrick DG, Gelernter J, Galea S, 2009a Modification of the association between serotonin transporter genotype and risk of posttraumatic stress disorder in adults by county-level social environment. Am. J. Epidemiol 169, 704–711. 10.1093/aje/kwn397 [PubMed: 19228812]

Koenen KC, Amstadter AB, Nugent NR, 2009b Gene-environment interaction in posttraumatic stress disorder: an update. J Trauma Stress 22, 416–426. [PubMed: 19743189]

Koenen KC, Harley R, Lyons MJ, Wolfe J, Simpson JC, Goldberg J, Eisen SA, Tsuang M, 2002 A twin registry study of familial and individual risk factors for trauma exposure and posttraumatic stress disorder. J. Nerv. Ment. Dis 190, 209–218. [PubMed: 11960081]

Koenen KC, Nugent NR, Amstadter AB, 2008 Gene-environment interaction in posttraumatic stress disorder: review, strategy and new directions for future research. EurArchPsychiatry Clin Neurosci 258, 82–96.

Koenen KC, Ratanatharathorn A, Ng L, McLaughlin KA, Bromet EJ, Stein DJ, Karam EG, Meron Ruscio A, Benjet C, Scott K, Atwoli L, Petukhova M, Lim CCW, Aguilar-Gaxiola S, Al-Hamzawi A, Alonso J, Bunting B, Ciutan M, de Girolamo G, Degenhardt L, Gureje O, Haro JM, Huang Y, Kawakami N, Lee S, Navarro-Mateu F, Pennell B-E, Piazza M, Sampson N, Ten Have M, Torres Y, C Viana M, Williams D, Xavier M, Kessler RC, 2017 Posttraumatic stress disorder in the World Mental Health Surveys. Psychol. Med 1–15. 10.1017/S0033291717000708

Koenen KC, Uddin M, Chang SC, Aiello AE, Wildman DE, Goldmann E, Galea S, 2011 SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. Depress.Anxiety 28, 639–647. [PubMed: 21608084]

Kolassa IT, Ertl V, Eckart C, Glockner F, Kolassa S, Papassotiropoulos A, de Quervain DJ, Elbert T, 2010 Association study of trauma load and SLC6A4 promoter polymorphism in posttraumatic stress disorder: evidence from survivors of the Rwandan genocide. J. Clin. Psychiatry 71, 543–547. 10.4088/JCP.08m04787blu [PubMed: 20441718]

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL, 1996 Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 274, 1527–1531. [PubMed: 8929413]

Li D, He L, 2007 Meta-analysis supports association between serotonin transporter (5-HTT) and suicidal behavior. Mol.Psychiatry 12, 47–54. [PubMed: 16969368]

Li G, Wang L, Cao C, Fang R, Hall BJ, Elhai JD, Liberzon I, 2019 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. Int. J. Psychol 10.1002/ijop.12614

Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von EE, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van DC, McLaughlin J, Paterson A, Wells G, Fortier I, Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N, 2009 STrengthening the REporting of Genetic Association studies (STREGA): an extension of the STROBE Statement. Ann.Intern.Med 150, 206–215. [PubMed: 19189911]

Liu L, Wang L, Cao C, Cao X, Zhu Y, Liu P, Luo S, Zhang J, 2017 Serotonin transporter 5-HTTLPR genotype is associated with intrusion and avoidance symptoms of DSM-5 posttraumatic stress disorder (PTSD) in Chinese earthquake survivors. Anxiety Stress Coping 1–10. 10.1080/10615806.2017.1420174

Logue MW, Amstadter AB, Baker DG, Duncan L, Koenen KC, Liberzon I, Miller MW, Morey RA, Nievergelt CM, Ressler KJ, Smith AK, Smoller JW, Stein MB, Sumner JA, Uddin M, 2015 The Psychiatric Genomics Consortium Posttraumatic Stress Disorder Workgroup: Posttraumatic Stress Disorder Enters the Age of Large-Scale Genomic Collaboration. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol 40, 2287–2297. 10.1038/npp.2015.118

Lotrich F, Pollock BG, 2004 Meta-analysis of serotonin transporter polymorphisms and affective disorders. Psychiatr.Genet 14, 121–129. [PubMed: 15318024]

Mehta D, Binder EB, 2012 Gene × environment vulnerability factors for PTSD: the HPA-axis. Neuropharmacology 62, 654–662. 10.1016/j.neuropharm.2011.03.009 [PubMed: 21439305]

Mellman TA, Alim T, Brown DD, Gorodetsky E, Buzas B, Lawson WB, Goldman D, Charney DS, 2009 Serotonin polymorphisms and posttraumatic stress disorder in a trauma exposed African American population. Depress.Anxiety 26, 993–997. [PubMed: 19842167]

Munafo MR, Clark TG, Roberts KH, Johnstone EC, 2006 Neuroticism mediates the association of the serotonin transporter gene with lifetime major depression. Neuropsychobiology. 53, 1–8. [PubMed: 16319503]

Nakamura M, Ueno S, Sano A, Tanabe H, 2000 The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. Mol.Psychiatry 5, 32–38. [PubMed: 10673766]

Navarro-Mateu Fernando, Escámez T, Koenen KC, Alonso J, Sánchez-Meca J, 2013 Meta-Analyses of the 5-HTTLPR Polymorphisms and Post-Traumatic Stress Disorder. PLOS One 8, e66227 10.1371/journal.pone.0066227 [PubMed: 23825531]

Navarro-Mateu F, Moran-Sanchez I, Alonso J, Tormo MJ, Pujalte MA, Garriga A, Aguilar-Gaxiola S, Navarro C, 2012 Cultural adaptation of the Latin American version of the World Health Organization Composite International Diagnostic Interview (WHO-CIDI) (v 3.0) for use in Spain. Gac.Sanit 27, 325–331. 10.1016/j.gaceta.2012.06.005 [PubMed: 22842058]

Navarro-Mateu F, Salmerón D, Vilagut G, Tormo MJ, Ruíz-Merino G, Escámez T, Júdez J, Martínez S, Koenen KC, Navarro C, Alonso J, Kessler RC, 2017 Post-Traumatic Stress Disorder and other mental disorders in the general population after Lorca's earthquakes, 2011 (Murcia, Spain): A cross-sectional study. PloS One 12, e0179690 10.1371/journal.pone.0179690 [PubMed: 28723949]

Navarro-Mateu F, Tormo M, Vilagut G, Alonso J, Ruiz-Merino G, Escamez T, Salmeron D, Judez J, Martinez S, Navarro C, 2013 Epidemiology and genetics of common mental disorders in the general population: the PEGASUS-Murcia project. BMJ Open 3, e004035 10.1136/bmjopen-2013-004035

Navarro-Mateu F, Tormo MJ, Salmeron D, Vilagut G, Navarro C, Ruiz-Merino G, Escamez T, Judez J, Martinez S, Kessler RC, Alonso J, 2015 Prevalence of Mental Disorders in the South-East of Spain, One of the European Regions Most Affected by the Economic Crisis: The Cross-Sectional PEGASUS-Murcia Project. PLOS One 10, e0137293 10.1371/journal.pone.0137293 [PubMed: 26394150]

Neria Y, Nandi A, Galea S, 2008 Post-traumatic stress disorder following disasters: a systematic review. Psychol. Med 38, 467–480. 10.1017/S0033291707001353 [PubMed: 17803838]

Norris FH, Friedman MJ, Watson PJ, 2002a 60,000 disaster victims speak: Part II. Summary and implications of the disaster mental health research. Psychiatry 65, 240–260. [PubMed: 12405080]

Norris FH, Friedman MJ, Watson PJ, Byrne CM, Diaz E, Kaniasty K, 2002b 60,000 disaster victims speak: Part I. An empirical review of the empirical literature, 1981–2001. Psychiatry 65, 207–239. [PubMed: 12405079]

Parsey RV, Hastings RS, Oquendo MA, Hu X, Goldman D, Huang Yung yu, Simpson N, Arcement J, Huang Yiyun, Ogden RT, Van Heertum RL, Arango V, Mann JJ, 2006 Effect of a Triallelic Functional Polymorphism of the Serotonin-Transporter-Linked Promoter Region on Expression of Serotonin Transporter in the Human Brain. Am. J. Psychiatry 163, 48–51. [PubMed: 16390888]

Rothman KJ, 1990 No adjustments are needed for multiple comparisons. Epidemiol. Camb. Mass 1, 43–46.

Silove D, Steel Z, 2006 Understanding community psychosocial needs after disasters: implications for mental health services. J PostgradMed 52, 121–125.

Smoller JW, 2016 The Genetics of Stress-Related Disorders: PTSD, Depression, and Anxiety Disorders. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol 41, 297–319. 10.1038/npp.2015.266

Takano A, Arakawa R, Hayashi M, Takahashi H, Ito H, Suhara T, 2007 Relationship between neuroticism personality trait and serotonin transporter binding. Biol.Psychiatry 62, 588–592. [PubMed: 17336939]

Thakur GA, Joober R, Brunet A, 2009 Development and persistence of posttraumatic stress disorder and the 5-HTTLPR polymorphism. J Trauma Stress 22, 240–243. [PubMed: 19444877]

The World Mental Health Survey Initiative, 2015 URL http://www.hcp.med.harvard.edu/wmhcidi/index.php (accessed 11.8.14).

Tian Y, Liu H, Guse L, Wong TKS, Li J, Bai Y, Jiang X, 2015 Association of Genetic Factors and Gene-Environment Interactions With Risk of Developing Posttraumatic Stress Disorder in a Case-Control Study. Biol. Res. Nurs 17, 364–372. 10.1177/1099800415588362 [PubMed: 26002549]

Walsh K, Uddin M, Soliven R, Wildman DE, Bradley B, 2014 Associations between the SS variant of 5-HTTLPR and PTSD among adults with histories of childhood emotional abuse: results from two African American independent samples. J. Affect. Disord 161, 91–96. 10.1016/j.jad.2014.02.043 [PubMed: 24751314]

Woud ML, Verwoerd J, Krans J, 2017 Modification of cognitive biases related to posttraumatic stress: A systematic review and research agenda. Clin. Psychol. Rev 54, 81–95. 10.1016/j.cpr.2017.04.003 [PubMed: 28445840]

Yehuda R, Hoge CW, McFarlane AC, Vermetten E, Lanius RA, Nievergelt CM, Hobfoll SE, Koenen KC, Neylan TC, Hyman SE, 2015 Post-traumatic stress disorder. Nat. Rev. Dis. Primer 1, 15057 10.1038/nrdp.2015.57

Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, Ellis SP, Goldman D, Mann JJ, 2006 Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. Am J Psychiatry 163, 1588–1593. [PubMed: 16946185]

Zhao M, Yang J, Wang W, Ma J, Zhang J, Zhao X, Qiu X, Yang X, Qiao Z, Song X, Wang L, Jiang S, Zhao E, Yang Y, 2017 Meta-analysis of the interaction between serotonin transporter promoter variant, stress, and posttraumatic stress disorder. Sci. Rep 7, 16532 10.1038/s41598-017-15168-0 [PubMed: 29184054]

Table 1:

Demographic, prior lifetime history of mental disorders, earthquake-related variables and triallelic and biallelic genotypes frequencies of Study participants by 12-month Post-traumatic Stress Disorder (PTSD) status.

Variables		No 12 PTSD N (%)	12m PTSD N (%)	p-value [#]
Sex				
	Male	154 (47.5)	4 (23.5)	
	Female	170 (52.5)	13 (76.5)	0.053
Age (Mean, SD)		47.22 (16.332)	48.00 (14.794)	0.85
Ethnicity				
	White/Caucasic	293 (90.4)	12 (70.6)	
	Not white/caucasic	31 (9.6)	5 (29.4)	**0.02**[&]
Marital status				
	Living with a partner	244 (75.3)	12 (70.6)	
	Not living with a partner	80 (24.7)	5 (29.4)	0.66
Education				
	Secondary or higher	123 (38.0)	3 (17.6)	
	Primary or lower	201 (62.0)	14 (82.4)	0.09
Employment				
	Working	172 (53.1)	4 (23.5)	
	Not working	152 (46.9)	13 (76.54)	**0.02**
Any prior lifetime PTSD		4 (1.2)	8 (47.1)	**< 0.005**
Number of any prior lifetime other anxiety disorders (Mean, SD)		0.10 (0.350)	0.53 (0.800)	**0.04**
Number of Any prior lifetime mood disorder (Mean, SD)		0.12 (0.341)	0.47 (0.514)	**0.01**
Number of any prior substance disorder (Mean, SD)		0.05 (0.274)	0.18 (0.529)	0.33
Number of any prior lifetime mental disorders (Mean, SD)		0.27 (0.677)	1.18 (1.334)	**0.01**
High Earthquake Exposure (HEE) [†]		154 (59.5)	13 (81.2)	**0.01**
High Emotional Impact (HEI) [‡]		189 (57.1)	14 (87.5)	**0.02**
Triallelic classification				
	S'S'	108 (33.3)	5 (29.4)	
	S'L'	154 (47.5)	7 (41.2)	
	L'L'	62 (19.1)	5 (29.4)	0.58
Biallelic classification				
	SS	96 (29.6)	5 (29.4)	
	SL	160 (49.4)	7 (41.2)	
	LL	68 (21.0)	5 (29.4)	0.68

[#] Chi-squared or Student-T test for categoric or continuous mesures respectivelly.

[‡] **Include:** student, homemaker, retired/disabled, unemployed and others diferent from working.

[†] **High Earthquake Exposure (HEE):** defined as those with an exposure score above the mean rate of the total sample;

‡**High Emòtional Impact (HEI):** defined as those with an Emotional impact above the mean rate of the total sample;

&**Fisher's exact test**

Table 2:

Association between of risk factors and analyses of gene-environment interactions on the risk of 12-month Post-traumatic Stress Disorder (PTSD) status after the exposure to Lorca's earthquakes, 2011 with a triallelic *5-HTTLPR* genotype approach.

	Model M1	Model M2	Model M3	Model M4 Interaction 1	Model M5 Interaction 2
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
High Earthquake Exposure (HEE) †	**4.42 (1.23 ; 15.81)** *	5.11 (0.86; 30.24)	4.59 (0.76; 27.42)	6.06 (0.85; 43.02)	3.61 (0.62; 21.01)
High Emotional Impact (HEI) ‡	**4.67 (1.04 ; 20.88)** *	3.07 (0.49; 19.21)	3.28 (0.48; 22.20)	0.17 (0.01; 2.43)	3.62 (0.48; 27.32)
Any prior lifetime PTSD	**71.11 (18.05; 280.10)** *	**70.38 (9.50; 521.58)** *	**62.67 (8.60; 456.70)** *	**176.49 (13.44; 2317.89)** *	**71.50 (7.41; 690.09)** *
Number of any prior lifetime other anxiety disorders	**3.74 (1.81 ; 7.76)** *	1.29 (0.26; 6.35)	1.21 (0.28; 5.27)	1.20 (0.26; 5.44)	0.01 (0.00; 1.37)
Number of any prior lifetime mood disorder	**4.97 (2.01 ; 12.28)** *	1.47 (0.35; 6.14)	1.65 (0.38; 7.15)	1.65 (0.41; 6.65)	1.81 (0.35; 9.42)
Number of any prior substance disorder	2.27 (0.85 ; 6.06)	0.80 (0.14; 4.67)	0.98 (0.17; 5.71)	0.82 (0.12; 5.51)	0.78 (0.09; 6.70)
Number of *Ĺ* alleles	1.32 (0.67 ; 2.60)	-	1.81 (0.65; 5.02)	0.12 (0.01; 1.42)	3.20 (0.91; 11.18)
Interaction: High Emotional Impact (HEI)-by-Number of *Ĺ* alleles	-	-	-	**33.83 (1.74; 657.86)** *	-
Interaction: OtherAnxiety-by-Number of *Ĺ* alleles	-	-	-	-	**45.69 (1.737; 1202.04)** *

Model M1: each row represents a simple logistic regression model with 12-month PTSD as the dependent variable.

Model M2: M1 adjusted by sociodemographic variables (student, homemaker, retired/disabled, unemployed and others diferent from working) and prior mental disorders.

Model M3: the number of *Ĺ* alleles is included in M2

Model M4 Interaction 1: M3 including the interaction term created with the product of HEI and the number of *Ĺ* alleles.

Model M5 Interaction 2 : M3 including the interaction term between the number of *Ĺ* alleles and the number of any prior lifetime other anxiety disorders

†**High Earthquake Exposure (HEE):** defined as those with an exposure score above the mean rate of the total sample.

‡**High Emotional Impact (HEI):** defined as those with an Emotional impact above the mean rate of the total sample.

*p-value < 0.05.