Research report

Association of TPH1, TPH2, and 5HTTLPR with PTSD and depressive symptoms

Armen K. Goenjian a,b,e,⁎, Julia N. Bailey c,d, David P. Walling b, Alan M. Steinberg a, Devon Schmidt b, Uma Dandekar d, Ernest P. Noble e

a UCLA/Duke University National Center for Child Traumatic Stress, Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles (UCLA), United States
b Collaborative Neuroscience Network, Garden Grove, CA 92845, United States
c Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, United States
d Epilepsy Genetics/Genomics Laboratories, VA GLAHS, Los Angeles, CA, United States
e Alcohol Research Center, Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, United States

Objective: To examine the potential contribution of the serotonin hydroxylase (TPH1 and TPH2) genes, and the serotonin transporter promoter polymorphism (5HTTLPR) to the unique and pleiotropic risk of PTSD symptoms and depressive symptoms.

Methods: Participants included 200 adults exposed to the 1988 Spitak earthquake from 12 multigenerational families (3 to 5 generations). Severity of trauma exposure, PTSD, and depressive symptoms were assessed using standard psychometric instruments. Pedigree-based variance component analysis was used to assess the association between select genes and the phenotypes.

Results: After adjusting for age, sex, exposure and environmental variables, there was a significant association of PTSD symptoms with the ‘t’ allele of TPH1 SNP rs2108977 (p<0.004), explaining 3% of the phenotypic variance. This allele also showed a non-significant trend for an association with depressive symptoms (p=0.08). Also, there was a significant association of PTSD symptoms and the ‘t’ allele of TPH2 SNP rs11178997 (p=0.03), explaining 4% of the variance. Depressive symptoms were significantly associated with the ‘s’ allele of 5HTTLPR (p=0.03), explaining 4% of the variance.

Limitations: Retrospective rating of exposure may have been subject to memory failure leading to misestimation of symptom severities. Second, findings may not be generalizable to other ethnic/racial populations.

Conclusion: To our knowledge, this is the first published report showing that variants in TPH1 and TPH2 genes constitute risk factors for PTSD symptoms. Additionally, the TPH1 gene may be associated pleiotropically with PTSD and depressive symptoms. The association of the ‘s’ allele of 5HTTLPR polymorphism with depression adds to similar findings from case–case control studies.

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Keywords: Genetics, PTSD, Tryptophan hydroxylase, Depression, Serotonin transporter

1. Introduction

1.1. Comorbidity of PTSD and depression

Comorbidity of PTSD and depression has been documented in numerous psychiatric epidemiologic studies (for example, see Breslau et al., 1991; Kessler et al., 1995). In the National...
Comorbidity Survey (Kessler et al., 1995), affective disorders were two to three times more likely to occur among those with PTSD compared to those without PTSD. Longitudinal studies after the Spitak earthquake in Armenia have shown significant comorbidity of PTSD and depressive symptoms among survivors (Goenjian et al., 2000; 2005). And in a family study the same population, Goenjian et al. (2008) found a moderate heritability of PTSD and depressive symptoms and a high genetic correlation (0.71) between the two phenotypes. Likewise, Koenen et al. (2008) in a study among Vietnam War veterans, found a high genetic correlation (0.77) between PTSD and major depression. In the study among the Spitak earthquake survivors the genetic correlation was statistically significantly different from 0, indicating pleiotropy (shared genes between two phenotypes), and significantly less than 1, implying the presence of unique genes for each phenotype.

1.2. Psychiatric genetics and the serotonergic system

Association studies of candidate genes with a phenotype are performed to determine if a particular gene variant has a direct effect on risk for the phenotype or if it is tightly linked in linkage disequilibrium to the gene which has a direct effect. The serotonergic system has been shown to play a role in controlling, arousal, sleep, anxiety, and depression (Jacobs, 1991; Lesch et al., 1996; Owens and Nemeroff, 1998). Serotonin (5HT) has been the target of various types of antidepressants, including tricyclics and serotonin reuptake inhibitors. These medications have been effectively used to treat both depression and PTSD symptoms (Blier and de Montigny, 1994; Davidson, 2000). Tryptophan hydroxylase (TPH) gene and serotonin transporter (5HTT) gene (SLC64A), have been the focus of many mood disorder studies (Gizatullin et al., 2006; Karg et al., 2011; Munafò et al., 2009; Risch et al., 2009; Serretti et al., 2002; Sun et al., 2004; Tsai et al., 2009; Van Den Bogaert et al., 2006). The TPH gene encodes tryptophan, the rate limiting enzyme in 5HT synthesis (Arrango et al., 2003; Harvey et al., 2004). Tryptophan is converted into 5hydroxytryptohan (5HTP) by the enzyme TPH, which then is converted to 5HT. The two isomorphisms of this compound are TPH1 (gene located on chromosome 11) and TPH2 (gene located on chromosome 12). Both are expressed in several brain regions, including the hippocampus, amygdala, frontal cortex, and hypothalamus. TPH2 expression is absent in the peripheral tissues, whereas TPH 1 controls most of the peripheral 5HT synthesis (Walther and Bader, 2003; Zill et al., 2007).

To our knowledge, there are no published reports of the association of TPH and PTSD. With regard to depression, some studies have shown the TPH gene to be associated with depression (Table 1). However, these findings have not been replicated in other studies (Cusin et al., 2001; Serretti et al., 2002).

A gene that may be related to both PTSD and depression in the serotonergic system is the 5HTT gene. This gene has been of interest because depression has been associated with reduced serotonin transporter sites in platelets and various brain regions, including the amygdala, prefrontal cortex, hypothalamus, and brainstem (Malison et al., 1998). 5HTTLPR is a 44-bp insertion/deletion functional polymorphism of the 5HTT gene located in the promoter region. The two main alleles are the short ‘s’ allele and the long ‘l’ allele. It is thought that the ‘s’ allele impairs transcriptional activity of 5-HTT and lowers biological activity of the transporter (Lesch et al., 1996) thereby increasing the risk for depression. With regard to the association of 5HTTLPR with PTSD, findings have been mixed (Table 2). Likewise, results of studies that have evaluated the association between 5HTTLPR and depression have been equivocal (see Table 3).

In order to assess for pleiotropy, a study should include subjects with an adequate degree of co-morbid PTSD and depressive symptoms. Prior studies in this population have shown a significant genetic correlation of both phenotypes (Goenjian et al., 2008) making the present sample appropri-

Table 1

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Sample</th>
<th>TPH1</th>
<th>TPH1</th>
<th>TPH1</th>
<th>TPH1</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun et al.</td>
<td>2004</td>
<td>Case-Control (females)</td>
<td>Taiwan</td>
<td>rs1799913</td>
<td>rs1800532</td>
<td>rs2108977</td>
<td>rs11178997</td>
<td>rs1386494</td>
<td>rs4570625</td>
<td>rs4290270</td>
</tr>
<tr>
<td>Zill et al.</td>
<td>2004</td>
<td>Case-Control</td>
<td>Taiwan</td>
<td></td>
<td></td>
<td>Asso. w. comorbid</td>
<td>MD + AD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gizatullin et al.</td>
<td>2006</td>
<td>Case-Control</td>
<td>Germany</td>
<td></td>
<td></td>
<td>Asso. w.</td>
<td>MD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Den Bogaert et al.</td>
<td>2006</td>
<td>Case-Control</td>
<td>Sweden</td>
<td></td>
<td></td>
<td>Asso. w.</td>
<td>MD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jokela et al.</td>
<td>2007</td>
<td>Cohort</td>
<td>Finland</td>
<td></td>
<td></td>
<td>No main effect</td>
<td>G/E</td>
<td>effect w. dep. symptoms (BDI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsai et al.</td>
<td>2009</td>
<td>Case-Control</td>
<td>Taiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Asso. w. MD</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: Asso. w. = association with; AD = anxiety disorders; dep. = depression; G/E = gene/environment; UP = unipolar depression; MD = major depression; Aff. Prob. = affective problems: MDD, dysthymia, anxiety, ADHD. Blank cells indicate that the SNPs were not reported.
ate to test for candidate genes. The purpose of the present study was to assess the potential contribution of serotonergic candidate genes TPH1, TPH2, and 5HTTLPR to the genetic risk for these phenotypes, uniquely and pleiotropically.

2. Methods

2.1. Subjects

Subject recruitment for this study has been previously described (Goenjian et al., 2008). A total of 200 adults (121 females, 79 males) from 12 multigenerational families exposed to the Spitak earthquake in Armenia participated in the study which was conducted approximately 14 years post-earthquake. Families consisted of three to five generations. Both the mean and modal number of subjects per family were 16, and the range was between 13 and 19. Subjects belonged to the same ethnic/racial (Caucasian Armenian) and religious group and had similar socioeconomic backgrounds. After obtaining IRB approval, subjects were given a full description of the study, including potential risks and benefits. Written informed consent was obtained for all participating subjects.

2.2. Instruments

An earthquake exposure profile was completed for each participant using a modified version of a DSM IV-based exposure questionnaire previously utilized in studies after disasters (Roussos et al., 2005). This instrument included information on objective experiences during the earthquake including destruction of residence, deaths of relatives, seeing dead bodies, being injured, and seeing someone else who was injured. Participants were also evaluated for subjective experiences during the earthquake. These items included fear during the earthquake, fear of getting badly injured or dying, and fear that someone else would be badly hurt or killed. Additionally, the questionnaire included items relating to pre- and post-earthquake exposure to traumatic experiences, pre- and post-earthquake psychiatric treatment, post-earthquake medical illness/treatment by subject or family member, treatment for such illness, post-earthquake psychiatric illness/treatment by family members, and adversities including lack of heat, electricity, food and financial difficulties. These items were rated on a five-point Likert scale, ranging from 0 = not at all to 4 = a whole lot.

Posttraumatic stress symptoms were evaluated using the UCLA Posttraumatic Stress Disorder Reaction Index (PTSD-R), a 22-item self-report scale based on DSM-IV PTSD criteria (Steinberg et al., 2004). This instrument assesses categories A (Exposure); B (Re-experiencing); C (Avoidance); and D (Hyper-arousal) symptoms. Frequency of symptom occurrence during the previous month is rated on a 5-point Likert scale, ranging from 0 = not at all, to 4 = most of the time. Psychometric properties of this scale have been reported, with high internal consistency reliability and validity (Steinberg et al., 2004). Depressive symptoms were evaluated using the Beck Depression Inventory (BDI). Psychometric properties for the BDI have been reported previously (Beck and Steer, 1984).  

2.3. Genomic processing

Buccal cell samples were taken from each participant in Armenia and transported to the University of California at Los Angeles (UCLA). Genomic DNA was extracted employing standard techniques. Three TPH1 and five TPH2 SNPs were investigated in this study. The TPH1 SNPs, rs2108977, rs1799913 and 1800532, were chosen due to prior evidence showing association with: comorbid major depression and anxiety (Sun et al., 2004), major depression (Gizatullin et al., 2006) and depressive symptoms (Jokela et al., 2007). The TPH2 SNPs, rs11178997, rs1386494, rs4570625, rs4290270, and rs17110747, were included due to prior findings indicating association between the various SNPs and major depression or affective problems (Nobile et al., 2009; Tsai et al., 2009; Van Den Bogaert et al., 2006; Zill et al., 2004).

These SNPs were genotyped using a 5’ nuclease assay to discriminate between the two alleles. Applied Biosystems Inc Taqman SNP Genotyping Assays were used for genotyping of: TPH1 — rs1799913, rs1800532, rs2108977, and TPH2 — rs11178997, rs1386494, rs4570625, rs4290270, and rs17110747. Polymerase chain reactions (PCR) were

Table 2
List of published association studies of 5HTTLPR polymorphism (s and l alleles) with PTSD.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Sample</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al.</td>
<td>2005</td>
<td>Case/control, clinic population Korea</td>
<td>Main effect (s allele ↑ risk)</td>
</tr>
<tr>
<td>Kilpatrick et al.</td>
<td>2007</td>
<td>Population based cohort, hurricane exposed, USA</td>
<td>No main effect; G/E 3 way interaction, s allele ↑ risk</td>
</tr>
<tr>
<td>Xie et al.</td>
<td>2009</td>
<td>Case-control, substance dependent PTSD subjects, USA</td>
<td>No main effect; G/E effect (s allele ↑ risk)</td>
</tr>
<tr>
<td>Koenen et al.</td>
<td>2009</td>
<td>Population based cohort, hurricane exposed, USA</td>
<td>No main effect; G/E effect (s allele can ↑ or ↓ risk)</td>
</tr>
<tr>
<td>Grabe et al.</td>
<td>2009</td>
<td>Population based cohort, Germany</td>
<td>Main effect (l allele ↑ risk)</td>
</tr>
<tr>
<td>Thakur et al.</td>
<td>2009</td>
<td>Cohort, ER accident cases, Canada</td>
<td>Main effect (l allele ↑ risk)</td>
</tr>
<tr>
<td>Kolassa et al.</td>
<td>2010</td>
<td>Cohort, Refugees, Rwanda</td>
<td>Main effect (l allele ↑ risk)</td>
</tr>
</tbody>
</table>

Abbreviations: G/E = gene environment.

Table 3
Meta-analyses of 5HTTLPR (s’ and l’ allele) and depression.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Method</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lotrich and Pollock</td>
<td>2004</td>
<td>Meta-analysis</td>
<td>OR 1.16, s allele risk factor</td>
</tr>
<tr>
<td>Lasky-Su et al.</td>
<td>2005</td>
<td>Meta-analysis</td>
<td>OR 1.11, s allele risk factor</td>
</tr>
<tr>
<td>Lopez-Leon et al.</td>
<td>2008</td>
<td>Meta-analysis</td>
<td>OR 1.11, s allele risk factor</td>
</tr>
<tr>
<td>Risch et al.</td>
<td>2009</td>
<td>Meta-analysis</td>
<td>No association</td>
</tr>
<tr>
<td>Munafò et al.</td>
<td>2009</td>
<td>Meta-analysis</td>
<td>Negligible findings; attributable to chance</td>
</tr>
<tr>
<td>Karg et al.</td>
<td>2011</td>
<td>Meta-analysis</td>
<td>s allele risk factor</td>
</tr>
</tbody>
</table>

Abbreviation: OR = odds ratio.
performed using 5-μL reaction volumes in 384-well plates with 5 ng of DNA. The standard protocol provided with the kit using Taqman Genotyping Master mix was followed. End point reads of fluorescence levels were obtained with an ABI 7900HT Sequence Detection System.

The short 5-HTTLPR was identified using a protocol modified from Lesch et al. (1996). Briefly, the forward primer was 5′-ATGCCGACCTAACCCCTAATGT-3′ (labeled with 6-carboxyfluorescein fluorophore), and the reverse primer was 5′GGACCGCAAGTGGCCGGA-3′, which yielded ampli- cons of 419 (l) or 376 (s) bp. The assay we used for the 5HTTLPR (Anchordoquy et al., 2003) is a modification of the method of Lesch et al. (1996). The primer sequences are from Gelenter et al. (1999). PCR was performed in a total volume of 20 μL, containing 100 ng of DNA; 10 nM of each primer; 1x buffer; 2 mM MgCl2; 10% DMSO (v/v); 2 U Ampli-taq Gold DNA polymerase (Applied Biosystems, Foster City, California); 200 μM of dATP, dCTP, and dTTP; 100 μM of dGTP; and 7-deaza-2′-dGTP. Cycling conditions consisted of 1) an initial 12 min denaturation at 94 °C; 2) touchdown from 65 °C to 55 °C decreasing by 0.5 °C per cycle with dena- turation for 30 s at 94 °C; varied annealing temperatures con- sisting of 30 s at 66 °C (2 cycles), then 65 °C (3 cycles), then 64 °C (3 cycles), followed by hybridization for 1 min at 72 °C; 3) 35 cycles with an annealing temperature of 63 °C and the same denaturation and hybridization parameters; and 4) a final extension for 20 min at 72 °C. The PCR products were electrophoresed on an ABI 3730 DNA analyzer (Applied Biosystems) with GeneScan LIZ500 size standard (Applied Biosystems). Data collection and analysis used GeneScan and Genemapper software (Applied Biosystems). Ambiguous genotypes were retyped. The call rates of genotypes were sat- isfactory, ranging between 88 and 100%. All PCR and geno- typing were done at UCLA GenoSeq Core.

2.4. Statistical analyses

Statistical genetic modeling was performed using a pedigree-based association method (Almasy and Blangero, 1998; Almasy et al., 2005; Amos, 1994) as implemented in SOLAR (Blangero and Almasy, 1996). This method has been used to analyze data in numerous psychiatric behavioral ge- netic family studies, e.g. Gur et al. (2007); Almasy et al. (2008); Carless et al. (2011). This quantitative genetic meth- od decomposes the phenotypic variance of a trait into its ad- ditive genetic and non-genetic components. This analysis includes a component for the candidate gene polymorphism in the general model for genetic and environmental effects individually on the quantitative PTSD and depression traits. The expected genetic DNA sharing between each relative pair is incorporated into the model via a kinship matrix times a variance component. Estimations of all the param- eters are obtained simultaneously using maximum likelihood methods. Nested models are then tested to determine signific- ance of parameters. For example, the likelihood of the models with and without the candidate gene component is compared to determine if the candidate gene polymorphism explains a significant portion of the expression of PTSD or de- pressive symptoms. Likewise, a component that includes the identical by descent (ibd) sharing of the marker can be incorporated into the model to test for co-segregation of the marker and trait or linkage.

We estimated that in this sample we have 80% power to detect variants that account for 4.6% of the trait variance and are in strong linkage disequilibrium (LD) with one of the genotyped markers. This calculation used a p-value of 0.025, which is equivalent to correcting the standard marginal p-value of 0.05 for two tests. For a variant that has an r-squared (the amount of linkage in the disequilibrium) of at least 0.85 with a genotyped marker, we reach 80% power accounting for 5.4% of the trait variance. On the basis of these calculations, genetic associations that account for less of the trait variations may not be detected because of the sample size.

Power of our current sample to detect a gene through linkage can be estimated by simulating genetic markers, assu- ming that the phenotype is influenced by a single bi- allelic quantitative trait locus (QTL) with a residual additive effect due to polygenes and that fully informative marker ge- notype data will be available. Simulations were performed using SOLAR (Almasy et al., 1998). We had 80% power to de- tect a lod score of 1 with a QTL single gene heritability over 0.3, and a lod score of 3 with a QTL heritability over 0.4. This means that we could detect a gene for PTSD in our cur- rent sample if one gene explained most of the 42% heritability.

3. Results

Subjects in this study were exposed to the severe destruc- tion, morbidity, and mortality of the Spitak earthquake, with a mean exposure score for the subjective experiences of PTSD (severe). With regard to their objective experiences during the earthquake, all subjects experienced the tremor and witnessed the destruction 90% saw dead bodies lying in the streets, and 92% saw people who were severely injured. The mean PTSD-RI score was 33.9 ± 13.2, with a range of 4–65. The mean BDI score was 24.4 ± 6.7 with a range of 12–44.

Covariates significantly associated with severity of PTSD symptoms included the following: sex (females had higher scores); witnessing death during the earthquake or its after- math; and exposure to pre-earthquake traumatic experi- ences. Covariates that were significantly associated with severity of depressive symptoms included: sex (females had higher scores); age (older subjects had higher scores); pre-earthquake traumatic experiences; and death of a family member. For the pleiotropic analyses, we adjusted each individ- ual outcome for their significant covariates prior to per- forming the bi-variate analyses.

After adjustment for sex, age, exposure and environmen- tal variables including adversities, the heritability rate for PTSD and depressive symptoms was respectively 0.41 and 0.73 (Table 4). The genetic correlation (ρg) of PTSD symp- toms with depressive symptoms was 0.71 (Table 4). This being significantly greater than 0 (ρg>0; p<0.0006) indicates that some genes are shared, and being significantly less than 1 (ρg<1; p<0.0001) indicates that not all genes are shared. Fig. 1 shows the scatter-gram of PTSD-RI and BDI scores. The Pearson correlation of PTSD-RI and BDI scores was r = 0.55. Since these were related individuals, their
phenotypic correlation does not account for the genetic relatedness.

TPH1 (Table 5): After adjusting for age, sex, exposure and environmental variables, the family based variance component analyses revealed a significant association of PTSD-RI with the ‘t’ allele of TPH1 SNP rs2108977 ($\chi^2 = 8.44; \text{df}=1; p<0.004$). This gene explained 3% of the variance in PTSD-RI scores. Linkage analysis of PTSD-RI scores of TPH1 rs2108977 revealed a lod score of 1.05. With regard to BDI and rs2108977, there was a trend ($p=0.08$) indicating more severe depressive symptoms among those with the ‘t’ allele.

TPH2 (Table 5): After adjusting for age, sex, exposure and environmental variables, analyses indicated a significant association of PTSD-RI and the ‘t’ allele of TPH2 SNP rs11178997 ($\chi^2 = 3.87; \text{df}=1; p=0.03$). This gene explained 4% of the variance of PTSD-RI scores. There was no association between rs11178997 and BDI scores. The remainder of the TPH1 and TPH2 SNPs tested did not show a significant association with both PTSD-RI and BDI scores; and the linkage scores were below 1.0.

5HTTLPR (Table 5): After adjusting for age, sex, exposure and environmental variables, analyses indicated a significant association of PTSD-RI and the ‘s’ allele of 5HTTLPR ($\chi^2 = 4.54; \text{df}=1; p=0.03$). This allele explained 4% of the variance of BDI scores. There was no significant association of either ‘s’ or ‘l’ allele and PTSD-RI scores.

4. Discussion

The aim of the present study was to determine the extent to which the observed comorbidity of PTSD and depressive symptoms and high genetic correlation ($\rho_g = 0.71$) between the two phenotypes among subjects exposed to the 1988 Spitak earthquake in Armenia was due to separate or similar candidate genes of the serotonergic system. The study differed from prior candidate gene studies that have used case or case–control designs in that the subjects for the present study were members of multigenerational (3 to 5 generations) families. The two main findings were the association of PTSD symptoms with TPH1 SNP rs2108977 ($p<0.004$), and TPH2 SNP rs11178997 ($p=0.03$). To our knowledge, this is the first published report showing the association of TPH genes with PTSD symptoms. The family study design allowed determination of the proportion of variance of PTSD symptoms due to the TPH1 gene (3%) and the TPH2 gene (4%).

Scheuch et al. (2007) found that rs11178997 reduced TPH2 transcriptional activity in neurons from rat raphe nuclei and in human small cell lung carcinoma cells. A possible mechanism underlying the present findings is that these alleles reduce the transcriptional activity of the two TPH enzymes, thereby reducing the production of intracellular serotonin resulting in vulnerability to PTSD symptoms. Indirect support for this proposal comes from clinical studies that have shown reduction of PTSD symptoms with serotonin re-uptake inhibitors. A third finding of the study was the association between depressive symptoms and the ‘s’ allele of the 5HTTLPR gene. This finding adds to prior similar findings in case/case–control studies. The proportion of the variance due to this gene was 4%.

With regard to the association of the TPH genes with depression, studies have shown that some TPH1 and TPH2 polymorphisms are associated with depression (Table 1). In the present study we did not replicate these findings. However, the TPH1 gene that was associated with PTSD (‘t’ allele of rs2108977) in this study, showed a trend for depression

\[ h^2 = \text{heritability}; \ \rho_g (\text{Rho G}) = \text{Genetic correlation}. \]

* Adjusted for age, sex, measured exposure and environmental variables.

<table>
<thead>
<tr>
<th>Genetic heritability</th>
<th>Bi-variate genetic correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>h² (se)</td>
<td>p</td>
</tr>
<tr>
<td>PTSD-RI (PTSD symptoms)</td>
<td>0.41 (0.18)</td>
</tr>
<tr>
<td>BDI (depressive symptoms)</td>
<td>0.73 (0.21)</td>
</tr>
<tr>
<td>$\rho_g$ (se)</td>
<td>p (RhoG = 0) = 0.0006</td>
</tr>
<tr>
<td></td>
<td>p (RhoG = 1) = 0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: h² = heritability; $\rho_g$ (Rho G) = Genetic correlation.

Table 4
Rates* of heritabilities and bi-variate genetic correlations for PTSD and depressive symptoms in 12 multi-generational families from Armenia exposed to the 1988 Spitak earthquake.

Fig. 1. Scattergram of PTSD-RI and BDI scores among the participants exposed to the 1988 Spitak earthquake in Armenia.

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ple, not screening for exposure among controls, as in the
estimated with 5HTTLPR) may be confounding factors. For exam-
existence of depression (which may be independently associ-
erature, including this study, may be dissimilar (e.g. insuffi-
der specific environmental conditions (Kilpatrick et al.,
errors. Possible publication bias against negative findings may
ation in such studies is that 5% of the results have type 1 er-
prior studies may have been false positives. A priori expecta-
findings (stratification bias). Finally, the positive findings in
likely due to genetic differences) may not produce comparable
ethnic/racial group population. Additionally, cohorts with
this study may be due to lack of adequate sample size to de-
northern Sweden. These two genes may be appropriate can-
be excluded. Elimination of depressed individuals who
risk factor for PTSD may have been more robust had non-
exposed subjects been excluded from the control group.
Similarly, adjusting for depression in the analyses for the
phenotypes have unique and shared genes. For example, in the
study by Lee et al. (2005) subjects who had major depression prior
to the trauma but not those who had it after the trauma
were excluded. Elimination of depressed individuals who
may have been at greater risk for developing PTSD may
have skewed the sample and led to missing important
genes that are pleiotropic for depression and PTSD. In the
study by Kilpatrick et al. (2007) among subjects exposed to
hurricanes in Florida, the results showed no main effect for
5HTTLPR and PTSD. However, these authors found a main
effect for trauma (hurricane exposure) and depression. They
also found a three way gene–environment interaction (5HTTLPR by high exposure by low social support) whereby
the ‘s’ allele was found to be a risk factor for PTSD and
major depression in the group with high exposure and low
social support. It remains unclear whether the significance
of the ‘s’ allele with PTSD would have emerged after account-
ing for depression and exposure. Since PTSD and depression
are often comorbid conditions and pleiotropic, future genetic
studies should utilize a design that includes assessment of
the independent effects of exposure, PTSD symptoms and
depressive symptoms.

With regard to the relation of 5HTTLPR gene and depres-
sion, Table 3 lists six meta-analyses. Three of the studies
reported a positive relation between the ‘s’ allele and depres-
sion, and three did not find such an association. The most re-
cent and largest meta-analysis by Karg et al. (2011) included
many of the studies reviewed in the other meta-analysis. The
positive findings in three of the studies indicate that the ‘s’ all-
le was a risk factor for depression, but had a small effect
size.

The results in the present study, using a family study de-
design also indicated a positive association between the ‘s’ all-
elle and depressive symptoms thus extending prior positive
findings in studies that have used case/case–control designs. The
phenotypic variance in depression due to the 5HTTLPR

Table 5
Association (p values) and linkage (lod scores) of polymorphisms of TPH1, TPH2 and 5HTTLPR with PTSD symptoms and depressive symptoms, adjusted for age, sex, exposure and measured environmental variables.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>SLC6A4</th>
<th>TPH1</th>
<th>TPH1</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
<th>TPH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTSD-RI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PTSD symptoms)</td>
<td>Association (p value)</td>
<td>0.96</td>
<td>0.69</td>
<td><strong>0.004</strong></td>
<td><strong>0.03</strong></td>
<td>0.89</td>
<td>0.94</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Linkage (lod score)</td>
<td>0</td>
<td>0.56</td>
<td>1.05</td>
<td>0.02</td>
<td>0.46</td>
<td>0.0</td>
<td>0.32</td>
<td>0</td>
</tr>
<tr>
<td>BDI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Depressive symptoms)</td>
<td>Association (p value)</td>
<td><strong>0.03</strong></td>
<td>0.85</td>
<td><strong>0.08</strong></td>
<td>0.77</td>
<td>0.82</td>
<td>0.87</td>
<td>0.72</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Linkage (lod score)</td>
<td>0.01</td>
<td>0.58</td>
<td>0.34</td>
<td>0</td>
<td>0.20</td>
<td>0.09</td>
<td>0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

(p = 0.08), and linkage analyses showed a lod score of 1.05.
In a case–control study among Taiwanese women, Sun et al.
(2004) found an association of this gene in group of patients
with co-morbid depression and anxiety. With regard to the
TPH2 gene, Van Den Bogaert et al. (2006) found the TPH2
rs11178997, that is associated with PTSD in this study, was
associated with unipolar depression among patients from
the independent effects of exposure, PTSD symptoms and
depressive symptoms, adjusted for age, sex, measured
environmental variables.
gene was 4%. This small effect-size may constitute one reason why the positive finding of the ‘s’ allele is not often replicated. Additionally, publication bias toward negative findings may have excluded true negative results from being published, thereby skewing the outcome of meta-analyses in favor of positive results.

The present study differs from the other candidate gene studies of 5HTTLPR and TPH among subjects with co-occurring PTSD and depression in several significant ways. First, as opposed to case and case–control studies, the present study was a multigenerational family study. Family studies control for genetic heterogeneity within a family where the likelihood of affected family members having the same causal variants is high. In case–control studies each case may be affected due to unique variants, making it difficult to establish a genetic association. Also, in the present study between family variances were reduced because the population was relatively homogenous.

Second, the families in this study were unascertained which permitted us to evaluate the heritability of both PTSD and depression independently. In case or case–control studies cases are typically ascertained. If a sample is ascertained for PTSD, such as when subjects with a known diagnosis are recruited from a clinic, independent analysis for depression may not be valid. This would be especially problematic if the diseases being investigated are related and share genes, as in the present study. Our study focused on unascertained family members to represent the general population in the region. Only two of these subjects were seeking treatment. Families were recruited based on family size and exposure to the Spitak earthquake and not based on any phenotypic symptom. This design allowed us to assess the association of both PTSD and depressive symptoms uniquely and pleiotropically. Another difference between ascertained and unascertained samples may be the severity of symptoms. Usually, a case is assumed to be more severe and that is why subjects may be seeking treatment. It is possible that genes involved for different severity level of symptoms vary from those who have less severe symptoms.

Third, in this study symptoms were measured on a continuous scale which allows for greater statistical power than symptoms measured dichotomously with a yes/no item response option (Bailey and Almasy, 1995). Decrease in power occurs when information is lost in the dichotomization of phenotypes. For example, individuals with many symptoms (but not enough for diagnosis) are considered as ‘unaffected’ and classified together with individuals with no symptoms. Most methods for analyzing dichotomous data assume an underlying quantitative distribution with latent threshold values that are arbitrary (Amos and de Andrade, 2001; Duggirala et al., 1997). Thus, individuals around the threshold value could be misclassified. Two prior important studies that measured heritability of PTSD symptoms, one among war veterans (True et al., 1993) and the other population based (Stein et al., 2002) used continuous (quantitative) scale to measure heritability.

Fourth, subjects in this study were exposed to similar, earthquake related, severe traumatic events contemporaneously, thereby reducing variance related to type, severity or timing of exposure. PTSD is one of the few psychiatric disorders where the presence of an external stressor is a sine qua non for the diagnosis. Reducing the variability in the dose and timing of exposure improves the reliability of the heritability measures and increases the likelihood of detecting genetic associations.

Limitations of the present study include the following. First, retrospective rating of exposure to the earthquake may have been subject to memory failure leading to misestimation of severities of phenotypes and heritability measures. Second, the subjects were ethnic Armenians thus the findings may not be generalizable. Third, the sample size may have been insufficient to detect genes with relatively very small effects.

In summary, the findings of an association between TPH1 SNP rs2108977 and TPH2 rs11187997 and PTSD symptoms suggest that the serotonergic system may be causally implicated in the onset and/or persistence of PTSD. Also, the association between depression and the ‘s’ allele of HTTLPR extends prior similar findings in studies that have used case and case–control designs. Even though the results did not demonstrate definite pleiotropy for the two phenotypes, the significant association of TPH1 gene (SNP rs2108977) with PTSD and the non-significant trend with depressive symptoms is suggestive that the gene may be pleiotropic for both phenotypes. Finding unique and shared genes for the two phenotypes may be helpful in establishing biologically defined illnesses and developing specific treatments. The present findings justify further family studies in a larger sample of traumatized individuals in other racial/ethnic groups to replicate the results.

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Conflict of interest
No conflict declared.

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References


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