The Leptin Gene rs7799039 Modulates the Prevalence of Posttraumatic Stress Disorder After an Earthquake in Han Chinese Adolescents

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ABSTRACT

Objectives: The study's aim was to examine the prevalence and severity of posttraumatic stress disorder (PTSD) longitudinally among high school students with different genotypes of the leptin gene (*LEP*) rs7799039 after the 2008 Wenchuan earthquake in China.

Methods: The symptoms of PTSD were measured by the PTSD Checklist-Civilian Version (PCL-C) based on *DSM-IV-TR* criteria in 462 students at 6, 12, and 18 months after the earthquake. The genotypes of *LEP* rs7799039 were identified by polymerase chain reaction–restriction fragment length polymorphism analyses in 2018 using genomic DNA prepared in 2008 and stored at −80°C and verified by DNA sequencing. The association of *LEP* genotypes with PTSD was then analyzed by various statistical methods.

Results: The AA homozygotes had higher prevalence of PTSD than the G allele carriers at 12 months (22.30% vs 10.53%, $P = .013$) and higher median (interquartile range [IQR]) PCL-C scores at 12 (27.00 [24.00–35.75] vs 26.00 [22.00–31.25], $P = .010$) and 18 months (27.00 [21.00–32.00] vs 24.00 [19.00–29.00], $P = .003$) post-earthquake among female subjects. Female students had higher PCL-C scores than male subjects at 6 and 12 months regardless of the genotypes but only among the AA homozygotes at 18 months (27.00 [21.00–32.00] vs 22.00 [18.00–26.00], $P = .000$). The potential risk factors for and predictors of PTSD severity differed at different time points during follow-up. *LEP* rs7799039 was a potential factor for PTSD at 12 months and a predictor of PTSD severity at 18 months post-earthquake.

Conclusions: An association of *LEP* rs7799039 with the prevalence and severity of PTSD in Chinese adolescents was observed. These results indicate that females with the *LEP* rs7799039 AA genotype had more severe PTSD characteristics compared to female G allele carriers, suggesting that psychosocial or pharmacologic managements may particularly be needed by these female subjects.

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Posttraumatic stress disorder (PTSD) is an anxiety disorder that develops with physiologic disturbances after people are exposed to life-threatening trauma, including violent attacks, war experiences, childhood abuses, sexual abuses, threatened death, or natural disasters.[1] The symptoms of PTSD comprise re-experiencing symptoms, numbing and avoidance symptoms, and hyperarousal symptoms. Although the pathophysiologic mechanism of PTSD has not yet been clarified, it is generally believed that both environmental factors and genetic background play important roles in the development of PTSD.[2]

Natural disasters, such as earthquakes,[3] have been shown to be risk factors for PTSD. Data from multiple sources at 6 to 7 months post-earthquake[4] indicated that the prevalence of PTSD was 35.7% in youth survivors aged 9–17 years. However, wide variations in PTSD prevalence have been reported after specific earthquakes, such as the 2008 Wenchuan earthquake in China.[5] The incidence of PTSD in adolescents was observed to range from 8.8% to 21.0% at 6 months post-earthquake, while PTSD prevalence was demonstrated to be from 1.3% to 40.1% at 12 months and from 1.6% to 13.5% at 18 months post-earthquake.[6–8] These variations might result from different characteristics of study population, the timing of assessments, and other environmental factors. However, genetic background was not analyzed by those studies, even though it had been shown to be associated with susceptibility to PTSD and might interact with environmental factors to influence the risk of PTSD.[9]

Leptin, a product of the *obese* gene (*ob* or *LEP*) produced by adipose tissues,[10] plays an important role in regulating energy metabolism by suppressing appetite.[11] Moreover, a strong relationship between leptin and disease characteristics has been well-documented in the literature.[12] A previous study[13] reported that serum leptin levels were significantly lower in rats under chronic stress than those in control rats. In addition, an anxious phenotype was observed in *LEP*-deficient (*ob*/*ob*) mice.[14] On the other hand, a longitudinal study in Americans[15] showed that leptin levels were positively correlated with PTSD severity. Moreover, von Känel et al[16] found that patients with PTSD caused by myocardial infarction had higher leptin levels than those without PTSD. Nevertheless, no significant differences in leptin levels were observed between PTSD patients and controls in a 3-month follow-up study.[17] Therefore, more

Clinical Points

- The relationship between posttraumatic stress disorder (PTSD) and leptin has been inconsistently reported. The mechanisms behind the inconsistent associations of leptin with PTSD have not been elucidated.
- The results of the present study suggest that *LEP* rs7799039 may interact with other potential factors for or predictors of PTSD.
- These findings may help explain the inconsistent associations of leptin with PTSD and pave the way for precise medical interventions for PTSD.

studies are needed to clarify the relationship between PTSD and leptin.

In recent years, significant progress has been made in understanding the genetic variations of *LEP* that can affect its concentrations, structures, or functions.[18] *LEP* rs7799039 single nucleotide polymorphism is a G-to-A transition at position 2548, the upstream of the ATG start site in the 5′ promoter region.[19] A previous study[20] suggested that the G2548A base substitution could particularly affect the transcription of *LEP*. It was also found that the change in transcriptional activity induced by *LEP* rs7799039 led to increased circulating leptin concentrations in subjects with the AA genotype.[21] Furthermore, *LEP* rs7799039 has been shown to be related to the risks of several diseases, such as obesity,[22] cancer,[23] metabolic disorder,[24] and malnutrition inflammation syndrome.[25] *LEP* rs7799039 was also shown to be associated with antipsychotic-induced weight gain.[26] However, it is unclear whether *LEP* rs7799039 is associated with PTSD.

In this study, we take a step further to analyze the cause of wide variations in PTSD prevalence post-trauma shown by previous studies and explore the possible mechanisms of PTSD. We hypothesized that *LEP* rs7799039 may interact with other factors longitudinally to influence PTSD during the course of recovery from traumatic stress. To test this hypothesis, we examined the prevalence and severity of PTSD, and their relationships with other factors, in high school students with different genotypes of *LEP* rs7799039 at 6, 12, and 18 months after the 2008 Wenchuan earthquake, measuring 8.0 on the Richter scale, that spread about 100,000 km^2, destroyed about 6.5 million homes, and influenced approximately 46 million persons. To the best of our knowledge, longitudinal changes in the prevalence and severity of PTSD, and their relationships with other factors, in subjects with different genotypes of *LEP* rs7799039 have not been previously explored.

METHODS

Study Population

This longitudinal research study was performed at 6, 12, and 18 months after the 2008 Wenchuan earthquake in a public high school that was 10 km away from the epicenter. Almost all buildings in this school were damaged during the earthquake. Because there were no deaths or severely injured individuals, no medical or psychological professionals were sent to the school, and the students were not transferred to other schools. Instead, the students studied and lived in temporary shelters for 15 months until the new dormitories and teaching buildings were built.

The students in grade 11 were chosen for this study because (1) they had studied there for 1 year and had acclimated to their studies and lives at this school and (2) they had enough time to finish the follow-up investigations before their graduations. Data for students were excluded if (1) questionnaires were not fully answered at any one time point during the follow-up, (2) more than 1 answer was made for any single-answer questions, and (3) blood samples were not provided. A total of 462 students (mean ± SD age = 16.9 ± 0.6 years) completed the study, all of whom were Han Chinese. All students and their guardians provided signed informed consent. This study was approved by the Human Ethics Committee of Sichuan University.

Measurements

Details of the survey were as previously described.[6] Briefly, in the first section, the survey covered demographic characteristics, including sex, age, number of family members, school residence status (living at school vs not), only-child status, parents' education levels, individual history of psychiatric illness, and family history of psychiatric illness. Trauma characteristics (both previous trauma experience and trauma endorsed during the earthquake) were evaluated by variables indicated in Table 1. To measure the symptoms of PTSD, the PTSD Checklist-Civilian Version[27] (PCL-C), which has been widely used in adolescents and has been proved to have high internal consistency,[28] was used in the second section of the survey.[29–31] The questionnaire is a 17-item self-report scale based on *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (*DSM-IV-TR*) criteria, with total scores between 17 and 85. A score of 38 was used as cutoff for diagnosis of PTSD.[32]

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood cells using a DNA Out Kit (Cat No: 3671-50; Tiandz; Mianyang, China) at 6 months after the 2008 Wenchuan earthquake and stored at −80°C. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to examine the polymorphism of *LEP* rs7799039 in 2018. Briefly, a 241-bp DNA fragment containing *LEP* rs7799039 was amplified by PCR using the following primers[33]: the forward primer, 5′-TTT CCT GTA ATT TTC CCG TGA G-3′; and the reverse primer, 5′-AAA GCA AAG ACA GGC ATA AAA A-3′. The 241-bp PCR products were digested overnight at 37°C by *HhaI* (Cat No: R0139S; New England Biolabs Inc; Ipswich, Massachusetts; 2018). The digested fragments were separated by electrophoresis on 3% agarose gel. The digested PCR products with the AA genotype had a single band of 241 bp, those with the

GG genotype had 2 bands of 173 bp and 68 bp, and those with the AG genotype had 3 bands of 241 bp, 173 bp, and 68 bp. The results of genotyping were verified by DNA sequencing.

Statistical Analyses

The χ^2 goodness-of-fit test was used to determine departures from the Hardy-Weinberg equilibrium. The χ^2 tests were employed to analyze differences in the distribution of *LEP* rs7799039 genotypes or allele frequencies between sexes as well as differences in PTSD prevalence between sexes, between the subjects with different genotypes, or among the subjects at different time durations (6, 12, and 18 months post-earthquake). Moreover, χ^2 tests were also utilized to assessed differences in trauma characteristics (both previous trauma experience and trauma endorsed during the earthquake) of study population with different *LEP* rs7799039 genotypes. Independent-sample Mann-Whitney *U* tests were performed to examine the differences in PCL-C scores between the subjects grouped by sex or genotypes of *LEP* rs7799039. Friedman *M* tests were employed to compare the differences in PCL-C scores at 6, 12, and 18 months post-earthquake. Multivariate linear regression analysis was implemented to determine predictors of PTSD severity. Binary logistic regressions were set up to identify potential factors associated with the prevalence of PTSD. All statistical tests in the current study were 2-tailed, and a *P* value ≤ .05 was considered significant.

RESULTS

Genotype and Allele Distribution of *LEP* rs7799039 in the Study Population

The genotype frequencies of *LEP* rs7799039 (AA = 266, AG = 166, and GG = 30) in the adolescents were in accordance with Hardy-Weinberg equilibrium in this study population ($P = .552$). There were no significant differences in the genotype distributions (AA = 118, AG = 70, and GG = 12 for male students vs AA = 148, AG = 96, and GG = 18 for female subjects; $P = .843$) and allele frequencies (A = 306 and G = 94 for male students vs A = 392 and G = 132 for female subjects, $P = .554$) of *LEP* rs7799039 between the male and the female subjects. Because of the limited number of subjects with the GG genotype, they were combined with the GA heterozygotes and defined as the G allele carriers for further analyses.

Trauma Characteristics of Subjects With Different Genotypes of *LEP* rs7799039

As shown in Table 1, previous trauma experience and trauma endorsed during the earthquake were estimated as described in the Measurements section. There were significant differences in damage to family property ($P = .016$) and direct exposures ($P = .027$) between the AA homozygotes and the G allele carriers. No significant differences were found in the other trauma characteristics between the AA homozygotes and the G allele carriers.

Table 1. Trauma Characteristics of the Subjects With Different Genotypes of *LEP* rs7799039[a]

Type of Trauma	AA Homozygotes (n = 266), n (%)	G Allele Carriers (n = 196), n (%)
Trauma Endorsed During the Earthquake		
Injuries to self		
No injury	246 (92.5)	186 (94.9)
Little injury	11 (4.1)	9 (4.6)
Minor injury	8 (3.0)	1 (0.5)
Moderate injury	1 (0.4)	0 (0.0)
Serious injury	0 (0.0)	0 (0.0)
Damage to family property[b]*		
No damage	48 (18.0)	27 (13.8)
Little damage	82 (30.8)	88 (44.9)
Damage by half	52 (19.5)	30 (15.3)
Major damage	72 (27.1)	38 (19.4)
Complete damage	12 (4.5)	13 (6.6)
Damage to family housing		
No damage	29 (10.9)	17 (8.7)
Little damage	67 (25.2)	60 (30.6)
Damage by half	56 (21.1)	34 (17.3)
Major damage	72 (27.1)	56 (28.6)
Complete damage	42 (15.8)	29 (14.8)
Family members' death or injury		
No	224 (84.2)	172 (87.8)
Yes	42 (15.8)	24 (12.2)
Direct exposures[b,c]*		
No	150 (56.4)	131 (66.8)
Yes	116 (43.6)	65 (33.2)
Previous Trauma Experiences		
Abuse experiences[d]		
No	228 (85.7)	173 (88.3)
Yes	38 (14.3)	23 (11.7)
Disasters experiences[e]		
No	134 (50.4)	82 (41.8)
Yes	132 (49.6)	114 (58.2)
Violence experiences[f]		
No	165 (62.0)	119 (60.7)
Yes	101 (38.0)	77 (39.3)
Family pressure[g]		
No	178 (66.9)	130 (66.3)
Yes	88 (33.1)	66 (33.7)
Study pressure[h]		
No	51 (19.2)	51 (26.0)
Yes	215 (80.8)	145 (74.0)
Emotional stress		
No	150 (56.4)	128 (65.3)
Yes	116 (43.6)	68 (34.7)
Pressure of social networking		
No	157 (59.0)	132 (67.3)
Yes	109 (41.0)	64 (32.7)
Pressure of resilience to environmental changes		
No	197 (74.1)	154 (78.6)
Yes	69 (25.9)	42 (21.4)

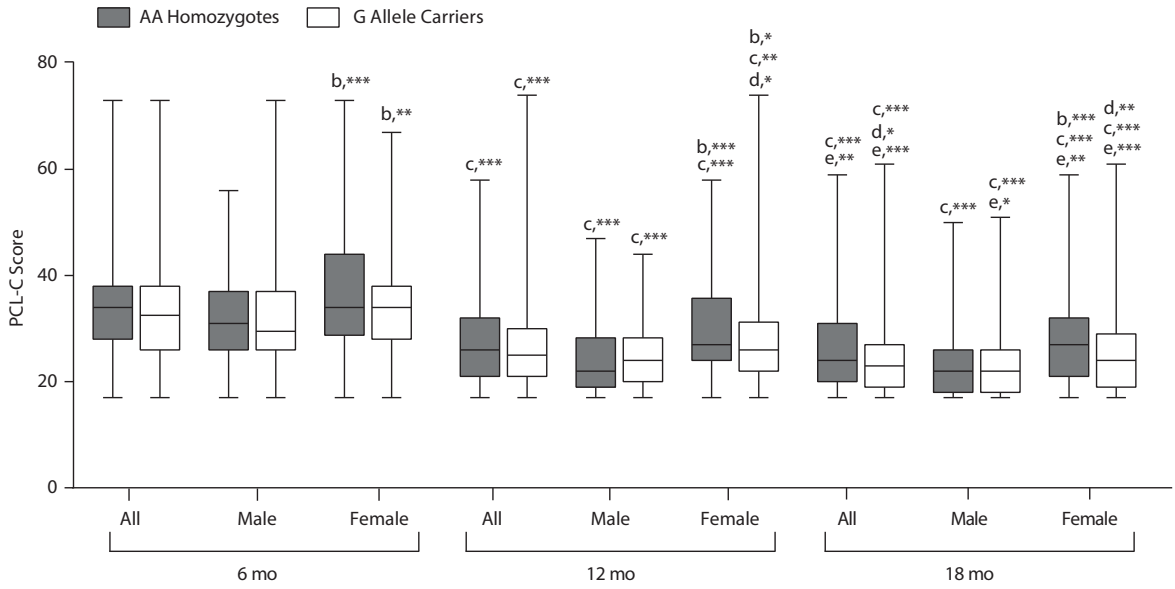
[a]Values expressed as n (%).
[b]Compared with the AA homozygotes (χ^2 tests).
[c]Exposures related directly to damage to family property, damage to family housing, or family members' death or injury.
[d]Including mental, physical, or sexual abuse experiences in one's life.
[e]Including experiences of car accidents, natural disasters, relatives and friends' deaths, and critical illness in one's life.
[f]Including experiences of animal attacks, assault, or witness of violence in one's life.
[g]Including tense parents' marriage, parents' health problems, and family economic pressure in one's life.
[h]Including pressure of study subjects, academic achievements, and future employments in one's life.
*$P < .05$.
Abbreviation: *LEP* = leptin gene.

Table 2. Prevalence of PTSD in Subjects With Different Genotypes of *LEP* rs7799039 at 6, 12, and 18 Months Post-Earthquake[a]

	Male		Female		All	
Time	AA Homozygotes	G Allele Carriers	AA Homozygotes	G Allele Carriers	AA Homozygotes	G Allele Carriers
6 mo	28 (23.72)	15 (18.29)	56 (37.84)[b*]	39 (34.21)[b*]	84 (31.57)	54 (27.56)
12 mo	6 (5.08)[c***]	2 (2.44)[c***]	33 (22.30)[b***,c**]	12 (10.53)[b*,c***,d*]	39 (14.67)[c***]	14 (7.14)[c***,d*]
18 mo	10 (8.47)[c**]	3 (3.66)[c**]	18 (12.16)[c***,e*]	9 (7.89)[c***]	28 (10.52)[c***]	12 (6.12)[c***]

[a]Values expressed as n (%).
[b]Compared with the male subjects of the same genotype at the same time post-earthquake (χ^2 tests).
[c]Compared with the subjects of the same genotype at 6 months post-earthquake (χ^2 tests).
[d]Compared with the subjects of the AA genotype at the same time post-earthquake (χ^2 tests).
[e]Compared with the subjects of the same genotype at 12 months post-earthquake (χ^2 tests).
*P < .05. **P < .01. ***P < .001.
Abbreviations: *LEP* = leptin gene, PTSD = posttraumatic stress disorder.

Figure 1. PCL-C Scores of Subjects With Different Genotypes of *LEP* rs7799039 at 6, 12, and 18 Months Post-Earthquake[a]



[a]All subjects: AA homozygotes, n = 266; G allele carriers, n = 196. Male subjects: AA homozygotes, n = 118; G allele carriers, n = 82. Female subjects: AA homozygotes, n = 148; G allele carriers, n = 114. Box length spans the interquartile range (25th to 75th percentile) of the data points, with the middle line indicating the median and whiskers indicating the minimum and maximum values for the given data set.
[b]Compared with the male subjects of the same genotype at the same time post-earthquake (Mann-Whitney *U* tests).
[c]Compared with the subjects of the same genotype at 6 months post-earthquake (Friedman *M* tests).
[d]Compared with the AA homozygotes of the same sex at the same month post-earthquake (Mann-Whitney *U* tests).
[e]Compared with the subjects of the same genotype at 12 months post-earthquake (Friedman *M* tests).
*P < .05, **P < .01, ***P < .001.
Abbreviations: *LEP* = leptin gene, PCL-C = PTSD Checklist-Civilian Version, PTSD = posttraumatic stress disorder.

Prevalence of PTSD in Subjects With Different Genotypes of *LEP* rs7799039 at 6, 12, and 18 Months Post-Earthquake

As displayed in Table 2, female students had significantly higher prevalence of PTSD than male subjects at 6 and 12 months but not at 18 months post-earthquake regardless of the genotypes of *LEP* rs7799039. Nevertheless, the AA homozygotes had significantly higher prevalence of PTSD than the G allele carriers at 12 months post-earthquake in female subjects (P = .013) but not in male students. Moreover, the prevalence of PTSD at 18 months post-earthquake was significantly lower than that at 12 months only in female AA homozygotes (P = .030) and not in female G allele carriers, although the prevalence at 12 and 18 months was significantly lower than that at 6 months regardless of sex and genotype of *LEP* rs7799039.

PCL-C Scores of Subjects With Different Genotypes of *LEP* rs7799039 at 6, 12, and 18 Months Post-Earthquake

Figure 1 demonstrates the PCL-C scores of subjects with different genotypes of *LEP* rs7799039 during the follow-up. The PCL-C scores in female students at 18 months were significantly lower than those at 6 or 12 months and also were significantly lower at 12 months than those at 6 months post-earthquake regardless of the genotypes of *LEP* rs7799039.

Table 3. Potential Factors Associated With Prevalence of PTSD in All Subjects at 6, 12, and 18 Months Post-Earthquake[a]

	6 mo		12 mo		18 mo	
Variable	Adjusted OR	95% CI	Adjusted OR	95% CI	Adjusted OR	95% CI
LEP rs7799039[b]	…	…	0.446*	0.220–0.905	…	…
Sex[c]	2.233***	1.421–3.508	5.445***	2.335–12.695	…	…
Previous trauma experience[d]	2.335**	1.362–4.004	…	…	…	…
Only-child[d]	…	…	…	…	5.475***	2.264–13.24
No. of family members	…	…	…	…	0.589*	0.347–1.000

[a]Binary logistic regressions were set up to identify potential factors associated with the prevalence of PTSD.
[b]0 = AA genotype, 1 = GG/GA genotype.
[c]0 = male, 1 = female.
[d]0 = no, 1 = yes.
*P < .05. **P < .01. ***P < .001.
Abbreviations: *LEP* = leptin gene, OR = odds ratio, PTSD = posttraumatic stress disorder.
Symbol: … = variable not entered into the binary logistic regressions model as potential factors for prevalence of PTSD were used.

Table 4. Predictors of PTSD Severity in All Subjects at 6, 12, and 18 Months Post-Earthquake[a]

	6 mo (adjusted R^2 = 0.070)		12 mo (adjusted R^2 = 0.122)		18 mo (adjusted R^2 = 0.095)	
Predictor	β	Partial Correlation	β	Partial Correlation	β	Partial Correlation
Sex	0.243***	0.245***	0.276***	0.282***	0.218***	0.222***
Previous trauma experience	0.121**	0.125**	0.122**	0.129**	0.109*	0.114*
Family members' death or injury	…	…	0.127**	0.134**	0.150***	0.155***
Damage to family property	…	…	0.104*	0.109*	…	…
LEP rs7799039	…	…	…	…	−0.104*	−0.109*
Age	…	…	…	…	0.106*	0.109*

[a]Multivariate linear regression analysis was implemented to determine predictors of PTSD severity.
*P < .05. **P < .01. ***P < .001.
Abbreviations: *LEP* = leptin gene, PTSD = posttraumatic stress disorder.
Symbols: β = standardized coefficient, … = variable not entered into the stepwise multiple linear regression model as predictors of PTSD severity were used.

On the other hand, there were significantly higher PCL-C scores in male subjects at 6 months compared to those at 12 months and 18 months regardless of genotype. However, higher PCL-C score at 12 months than at 18 months in male subjects was observed only in G allele carriers, not in AA homozygotes. Female subjects had higher PCL-C scores than male students at 6 and 12 months post-earthquake in both the AA homozygotes and the G allele carriers of *LEP* rs7799039. Nevertheless, female subjects had higher PCL-C scores than male students at 18 months post-earthquake only in the AA homozygotes (P = .000) and not in the G allele carriers. Moreover, the AA homozygotes had higher levels of PCL-C scores than the G allele carriers at 12 (P = .010) and 18 months (P = .003) post-earthquake in female subjects but not in male students.

Potential Factors Associated With the Prevalence of PTSD in All Subjects at 6, 12, and 18 Months Post-Earthquake

Table 3 shows the potential factors associated with the prevalence of PTSD in all subjects at 6, 12, and 18 months post-earthquake using binary logistic regressions. The results demonstrated that the adjusted potential factors associated with the prevalence of PTSD were sex and previous trauma experience at 6 months, while sex and *LEP* rs7799039 were potential factors associated with PTSD at 12 months post-earthquake. Moreover, only-child status and number of family members were potential factors associated with PTSD at 18 months post-earthquake.

Predictors of PTSD Severity in All Subjects at 6, 12, and 18 Months Post-Earthquake

Table 4 presents the further multivariate estimate results of independent predictors of severity of PTSD in the follow-up period. The stepwise multiple linear regression analyses revealed that sex and previous trauma experience were predictors of PCL-C scores at 6 months post-earthquake, which accounted for 6.0% and 1.5% of the total variances, respectively. Sex, previous trauma experience, family members' death or injury, and damage to family property were predictors of PCL-C scores at 12 months post-earthquake, which accounted for 8.0%, 2.1%, 1.4%, and 1.0% of the total variances, respectively. At 18 months post-earthquake, sex, previous trauma experience, family members' death or injury, *LEP* rs7799039, and age accounted for 3.8%, 1.5%, 2.4%, 1.1%, and 1.2% of the total variances, respectively.

DISCUSSION

To our best knowledge, the present study represents the first effort to longitudinally explore the interplay of *LEP* rs7799039 and other potential factors or predictors with the prevalence and severity of PTSD after stress. The featured findings include the following: (1) The AA homozygotes had higher prevalence of PTSD at 12 months (Table 2) and higher PCL-C scores at 12 and 18 months (Figure 1) post-earthquake than the G allele carriers in female students but not in the male subjects. (2) PTSD prevalence at 18 months was lower than that at 12 months only in female AA

homozygotes (Table 2). Male students had higher PCL-C scores at 12 months than those at 18 months in G allele carriers but not in AA homozygotes (Figure 1). (3) Female students had higher PCL-C scores than male subjects at 18 months in the AA homozygotes but not in the G allele carriers (Figure 1). (4) The potential factors associated with PTSD and predictors of PTSD severity were different at different times post-earthquake. *LEP* rs7799039 was one of the potential factors associated with PTSD prevalence at 12 months (Table 3) and one of the predictors of PTSD severity at 18 months (Table 4) post-earthquake. These results suggest that interplay did occur longitudinally among *LEP* rs7799039 and other potential factors or predictors to affect the prevalence and severities of PTSD in this population. Moreover, the interplay functioned in a time-course and sex-dependent manner. These findings may be involved in the explanations of the inconsistent relationships between PTSD[34] and leptin reported previously,[17] although only *LEP* rs7799039 was adopted as a marker of leptin in the present investigation.

It was reported that female subjects had higher prevalence and worse severities of PTSD than male subjects in an adolescent population characterized with a greater frequency of rape in the female subjects[35] and in an adult population with lower verbal ability in early childhood.[36] It was also reported[37] that male subjects had higher prevalence of PTSD than female subjects due to the level of combat exposure in the male subjects. Nevertheless, it was observed[38] that there were no significant differences in the development of PTSD between women and men when their baseline characteristics were matched. However, genetic background was not analyzed in these studies. In the present study, the PTSD prevalence of the female subjects was significantly higher than the male subjects at 6 months and 12 months post-earthquake in either AA homozygotes or G allele carriers (Table 2). Female adolescents also had higher PCL-C scores than did male students at 18 months in the AA homozygotes but not in the G allele carriers, although the female students had higher PCL-C scores than the male subjects regardless of the genotypes of *LEP* rs7799039 at 6 and 12 months post-earthquake (Figure 1). Additionally, sex was a potential factor associated with PTSD together with previous trauma experience at 6 months or with *LEP* rs7799039 at 12 months but not at 18 months post-earthquake (Table 3). Meanwhile, sex accounted for 6.0% of the total variance of PCL-C scores at 6 months, 8.0% at 12 months, and 3.8% at 18 months post-earthquake (Table 4). These results suggest that sex may interact differently with other potential factors or predictors including *LEP* rs7799039 to affect PTSD occurrence or development at different times after stress. In fact, it was shown that the AA genotype of *LEP* rs7799039 was associated with increased estrogen levels.[39] A previous study[40] has indicated that the administration of conjugated estrogens and antiandrogens could cause an increase in serum leptin levels in the second, fourth, sixth, and ninth months after administration in women with polycystic ovary syndrome. In addition, estrogen has also been shown to be positively related to an increased serum leptin concentration and a higher leptin mRNA expression in adipose tissue of immature rats.[41] Therefore, it is likely that the overall outcome of the interaction between hormones and *LEP* rs7799039 may contribute to the sex differences in prevalence and PCL-C scores observed in the present study.

Leptin may play an important role in the occurrence and development of PTSD. Significantly higher serum leptin levels were observed in PTSD patients experiencing a major earthquake.[34] The levels of serum leptin were also found to be positively associated with the severity of PTSD.[15] On the other hand, *LEP* rs7799039 was found to be a regulator of the expression of *LEP*.[20] In fact, AA homozygotes exhibited higher expression levels of leptin compared with GG homozygotes and AG heterozygotes in obese children.[21] Taken together, the changes in *LEP* expression and their subsequent variations in serum leptin levels might be one of the mechanisms in the association of *LEP* rs7799039 and PTSD observed in the present study. However, no associations were detected between the levels of leptin and *LEP* rs7799039 in pregnant women.[22] Besides, AA homozygotes were discovered to have a lower level of leptin than G allele carriers in obese girls.[42] Therefore, other mechanisms, such as linkage disequilibrium and haplotype block, may be involved in the association of PTSD with *LEP* rs7799039 observed in the present study. Since the present study was an investigation of only the relationship between *LEP* rs7799039 and PTSD, more studies are needed to put the present data in a broader context to explore the mechanism and implication of this complex relationship.

There were several limitations of the present study. First, the serum leptin levels were not measured, although there still are advantages to testing the associations without checking proteins because the changes in proteins do not constitute all of the mechanisms of the associations between genetic mutations and phenotypes, and other mechanisms such as linkage disequilibrium and haplotype block may be involved. Second, PTSD was measured only by the PTSD Checklist-Civilian Version. The PTSD patients defined in the present study might not be the same as clinical patients. This possibility should be considered when the results of the present study are explained.

In brief, the results of the present study indicate that the association of *LEP* rs7799039 with the prevalence and severity of PTSD was time course–dependent and affected by sex in Han Chinese adolescents. The female subjects with the *LEP* rs7799039 AA genotype had more severe PTSD characteristics. This finding suggests the interplay of *LEP* rs7799039 with other potential factors related to and predictors of PTSD occurrence and development. This interplay may be among the mechanisms of the inconsistent associations of leptin with PTSD reported previously and might pave the way for precise medical interventions for PTSD among adolescents with different genetic backgrounds after experiencing traumatic events. Psychosocial or pharmacologic treatment may particularly be needed by female subjects with the *LEP* rs7799039 AA genotype.

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