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The preliminary investigation of orexigenic hormone gene polymorphisms on posttraumatic stress disorder symptoms

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ABSTRACT

Orexigenic hormones are a group of hormones that can up-regulate appetite. Current studies have shown that orexigenic hormones also play important roles in stress responses and may be implicated in regulation of fear memory. However, these conclusions lack evidence from human studies. In this study, we examined associations between orexigenic hormone genes and fear-related mental disorders by investigating main, G × E, and G × G effects of ghrelin and orexin gene single nucleotide polymorphisms (SNPs) on human posttraumatic stress disorder (PTSD) symptoms in 1134 Chinese earthquake survivors. SNPs Leu72Met of the *GHRL* gene (rs696217), Ile408Val of the *HCRTR1* gene (rs2271933) and Val308Ile of the *HCRTR2* gene (rs2653349) were genotyped. None of the SNPs showed significant main or G × E effects. However, a significant interaction effect between *GHRL* rs696217 and *HCRTR1* rs2271933 was found to predict the PTSD Checklist (PCL-5) total score (P = 0.007). Further analysis revealed different interaction patterns in males and females. For females, the rs2271933 G allele was associated with an increased PCL-5 total score (B = 2.59, P = 0.024) when the rs696217 genotype TT/TG was present. For males, the rs696217 T allele is associated with an increased PCL-5 total score (B = 3.62, P = 0.040) when the rs2271933 genotype GG/GA was present. These current findings expand our knowledge of physiological function of the orexigenic hormone system, and suggest its involvement in development of fear-related mental disorders such as PTSD.

1. Introduction

Orexigenic hormones are a group of hormones that regulate appetite. The main functions of orexigenic hormones include regulation of feeding behaviors, metabolism, and sleep-wake cycles. Recently, increasing evidence has shown that orexigenic hormones are also involved in regulation of stress through complex mechanisms (Mustafa, 2018) and may play a role in physiopathology of stress-related mental disorders (Labarthe et al., 2014; Lutter et al., 2008).

Ghrelin is a 28-amino acid peptide modified by an eight carbon fatty acid at its third residue (Ueno et al., 2005), and it is one of the key orexigenic hormones. Most ghrelin is produced in the stomach, but some neurons in the hypothalamic arcuate nucleus (ARC) express ghrelin in the central nervous system (CNS) (Kojima et al., 1999). Ghrelin works through activation of the ghrelin receptor (GHS-R), and primary physiological functions include stimulating growth hormone (Kojima et al., 1999), influencing food intake and energy homeostasis (Nakazato et al., 2001) and regulating the sleep-wake cycle (Steiger, 2007). Other studies have shown that ghrelin is also involved in regulation of stress responses and in stress-related mental disorders (Wittekind and Kluge, 2015). Animal studies demonstrate that the plasma ghrelin levels increase after chronic social defeat stress (CSDS), and ghrelin signaling can decrease depression-like behavior in mice (Lutter et al., 2008). Similarly, in humans psychological stress induces an increase in plasma levels of ghrelin (Rouach et al., 2007), however, studies on the association between ghrelin and depression have yielded mixed results (Kluge et al., 2009; Matsuo et al., 2012; Nakashima et al., 2008). Furthermore, a recent study suggested that a stress-related

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increase in ghrelin is involved in stress-associated enhancement of fear memories (Meyer et al., 2015). Repeated activation of ghrelin receptors in the basolateral complex of the amygdala (BLA) has fear-enhancing effects and may contribute to trauma-related affective disorders (Meyer et al., 2015).

Orexin is another orexigenic hormone that includes two subtypes, orexin-A and orexin-B. Orexin-A and orexin-B are 33- and 28-residue peptides, respectively. They function by activating corresponding G protein-coupled receptors, OX_1 and OX_2. In the CNS, orexin neurons are found only in the lateral hypothalamus (LH) and project to several brain areas including the hippocampus, medial prefrontal cortex, and amygdala. In addition to regulation of feeding, metabolism, wakefulness and sleep, the orexin system is also involved in the stress response through activation of the hypothalamus-pituitary-adrenal (HPA) axis (Kukkonen, 2013). Studies in rodents suggest that orexins may play a role in pathogenesis of stress-related affective disorders such as depression and anxiety (Allard et al., 2007; Feng et al., 2008; Suzuki et al., 2005). Finally, considerable empirical evidence suggests that the OX_1 receptor plays an important role in formation and extinction of fear memories (Sears et al., 2013; Soya et al., 2013), which was also highlighted in a recent review (Flores et al., 2015).

While multiple animal studies suggest involvement of both ghrelin and orexin in modulation of fear memories (Flores et al., 2015; Meyer et al., 2015), and thus may play important roles in pathophysiology of fear-related mental disorders, relatively few studies have investigated the relationship between orexigenic hormones and mental disorders in humans.

Posttraumatic stress disorder (PTSD) is a mental disorder that develops after an individual experiences a traumatic event, and abnormal regulation of fear memory is one of the prime explanations for the pathophysiology of PTSD (Shalev et al., 2017). Therefore, PTSD may be a good candidate to examine associations between these orexigenic hormones and mental disorders related to abnormal fear memory. Interactions between the ghrelin and orexin systems have been identified in regulating key biological processes that might play key role in PTSD development like sleep-wake cycle and even in depression-like symptoms development (García-García et al., 2014; Lutter et al., 2008). The biological functions of ghrelin may rely on the presence of orexin, and ghrelin levels can directly modulate orexin activity by increasing orexin neuron excitability (García-García et al., 2014). Although both ghrelin and orexin systems are involved in fear regulation, their interaction in context of fear learning and trauma-related disorders like PTSD have not been examined to date. Examining genetic variants in both ghrelin and orexin genes in the same individuals is one step in identifying these potential interactions, and indeed it has been Suggested in the literature that the gene-gene interactions play an important role in the development and of vulnerability of PTSD (Broekman et al., 2007; Cordell, 2009; Frazer et al., 2009). This was further supported by recent empirical studies (Hemmings et al., 2013; Zhang et al., 2018). Through investigation of ghrelin and orexin gene-gene interaction effects on PTSD symptoms, we aimed to determine whether interactions between these hormones are involved in regulation of fear memory and fear-related mental disorders.

Previous human genetic studies of mental disorders have focused on several specific SNPs of orexigenic hormone genes, previously linked to mental health disorders like depression, schizophrenia and anxiety disorders (Annerbrink et al., 2011; Fukunaka et al., 2007; Nakashima et al., 2008; Rainero et al., 2011; Suchankova et al., 2017). For ghrelin gene *GHRL*, Leu72Met of the *GHRL* gene (rs696217) has been reported most frequently. The *GHRL* gene encodes the ghrelin preproprotein, and the rs696217 SNP was previously found to be related to alcohol consumption and depression symptoms (Nakashima et al., 2008; Suchankova et al., 2017). For orexin, Ile408Val of the OX1 receptor HCRTR1 gene (rs2271933), and Val308Ile of the OX2 receptor HCRTR2 gene (rs2653349), have been studied most often and allele frequencies are well established (Thompson et al., 2017). *HCRTR1* Ile408Val has been linked to major mood disorders and schizophrenia (Fukunaka et al., 2007; Rainero et al., 2011), and *HCRTR2* Val308Ile was shown to be associated with risk of panic disorder in females (Annerbrink et al., 2011). In this study, we genotyped these three SNPs in a predominantly adult Chinese cohort who exposed to the trauma of the 2008 Wenchuan earthquake.

We aimed to address the following questions: (1) How do these genetic polymorphisms of ghrelin and orexin independently affect human PTSD symptoms? (2) Do interactions between genetic polymorphisms of ghrelin and orexin affect human PTSD symptoms? Based on previous evidences, we hypothesized that interactions between genetic polymorphisms of ghrelin and orexin may impact human PTSD symptoms.

2. Materials and methods

2.1. Sample and procedure

Our participants were recruited from a large rebuilt community in Hanwang Town, Sichuan Province, China. In 2008, a terrible earthquake rated 8.0 almost destroyed this town, with over 5000 killing inhabitants there. Our survey was conducted five and a half years after the earthquake. Participates over 16 years old and personally experienced the earthquake were included in the survey. Individuals with diagnosed mental retardation history of pharmacological treatments or major psychiatry history (e.g. schizophrenia and organic mental disorders) were excluded. Before the participants completed self-reported questionnaires, all of them were told the aims of our study and signed a written informed consent. After they completed the questionnaires, peripheral blood was collected by nurse and processed for genotyping. A total of 1140 subjects with both clinical and genotyping data were initially included. We further excluded 6 subjects due to missing age information, resulting in total of 1134 subjects. In the final sample, there are 361 males and 773 females. Mean age of this sample is 48.1 years old (16–73, SD = 10.0). The quartiles for age were 42, 48 and 56 years old. This study protocol was approved by the Institutional Review Board of Institute of Psychology, Chinese Academy of Sciences.

2.2. Measurements

Earthquake-related trauma exposures were assessed by a 10-item self-report questionnaire. Participants were asked whether they experienced (1) being trapped under rubble; (2) being injured; (3) being disabled due to injuries; (4) participating in rescue efforts; (5) witnessing a death of someone; (6) exposure to mutilated bodies; (7) traumatic death of a family member; (8) traumatic injury of a family member; (9) traumatic death of a friend or neighbour; and (10) losing livelihood due to the disaster. Each item was rated with either 0 (no) or 1 (yes). The total score were summed as the severity of trauma exposure.

PTSD symptoms were assessed with PTSD Checklist for DSM-5 (PCL-5; Blevins et al., 2015). The PCL-5 is a 20-item self-report checklist of DSM-5 defined PTSD symptoms. The items of the PCL-5 were focused on the earthquake in particular. Each item is rated on the five-point Likert scale to reflect severity of a particular symptom from 0 (not at all) to 4 (extremely) during the past month. The psychometric properties of the PCL-5 have been widely reported (Bovin et al., 2016). The Chinese version of PCL-5 was adapted by translation and back translation, and has been previously used in traumatized Chinese sample (Wang et al., 2017). In this current sample, the checklist demonstrated good reliability (Cronbach's $\alpha = 0.95$).

2.3. Genotype and quality control

The genotyping was conducted by a custom-by-design 2 × 48-Plex SNPscan™Kit (Genesky Biotechnologies Inc., Shanghai, China). For each

participant, 10 ng DNA were amplified by PCR, and the following genotyping was conducted based on multiplex fluorescence PCR for rs696217, rs2271933 and rs2653349. For quality control of samples, we removed subjects with call rate < 0.8. Quality controls of SNPs were performed by excluding SNPs with call rate < 0.95 or minor allele frequency (MAF) < 0.01 or Hardy-Weinberg equilibrium (HWE) test P < 0.05. All the subjects and SNPs passed the quality control.

2.4. Statistical analysis

All the statistical analyses were performed using R 3.2.3. All the linear regression models utilized an additive model (minor allele count for each SNP) and *t*-test *P*-values for linear regression were two-tailed. The SNPs were designated according to the number of minor alleles in all the regression equations. Therefore, we coded GG/TG/TT as 0/1/2 for rs696217, AA/GA/GG as 0/1/2 for rs2271933 and GG/GA/AA as 0/1/2 for rs2653349. For single SNP-based analysis, we firstly utilized a linear regression model including SNP, sex, age and trauma exposure score as independent variables and the PCL-5 total score as the dependent variable. Then the interaction effect between SNP and trauma exposure was further added. In the second step, the interactions between the *GHRL* and *HCRTR1* gene, the *GHRL* and *HCRLR2* gene were examined. For gene–gene interaction analysis, we employed a linear regression model including SNPs, sex, age, trauma exposure and SNP–SNP interaction between ghrelin and orexin genes as independent variables and PCL-5 total score as the dependent variable. After the gene–gene interaction analysis, we performed simple effect analysis by dividing full sample according to the genotypes of one SNP and using the same linear regression model to analyze the effect of another SNP to PTSD symptoms. The gene–gene interaction analyses were performed in the whole sample, male and female subsamples respectively. For multiple comparison correction, we performed permutation test for the significant results.

3. Results

Mean earthquake-related trauma exposure score of our sample was 3.5 (SD = 1.8; range: 0–10), which reflects a medium level of earthquake-related trauma exposure in this sample. The PCL-5 total score and the distributions of rs696217, rs2271933 and rs2653349 in our sample were shown in Table 1. The distribution of the PCL total score is presented in Fig. 1.

The main effects and G × E effects of the SNPs on PTSD symptom severity were examined first using linear regression. In the regression analyses none of the SNPs showed significant main effect or interaction effect with trauma exposure (see Table 2). For the gene-gene interaction analysis, a significant interaction effect between the *GHRL* rs696217 and *HCRTR1* rs2271933 (B = 2.89, P = 0.007 and $P_{permutation}$ = 0.007) was found to be associated with PTSD symptoms in the whole sample (see Table 3). There were trends with the same direction for this interaction effect separately for male (B = 4.02, P = 0.058, $P_{permutation}$ = 0.059) and female (B = 2.38, P = 0.053, $P_{permutation}$ = 0.053) subsamples (see Supplementary materials). There was no interaction between the *GHRL* rs696217 and *HCRLR2* rs2653349 (P = 0.082). Simple effect analyses further revealed the different interaction patterns in males and females. For females, the rs2271933 G allele was associated with an increased PCL-5 total score (B = 2.59, P = 0.024, $P_{permutation}$ = 0.024) when the rs696217 genotype TT/TG was present (see Table 4). While for males, the rs696217 T allele is associated with an increased PCL-5 total score (B = 3.62, P =

Table 1
PTSD symptom severity in different genotypes.

SNP	Genotype	PCL mean score	SD	Range
Total sample		18.8	13.5	1–77
rs696217	G/G (n = 728)	18.5	13.4	1–69
	G/T (n = 361)	19.9	13.8	1–77
	T/T (n = 45)	14.4	11.0	1–58
rs2271933	A/A (n = 590)	18.7	13.2	1–69
	G/A (n = 465)	18.7	13.5	1–77
	G/G (n = 79)	19.6	15.0	1–73
rs2653349	G/G (n = 988)	18.9	13.3	1–73
	G/A (n = 141)	17.8	14.7	1–77
	A/A (n = 5)	15.9	14.9	3–41

Note. N = 1134.

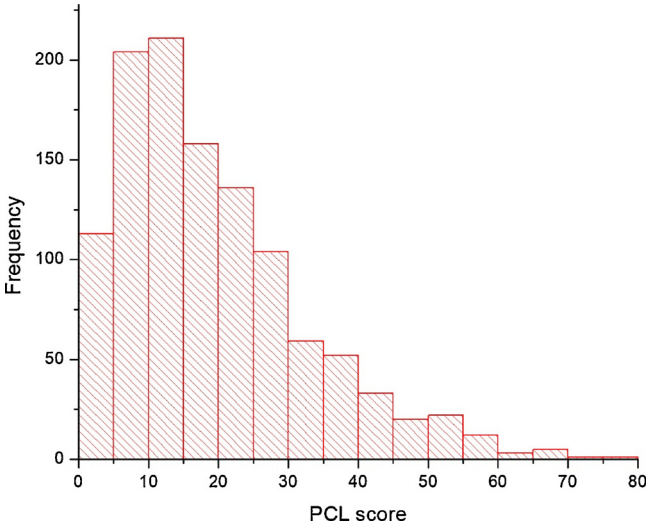

Fig. 1. Distribution of PCL score in our sample.
Note. Quartiles of the PCL score are 9, 16 and 26.

Table 2
Main and G × E effects of ghrelin and orexin genes on PTSD symptom severity.

SNP	Allel[a]	MAF	G × E[b]	B	SE	*t*	*P*
rs696217	G/T	0.20	N	0.30	0.66	0.46	0.644
			Y	−1.14	1.39	−0.82	0.414
			inter	0.43	0.36	1.17	0.241
rs2271933	A/G	0.27	N	0.48	0.59	0.81	0.418
			Y	0.14	1.28	0.11	0.914
			inter	0.10	0.32	0.30	0.762
rs2653349	G/A	0.07	N	−0.73	1.05	−0.69	0.490
			Y	−1.77	2.13	−0.83	0.404
			inter	0.30	0.52	0.57	0.571

Note. N = 1134. MAF: minor allele frequency. G × E: gene-environment interaction (SNP × trauma exposure). We coded GG/TG/TT as 0/1/2 for rs696217, AA/GA/GG as 0/1/2 for rs2271933 and GG/GA/AA as 0/1/2 for rs2653349 respectively. Sex was coded: 0 = male; 1 = female.

[a] Major/minor.

[b] N: main effect of SNP based on lineal regression without G × E; Y: main effect of SNP based on lineal regression with G × E; inter: results for interaction effect based on lineal regression with G × E.

Table 3
The gene–gene interaction effect between rs696217 and rs2271933 on PTSD symptom severity in the whole sample.

Predictor	B	SE	*t*	*P*	$P_{permutation}$
Sex	4.18	0.80	5.21	2.24e-07	
Age	0.37	0.04	9.93	< 2e-16	
Trauma exposure	2.20	0.20	10.75	< 2e-16	
rs696217	−1.24	0.87	−1.42	0.155	
rs2271933	−0.65	0.73	−0.89	0.373	
rs696217 × rs2271933	2.89	1.07	2.70	0.007	0.007

Note. N = 1134. We coded GG/TG/TT as 0/1/2 for rs696217 and AA/GA/GG as 0/1/2 for rs2271933 respectively. Sex was coded: 0 = male; 1 = female.

Table 4
Linear regression analysis of rs2271933 in rs696217 TT/TG set and rs696217 GG set, respectively.

Sex	rs696217 genotype	Sample size	B	SE	*t*	*P*	$P_{permutation}$
ALL	TT/TG	406	2.30	1.01	2.26	0.024	0.024
	GG	728	−0.56	0.73	−0.77	0.441	
Female	TT/TG	284	2.59	1.14	2.27	0.024	0.024
	GG	489	0.08	0.87	0.09	0.927	
Male	TT/TG	122	1.43	2.07	0.69	0.492	
	GG	239	−2.09	1.34	−1.56	0.120	

Note. We coded AA/GA/GG as 0/1/2 for rs2271933.

Table 5
Linear regression analysis of rs696217 in rs2271933 GG/GA set and rs2271933 AA set, respectively.

Sex	rs2271933 genotype	Sample size	B	SE	*t*	*P*	$P_{permutation}$
ALL	GG/GA	544	1.85	0.95	1.94	0.053	0.053
	AA	590	−1.11	0.90	−1.23	0.220	
Female	GG/GA	362	1.01	1.14	0.88	0.377	
	AA	411	−1.49	1.02	−1.46	0.145	
Male	GG/GA	182	3.62	1.75	2.07	0.040	0.040
	AA	179	−0.11	1.82	−0.06	0.954	

Note. We coded GG/TG/TT as 0/1/2 for rs69621.

0.040, $P_{\text{permutation}} = 0.040$) when the rs2271933 genotype GG/GA was present (see Table 5).

4. Discussion

This study examined the associations between three genetic polymorphisms in orexigenic hormones and human PTSD symptoms. Neither ghrelin nor orexin SNPs alone directly affected PTSD symptom severity. There were also no G × E effects of these SNPs in interaction with trauma exposure. However, our results revealed that orexigenic hormone genes may be linked to PTSD symptoms development through a gene-gene interaction between the *GHRL* and *HCRTR1* genes. *GHRL* Leu72Met (rs2271933) and *HCRTR1* Ile408Val (rs696219) interact to predict PTSD symptoms in males and females, differentially. For females, the minor allele of rs2271933 is a risk factor for developing PTSD symptoms when the rs696217 genotype TT/TG is present. While for males, the rs696217 minor allele can be a risk factor when the rs2271933 genotype GG/GA is present.

To our knowledge, only a single study has examined associations between orexigenic hormone genetic polymorphisms and human PTSD so far. Solovieff et al., 2014 found no associations between SNPs of the neuropeptide Y gene and PTSD symptoms/diagnosis. In the current study, we also failed to detect significant effects of a single gene. However, the gene-gene interaction effect revealed here suggests that multiple orexigenic hormones, might together affect the development of PTSD symptoms. Interactions between the ghrelin and orexin systems have been reported in many animal studies. Antidepressant-like effects of ghrelin were blocked in mice lacking orexin (Lutter et al., 2008), and orexin-induced feeding behaviors were found to depend on the ghrelin system. In addition, orexin neurons were reported to stimulate consummatory behaviors through a ghrelin orexigenic pathway (Ma et al., 2007). Our findings further extend evidence for interactions between ghrelin and orexin in modulating human PTSD symptoms.

It is important to acknowledge that we bring here evidence for a ghrelin-orexin interaction on a gene level and not on a functional level. According to a previous study, T alleles of *GHRL* rs696217 are associated with higher human plasma ghrelin levels (Zavarella et al., 2008). For the *HCRTR1* gene, a previous literature predicted that rs2271933 may be implicated in G protein and/or other protein coupling of orexin receptors that cause functional changes (Obenauer et al., 2003). Therefore, these findings suggest that the gene-gene interaction between rs696217 and rs2271933 may represent a functional interaction. A functional interaction between ghrelin and orexin in terms of gene expression has been found and the neurophysiological basis for this interaction has been elucidated in animals (Adamantidis and de Lecea, 2008, 2009). Ghrelin can directly modulate orexin activity by increasing neuronal excitability (García-García et al., 2014). However, more evidence for a functional interaction between ghrelin and orexin in humans is required for a clear understanding of the role of the orexigenic hormone system.

Existing evidence indicates that interactions between ghrelin and orexin may affect PTSD symptoms through modulation of fear learning and extinction in the BLA (Flores et al., 2014; Meyer et al., 2015). The BLA is a key brain region responsible for processing fear memory (LeDoux, 2009). According to a recent neuropharmacological study (Meyer et al., 2015), pharmacological activation of ghrelin receptors in the BLA can enhance fear learning. In contrast, when BLA ghrelin receptors are blocked, stress-related enhancement of fear memory is abolished during repeated stress. These results suggest that ghrelin signaling in the BLA is necessary for stress-related enhancement of fear memory. Other stress hormones are not sufficient to mediate this effect in the absence of heightened ghrelin signaling. Our findings in females are consistent with these conclusions. The minor allele of the OX_1 receptor SNP is a risk factor for developing PTSD symptoms only in those with high ghrelin signaling (rs696217 T allele carriers). In addition, studies on orexin support modulation of fear memory by OX_1 receptor signaling in the BLA. Injection of an OX_1 receptor antagonist into the BLA can rescue contextual and cued fear extinction, whereas OX_2 receptor blockade does not modify fear extinction (Flores et al., 2014). These results suggest that only OX_1 receptor blockade can facilitate activation of pro-extinction circuits in the amygdala, and might explain why we did not detect a significant interaction between ghrelin and the OX_2 receptor in the current study. As suggested by our current findings, future studies focused on interactions between ghrelin and OX_1 signals in the BLA are needed to help understand modulation of fear memory.

It is noteworthy that we observed different gene interaction patterns in males and females. Effects of orexigenic hormones on psychopathology often have been reported to be sexually dimorphic. For ghrelin, ghrelin administration only produces antidepressant-like effects in males (Kluge et al., 2011). In the same study, changes in stress-related hormone secretion after ghrelin administration also showed sexual dimorphism. Plasma cortisol decreased to levels that were significantly lower in the ghrelin-treated group compared with the placebo group only in male patients. In addition, the GHRL rs696217 genotype was reported to be differentially associated with plasma biochemical parameters (e.g., leptin levels) in males and females (Takezawa et al., 2009). As for orexin, expression of orexin genes in the CNS has been shown to be sexually dimorphic (Jöhren et al., 2002). It was also reported that sex hormone can modulate influences of orexin on fearfulness in mice (Easton et al., 2006). In addition, the orexin receptor gene has been reported associated with panic disorder only in female subjects (Annerbrink et al., 2011).

There are meaningful limitations to the present findings that have to be carefully considered. The main one is obviously the fact that three specific SNP's have been studied as priori or candidate genes, and not undergone genome–wide significance tests. For this reason, and in absence of large replication cohort, the current results have to be considered as preliminary, awaiting future replications. Furthermore, we did not perform experiments to examine biological functions of the SNPs. To provide some more information for the interacted SNPs, we performed bioinformatics analysis to discover the possible functions of the SNPs. The results indicated that rs696217 is a putative eQTL of GHRL and rs2271933 (corresponding to I408 V) may affect protein-binding of human HCRTR1 protein (Supplementary materials). Further evidence addressing this issue is still needed. For example, levels of

central ghrelin and orexin in PTSD patients are not known. In future studies, measurements of plasma ghrelin and orexin levels may be helpful in explaining mechanisms of the interaction between the ghrelin and orexin systems. Finally the effects of agonists or antagonists of ghrelin/orexin receptors on human PTSD symptoms should be examined in future clinical trials.

5. Conclusion

This is the first study to examine associations between orexigenic hormones (ghrelin, orexin) and PTSD symptoms in humans. We found that an interaction between genetic polymorphisms of the *HCRTR1* and *GHRL* genes affects PTSD symptom severity. This study expands our knowledge of physiological function of the orexigenic hormone system and provides novel potential targets for treatment of fear-related mental disorders. Currently, clinical trials based on orexigenic hormones focus primarily on intervention of depression (Flores et al., 2015; Kluge et al., 2011). Current finding suggest effects of orexigenic agent should be also assessed among patients with fear related mental disorders.

Conflict of interest

This manuscript does not represent any conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.09.042.

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