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ORIGINAL ARTICLE

Posttraumatic Stress Disorder Augments Plasma Triglycerides in TT Homozygotes of rs495225 at Growth Hormone Secretagogue Receptor Gene

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Abstract

Posttraumatic stress disorder (PTSD) and growth hormone secretagogue receptor (GHSR) were reported to be associated with plasma lipid and glucose levels. However, interplays of PTSD with GHSR on plasma lipid and glucose levels have not been explored yet. This study was to investigate the interplays of PTSD and *GHSR* rs495225 on plasma glucose and lipid profiles. A total of 709 high school students were recruited at 6 months after the 2008 Wenchuan earthquake. Variants of *GHSR* rs495225 were identified by polymerase chain reaction-restriction fragment length polymorphism analyses and verified by DNA sequencing. The PTSD Checklist Civilian Version (PCL-C) was used to assess PTSD. There was no significant difference of PTSD prevalence between the TT homozygotes and the C allele carriers. However, the students with PTSD had significantly lower levels of glucose, insulin and homeostasis model assessment of insulin resistance (HOMA-IR) than the students without PTSD in the C allele carriers of *GHSR* rs495225 after the adjustment for age, gender and body mass index (BMI), but higher levels of TG and TG/HDL-C in the TT homozygotes. Meanwhile, the TT homozygotes had lower levels of HDL-C than the C allele carriers in the students without PTSD, but higher levels of insulin and HOMA-IR in the subjects with PTSD. After the adjustment of age and gender, and additional adjustment for BMI, the results were not changed except the difference of insulin was only a tendency ($p = 0.054$) after the additional adjustment for BMI. PTSD may augment TG levels and the related lipid ratio TG/HDL-C in the TT homozygotes of *GHSR* rs495225 but decrease the levels of glucose, insulin and HOMA-IR in the C allele carriers.

Keywords PTSD · *GHSR* · rs495225 · Lipid · Insulin

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Introduction

It was reported that psychological distresses were increased after natural disasters (Kulkarni and Pole 2008; Wahlström et al. 2009). Among the wide range of the consequences of disasters, posttraumatic stress disorder (PTSD) was documented to be probably the most frequent psychopathology in the aftermaths of disasters (Galea et al. 2005; Iii et al. 2006). PTSD is a mental illness after exposures to a traumatic stressor, which has three symptom clusters: the re-experiencing cluster, the numbing cluster and the miscellaneous symptoms such as exaggerated startle, sleep disturbance, and memory impairment or trouble concentrating (McNally 2003). Previous researches have demonstrated that PTSD is associated with poor physical health, including the elevated prevalence of cardiovascular disease (CVD) (Dedert et al. 2010). Meanwhile, obesity, diabetes and abnormalities of plasma lipid profiles, including increased levels of plasma triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and decreased levels of high-density lipoprotein cholesterol (HDL-C) are the major traditional risk factors of CVD (Baigent et al. 2005; Mihaylova et al. 2012). Moreover, previous studies reported that participants with PTSD had significantly higher body mass index (BMI) (Dobie et al. 2004; Vieweg et al. 2007) and morbidity of diabetes (Lukaschek et al. 2013; Vancampfort et al. 2016), higher levels of TG, TC and LDL-C, and lower levels of HDL-C (Kagan et al. 1999; Karlović et al. 2004; Vilibić et al. 2014) when compared with the control subjects. However, the associations are still controversial since some studies reported no associations between PTSD and BMI (Barber et al. 2011; Kozaric-Kovacic et al. 2009), diabetes (Goodwin and Davidson 2005) or lipid profiles (Jendricko et al. 2009; Tochigi et al. 2002). The mechanism of these controversies has not been elucidated yet. Nevertheless, genetic backgrounds were not included in these studies, which are believed to be closely related to not only PTSD but also these metabolic parameters.

Growth hormone secretagogue receptor (GHSR), also known as ghrelin receptor, was first cloned in human pituitary and hypothalamus (Howard et al. 1996). It is implicated in a diverse range of physiological processes, such as the release of growth hormone, modulation of food intake and energy metabolism, regulation of pancreatic function, influences on glucose and lipid metabolism, and cell protection in the nervous and the cardiovascular systems (Beiras-Fernandez et al. 2010; Date et al. 2002; Hosoda et al. 2006; Jiang et al. 2006; Sun et al. 2004). The GHSR gene (*GHSR*) is located on chromosome 3q26.31 and encodes a protein that belongs to the family of G protein-coupled receptors (Howard et al. 1996). The gene consists of 2 exons separated by 1 intron. The polymorphism of *GHSR* rs495225 (NG_021159.1:g.5214C>T) is on the exon 1 with a cytosine (C) to thymine (T) transition at base 171 (c.171T>C) (Liu et al. 2011). Previous studies indicated that this polymorphism was associated with obesity (Wang et al. 2004) and type 2 diabetes (Vartiainen et al. 2004). Wang et al. (2004) found that the frequency of the 171T allele of *GHSR* rs495225 was higher in obese subjects (75.0%) than in the underweight individuals (70.2%). Vartiainen et al.

(2004) showed that the subjects with the CC genotype of *GHSR* rs495225 had higher area under the insulin curve values than the T allele carriers in an oral glucose tolerance test (OGTT) (Vartiainen et al. 2004). However, no unequivocal association was found between *GHSR* rs495225 and the measures of obesity or type 2 diabetes in a study conducted in 507 middle-aged overweight persons with impaired glucose tolerance (Mager et al. 2008). Still, the mechanism of these equivocal reports has not been interpreted. In addition, the association of this polymorphism with plasma lipid profiles has not been reported yet.

To explain the above discrepancies of the association of metabolic parameters with PTSD or *GHSR* rs495225, we hypothesized that *GHSR* rs495225 might interact with PTSD and diversify the associations of PTSD with the CVD-related metabolic parameters. To test our hypothesis in the present study, the effects of PTSD on the CVD-related metabolic parameters were analyzed in high school students with different genotypes of *GHSR* rs495225. These students were enrolled at 6 months after the 2008 Wenchuan earthquake, a devastating one rating 8.0 on the Richter scale occurred on May 12, 2008. The earthquake spread about 100,000 km^2, destroyed about 6.5 million homes and influenced approximately 46 million persons. Official figures stated that 69,277 were confirmed dead, 374,643 injured, and 17,923 were missing (https://www.csi.ac.cn/sichuan/index080512001.htm; website in Chinese). Lipid ratios were also included in the current work because studies had demonstrated that the lipid ratios might be superior to conventional lipid parameters as predictors of CVD, including TG/HDL-C, TC/HDL-C and LDL-C/ HDL-C (Hsia et al. 2006; Millan et al. 2009; Shai et al. 2004).

Materials and Methods

Study Population

This study population was enrolled at 6 months after Wenchuan earthquake in a boarding high school (grades 11–12), which was located only 10 KM away from the epicenter and severely destroyed by the earthquake. This school was selected for two reasons. The first one is that it is a public school with common characteristics of all local schools. The characteristics of the students were representative of and comparable to all students in this region. The second reason is that there was no death or heavy injury in this school and the students did not receive any therapeutic interventions or psychological help. The students were not dispersed to other schools, but lived and studied in temporary houses for 15 months until the school was rebuilt. Of all the 746 students enrolled, 709 students were finally included in the present study. The other students were excluded because (1) they did not complete all the questionnaires as requested in the survey and (2) their blood were not sampled because of personal reasons and (3) they had diseases, medications or other interferences that influenced plasma lipid profiles or PTSD. All the 709 students were Chinese Han. Written Consents were provided by the students and their guardians. This study was approved by the Human Ethics Committee of Sichuan University.

Measurements

The measurements consisted of two parts and were carried out at 6 months after the earthquake. The first part was used to assess demographic characteristics including gender, age, height and weight. Body mass index (BMI) was calculated by dividing weight in kilograms (kg) by height in meters (m) squared (kg/m^2) for each participant. In the second part, PTSD was assessed using the PTSD Checklist-Civilian Version (PCL-C)(Blanchard et al. 1996), which is a self-report 17-item symptom scale that corresponds to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria (APA, 2000). Each item of PCL-C was scored from 1 to 5, and the total score ranged from 17 to 85. A total score of 50 was used to be the cutoff to classify whether an adolescent has clinically significant PTSD symptoms or not in this study. The students with PCL-C scores of 50 and more were designated as subjects with PTSD and less than 50 as subjects without PTSD. The PCL-C has been shown to have high internal consistency (Cook et al. 2005), and has been commonly used with adolescents (Barnes et al. 2005; Calderoni et al. 2006; Liu et al. 2010). All questionnaires were pretested with students in the school who had been exposed to the earthquake.

DNA Extraction and Genotyping

Genomic DNAs were prepared from peripheral blood cells at 6 months after the earthquake using a DNAout kit (Tiandz, china) and stored at -80°C for further uses. The polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method was used to screen the genotype of *GHSR* rs495225 in 2016 using the stored genomic DNAs. A 593-bp DNA fragment containing the polymorphic site was amplified by PCR using the following primer sequences: forward primer, 5′-CGGGGTTCAACCTCACACT-3′, reverse primer, 5′-AGAGCGCAC CGCAA ACTC-3′. The PCR conditions were denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 35 s, annealing at 63°C for 20 s, extension at 72°C for 40 s and a final extension at 72°C for 10 min. The genotypes of *GHSR* rs495225 were identified by restriction digestion with LweI (Fermentas, Vilnius, Lithuania) and electrophoreses on 3.0% agarose gel, and verified by DNA sequencing.

Blood Collection and Laboratory Analyses

Twelve-hour fasting venous bloods were sampled between 7:00 a.m. and 8:00 a.m. at 6 months after the earthquake. Laboratory analyses were carried out after the sampling. Enzymatic methods were used to assess the plasma concentrations of TG, TC and glucose. LDL-C concentrations were quantified by the polyvinyl sulfate precipitation method. HDL-C concentrations were measured enzymatically after phosphotungstic-Mg^{2+} precipitation of apolipoprotein B-containing lipoproteins. Insulin concentrations were determined by electrochemical luminescence with a Roche E170 Analyzer. Homeostasis model assessment of insulin resistance

(HOMA-IR) was calculated by glucose (mmol/L) × insulin (mIU/L)/22.5. TG/HDL-C, TC/HDL-C and LDL-C/HDL-C were calculated. All biochemical parameters were measured three times and the average values were used for statistical analyses.

Statistical Analyses

Data were expressed as mean ± SD unless otherwise stated. Statistical analyses were carried out in 2016 using the data of anthropometric, biochemical and psychological characteristics obtained at 6 months after the earthquake, and the data of genetic characteristics obtained in 2016. The χ^2 goodness-of-fit test was used to identify significant departures from the Hardy–Weinberg equilibrium. Chi-square was used to examine the differences in genotypic distribution and PTSD prevalence. One-way analysis of variance (ANOVA) was applied to analyze the differences of variables between the subjects with different genotypes or the subjects with and without PTSD. Analyses of covariance (ANCOVA) with age and gender or with age, gender and BMI as covariates were used to analyze the differences of TG, TC, LDL-C, HDL-C, TG/HDL-C, TC/HDL-C, LDL-C/HDL-C, glucose, insulin and HOMA-IR in subjects with and without PTSD or/and with different genotypes of *GHSR* rs495225. Age, gender and BMI were used as covariates because impacts of these variables were observed on plasma lipid and glucose concentrations (Bijari et al. 2015; Guzzaloni et al. 1991; Polac et al. 2003; Wakabayashi 2014). Statistical significance was defined as $p \leq 0.05$.

Results

Distribution of the Genotypes of *GHSR* rs495225

As Fig. 1 shows, the digestion of the PCR products with the CC genotype yielded two bands of 151 and 442 bp, the TT genotype yielded only one band of 593 bp, and the CT genotype yielded three bands of 151, 442 and 593 bp.

Table 1 shows the genotype frequencies of *GHSR* rs495225 in the subjects of the present study. No departure was found from Hardy–Weinberg equilibrium in the study population ($p = 0.253$). Moreover, no significant gender differences were found in the genotype frequencies of *GHSR* rs495225 ($p = 0.498$).

Prevalence of PTSD in the Subjects with Different Genotypes of *GHSR* rs495225

Table 2 presents the prevalence of PTSD in the students with different *GHSR* rs495225 genotypes. Due to the relatively small number of subjects with the CC genotype, the CC homozygotes and the CT heterozygotes were ascribed to one group, designated as the C allele carriers and presented as CX in the tables. There was no significant difference of PTSD prevalence between the TT homozygotes and the C allele carriers in the all subjects, the male subjects or the female subjects. In addition, the female subjects

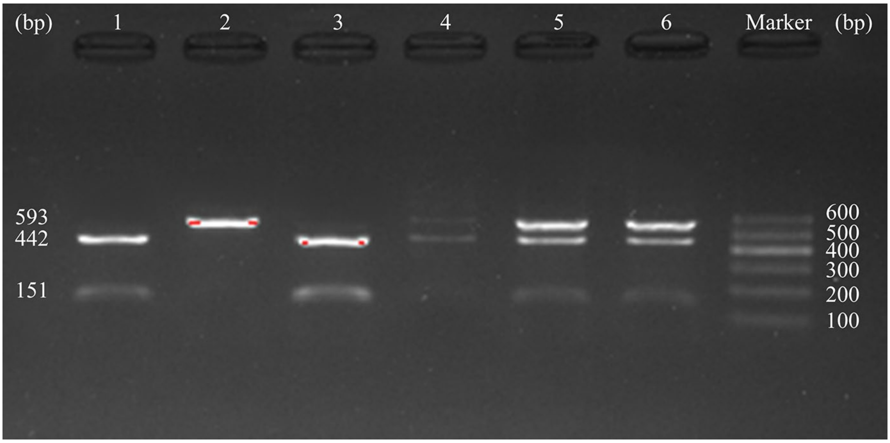

Fig. 1 Genotyping electrophoretogram of *GHSR* rs495225. Marker: DNA marker; 1, 3: CC; 2: TT; 4, 5, 6: CT

Table 1 Distribution of the genotypes of *GHSR* rs495225

Genotype	Total *n* (%)	Hardy–Weinberg *p*	Males *n* (%)	Females *n* (%)	p^a
CC	98 (13.8)	0.253	44 (14.1)	54 (13.6)	0.498
CT	312 (44.0)		144 (46.2)	168 (42.3)	
TT	299 (42.2)		124 (39.7)	175 (44.1)	

Data are expressed as *n* (%)

[a]Males vs. females by Chi-square tests

had significantly higher prevalence of PTSD than the male subjects in both the TT homozygotes ($p=0.004$) and the C allele carriers ($p=0.018$).

Anthropometric and Biochemical Characteristics of the Subjects With or Without PTSD

As presented in Table 3, the subjects with PTSD had significantly higher BMI, TG, TC levels and TG/HDL-C than the subjects without PTSD. After the adjustment of age and gender, the BMI, TG levels and TG/HDL-C of the PTSD subjects were still significantly higher than those of the subjects without PTSD. After the additional adjustment for BMI, no significant difference was found between the subjects with or without PTSD.

Table 2 Prevalence of PTSD in the subjects with different genotypes of *GHSR* rs495225

Genotype	All			Males			Females		
	With PTSD	Without PTSD	OR (95% CI)	With PTSD	Without PTSD	OR (95% CI)	With PTSD	Without PTSD	OR (95% CI)
TT	33 (11.0)	266 (89.0)	1.09 (0.67 ~ 1.76)	6 (4.80)	118 (95.2)	0.75 (0.27 ~ 2.04)	27 (15.4) ##	148 (84.6)	1.17 (0.67 ~ 2.05)
CX	42 (10.2)	368 (89.8)		12 (6.40)	176 (93.6)		30 (13.5) #	192 (86.5)	

Data are expressed as *n* (%)

CX C allele carriers, *TT* TT homozygotes, *OR* odds ratio, *CI* confidence interval

#$p<0.05$, ##$p<0.01$ when compared with that of the male students

Table 3 Anthropometric and biochemical characteristics of the subjects with or without PTSD

Variables	Without PTSD	With PTSD	*p*-value	ANCOVA, *p*-value*	ANCOVA, *p*-value[&]
Gender: female, *n* (%)	340 (53.6)	57 (76.0)	**0.000**	–	–
Age, year	16.9 ± 0.58	17.0 ± 0.61	0.171	–	–
BMI, kg/m^2	20.2 ± 2.22	21.4 ± 2.69	**0.000**	**0.000**	–
TG, mmol/L	1.10 ± 0.40	1.29 ± 0.66	**0.000**	**0.011**	0.091
TC, mmol/L	3.57 ± 0.56	3.74 ± 0.64	**0.014**	0.142	0.336
LDL-C, mmol/L	1.66 ± 0.47	1.74 ± 0.59	0.156	0.300	0.752
HDL-C, mmol/L	1.41 ± 0.28	1.41 ± 0.29	0.994	0.515	0.813
TG/HDL-C	0.82 ± 0.39	0.99 ± 0.66	**0.001**	**0.008**	0.105
TC/HDL-C	2.61 ± 0.57	2.74 ± 0.67	0.057	0.078	0.580
LDL-C/HDL-C	0.23 ± 0.46	1.29 ± 0.53	0.314	0.275	0.987
Glucose, mmol/L	5.08 ± 0.43	5.00 ± 0.47	0.137	0.486	0.299
Insulin, mIU/L	11.8 ± 5.21	13.1 ± 8.06	0.057	0.460	0.670
HOMA-IR	2.67 ± 1.24	2.95 ± 2.04	0.091	0.466	0.669

Bold values indicate the significant difference *p* value

Data are expressed as mean ± SD

*Analyses of covariance with the adjustment for age, gender

[&]Analyses of covariance with the adjustment for age, gender and BMI

Anthropometric and Biochemical Characteristics of the Subjects With Different Genotypes of *GHSR* rs495225

As presented in Table 4, the plasma levels of HDL-C were significantly lower in the TT homozygotes than that in the C allele carriers. Moreover, the TG/HDL-C and the plasma levels of insulin were significantly higher in the TT homozygotes when compared with those in the C allele carriers. After the adjustment of age and gender, the TT homozygotes only showed lower HDL-C levels and higher TG/HDL-C than the C allele carriers. After the additional adjustment for BMI, the HDL-C levels was still significantly lower while TG/HDL-C, TC/HDL-C and insulin levels were significantly higher in the TT homozygotes than those in the C allele carriers.

Interplays of PTSD with *GHSR* rs495225 on Anthropometric and Biochemical Characteristics

Table 5 presents the interplays of PTSD with *GHSR* rs495225 on anthropometric and biochemical characteristics of the subjects in the present study. The plasma levels of HDL-C was significantly lower in the TT homozygotes without PTSD than that in the C allele carriers without PTSD. In addition, both the plasma levels of insulin and HOMA-IR were significantly higher in the TT homozygotes with PTSD than those in the C allele carriers with PTSD. After the adjustment of age

Table 4 Anthropometric and biochemical characteristics of the subjects with different genotypes of *GHSR* rs495225

Variables	CX	TT	*p* value	ANCOVA, *p* value*	ANCOVA, *p* value&
Gender: female, *n* (%)	226 (54.1)	175 (58.5)	0.246	–	–
Age, year	16.9 ± 0.59	16.9 ± 0.59	0.982	–	–
BMI, kg/m^2	20.3 ± 2.18	20.8 ± 2.47	0.925	0.895	–
TG, mmol/L	1.09 ± 0.43	1.15 ± 0.45	0.077	0.141	0.121
TC, mmol/L	3.60 ± 0.57	3.58 ± 0.58	0.712	0.467	0.474
LDL-C, mmol/L	1.66 ± 0.46	1.67 ± 0.52	0.852	0.952	0.931
HDL-C, mmol/L	1.43 ± 0.29	1.38 ± 0.26	**0.018**	**0.009**	**0.007**
TG/HDL-C	0.81 ± 0.41	0.88 ± 0.44	**0.028**	**0.045**	**0.032**
TC/HDL-C	2.59 ± 0.55	2.67 ± 0.62	0.053	0.062	**0.043**
LDL-C/HDL-C	1.21 ± 0.44	1.27 ± 0.51	0.138	0.135	0.110
Glucose, mmol/L	5.08 ± 0.43	5.06 ± 0.44	0.617	0.790	0.798
Insulin, mIU/L	11.5 ± 4.99	12.4 ± 6.28	**0.032**	0.061	**0.045**
HOMA-IR	2.62 ± 1.20	2.81 ± 1.53	0.059	0.102	0.079

Bold values indicate the significant difference *p* value

Data are expressed as mean ± SD

BMI body mass index, *TG* triglycerides, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment-insulin resistance, *ANCOVA* analysis of covariance

*Analyses of covariance with the adjustment for age, gender

&Analyses of covariance with the adjustment for age, gender and BMI

and gender, the results were not changed. After the additional adjustment for BMI, the HDL-C levels was significantly lower in the TT homozygotes than that in the C allele carriers only in the subjects without PTSD. HOMA-IR was significantly higher in the TT homozygotes than that in the C allele carriers in the PTSD subjects.

In the C allele carriers, the PTSD subjects showed significantly higher BMI and lower glucose levels than the subjects without PTSD. While in the TT homozygotes, BMI, TG, TG/HDL-C, insulin and HOMA-IR were significantly higher in the PTSD subjects than those in subjects without PTSD. After the adjustment of age and gender, the results were changed. After the additional adjustment for BMI, the PTSD subjects had significantly lower glucose levels, insulin levels and HOMA-IR than the subjects without PTSD only in the C allele carriers, while the PTSD subjects had significantly higher TG levels and TG/HDL-C than the subjects without PTSD in the TT homozygotes.

Table 5 Interplays of PTSD with GHSR rs495225 on anthropometric and biochemical characteristics

Variables	Group	Without PTSD	With PTSD	*p* value[b]	ANCOVA, *p* value[b,*]	ANCOVA, *p* value[b,&]
Gender: female, *n* (%)	CX	192 (52.2)	30 (71.4)	**0.018**	–	–
	TT	148 (55.6)	27 (81.8)	**0.004**	–	–
	p value[a]	0.388	0.296			
	ANCOVA, *p* value[a,*]	–	–			
	ANCOVA, *p* value[a,&]	–	–			
Age, year	CX	16.9 ± 0.58	16.9 ± 0.62	0.589	–	–
	TT	16.8 ± 0.59	17.0 ± 0.61	0.148	–	–
	p value[a]	0.812	0.508			
	ANCOVA, *p* value[a,]	–	–			
	ANCOVA, *p* value[a,&]	–	–			
BMI, kg/m^2	CX	20.2 ± 2.13	21.1 ± 2.42	**0.013**	**0.037**	–
	TT	20.1 ± 2.35	21.8 ± 2.99	**0.000**	**0.001**	–
	p value[a]	0.678	0.276			
	ANCOVA, *p* value[a,*]	0.552	0.306			
	ANCOVA, *p* value[a,&]	–	–			
TG, mmol/L	CX	1.08 ± 0.41	1.16 ± 0.60	0.294	0.714	0.868
	TT	1.11 ± 0.40	1.45 ± 0.71	**0.000**	**0.001**	**0.008**
	p value[a]	0.359	0.058			
	ANCOVA, *p* value[a,*]	0.513	0.084			
	ANCOVA, *p* value[a,&]	0.435	0.157			
TC, mmol/L	CX	3.58 ± 0.57	3.73 ± 0.58	0.097	0.322	0.482
	TT	3.56 ± 0.56	3.75 ± 0.72	0.069	0.268	0.493
	p value[a]	0.632	0.906			
	ANCOVA, *p* value[a,*]	0.451	0.967			
	ANCOVA, *p* value[a,&]	0.492	0.786			
LDL-C, mmol/L	CX	1.65 ± 0.46	1.75 ± 0.51	0.201	0.327	0.574
	TT	1.66 ± 0.50	1.73 ± 0.68	0.475	0.622	0.930
	p value[a]	0.808	0.891			
	ANCOVA, *p* value[a,*]	0.883	0.811			
	ANCOVA, *p* value[a,&]	0.784	0.723			

Table 5 (continued)

Variables	Group	Without PTSD	With PTSD	p value[b]	ANCOVA, p value[b,*]	ANCOVA, p value[b,&]
HDL-C, mmol/L	CX	1.43 ± 0.29	1.45 ± 0.29	0.604	0.945	0.570
	TT	1.38 ± 0.26	1.36 ± 0.29	0.564	0.282	0.761
	p value[a]	**0.045**	0.153			
	ANCOVA, p value[a,*]	**0.030**	0.157			
	ANCOVA, p value[a,&]	**0.017**	0.217			
TG/HDL-C	CX	0.80 ± 0.39	0.86 ± 0.61	0.366	0.597	0.905
	TT	0.85 ± 0.39	1.15 ± 0.69	**0.000**	**0.001**	**0.013**
	p value[a]	0.163	0.058			
	ANCOVA, p value[a,*]	0.217	0.077			
	ANCOVA, p value[a,&]	0.151	0.144			
TC/HDL-C	CX	2.58 ± 0.55	2.65 ± 0.60	0.427	0.490	0.968
	TT	2.65 ± 0.60	2.86 ± 0.74	0.060	0.073	0.402
	p value[a]	0.130	0.178			
	ANCOVA, p value[a,*]	0.145	0.218			
	ANCOVA, p value[a,&]	0.083	0.363			
LDL-C/HDL-C	CX	1.21 ± 0.44	1.25 ± 0.45	0.539	0.518	0.999
	TT	1.26 ± 0.49	1.33 ± 0.63	0.437	0.373	0.964
	p value[a]	0.184	0.526			
	ANCOVA, p value[a,*]	0.184	0.580			
	ANCOVA, p value[a,&]	0.116	0.746			
Glucose, mmol/L	CX	5.09 ± 0.44	4.93 ± 0.34	**0.024**	**0.046**	**0.022**
	TT	5.06 ± 0.42	5.08 ± 0.59	0.770	0.185	0.227
	p value[a]	0.307	0.177			
	ANCOVA, p value[a,*]	0.383	0.122			
	ANCOVA, p value[a,&]	0.413	0.141			
Insulin, mIU/L	CX	11.5 ± 5.00	11.3 ± 5.29	0.793	0.204	**0.044**
	TT	12.1 ± 5.52	15.3 ± 10.3	**0.006**	**0.032**	0.216
	p value[a]	0.203	**0.034**			
	ANCOVA, p value[a,*]	0.304	**0.028**			
	ANCOVA, p value[a,&]	0.215	0.054			

Table 5 (continued)

Variables	Group	Without PTSD	With PTSD	p value[b]	ANCOVA, p value[b,*]	ANCOVA, p value[b,&]
HOMA-IR	CX	2.63 ± 1.20	2.50 ± 1.22	0.509	0.122	**0.020**
	TT	2.72 ± 1.31	3.51 ± 2.67	**0.005**	**0.019**	0.139
	p value[a]	0.353	**0.032**			
	ANCOVA, p value[a,*]	0.493	**0.025**			
	ANCOVA, p value[a,&]	0.376	**0.046**			

Bold values indicate the significant difference p value

Data are expressed as mean ± SD

*Analyses of covariance with the adjustment for gender, age

[&]Analyses of covariance with the adjustment for gender, age and BMI

[a]Comparisons of those between the TT homozygotes and the C allele carriers in the subjects with or without PTSD

[b]Comparisons of those between the subjects with or without PTSD in the TT homozygotes or the C allele carriers

Discussion

The association of metabolic parameters with PTSD or *GHSR* rs495225 have explored by others (Barber et al. 2011; Dobie et al. 2004; Goodwin and Davidson 2005; Jendricko et al. 2009; Kagan et al. 1999; Karlović et al., 2004; Kozaric-Kovacic et al. 2009; Lukaschek et al. 2013; Mager et al. 2008; Tochigi et al. 2002; Vancampfort et al. 2016; Vartiainen et al. 2004; Vieweg et al. 2007; Vilibić et al. 2014; Wang et al. 2004). However, the results were not consistent. The mechanism of the controversies has not been clarified yet. The present study showed that the subjects with PTSD had significantly higher BMI, TG, TC and TG/HDL-C than the subjects without PTSD (Table 3). These findings are in accordance with the results of the prior investigations in which veterans with chronic PTSD showed higher TG and TC levels (Kagan et al. 1999; Karlovic et al. 2004). Nevertheless, these previous studies did not include the analysis of the lipid ratios, which were reported to be superior to conventional lipids parameters as predictors of CVD (Hsia et al. 2006; Millan et al. 2009; Shai et al. 2004). Furthermore, the results of the present study showed that the significant differences of BMI, TG and TG/HDL-C between the subjects with and without PTSD still existed after the adjustment for age and gender. However, no significant difference was found after the additional adjustment for BMI (Table 3), an important factor reported to be associated with lipid profiles (Pereira and Power 2013; Shamai et al. 2011). These results suggest that BMI may be one of the factors mediating the difference of the lipid profiles between the subjects with and without PTSD.

Previous studies showed that the CC homozygotes had higher area under the insulin curve values than the T allele carriers after OGTT in patients from the Oulu Project Elucidating Risk of Atherosclerosis study (Vartiainen et al. 2004). However, no significant differences were observed of type 2 diabetes related parameters between the participants with different *GHSR* rs495225 genotypes in individuals with impaired glucose tolerance (Mager et al. 2008). In the present study, no significant difference of plasma glucose levels was observed between the TT homozygotes and the C allele carriers of *GHSR* rs495225 regardless of the adjustment for age and gender or the additional adjustment for BMI. However, the TT homozygotes were found to have significantly higher levels of insulin than the C allele carriers only after the adjustment for age, gender and BMI. These results suggest that age, gender and BMI are the confounding factors affecting the association of *GHSR* rs495225 with plasma levels of insulin. This finding may be one of the explanations for the previous inconsistent reports in the literature. It should also be considered that the participants of the present study were healthy young adolescents with normal glucose and insulin levels.

Although discrepancies have been reported about the associations between *GHSR* rs495225 and obesity or diabetes (Mager et al. 2008; Vartiainen et al. 2004; Wang et al. 2004), the association of this polymorphism has not been explored with plasma lipid profiles. Meanwhile, the TT homozygotes were found to have significantly lower levels of HDL-C and higher levels of TG/HDL-C and TC/HDL-C than the C allele carriers after the adjustment for age, gender and BMI (Table 4). These results demonstrate for the first time that *GHSR* rs495225 is associated with plasma levels of HDL-C and its related lipid ratios.

When both PTSD and the polymorphism of *GHSR* rs495225 were taken into consideration, the TT homozygotes were detected to have lower levels of HDL-C than the C allele carriers in the students without PTSD, but higher levels of insulin and HOMA-IR in the subjects with PTSD even after the adjustment of age and gender and additional adjustment for BMI. Furthermore, the PTSD subjects had lower levels of glucose than the subjects without PTSD in the C allele carriers, but higher levels of TG, TG/HDL-C, insulin and HOMA-IR in the TT homozygotes even after the adjustment of age and gender. After the additional adjustment for BMI, the PTSD subjects had significantly lower levels of glucose, insulin and HOMA-IR in the C allele carriers, and higher levels of TG and TG/HDL-C in the TT homozygotes (Table 5). These results indicated that the associations between PTSD and metabolic parameters were not only affected by age, gender and BMI, but also influenced by *GHSR* rs495225.

Although the polymorphism of *GHSR* rs495225 is located within the transmembrane domain of the coded protein, it does not alter the amino acid sequence (Liu et al. 2011). The related functions of the encoded protein should not be changed. Therefore, other mechanisms such as linkage dysequilibrium with functional polymorphisms need to take into account in the underlying mechanism of the effects of *GHSR* rs495225 observed in the present study. Another mechanism can also be considered that *GHSR* rs495225 may change binding capabilities for transcription factors, leading to altered expression levels of the protein product.

There were some limitations in the present study. Firstly, the subjects in the present study were healthy young students. This needs to take into account for the explanation and implication of the results in other populations. Second, plasma levels of GHSR were not measured.

Conclusion

Taken all above together, the conclusion may be made that there may be some interplays of PTSD with *GHSR* rs495225 on plasma metabolic parameters, together with age, gender and BMI. PTSD subjects had significant higher levels of TG and TG/HDL-C in the TT homozygotes, but lower levels of glucose, insulin and HOMA-IR in the C allele carries. The present finding may be one of the explanations for the discrepancies of the association of metabolic parameters with PTSD or *GHSR* rs495225 reported before and pave the way to precision medical intervention to reduce risks of metabolic diseases in young subjects suffering from PTSD with different genotypes of *GHSR* rs495225.

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Author Contributions Ding Zhi Fang was responsible for conception, study design, data interpretation and manuscript finalization. Mi Su was responsible for lab experiments, statistical analysis and manuscript preparation. Yan Jun Si and Qi Wei Guo were involved in lab experiments and statistical analysis. Mei Yang, Xu Chen and Jia Lin were involved in evolving the ideas and revising the manuscript. All authors have contributed to and have approved the final manuscript.

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References

Baigent C et al (2005) Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 366:1267–1278. https://doi.org/10.1016/s0140-6736(05)67394-1

Barber J, Bayer L, Pietrzak RH, Sanders KA (2011) Assessment of rates of overweight and obesity and symptoms of posttraumatic stress disorder and depression in a sample of operation enduring freedom/operation Iraqi freedom veterans. Milit Med 176:151–155

Barnes VA, Treiber FA, Ludwig DA (2005) African-American adolescents' stress responses after the 9/11/01 terrorist attacks. J Adolesc Health 36:201–207. https://doi.org/10.1016/j.jadohealth.2004.02.035

Beiras-Fernandez A et al (2010) Altered myocardial expression of ghrelin and its receptor (GHSR-1a) in patients with severe heart failure. Peptides 31:2222–2228. https://doi.org/10.1016/j.peptides.2010.08.019

Bijari B, Taheri F, Chahkandi T, Kazemi T, Namakin K, Zardast M (2015) The relationship between serum lipids and obesity among elementary school in Birjand: a case control study. J Res Health Sci 15:83–87

Blanchard EB, Jones-Alexander J, Buckley TC, Forneris CA (1996) Psychometric properties of the PTSD Checklist (PCL). Behav Res Therapy 34:669–673

Calderoni ME, Alderman EM, Silver EJ, Bauman LJ (2006) The mental health impact of 9/11 on inner-city high school students 20 miles north of Ground Zero. The Journal of adolescent health 39:57–65. https://doi.org/10.1016/j.jadohealth.2005.08.012

Cook JM, Elhai JD, Arean PA (2005) Psychometric properties of the PTSD Checklist with older primary care patients. J Trauma Stress 18:371–376. https://doi.org/10.1002/jts.20038

Date Y et al (2002) Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. Diabetes 51:124–129

Dedert EA, Calhoun PS, Watkins LL, Sherwood A, Beckham JC (2010) Posttraumatic stress disorder, cardiovascular, and metabolic disease: a review of the evidence. Ann Behav Med 39:61–78. https://doi.org/10.1007/s12160-010-9165-9

Dobie DJ, Kivlahan DR, Maynard C, Bush KR, Davis TM, Bradley KA (2004) Posttraumatic stress disorder in female veterans: association with self-reported health problems and functional impairment. Arch Intern Med 164:394–400. https://doi.org/10.1001/archinte.164.4.394

Galea S, Nandi A, Vlahov D (2005) The epidemiology of post-traumatic stress disorder after disasters. Epidemiol Rev 27:78–91. https://doi.org/10.1093/epirev/mxi003

Goodwin RD, Davidson JR (2005) Self-reported diabetes and posttraumatic stress disorder among adults in the community. Prev Med 40:570–574

Guzzaloni G, Moreni G, Ardizzi A, Moro D, Grugni G, Morabito F (1991) Correlation between lipid pattern and body mass index in obesity. Minerva Med 82:339–344

Hosoda H, Kojima M, Kangawa K (2006) Biological, physiological, and pharmacological aspects of ghrelin. J Pharmacol Sci 100:398–410

Howard AD et al (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. Science (New York, NY) 273:974–977

Hsia SH, Pan D, Berookim P, Lee ML (2006) A population-based, cross-sectional comparison of lipid-related indexes for symptoms of atherosclerotic disease. Am J Cardiol 98:1047–1052. https://doi.org/10.1016/j.amjcard.2006.05.024

Iii JL, Hahn H, Martin A (2006) 9/11: mental health in the wake of terrorist attacks. BMJ 333:219

Jendricko T, Vidović A, Grubisić-Ilić M, Romić Z, Kovacić Z, Kozarić-Kovacić D (2009) Homocysteine and serum lipids concentration in male war veterans with posttraumatic stress disorder. Prog Neuropsychopharmacol Biol Psychiatr 33:134–140

Jiang H, Betancourt L, Smith RG (2006) Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. Molecular endocrinology (Baltimore, Md) 20:1772–1785. https://doi.org/10.1210/me.2005-0084

Kagan BL, Leskin G, Haas B, Wilkins J, Foy D (1999) Elevated lipid levels in Vietnam veterans with chronic posttraumatic stress disorder. Biol Psychiatr 45:374–377

Karlović D, Buljan D, Martinac M, Marcinko D (2004) Serum lipid concentrations in Croatian veterans with post-traumatic stress disorder, post-traumatic stress disorder comorbid with major depressive disorder, or major depressive disorder. J Korean Med Sci 19:431–436

Karlovic D, Martinac M, Buljan D, Zoricic Z (2004) Relationship between serum lipid concentrations and posttraumatic stress disorder symptoms in soldiers with combat experiences. Acta Med Okayama 58:23–27

Kozaric-Kovacic D, Ilic MG, Romic Z, Vidovic A, Jendricko T, Pivac N (2009) Body mass index in male Caucasian veterans with or without posttraumatic stress disorder Prog Neuro-Psychopharmacol. Biol Psychiatr 33:1447–1450. https://doi.org/10.1016/j.pnpbp.2009.07.026

Kulkarni M, Pole N (2008) Psychiatric distress among Asian and European American Survivors of the 1994 Northridge Earthquake. J Nervous Mental Dis 196:597–604. https://doi.org/10.1097/NMD.0b013e3181813290

Liu B, Garcia EA, Korbonits M (2011) Genetic studies on the ghrelin, growth hormone secretagogue receptor (GHSR) and ghrelin O-acyl transferase (GOAT) genes. Peptides 32:2191–2207. https://doi.org/10.1016/j.peptides.2011.09.006

Liu ZY, Yang YF, Ye YL, Zeng ZQ, Xiang YJ, Yuan P (2010) One-year follow-up study of post-traumatic stress disorder among adolescents following the Wen-Chuan earthquake in China. Biosci Trends 4:96–102

Lukaschek K et al (2013) Relationship between posttraumatic stress disorder and Type 2 Diabetes in a population-based cross-sectional study with 2970 participants. J Psychosomat Res 74:340–345. https://doi.org/10.1016/j.jpsychores.2012.12.011

Mager U et al (2008) Variations in the ghrelin receptor gene associate with obesity and glucose metabolism in individuals with impaired glucose tolerance. PloS ONE 3:e2941. https://doi.org/10.1371/journal.pone.0002941

McNally RJ (2003) Progress and controversy in the study of posttraumatic stress disorder. Annu Rev Psychol 54:229–252. https://doi.org/10.1146/annurev.psych.54.101601.145112

Mihaylova B et al (2012) The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet 380:581–590. https://doi.org/10.1016/s0140-6736(12)60367-5

Millan J et al (2009) Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manage 5:757–765

Pereira SMP, Power C (2013) Life course body mass index, birthweight and lipid levels in mid-adulthood: a nationwide birth cohort study. Eur Heart J 34:1215–1224. https://doi.org/10.1093/eurheartj/ehs333

Polac I, Stachowiak G, Jedrzejczyk S, Stetkiewics T, Sobieszczanska S, Pertynski T (2003) Hemostatic variables, carbohydrate metabolism and lipid profile in women with low body mass index. Gynecol Endocrinol 17:151–157

Shai I et al (2004) Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. Circulation 110:2824–2830. https://doi.org/10.1161/01.cir.0000146339.57154.9b

Shamai L et al (2011) Association of body mass index and lipid profiles: evaluation of a broad spectrum of body mass index patients including the morbidly obese. Obes Surg 21:42–47. https://doi.org/10.1007/s11695-010-0170-7

Sun Y, Wang P, Zheng H, Smith RG (2004) Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci USA 101:4679–4684. https://doi.org/10.1073/pnas.0305930101

Tochigi M et al (2002) Serum cholesterol, uric acid and cholinesterase in victims of the Tokyo subway sarin poisoning: a relation with post-traumatic stress disorder. Neurosci Res 44:267–272

Vancampfort D et al (2016) Type 2 diabetes among people with posttraumatic stress disorder: systematic review and meta-analysis. Psychosom Med 78:465–473. https://doi.org/10.1097/psy.0000000000000297

Vartiainen J, Poykko SM, Raisanen T, Kesaniemi YA, Ukkola O (2004) Sequencing analysis of the ghrelin receptor (growth hormone secretagogue receptor type 1a) gene. Eur J Endocrinol 150:457–463

Vieweg WV, Julius DA, Bates J, Quinn JF 3rd, Fernandez A, Hasnain M, Pandurangi AK (2007) Posttraumatic stress disorder as a risk factor for obesity among male military veterans. Acta Psychiatr Scand 116:483–487. https://doi.org/10.1111/j.1600-0447.2007.01071.x

Vilibić M, Jukić V, Pandžićsakoman M, Bilić P, Milošević M (2014) Association between total serum cholesterol and depression, aggression, and suicidal ideations in war veterans with posttraumatic stress disorder: a cross-sectional study. Croat Med J 55:520–529

Wahlström L, Michélsen H, Schulman A, Backheden M (2009) Psychological distress and sick leave in Swedish survivors of the 2004 tsunami: a comparison with a population sample. J Nerv Ment Dis 922:918. https://doi.org/10.1097/NMD.0b013e3181c29a60

Wakabayashi I (2014) Influence of age and gender on lipid accumulation product and its relation to diabetes mellitus in Japanese. Clin Chim Acta 431:221–226. https://doi.org/10.1016/j.cca.2014.02.002

Wang HJ et al (2004) Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. J Clin Endocrinol Metabol 89:157–162. https://doi.org/10.1210/jc.2003-031395