Possible Association of Polymorphisms in Ubiquitin Specific Peptidase 46 Gene with Post-Traumatic Stress Disorder

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Research article

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Abstract

Background: Dynamic proteolysis, through the ubiquitin-proteasome system, has an important role in DNA transcription and cell cycle, and is considered to modulate cell stress response and synaptic plasticity. We investigated whether genetic variants in the ubiquitin carboxyl-terminal hydrolase 46 (USP46) would be associated with posttraumatic stress disorder (PTSD) in people with exposure to combat trauma using a case-control candidate gene association design.

Methods: Korean male veterans exposed to the Vietnam War were grouped into those with (n = 128) and without (n = 128) PTSD. Seven tagging SNPs of USP46 were selected, and single-marker and haplotype-based association analyses were performed. All analyses were adjusted for sociodemographic factors and levels of combat exposure severity and alcohol problem.

Results: One single-marker (rs2244291) showed nominal evidence of association with PTSD status and with the ‘re-experiencing’ cluster, although the association was not significant after Bonferroni correction. No significant association with the other SNPs or the haplotypes was detected.

Conclusion: The present finding suggests preliminarily that genetic vulnerability regarding the ubiquitin-proteasome system may be related to fear memory processes and the development of PTSD symptoms after trauma exposure. Further studies with a larger sample size will be needed to examine the role of the ubiquitin-proteasome system including USP46 in PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is a chronic and debilitating condition with characteristic symptoms, including re-experience of fear memory and severe anxiety as long-term responses to life-threatening traumatic exposure [1]. However, not all people who are exposed to trauma develop PTSD. For instance, only around 10–20% of veterans exposed to combat trauma develop PTSD [2–4]. The reason why certain individuals are more likely to develop PTSD than others after similar trauma exposure has not been elucidated. The molecular determinants of individual differences in vulnerability or resilience to stressors are still not well understood. Twin studies have shown that PTSD is moderately heritable, with approximately 40% of the variance in PTSD attributable to genetic variance [5–7]. Accumulating evidence shows that genetic factors contribute to the PTSD susceptibility among people who have experienced trauma [8, 9].

The ubiquitin-proteasome system is a novel biological candidate for the pathophysiology of neuropsychiatric disorders such as PTSD. Regulated proteolysis via the ubiquitin-proteasome system is essential for cell-cycle control and DNA transcription, and is a dynamic and reversible process, governed by the activity of deubiquitinating enzymes, including ubiquitin specific peptidase (USP) [10]. The ubiquitin-dependent processes are considered to play a crucial role in synaptic development and long-term synaptic plasticity in neural circuits [11–15]. Substantial evidence from animal studies suggest that ubiquitin-mediated proteolysis is an important regulation process for fear memory formation and reconsolidation [16–19]. A study in male rats using a fear conditioning task demonstrated that USP14, a proteasome deubiquitinating enzyme, is critical for fear memory consolidation in the amygdala [20]. As the neurobiological mechanisms underlying fear memory formation can be critical for the development and recovery of fear-related disorders, the ubiquitin-mediated proteolysis is an interesting target in the pathophysiology of PTSD. To date, little is known about the genetic evidence of the role of ubiquitin-proteasome system in clinical samples of individuals with PTSD.

There is a growing interest in the possible role of ubiquitin carboxyl-terminal hydrolase 46 (USP46), a deubiquitinating enzyme that is widely expressed throughout the brain and it is enriched at synapses in the central nervous system [14]. In animal studies, USP46 has been implicated in regulating the GABAergic system and Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamatergic system, which are important for inter-neuronal communication and higher brain functions such as learning and memory [21–24]. Notably, Ebihara and colleagues reported that Uspl4 knockout mice display shortened immobility times in the tail suspension test [24] and long-term memory deficits in the object recognition test [25]. Furthermore, in humans, a genetic association between a haplotype of USP46 and major depression was reported in a Japanese population [26]. No study has been conducted so far to examine the association between the USP46 gene and PTSD.

We investigated whether the genetic variants of the USP46 would be associated with chronic PTSD status in Korean male veterans with exposure to combat trauma using a case-control candidate gene association design. Our main hypothesis was that susceptibility to PTSD might be associated with genetic polymorphisms of the USP46.

Methods

Participants and procedure

According to the DSM-IV-TR diagnostic criteria [1] for PTSD, 128 subjects with PTSD and 128 (non-PTSD) controls were recruited from a psychiatric outpatient clinic at the Veterans Health Service (VHS) Medical Center. All subjects were of Korean ethnicity and male veterans who had served on active duty during the Vietnam War. Individuals with a history of head trauma, organic brain syndrome including cerebrovascular accidents or dementia, major psychiatric disorders including psychosis or bipolar disorder, or substance dependence other than alcohol and
nicotine were excluded. The study was approved by the institutional review board of the VHS Medical Center, South Korea (BOHUN 2016-02-007). All subjects gave their written informed consent before participating in this study.

**Measures**

For assessing PTSD, we used the Clinician-Administered PTSD Scale (CAPS), a structured clinical interview, which is considered the gold standard for diagnosing PTSD [27, 28]. The diagnosis of PTSD was determined by symptom frequency and intensity based on the liberal scoring rule of the CAPS [29]. In addition, the Combat Exposure Scale (CES), a self-reporting scale, was administered for measuring the level of wartime traumatic stressors experienced by the combatants [30]. The total CES scores were divided into five categories of combat exposure: light (1–8), light–moderate (9–16), moderate (17–24), moderate–heavy (25–32), and heavy (33–41). The Alcohol Use Disorders Identification Test (AUDIT) was also used to assess hazardous and harmful alcohol use [31].

**Genotyping**

Seven tagging single nucleotide polymorphisms (SNPs) in the USP46 (rs346005, rs10034164, rs2244291, rs12646800, rs6554557, rs17675844, and rs10517263) were selected based on a prior genetic association study in a Japanese population [26]. Subjects donated a blood sample through venipuncture, and the DNA of each subject was isolated using standard techniques. The genotyping procedures were carried out using single base primer extension assay using the ABI PRISM SNaPShot multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's recommendations. The forward and reverse primer pairs used for the SNaPshot assay and genetic information for all tested SNPs are presented in Table S1 in Additional file 1 (Supplementary information). Analysis was performed using Genemapper software (version 3.0; Applied Biosystems) in the DNA Link, Inc. (Seoul, South Korea).

**Data Analyses**

Demographic and clinical characteristics between subjects with and without PTSD were compared using $\chi^2$-test or Student’s $t$-test on the Statistical Package for the Social Sciences version 25.0 (SPSS Inc., Chicago, IL, USA). The Hardy–Weinberg equilibrium for each SNP in the control group was calculated by $\chi^2$-test.

Single-marker analyses were performed using the R package SNPassoc [32]. Between-group comparisons of genotype frequency differences for diagnostic status were performed by logistic regression analysis considering different genetic inheritance models. The outcome variable was analyzed yielding odds ratios (ORs) with 95% confidence intervals (CIs) and $p$-values. Associations between haplotype distributions and PTSD status were examined using the 'haplo.score' function of R package haplo.stats [33]. Haploblock structure and linkage disequilibrium (LD) patterns obtained from the seven SNPs were constructed using the Haploview ver. 4.2 (http://www.broad.mit.edu/mpg/haplovie). In further analysis, we conducted linear regression analyses for three clusters (re-experiencing, avoidance, and hyperarousal) of PTSD symptoms considering PTSD as continuous phenotypes. All analyses were adjusted for demographic factors including age, education year, socio-economic status, and marital status; the 5 levels of CES, and AUDIT scores (harmful alcohol drinking). In all analyses, $p$-value of less than 0.05 was considered as nominally significant (uncorrected $p < 0.05$). The statistical threshold was corrected using the Bonferroni method for the total number of SNPs ($\alpha = 0.05/7 = 0.0071$).

**Results**

The demographic and clinical characteristics of subjects with and without PTSD are presented in Table 1. The groups with and without PTSD were not significantly different in terms of age, education level, marital status, and socioeconomic status. For combat exposure, the distribution of the five CES categories showed a significant difference between PTSD and non-PTSD groups ($\chi^2 = 48.54, df = 4, p < 0.001$), with a higher proportion of heavy trauma experience in subjects with PTSD than those without PTSD. For alcohol problem, subjects with PTSD had significantly harmful alcohol consumption based on the AUDIT score, compared to those without PTSD ($11.66 \pm 10.92$ vs. $6.84 \pm 7.53, p < 0.001$).
Table 1
Demographic and clinical characteristics of the study participants

|                           | non-PTSD (N = 128) | PTSD (N = 128) | T or $\chi^2$ | $P_\cdot$ |
|---------------------------|--------------------|----------------|---------------|-----------|
| Age                       | 62.92 ± 4.32       | 63.15 ± 3.55   | 0.46          | 0.647     |
| Education(years)          | 10.53 ± 3.12       | 10.38 ± 2.83   | -0.40         | 0.690     |
| Marital status:           |                    |                |               |           |
| Married / others, n       | 119/9              | 110/18         | 3.35          | 0.067     |
| Socioeconomic status:     |                    |                |               |           |
| Low/Medium/High, n        | 45/63/20           | 44/59/25       | 0.70          | 0.705     |
| Combat Exposure           |                    |                |               |           |
| Light / Light-moderate / Moderate / Moderate-heavy / Heavy, n | 38/48/30/11/1 | 6/29/60/26/7 | 48.54       | < 0.001   |
| AUDIT score               | 6.84 ± 7.53        | 11.66 ± 10.92  | 4.11          | < 0.001   |
| Total CAPS                | 8.84 ± 11.39       | 62.85 ± 22.27  | 24.42         | < 0.001   |
| Re-experiencing           | 4.16 ± 7.38        | 20.72 ± 8.56   | 16.58         | 0.001     |
| Avoidance                 | 1.57 ± 3.34        | 21.46 ± 10.18  | 21.00         | < 0.001   |
| Hyperarousal              | 3.19 ± 4.18        | 20.67 ± 8.15   | 17.48         | < 0.001   |

The allelic distributions of the seven SNPs in the control group were in accordance with the Hardy–Weinberg equilibrium (Table S2 in Additional file 1). In single-marker analyses under multiple genetic models, only one single-marker (rs2244291) showed a significant association at the nominal significance level of 5% ($p = 0.0193$ in overdominant model and $p = 0.0497$ in codominant model), but the association did not remain significant after stringent correction for multiple comparisons (Table 2). For the other SNPs in the *USP46* region, no significant association was found between the groups (Table 2).
| SNP         | Model          | Genotype | non-PTSD | PTSD       | OR (95% CI) | P-value | AIC     |
|-------------|----------------|----------|----------|------------|-------------|---------|---------|
| rs346005    | Codominant     | A/A      | 42 (35.9%) | 35 (29.9%) | 1.00        | 0.4781  | 279.1   |
|             |                | A/C      | 49 (41.9%) | 61 (52.1%) | 1.33 (0.67–2.65) |         |         |
|             |                | C/C      | 26 (22.2%) | 21 (17.9%) | 0.84 (0.36–1.96) |         |         |
|             | Dominant       | A/A      | 42 (35.9%) | 35 (29.9%) | 1.00        | 0.6570  | 278.4   |
|             |                | A/C-C/C  | 75 (64.1%) | 82 (70.1%) | 1.16 (0.61–2.19) |         |         |
|             | Recessive      | A/A-A/C  | 91 (77.8%) | 96 (82.1%) | 1.00        | 0.3719  | 277.8   |
|             |                | C/C      | 26 (22.2%) | 21 (17.9%) | 0.71 (0.34–1.50) |         |         |
|             | Overdominant   | A/A-C/C  | 68 (58.1%) | 56 (47.9%) | 1.00        | 0.2504  | 277.3   |
|             |                | A/C      | 49 (41.9%) | 61 (52.1%) | 1.42 (0.78–2.60) |         |         |
|             | log-Additive   | -        | 117 (50.0%) | 117 (50.0%) | 0.96 (0.63–1.45) | 0.8312  | 278.6   |
| rs10034164  | Codominant     | T/T      | 85 (71.4%) | 87 (73.7%) | 1.00        | 0.3382  | 283.2   |
|             |                | C/T      | 31 (26.1%) | 26 (22.0%) | 0.59 (0.29–1.21) |         |         |
|             |                | C/C      | 3 (2.5%)   | 5 (4.2%)   | 1.05 (0.21–5.18) |         |         |
|             | Dominant       | T/T      | 85 (71.4%) | 87 (73.7%) | 1.00        | 0.1926  | 281.7   |
|             |                | C/T-C/C  | 34 (28.6%) | 31 (26.3%) | 0.64 (0.32–1.26) |         |         |
|             | Recessive      | T/T-C/T  | 116 (97.5%) | 113 (95.8%) | 1.00        | 0.8017  | 283.4   |
|             |                | C/C      | 3 (2.5%)   | 5 (4.2%)   | 1.22 (0.43–3.13) |         |         |
|             | Overdominant   | T/T-C/C  | 88 (73.9%) | 92 (78.0%) | 1.00        | 0.1412  | 281.3   |
|             |                | C/T      | 31 (26.1%) | 26 (22.0%) | 0.59 (0.29–1.20) |         |         |
|             | log-Additive   | -        | 119 (50.2%) | 118 (49.8%) | 0.75 (0.43–1.32) | 0.3227  | 282.4   |
| rs2244291   | Codominant     | A/A      | 86 (67.2%) | 73 (57.0%) | 1.00        | *0.0497 | 307.1   |
|             |                | A/G      | 33 (25.8%) | 50 (39.1%) | 1.95 (1.06–3.59) |         |         |
|             |                | G/G      | 9 (7.0%)   | 5 (3.9%)   | 0.61 (0.16–2.32) |         |         |
|             | Dominant       | A/A      | 86 (67.2%) | 73 (57.0%) | 1.00        | 0.0765  | 308.0   |
|             |                | A/G-G/G  | 42 (32.8%) | 55 (43.0%) | 1.68 (0.94–3.00) |         |         |
|             | Recessive      | A/A-A/G  | 119 (93.0%) | 123 (96.1%) | 1.00        | 0.2613  | 309.9   |
|             |                | G/G      | 9 (7.0%)   | 5 (3.9%)   | 0.48 (0.13–1.77) |         |         |
|             | Overdominant   | A/A-G/G  | 95 (74.2%) | 78 (60.9%) | 1.00        | *0.0193 | 305.6   |
|             |                | A/G      | 33 (25.8%) | 50 (39.1%) | 2.03 (1.11–3.69) |         |         |
|             | log-Additive   | -        | 128 (50.0%) | 128 (50.0%) | 1.29 (0.80–2.07) | 0.2952  | 310.0   |
| rs12646800  | Codominant     | C/C      | 107 (89.9%) | 102 (86.4%) | 1.00        | 0.7025  | 283.3   |
|             |                | C/T      | 12 (10.1%) | 16 (13.6%) | 1.20 (0.47–3.10) |         |         |
|             | log-Additive   | -        | 119 (50.2%) | 118 (49.8%) | 1.20 (0.47–3.10) | 0.7025  | 283.3   |
| rs6554557   | Codominant     | A/A      | 85 (71.4%) | 86 (73.5%) | 1.00        | 0.3471  | 281.3   |
|             |                | A/C      | 31 (26.1%) | 27 (23.1%) | 0.59 (0.29–1.22) |         |         |
|             |                | C/C      | 3 (2.5%)   | 4 (3.4%)   | 0.69 (0.13–3.69) |         |         |
|             | Dominant       | A/A      | 85 (71.4%) | 86 (73.5%) | 1.00        | 0.1484  | 279.4   |

OR, odds ratio; CI, confidence interval; AIC, akaike information criterion of each genetic model; * p < 0.05
| SNP          | Model      | Genotype      | non-PTSD | PTSD      | OR (95% CI) | P-value | AIC  |
|-------------|------------|---------------|----------|-----------|-------------|---------|------|
| rs17675844  | Codominant | A/C-C/C       | 34 (28.6%) | 31 (26.5%) | 0.60 (0.30–1.20) | 0.8020 | 281.4 |
|             |            | A/A-A/C       | 116 (97.5%) | 113 (96.6%) | 1.00 |        | 279.5 |
|             |            | C/C           | 3 (2.5%) | 4 (3.4%) | 0.81 (0.15–4.24) |        |      |
|             | Overdominant | A/A-C/C      | 88 (73.9%) | 90 (76.9%) | 1.00 | 0.1650 | 279.5 |
|             |            | A/C           | 31 (26.1%) | 27 (23.1%) | 0.61 (0.30–1.24) |        |      |
|             | log-Additive | -             | 119 (50.4%) | 117 (49.6%) | 0.68 (0.38–1.21) | 0.1911 | 279.7 |
| rs10517263  | Codominant | A/A           | 101 (86.3%) | 94 (81.7%) | 1.00 | 0.2047 | 276.3 |
|             |            | C/A           | 15 (12.8%) | 20 (17.4%) | 2.13 (0.92–4.91) |        |      |
|             |            | C/C           | 1 (0.9%) | 1 (0.9%) | 1.02 (0.03–35.28) |        |      |
|             | Dominant   | A/A           | 101 (86.3%) | 94 (81.7%) | 1.00 | 0.0825 | 274.5 |
|             |            | C/A-C/C       | 16 (13.7%) | 21 (18.3%) | 2.05 (0.91–4.64) |        |      |
|             | Overdominant | A/A-C/A      | 116 (99.1%) | 114 (99.1%) | 1.00 | 0.9888 | 277.5 |
|             |            | C/C           | 1 (0.9%) | 1 (0.9%) | 0.98 (0.03–33.44) |        |      |
|             | Recessive  | A/A-C/A       | 116 (99.1%) | 114 (99.1%) | 1.00 | 0.0749 | 274.3 |
|             |            | C/C           | 1 (0.9%) | 1 (0.9%) | 0.98 (0.03–33.44) |        |      |
|             | log-Additive | -             | 117 (50.4%) | 115 (49.6%) | 1.86 (0.87–4.00) | 0.1061 | 274.9 |
|             | rs10517263 | C/C           | 93 (78.8%) | 97 (82.2%) | 1.00 | 0.3338 | 281.7 |
|             |            | G/C           | 24 (20.3%) | 20 (16.9%) | 0.56 (0.25–1.23) |        |      |
|             |            | G/G           | 1 (0.8%) | 1 (0.8%) | 0.65 (0.04–11.49) |        |      |
|             | Dominant   | C/C           | 93 (78.8%) | 97 (82.2%) | 1.00 | 0.1394 | 279.7 |
|             |            | G/C-G/G       | 25 (21.2%) | 21 (17.8%) | 0.56 (0.26–1.22) |        |      |
|             | Overdominant | C/C-G/C      | 117 (99.2%) | 117 (99.2%) | 1.00 | 0.8291 | 281.8 |
|             |            | G/G           | 1 (0.8%) | 1 (0.8%) | 0.73 (0.04–12.88) |        |      |
|             | Recessive  | C/C-G/C       | 117 (99.2%) | 117 (99.2%) | 1.00 | 0.1467 | 279.8 |
|             |            | G/G           | 1 (0.8%) | 1 (0.8%) | 0.73 (0.04–12.88) |        |      |
|             | Overdominant | C/C-G/G      | 94 (79.7%) | 98 (83.1%) | 1.00 | 0.1579 | 279.9 |
|             | log-Additive | -             | 118 (50.0%) | 118 (50.0%) | 0.60 (0.30–1.23) |        |      |

OR, odds ratio; CI, confidence interval; AIC, akaike information criterion of each genetic model; * p < 0.05

The analysis of LD and haplotype block for the *USP46* revealed one haplotype block (Fig. 1). In haplotype analyses, the permutation test of the seven SNP haplotypes showed no significant difference in the estimated haplotype frequency distributions between both groups (Table 3).
Table 3
The effects of USP46 Haplotype on the affected status of PTSD

| Block 1 | rs346005 | rs10034164 | rs2244291 | rs12646800 | rs6554557 | rs17675844 | rs10517263 |
|---------|---------|-----------|-----------|------------|-----------|------------|------------|
| C       | C       | A         | C         | C          | A         | G          | 0.0997     |
| 0.0718  | -1.3480 | 0.1777    | 0.1856    |
| C       | T       | A         | C         | A          | A         | C          | 0.1284     |
| 0.1278  | -1.1957 | 0.2318    | 0.2389    |
| C       | T       | G         | C         | A          | A         | C          | 0.5052     |
| 0.2355  | 0.8983  | 0.9007    |
| C       | A       | T         | A         | A          | C         |            |            |
| 0.0521  | 0.3821  | 0.7024    | 0.7084    |
| C       | T       | G         | C         | A          | C         | C          |            |
| 0.0845  | 1.3005  | 0.1934    | 0.1996    |

Supplementary Table 3

| rs346005 | rs10034164 | rs2244291 | rs12646800 | rs6554557 | rs17675844 | rs10517263 |
|----------|------------|-----------|------------|-----------|------------|------------|
| C        | C          | A         | C          | C         | A          | G          | 0.0997     |
| 0.0718   | -1.3480    | 0.1777    | 0.1856     |
| C        | T          | A         | C          | A         | A          | C          | 0.1284     |
| 0.1278   | -1.1957    | 0.2318    | 0.2389     |
| C        | T          | G         | C          | A         | A          | C          | 0.5052     |
| 0.2355   | 0.8983     | 0.9007    |
| A        | T          | A         | C          | A         | A          | C          |            |
| 0.0521   | 0.3821     | 0.7024    | 0.7084     |
| C        | T          | G         | C          | A         | C          | C          |            |
| 0.0845   | 1.3005     | 0.1934    | 0.1996     |

In further analyses considering PTSD as continuous phenotypes, the rs2244291, which was shown to be nominally significantly associated with PTSD status in the main analysis, was associated with the ‘re-experiencing’ cluster of PTSD symptoms (p = 0.014 in overdominant model and p = 0.041 in codominant model) (Table 4).
Table 4

The effects of USP46 rs2244291 on three clusters of PTSD symptom

| Cluster         | Model      | Genotype | n  | Mean(S.E)   | Mean difference (95% CI) | p-value | AIC  |
|-----------------|------------|----------|----|-------------|--------------------------|---------|------|
|                 |            |          |    |             |                          |         |      |
| Re-experiencing | Codominant | A/A      | 159| 11.56(0.09) |                          | 0.041*  | 1902 |
|                 |            | A/G      | 83 | 14.71(1.32) | 3.10 (0.50 ~ 5.70)       |         |      |
|                 |            | G/G      | 14 | 8.93(2.90)  | -1.54 (-6.91 ~ 3.83)     |         |      |
|                 | Dominant   | A/A      | 159| 11.56(0.89) |                          | 0.054   | 1902 |
|                 |            | A/G-G/G  | 97 | 13.88(1.21) | 2.46 (-0.03 ~ 4.95)      |         |      |
|                 | Recessive  | A/A-A/G  | 242| 12.64(0.74) |                          | 0.326   | 1905 |
|                 | Overdominant | A/A-G/G | 173| 11.35(0.85) |                          | 0.014*  | 1900 |
|                 |            | A/G      | 83 | 14.71(1.32) | 3.24 (0.68 ~ 5.79)       |         |      |
|                 | log-Additive | -      | -  | -           |                          | 0.234   | 1905 |
| Avoidance       | Codominant | A/A      | 159| 11.04(1.01) |                          | 0.603   | 1986 |
|                 |            | A/G      | 83 | 12.69(1.34) | 1.45 (1.62 ~ 4.51)       |         |      |
|                 |            | G/G      | 14 | 9.93(3.29)  | -0.73 (-7.06 ~ 5.61)     |         |      |
|                 | Dominant   | A/A      | 159| 11.04(1.01) |                          | 0.443   | 1984 |
|                 |            | A/G-G/G  | 97 | 12.29(1.24) | 1.14 (-1.78 ~ 4.07)      |         |      |
|                 | Recessive  | A/A-A/G  | 242| 11.61(0.81) |                          | 0.693   | 1985 |
|                 | Overdominant | A/A-G/G | 173| 10.95(0.96) |                          | 0.326   | 1984 |
|                 |            | A/G      | 83 | 12.69(1.34) | 1.51 (-1.50 ~ 4.52)      |         |      |
|                 | log-Additive | -      | -  | -           |                          | 0.635   | 1985 |
| Hyperarousal    | Codominant | A/A      | 159| 11.36(0.87) |                          | 0.407   | 1903 |
|                 |            | A/G      | 83 | 13.06(1.15) | 1.77 (-0.84 ~ 4.38)      |         |      |
|                 |            | G/G      | 14 | 11.71(3.30) | 1.12 (-4.27 ~ 6.51)      |         |      |
|                 | Dominant   | A/A      | 159| 11.36(0.87) |                          | 0.186   | 1901 |
|                 |            | A/G-G/G  | 97 | 12.87(1.09) | 1.68 (-0.80 ~ 4.17)      |         |      |
|                 | Recessive  | A/A-A/G  | 242| 11.94(0.70) |                          | 0.862   | 1903 |
|                 | Overdominant | A/A-G/G | 173| 11.39(0.84) |                          | 0.201   | 1902 |
|                 |            | A/G      | 83 | 13.06(1.15) | 1.68 (-0.89 ~ 4.24)      |         |      |
|                 | log-Additive | -      | -  | -           |                          | 0.252   | 1902 |

Cluster, PTSD symptom cluster; S.E, standard errors for each genotype; CI, confidence interval; AIC, Akaike information criterion of each genetic model; * p < 0.05

Discussion

The present study examined a genetic association between the USP46 genetic variants and chronic PTSD among Korean male combat veterans. Single-marker analysis resulted in a nominally significant association only for rs2244291 with PTSD status, although the association did not remain significant after stringent correction for multiple comparisons. In addition, the rs2244291 was found to be associated with the ‘re-experiencing’ cluster of PTSD symptoms. The present finding suggests preliminarily that some underlying genetic vulnerability regarding the
The ubiquitin-proteasome system such as USP46 may be related to fear memory processes and the development of some PTSD symptoms after trauma exposure.

To the best of our knowledge, the present study is the first to investigate the possible genetic association of the deubiquitinating enzyme in genetic susceptibility for PTSD. There is indirect evidence supporting the role of USP46 in PTSD and fear memory processes. In animal studies, Ebihara and colleagues suggested that Usp46 might be a quantitative trait gene responsible for immobility time reflecting behavioral despair under inescapable stress conditions [23]. They showed that Usp46 knockout mice exhibited shorter immobility times in the tail-suspension test, assessing depression-like behavior; reduced sucrose consumption in the sucrose preference test, assessing anhedonia-like symptoms; and lower locomotor activity levels in the open field test, assessing exploratory behavior and anxiety compared to wild type mice [25], which suggests the involvement of Usp46 in stress-related phenotypes. In addition, ubiquitin-mediated protein degradation has been shown as important regulatory process in consolidation and extinction of memory in animal studies [16, 34, 35]. Recent in vitro and in vivo findings showed that ubiquitin carboxyl-terminal hydrolase 46 regulates glutamatergic receptor ubiquitination and turnover, as well as the strength of synaptic transmission, which suggest the involvement of USP46 in synaptic plasticity and fear memory processes [22, 36]. These findings are compatible with our finding that the USP46 rs2244291 is associated with the ‘re-experiencing (having sudden and intrusive traumatic memories)’ cluster, the core PTSD symptom, when considering that re-experiencing of the traumatic event is closely related with abnormalities in fear memory processes including conditioning, reconsolidation and extinction of fear memory [37, 38].

However, since no significant association with PTSD for the SNPs or the haplotype in the USP46 region was detected after stringent correction for multiple comparisons in this Korean population, the present findings should be interpreted cautiously and preliminarily until confirmed. One possible reason for a weak association is that any one genetic polymorphism may confer a small genetic contribution to PTSD due to multifactorial polygenic involvement in the pathophysiology of PTSD. The present negative findings in the main analysis should not be interpreted as conclusive for no association because the present sample might be too small for adequate statistical power to detect genetic variants with extremely small effect. Further in vivo studies in a larger sample as well as in vitro functional assays of USP genes might shed new light on the role of ubiquitination in synaptic plasticity and brain function.

The strength of the present genetic association study is that case (trauma-exposed PTSD subjects) – control (trauma-exposed non-PTSD controls) design was applied for a relatively homogenous sample with exposure to similar trauma in a racially uniform population. However, limitations of this study should be noted. First, although our subjects are likely to comprise a more homogeneous sample with similar age and a single ethnic origin, the present study can only be regarded as a preliminary study in the Korean elderly population. Therefore, it should be replicated in larger sample sets, including populations with diverse ages and different ethnic backgrounds. Second, environmental factors such as early-life trauma were not controlled. Considering possible gene-environment interactions, some environments may have confounding effects that influence chronic PTSD status.

Conclusion

In summary, we investigated the clinical relevance of the genetic factors in the USP46 using a case-control association design in Korean male veterans with or without PTSD after exposure to combat trauma. One single-marker (rs2244291) showed nominal evidence of association with PTSD status and with the ‘re-experiencing’ cluster, although the association was not significant after Bonferroni correction. The present findings suggest preliminarily that USP46 rs2244291 may potentially be involved in fear memory processes and the development of PTSD after exposure to traumatic events. Further research in large cohorts is needed to better understand the role of ubiquitin-proteasome system in genetic susceptibility to PTSD.

Supplementary Information

Additional file 1 Table S1. Characteristics of SNP markers on the USP46 gene in the non-PTSD group; Table S2. Primer sequences used in the analysis of USP46 SNPs

Abbreviations

USP46 ubiquitin carboxyl-terminal hydrolase 46; PTSD:posttraumatic stress disorder; USP:ubiquitin specific peptidase; GABA:gamma-Aminobutyric acid; AMPA:Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; VHS:Veterans Health Service; CAPS:Clinician-Administered PTSD Scale; CES:Combat Exposure Scale; AUDIT:Alcohol Use Disorders Identification Test; SNPs:single nucleotide polymorphisms; LD:linkage disequilibrium;

Declarations

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Author’s contributions
Drs. JIK and TYK designed the study, and Drs. TYK, JHC, and HSS collected the data. Drs. JHS, JIK, SJK, and HSS undertook the statistical analyses, and interpreted the findings, and Drs. JHS and JIK prepared the manuscript. All authors contributed to, and approved, the final manuscript.

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Availability of data and materials
Not applicable

Ethics approval and consent to participate
The study was approved by the institutional review board of the VHS Medical Center, South Korea (BOHUN 2016-02-007). The study followed the 1964 Helsinki declaration and its subsequent revisions. All subjects gave their written informed consent before participating in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests

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**Figures**

Figure 1

Haploblock structure and linkage disequilibrium for the non-PTSD group from tagging SNPs of USP46. The color scheme is based on r2 value.

**Supplementary Files**

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