Associations of Ubiquitin–Specific Protease Genes with Resilience and Social Anxiety in Healthy Youths

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ABSTRACT

Objective : Dynamic proteolysis, through the ubiquitin-proteasome system, is an important molecular mechanism for the constant regulation of synaptic plasticity and stress responses in humans. In this study, we examined whether genetic variants in the ubiquitin-specific peptidase (USP) genes were associated with psychological traits of resilience and susceptibility to neuropsychiatric disorders for each gender.

Methods : A total of 344 Korean healthy youths (190 males, 154 females) were included in the study. A genotyping of rs2241646 of USP2 and rs346006 of USP46 was performed. The Connor–Davidson Resilience Scale and Brief Fear of Negative Evaluation Scale were administered for measuring trait resilience and social anxiety, respectively. The genetic associations of the USP variants were tested using multiple analyses of covariance with psychological traits as dependent variables after controlling for age in each gender.

Results : For USP2 rs2241646, women with the TT genotype showed significantly higher resilience and lower social anxiety, as compared to those carrying the C allele. There were no associations between USP46 rs346006 and the psychological traits in both genders.

Conclusions : The present study showed a possible genetic association between the USP2 rs2241646 and stress resilience and trait anxiety in women. The findings suggest that ubiquitin-proteasome system may be related to the resilience and susceptibility to stress-related neuropsychiatric disorders such as anxiety disorders, possibly through the regulation of dynamic proteolysis responses to stress. (Anxiety and Mood 2019; 15(2):122-126)

KEY WORDS : Resilience · Social anxiety · Ubiquitin-proteasome system · Ubiquitin-specific peptidase (USP) · Genetic association study.

Introduction

The ubiquitin–proteasome system, the major proteolytic pathway which selectively degrades ubiquitin tagged proteins and modulate protein homeostasis and synaptic plasticity, is a promising biological mechanism underlying neuropsychiatric disorders. Regulated proteolysis via the ubiquitin-proteasome system is a dynamic and reversible process, governed by the activity of deubiquitinating enzymes, including ubiquitin-specific peptidase (USP). The ubiquitin-dependent processes are considered to play a crucial role in synaptic development and long-term synaptic plasticity in neural circuits.

Alteration in ubiquitin–proteasome system has been revealed to be associated with neurodegenerative diseases such as Alzheimer’s dementia as well as neurodevelopmental diseases such as autism spectrum disorder and schizophrenia. In addition, recent genetic studies showed that dysregulated ubiquitin–proteasome system is involved in the pathophysiology of depressive disorders. The studies suggested that USP46 molecule is involved in the pathophysiology of depressive disorders through the circadian clock system and GABAergic system. Although genetic factors related to ubiquitin–proteasome system have been less well studied in human stress responses, they are interesting target of resilient and vulnerable traits to stress-related neuropsychiatric disorders such as autism spectrum disorder.
as depression.

Stress resilience, the psychologically adaptive ability in response to stress, has been suggested as an important protective factor against development of psychiatric disorders in response to stressful experiences. Fear of negative evaluation (FNE), a psychological factor reflecting trait social anxiety about evaluation by others, and distress due to expectation of others’ negative evaluation, is a vulnerability factor to stress-responsive factor against development of psychiatric disorders in response to stress, has been suggested as an important protective factor against development of psychiatric disorders in response to stressful experiences. Fear of negative evaluation (FNE), a psychological factor reflecting trait social anxiety about evaluation by others, and distress due to expectation of others’ negative evaluation, is a vulnerability factor to stress.

We investigated whether genetic variants of the USP2 and USP46, which have been previously reported to be associated with circadian rhythm and depression, could serve as potential candidates to explain psychological traits regarding stress resilience and fear of negative evaluation in healthy Korean youths.

Subjects and Methods

Participants

A total of 361 Korean healthy volunteers (197 males, 164 females) were recruited for the present study through advertisements. They were all interviewed by trained psychiatrists, and subjects with any current or previous Axis I psychiatric disorders according to the DSM-IV-TR diagnostic criteria or subjects with neurologic disorders or a family history of psychiatric disorders in first-degree relatives were excluded. Seventeen subjects were excluded through psychiatric screening and genotyping failure from their saliva samples. Finally, 344 subjects (190 males, 154 females) with complete data sets and genotype analysis were included. All participants provided written informed consent prior to starting the study. The study protocol was approved by the Institutional Review Board of Severance Hospital (Severance IRB 4-2010-0577).

Measures of stress-related psychological traits

To assess stress resilience, we used the Korean version of Connor-Davidson Resilience Scale (CD-RISC), which is a reliable self-report for measuring resilience trait. The CD-RISC is composed of 25 items that are each rated on a five-point Likert scale ranging from 0 (rarely true) to 4 (true nearly all of the time). The items include the ability to adapt to change or to bounce back from challenges, and the extent of the individual’s social support network.

To assess the psychological trait of social anxiety as a susceptibility factor for psychiatric disorders, fear of negative evaluation was assessed using the Korean version of Brief FNE scale. The scale measures apprehension about, avoidance of, and expectations regarding negative evaluation by others. It contains 12 items rated on 6-point Likert scales ranging from 0=“not at all characteristic of me” to 5=“extremely characteristic of me.” Its reliability and validity have been established in undergraduate populations.

SNP selection and genotyping

We selected a single-nucleotide polymorphism (SNP) of USP2 gene, which has been known to play important roles in regulating circadian rhythms and depression, and an SNP of USP46 gene, which has been known to be associated with depressive disorders, as candidates. Based on previous genetic studies in humans and minor allele frequencies (MAFs, above 0.05) in Japanese and Han Chinese population based on 1,000 Genome database, the rs2241646 of USP2 and rs346005 of USP46 were genotyped in the present study. The DNA of the subjects was isolated from saliva collected in Oragene self-collection kit (tube format “OG-500”) using standard techniques. Genotyping of the SNPs was performed by the single-base primer extension assay using ABI PRISM® SNaPShot® Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer’s instructions. The genotyping was provided by genetic analysis service at the DNA Link, Inc. (Seoul, South Korea). Analysis was performed using Genemapper software (version 3.0; Applied Biosystems).

Statistical analyses

All statistical analyses were conducted using the Statistical Package for the Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, IL, USA). Demographic characteristics and the genotype frequencies in men and women were compared using Student’s t-test, χ²-test, and Fisher’s exact test. The Hardy–Weinberg equilibrium was also calculated by χ²-test. In the genotype-based analyses, we used dominant model for the rs2241646 of USP2 and additive model for and rs346005 of USP46. Multiple analyses of covariance (MANCOVA) were performed to compare psychological traits between genotypes, with total scores of the CD-RISC and brief FNE as dependent variables, genotypes of USP2 or USP46 as fixed factors, and age as a covariate. The MANCOVA was conducted separately for each gender since gender differences have been reported in stress responses and vulnerability to psychiatric disorders. Significance level was set at p<0.05, and all tests were two-tailed.
Results

Demographic characteristics and genotype frequency of subjects are summarized in Table 1. The mean age of the participants was 21.61 years (standard deviation; SD=2.16 years) for males and 20.90 years (SD=2.14 years) for females. There were significant differences in resilience scores between men and women ($p=0.023$).

The allelic distributions of the USP2 and USP46 polymorphisms were in accordance with the Hardy–Weinberg equilibrium ($\chi^2=0.631, p=0.427$, and $\chi^2=0.067, p=0.796$, respectively). For the rs2241646 of the USP2 gene, the most prevalent genotype was TT (N=229, 66.6%) followed by TC (N=105, 30.5%), and CC (N=10, 2.9%). For the rs346005 of USP46, the most prevalent genotype was AC (N=173, 50.3%), followed by AA (N=101, 29.4%) and CC (N=70, 20.3%).

Results from MANCOVA for the USP2 and USP46 polymorphisms are presented in Table 2 and Table 3, respectively. Among women, the MANCOVA showed a significant overall effect of USP2 rs2241646 genotypes (Wilks $\lambda=0.941, F(4,150)=4.661, p=0.011$). Women with the TT genotype of the rs2241646 showed significantly higher resilience and lower FNE score compared to those carrying the C allele (Table 2). There were no associations of USP46 rs346005 with CD-RISC or brief FNE scores in both genders (Table 3).

Discussion

The present study examined whether genetic variants in the ubiquitin-related genes were associated with resilience and fear of negative evaluation that could be an important psychological trait regarding stress resilience and vulnerability to neuropsychiatric disorders. Our results in Korean young women showed a genetic association of the USP2 rs2241646 with stress resilience and fear of negative evaluation. The finding provides evidence for a possible regulatory role of the USP2 deubiquitinating enzyme in synaptic plasticity and stress-related neuropsychiatric traits in women.

The balance between protein synthesis and proteasomal degradation is known to play a crucial role in regulating neural plasticity at potentiated synapses and long-term synaptic alterations. Furthermore, emerging evidence suggests that dynamic ubiquitination by deubiquitinating enzymes, including USP2, plays an important role in regulating the stability of...
circadian clock protein functions, circadian plasticity and circadian behavior. Since the circadian system is essential for adaptation to stressors and regulation of stress responses, a key biological component of circadian clocks such as USP2 may be involved in regulating circadian rhythm against stressful situations and developing stress-related neuropsychiatry disorders. In particular, USP2 has been found to be involved in interacting with core clock proteins and modulating light-dependent resetting of the circadian clock in mice. A human genome-wide association study on rumination phenotype in a European population showed that USP2 ranked on top five hits for reflection style of responding to stress although no gene survived after correction for multiple testing. In addition, an animal study with acutely stressed rats demonstrated that USP2-related signaling is a key pathway in the underlying mechanism of stress-induced hippocampus-dependent cognitive processes. Based on previous findings, the present association of the USP2 rs2241646 with stress resilience and fear of negative evaluation suggests that USP2 may be involved in biological mechanisms of resilience and vulnerability to neuropsychiatric disorders through the regulation of dynamic proteolysis processes and neuroplasticity to stress.

Several limitations of the present study should be noted. First, the finding of the USP2 can only be regarded as a preliminary in the Korean young population. It should be replicated in larger sample sets, including populations with diverse ages and different ethnic backgrounds. Second, since the present sample may have been too small for adequate statistical power to detect small genetic effect, present negative finding of USP46 should not be interpreted as conclusive. Third, only one polymorphism in each gene of USP2 and USP46 was chosen for this study. One genetic polymorphism may only confer a small genetic contribution to stress-related psychological traits due to multi-factorial polygenic involvement in the stress responses and individual personality traits. Therefore, further studies for additional polymorphisms of ubiquitin-related genes may be necessary to determine the roles of the ubiquitin-proteasome system more definitively in resilience and vulnerability to stress. Fourth, we assessed psychological traits regarding stress resilience and vulnerability with only two self-rating scales of CD-RISC and brief FNE. More comprehensive assessment of psychological traits related to stress responses is required to better represent resilience and vulnerability to stress-related neuropsychiatric disorders. Finally, environmental factors such as early-life trauma which could affect individuals' stress resilience and vulnerability were not controlled. Considering possible gene-environment interactions, some environmental factors may have confounding effects that influence gene expression and stress responses.

**Conclusion**

The present study showed a genetic association of the USP2 rs2241646 with stress resilience and fear of negative evaluation, suggesting that USP2 may be involved in resilience and vulnerability to stress-related neuropsychiatric disorders such as anxiety disorders through the regulation of dynamic proteolysis processes. The biological mechanisms of ubiquitin-proteasome system underlying the stress resilience and vulnerability should be further elucidated.

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