Anxiety and Mood Vol 15, No 2 / pISSN 2586-0151 / eISSN 2586-0046
https://doi.org/10.24986/anxmod.2019.15.2.122

ORIGINAL ARTICLE

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

Jun Ho Seo, MD, Chun Il Park, MD, Se Joo Kim, MD, PhD, and Jee In Kang, MD, PhD

Institute of Behavioral Science in Medicine & Department of Psychiatry, Yonsei University College of Medicine, Seoul, Korea

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* rs346005 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.[1] 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*).[2] The ubiquitin-dependent processes are considered to play a crucial role in synaptic development and long-term synaptic plasticity in neural circuits.[3-8]

Alteration in ubiquitin-proteasome system has been revealed to be associated with neurodegenerative diseases such as Alzheimer's dementia[9,10] as well as neurodevelopmental diseases such as autism spectrum disorder[11,12] and schizophrenia.[13,14] In addition, recent genetic studies showed that dysregulated ubiquitin–proteasome system is involved in the pathophysiology of depressive disorders.[15-18] The studies suggested that USP46 molecule is involved in the pathophysiology of depressive disorders through the circadian clock system[15] and GABAergic system.[16] 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

Received : June 19, 2019 / **Revised :** August 7, 2019
Accepted : September 9, 2019

Address for correspondence
Jee In Kang, M.D., Ph.D., Institute of Behavioral Science in Medicine & Department of Psychiatry, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel : +82-2-2228-1620, Fax : +82-2-313-0891
E-mail : jeeinkang@yuhs.ac

Address for correspondence
Se Joo Kim, M.D., Ph.D., Institute of Behavioral Science in Medicine & Department of Psychiatry, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel : +82-2-2228-1620, Fax : +82-2-313-0891
E-mail : kimsejoo@yuhs.ac

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.[19] 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-related neuropsychiatric disorders such as anxiety[20] and depressive disorders.[21,22]

We investigated whether genetic variants of the *USP2* and *USP46*, which have been previously reported to be associated with circadian rhythm[23-25] and depression,[15-18] 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[26] 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.[27,28] 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).[29] 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.[29]

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.[30,31] The scale measures apprehension about, avoidance of, and expectations regarding negative evaluation by others.[30,32] 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.[30,32]

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,[24,25] and an SNP of *USP46* gene, which has been known to be associated with depressive disorders,[15] 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*[33] and rs346005 of *USP46*[15] 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, χ^2-test, and Fisher's exact test. The Hardy–Weinberg equilibrium was also calculated by χ^2-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 (χ^2=0.631, p=0.427, and χ^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 λ=0.941, $F_{(2,150)}$=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).

Table 1. Demographic characteristics of the subjects (mean±standard deviation)

	Male (n=190)	Female (n=154)	p
Age (years)	21.61±2.16	20.90±2.14	0.003
Educational years	13.85±1.43	14.13±1.64	0.093
Resilience (CD-RISC)	67.09±12.55	64.12±11.21	0.023
Brief FNE	32.92±7.71	32.08±7.97	0.322
Genotypes (%)			
USP2 rs2241646			
TT	119 (62.6)	110 (71.4)	
TC	67 (35.3)	38 (24.7)	0.084
CC	4 (2.1)	6 (3.9)	
USP46 rs346005			
AA	54 (28.4)	47 (30.5)	
CA	98 (51.6)	75 (48.7)	0.870
CC	38 (20.0)	32 (20.8)	

CD-RISC : Connor–Davidson Resilience Scale, FNE : Fear of Negative Evaluation, FNE : Brief Fear of Negative Evaluation Scale

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.[34] Furthermore, emerging evidence suggests that dynamic ubiquitination by deubiquitinating enzymes, including *USP2*, plays an important role in regulating the stability of

Table 2. Multivariate analysis comparing resilience and fear of negative evaluation for *USP2* single-nucleotide polymorphism rs2241646 (mean±standard deviation) after controlling for age

	Male (n=190)		p	Female (n=154)		p
	TT (n=119)	CT+CC (n=71)		TT (n=110)	CT+CC (n=44)	
MANCOVA	Wilks λ=0.999, $F_{(2,186)}$=0.062, p=0.940			Wilks λ=0.941, $F_{(2,150)}$=4.661, p=0.011		
Resilience	67.29±12.72	66.75±12.34	0.725	65.28±10.76	61.20±11.91	0.039*
Brief FNE	32.87±7.37	33.00±8.31	0.943	31.05±8.00	34.63±7.40	0.012*

* : p<0.05. FNE : Fear of Negative Evaluation

Table 3. Multivariate analysis comparing resilience and fear of negative evaluation for *USP46* single-nucleotide polymorphism rs346005 (mean±standard deviation) after controlling for age

	Male (n=190)			p	Female (n=154)			p
	AA (n=54)	CA (n=98)	CC (n=38)		AA (n=47)	CA (n=75)	CC (n=32)	
MANCOVA	Wilks λ=0.991, $F_{(4,370)}$=0.434, p=0.784				Wilks λ=0.982, $F_{(4,298)}$=0.692, p=0.598			
Resilience	68.16±11.60	67.09±13.11	65.58±12.52	0.834	64.02±11.66	64.84±11.25	62.56±10.62	0.643
Brief FNE	34.07±7.81	32.28±7.61	32.95±7.85	0.512	32.49±7.72	31.36±8.04	33.15±8.25	0.339

FNE : Fear of Negative Evaluation

circadian clock protein functions, circadian plasticity and circadian behavior.[23-25] Since the circadian system is essential for adaptation to stressors and regulation of stress responses,[35,36] 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.[24] 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.[37] 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.[38] 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[39] 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.

■ **Acknowledgement**

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (NRF-2018R1A2B2007714). The funding source did not give any influences on the study design, data collection, analysis and interpretation of data, the writing of the report, and the decision to submit the article for publication.

REFERENCES

1. Cheon S, Dean M, Chahrour M. The ubiquitin proteasome pathway in neuropsychiatric disorders. Neurobiol Learn Mem 2018.
2. Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin-proteasome system. Nat Rev Drug Discov 2006;5:596-613.
3. Dong C, Bach SV, Haynes KA, Hegde AN. Proteasome modulates positive and negative translational regulators in long-term synaptic plasticity. J Neurosci 2014;34:3171-3182.
4. Li Q, Korte M, Sajikumar S. Ubiquitin-Proteasome System Inhibition Promotes Long-Term Depression and Synaptic Tagging/Capture. Cereb Cortex 2016;26:2541-2548.
5. Cai F, Frey JU, Sanna PP, Behnisch T. Protein degradation by the proteasome is required for synaptic tagging and the heterosynaptic stabilization of hippocampal late-phase long-term potentiation. Neuroscience 2010;169:1520-1526.
6. Kowalski JR, Juo P. The role of deubiquitinating enzymes in synaptic function and nervous system diseases. Neural Plast 2012;2012:892749.
7. Todi SV, Paulson HL. Balancing act: deubiquitinating enzymes in the nervous system. Trends Neurosci 2011;34:370-382.
8. Hegde AN. The ubiquitin-proteasome pathway and synaptic plasticity. Learn Mem 2010;17:314-327.
9. Lam YA, Pickart CM, Alban A, Landon M, Jamieson C, Ramage R, et al. Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. Proc Natl Acad Sci U S A 2000;97:9902-9906.
10. Gadhave K, Bolshette N, Ahire A, Pardeshi R, Thakur K, Trandafir C, et al. The ubiquitin proteasomal system: a potential target for the management of Alzheimer's disease. J Cell Mol Med 2016;20:1392-1407.
11. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 2009;459:569-573.
12. Louros SR, Osterweil EK. Perturbed proteostasis in autism spectrum disorders. J Neurochem 2016;139:1081-1092.
13. Chang S, Fang K, Zhang K, Wang J. Network-Based Analysis of

Schizophrenia Genome-Wide Association Data to Detect the Joint Functional Association Signals. PLoS One 2015;10:e0133404.
14. Lin CY, Sawa A, Jaaro-Peled H. Better understanding of mechanisms of schizophrenia and bipolar disorder: from human gene expression profiles to mouse models. Neurobiol Dis 2012;45:48-56.
15. Fukuo Y, Kishi T, Kushima I, Yoshimura R, Okochi T, Kitajima T, et al. Possible association between ubiquitin-specific peptidase 46 gene and major depressive disorders in the Japanese population. J Affect Disord 2011;133:150-157.
16. Imai S, Mamiya T, Tsukada A, Sakai Y, Mouri A, Nabeshima T, et al. Ubiquitin-specific peptidase 46 (Usp46) regulates mouse immobile behavior in the tail suspension test through the GABAergic system. PLoS One 2012;7:e39084.
17. Tomida S, Mamiya T, Sakamaki H, Miura M, Aosaki T, Masuda M, et al. Usp46 is a quantitative trait gene regulating mouse immobile behavior in the tail suspension and forced swimming tests. Nat Genet 2009;41:688-695.
18. Boo YJ, Park CI, Kim HW, Kim SJ, Kang JI. Possible Association of the Ubiquitin-Specific Peptidase 46 Gene (USP46) with Affective Temperamental Traits in Healthy Korean Volunteers. Psychiatry Investig 2019;16:87-92.
19. Hjemdal O, Aune T, Reinfjell T, Stiles TC, Friborg O. Resilience as a predictor of depressive symptoms: a correlational study with young adolescents. Clin Child Psychol Psychiatry 2007;12:91-104.
20. Collins KA, Westra HA, Dozois DJ, Stewart SH. The validity of the brief version of the Fear of Negative Evaluation Scale. J Anxiety Disord 2005;19:345-359.
21. Sato T, McCann D, Ferguson-Isaac C. Sociotropy-autonomy and situation-specific anxiety. Psychol Rep 2004;94:67-76.
22. O'Connor LE, Berry JW, Weiss J, Gilbert P. Guilt, fear, submission, and empathy in depression. J Affect Disord 2002;71:19-27.
23. Stojkovic K, Wing SS, Cermakian N. A central role for ubiquitination within a circadian clock protein modification code. Front Mol Neurosci 2014;7:69.
24. Yang Y, Duguay D, Bedard N, Rachalski A, Baquiran G, Na CH, et al. Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. Biol Open 2012;1:789-801.
25. Yang Y, Duguay D, Fahrenkrug J, Cermakian N, Wing SS. USP2 regulates the intracellular localization of PER1 and circadian gene expression. J Biol Rhythms 2014;29:243-256.
26. First MB, Spitzer R, L GM, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders, Clinical Version (SCID-CV). Washington, D.C. : American Psychiatric Press, Inc.;1996.
27. Baek HS, Lee KU, Joo EJ, Lee MY, Choi KS. Reliability and validity of the korean version of the connor-davidson resilience scale. Psychiatry Investig 2010;7:109-115.
28. Jung YE, Min JA, Shin AY, Han SY, Lee KU, Kim TS, et al. The Korean version of the Connor-Davidson Resilience Scale: an extended validation. Stress Health 2012;28:319-326.
29. Connor KM, Davidson JR. Development of a new resilience scale: the Connor-Davidson Resilience Scale (CD-RISC). Depress Anxiety 2003;18:76-82.
30. Leary MR. A brief version of the Fear of Negative Evaluation Scale. Personality and Social Psychology Bulletin 1983;9:371-375.
31. Lee JY, Choi CH. A Study of the Reliability and the Validity of the Korean versions of Social Phobia Scales (K-SAD, K-FNE). Korean Journal of Clinical Psychology 1997;16:251-264.
32. Weeks JW, Heimberg RG, Fresco DM, Hart TA, Turk CL, Schneier FR, et al. Empirical validation and psychometric evaluation of the Brief Fear of Negative Evaluation Scale in patients with social anxiety disorder. Psychol Assess 2005;17:179-190.
33. Jin HS, Hong KW, Lim JE, Hwang SY, Lee SH, Shin C, et al. Genetic variations in the sodium balance-regulating genes ENaC, NEDD4L, NDFIP2 and USP2 influence blood pressure and hypertension. Kidney Blood Press Res 2010;33:15-23.
34. Cajigas IJ, Will T, Schuman EM. Protein homeostasis and synaptic plasticity. EMBO J 2010;29:2746-2752.
35. Karatsoreos IN, McEwen BS. Psychobiological allostasis: resistance, resilience and vulnerability. Trends Cogn Sci 2011;15:576-584.
36. McClung CA. Circadian genes, rhythms and the biology of mood disorders. Pharmacol Ther 2007;114:222-232.
37. Eszlari N, Millinghoffer A, Petschner P, Gonda X, Baksa D, Pulay AJ, et al. Genome-wide association analysis reveals KCTD12 and miR-383-binding genes in the background of rumination. Transl Psychiatry 2019;9:119.
38. Li C, Zhang J, Xu H, Chang M, Lv C, Xue W, et al. Retigabine ameliorates acute stress-induced impairment of spatial memory retrieval through regulating USP2 signaling pathways in hippocampal CA1 area. Neuropharmacology 2018;135:151-162.
39. Kyoung MH, Min JA, Chae JH. Relationship of Affective Symptoms and Resilience with Childhood Abuse in Patients with Depressive or Anxiety disorders. Anxiety and Mood 2013;9:68-73.