Journal of Human Genetics (2011) 56, 806–809

www.nature.com/jhg

SHORT COMMUNICATION

Support for association between the Ser205Leu polymorphism of *p75^{NTR}* and major depressive disorder

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Altered neurotrophin functions have been implicated in major depressive disorder (MDD). Previously, we reported an association between MDD and a missense polymorphism (Ser205Leu: rs2072446) of the gene encoding the p75 neurotrophin receptor (*p75^{NTR}*). However, contradictive negative results have also been reported. This study tried to replicate the association in an independent sample. Subjects were 668 patients with MDD and 1130 healthy controls. The proportion of individuals carrying the Leu205 allele was significantly decreased in the patients than in the controls (χ^2=5.3, d.f.=1, *P*=0.021, odds ratio (OR) 0.74, 95% confidential interval (CI) 0.58–0.96). When allele frequencies were compared, the Leu205 allele was significantly reduced in the patients than in the controls (χ^2=4.4, d.f.=1, *P*=0.037, OR 0.78, 95% CI 0.61–0.99). When men and women were examined separately, there was a significant difference in genotype and allele distributions in women (genotype: χ^2=8.3, d.f.=1, *P*=0.0039, OR 0.60, 95% CI 0.43–0.85; allele: χ^2=7.3, d.f.=1, *P*=0.0069, OR 0.64, 95% CI 0.47–0.89), but not in men. The present study provided support for the previously reported association between the Ser205Leu polymorphism of the p75^{NTR} gene and MDD, indicating that the Leu205 allele has a protective effect against the development of MDD, particularly in women.
Journal of Human Genetics (2011) **56,** 806–809; doi:10.1038/jhg.2011.107; published online 22 September 2011

Keywords: association study; major depressive disorder; neurotrophin; p75^{NTR}; Ser205Leu polymorphism

INTRODUCTION

Neurotrophins such as nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin 3 and neurotrophin 4/5 are essential to several aspects of neuronal activities including proliferation, growth, differentiation and regeneration.[1] Molecules participating in this signaling pathway have been implicated in several neuropsychiatric diseases. For example, decreased levels of BDNF have been reported in peripheral blood and hippocampus of postmortem brains of patients with major depressive disorder (MDD).[2] A genetic variant of BDNF was reported to be associated with MDD in a recent meta-analysis.[3] In stress response and stress-related psychiatric diseases, such as MDD, glucocorticoids are key molecules and have substantial interaction with BDNF function.[4] Induced by chronic stress, excessive glucocorticoids decrease BDNF levels and damage hippocampus and other brain areas, which is considered as a risk of the development of MDD.[4]

In this context, p75^{NTR} could have an important role in neuropsychiatric diseases, and its functional polymorphism might modulate the risk.[5] p75^{NTR} was identified as a low-affinity receptor for neurotrophins, and cloned as a type I transmembrane protein, with its molecular weight of 75 kDa. Previously, we identified the Ser205Leu missense polymorphism of *p75^{NTR}* and its association with MDD in a Japanese sample.[6] The frequency of the minor allele (Leu205) was significantly decreased in patients compared with controls ($P<0.05$, odds ratio (OR) 0.54, 95% confidential interval (CI) 0.31–0.94), suggesting that this variant may have a protective effect against the development of MDD. However, a subsequent study in a Han Chinese population failed to obtain evidence for the association.[7] This study tried to replicate the association in an independent larger sample.

MATERIALS AND METHODS

Subjects were 668 patients with MDD (668 patients: mean age ± s.d.: 49.5 ± 15.9 years: median 49 years: range 17–89 years; 282 males: mean age ± s.d.: 44.8 ± 13.9 years: median 41.5 years: range 18–88 years; 386 females: mean age ± s.d.: 52.8 ± 16.5 years: median 54 years: range 17–89 years) and 1130 healthy controls (1130 controls: mean age ± s.d.: 45.8 ± 16.2 years: median 43 years: range 18–88 years; 385 males: mean age ± s.d.: 44.7 ± 17.3 years: median 39 years: range 18–88 years; 745 females: mean age ± s.d.:

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Received 23 May 2011; revised 16 August 2011; accepted 20 August 2011; published online 22 September 2011

Table 1 Genotype and allele distributions of the Ser205Leu polymorphism in *p75^NTR^* for patients with MDD and controls

	Genotype count (frequency)									*Allele count (frequency)*						*Dominant model count (frequency)*						*HWE*	
	Total	*S/S*		*S/L*		*L/L*		χ^2 *(d.f.=2)*	P	*S*		*L*		χ^2 *(d.f.=1)*	P	*S/S*		*S/L or L/L*		χ^2 *(d.f.=1)*	P	χ^2 *(d.f.=1)*	*HWE*-P
Total																							
MDD	668	563	(0.84)	100	(0.15)	5	(0.01)	5.9	0.053	1226	(0.92)	110	(0.08)	4.4	0.037	563	(0.84)	105	(0.16)	5.3	0.021	0.058	0.81
Control	1130	903	(0.80)	220	(0.19)	7	(0.01)			2026	(0.90)	234	(0.10)			903	(0.80)	227	(0.20)			2.7	0.10
Men																							
MDD	282	228	(0.81)	52	(0.18)	2	(0.01)	0.89	0.64	508	(0.90)	56	(0.10)	0.015	0.90	228	(0.81)	54	(0.19)	0.075	0.78	0.27	0.60
Control	385	308	(0.80)	76	(0.20)	1	(0.00)			692	(0.90)	78	(0.10)			308	(0.80)	77	(0.20)			2.7	0.10
Women																							
MDD	386	335	(0.87)	48	(0.12)	3	(0.01)	8.6	0.014	718	(0.93)	54	(0.07)	7.3	0.0069	335	(0.87)	51	(0.13)	8.3	0.0039	0.76	0.38
Control	745	595	(0.80)	144	(0.19)	6	(0.01)			1334	(0.90)	156	(0.10)			595	(0.80)	150	(0.20)			0.72	0.40

Abbreviations: HWE, Hardy–Weinberg equilibrium; MDD, major depressive disorder.

Table 2 Genotype and allele distributions of the Ser205Leu polymorphism in *p75^NTR^* for patients with MDD and controls in the present and previous studies

	Genotype count (frequency)									*Allele count (frequency)*						*Dominant model count (frequency)*						*HWE*	
	Total	*S/S*		*S/L*		*L/L*		χ^2 *(d.f.=2)*	P	*S*		*L*		χ^2 *(d.f.=1)*	P	*S/S*		*S/L or L/L*		χ^2 *(d.f.=1)*	P	χ^2 *(d.f.=1)*	*HWE*-P
Kunugi *et al.*[6]																							
MDD	164	143	(0.87)	21	(0.13)	0	(0.00)	5.4	0.068	307	(0.94)	21	(0.06)	4.8	0.028	143	(0.87)	21	(0.13)	4.2	0.040	0.77	0.38
Control	164	129	(0.79)	33	(0.20)	2	(0.01)			291	(0.89)	37	(0.11)			129	(0.79)	35	(0.21)			0.0046	0.95
Gau et al.[7]																							
MDD	226	178	(0.79)	46	(0.20)	2	(0.01)	0.35	0.84	402	(0.89)	50	(0.11)	0.00013	0.99	178	(0.79)	48	(0.21)	0.010	0.922	0.27	0.60
Control	394	309	(0.78)	83	(0.21)	2	(0.01)			701	(0.89)	87	(0.11)			309	(0.78)	85	(0.22)			2.1	0.15
Present study																							
MDD	668	563	(0.84)	100	(0.15)	5	(0.01)	5.9	0.053	1226	(0.92)	110	(0.08)	4.4	0.037	563	(0.84)	105	(0.16)	5.3	0.021	0.058	0.81
Control	1130	903	(0.80)	220	(0.19)	7	(0.01)			2026	(0.90)	234	(0.10)			903	(0.80)	227	(0.20)			2.7	0.10

Abbreviations: HWE, Hardy–Weinberg equilibrium; MDD, major depressive disorder.

46.3 ± 15.6 years: median 47 years: range 19–86 years) recruited around the Tokyo Metropolitan area. Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edn[8] on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from the same geographical area. They were interviewed using the Japanese version of the Mini International Neuropsychiatric Interview (MINI.)[9,10] by a research psychiatrist to rule out any axis I psychiatric disorders, and individuals with a current or past history of psychiatric treatment were excluded. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence or substance abuse. However, we did not exclude MDD patients with comorbid anxiety syndromes. Among the total 668 patients, 49% had a single episode, 49% recurrent episodes and the remaining patients being unclassified. With regard to history of admission, 19% had a history of admission to a psychiatric hospital and the remaining 81% did not have such a history. All subjects were biologically unrelated Japanese. The present sample was independent of our previous sample.[6] The study protocol was approved from the ethics committee of the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

The rs2072446 genotyping was performed by the TaqMan 5′-exonuclease allelic discrimination assay (Assay ID C__15870920_10, Applied Biosystems (Foster City, CA, USA)) according to the manufacturer's instructions.

Deviations of genotype distributions from the Hardy–Weinberg equilibrium were assessed with the χ^2-test for goodness of fit. Genotype and allele distributions were compared between the patients and controls by using the χ^2-test for independence. These tests were performed with the SPSS software ver.11 (SPSS Japan, Tokyo, Japan).

RESULTS

Genotype and allele distributions of the Ser205Leu polymorphism in the patients and controls are shown in Table 1. The genotype distributions for both the groups were in Hardy–Weinberg equilibrium. For these non-synonymous polymorphisms, there was a difference at a trend level in the genotype distribution between the patients and controls (χ^2=5.9, d.f.=2, P=0.053; Table 1). When allele frequencies were compared, the Leu205 allele was significantly reduced in the patients than in the controls (χ^2=4.4, d.f.=1, P=0.037, OR 0.78, 95% CI 0.61–0.99; Table 1). As there were only 12 individuals homozygous for the minor allele (Leu205), individuals homozygous for this allele and those heterozygous were combined when we compared the genotype distribution between the patients and controls. The proportion of individuals carrying the Leu205 allele was significantly decreased in the patients than in the controls (χ^2=5.3, d.f.=1, P=0.021, OR 0.74, 95% CI 0.58–0.96; Table 1). When we further performed stratified analysis of the data by sex, there was a significant difference in genotype (χ^2=8.6, d.f.=2, P=0.014) and allele (χ^2=7.3, d.f.=1, P=0.0069, OR 0.64, 95% CI 0.47–0.89) distributions between the patients and controls in women, but not in men (genotype: χ^2=0.89, d.f.=2, P=0.64; allele: χ^2=0.015, d.f.=1, P=0.90, OR 0.98, 95% CI 0.68–1.40). The proportion of individuals carrying the Leu205 allele was significantly decreased in the patients than in the controls in women (χ^2=8.3, d.f.=1, P=0.0039, OR 0.60, 95% CI 0.43–0.85), but not in men (χ^2=0.075, d.f.=1, P=0.78, OR 0.95, 95% CI 0.64–1.40; Table 1).

DISCUSSION

In the present study, we obtained evidence supporting the association between the Ser205Leu polymorphism of $p75^{NTR}$ and MDD in a larger sample than our previous study. [6] The frequency of the minor allele (Leu205) was significantly decreased in the patients compared with the controls, suggesting that the Leu205 has a protective effect against the development of MDD. In particular, we showed a significant association between this polymorphism and MDD in women in our Japanese sample. In contrast, we did not observe such an association in men. In a recent study about neonatal NMDAR-mediated excitotoxic brain injury, lesion size was significantly increased in $p75^{NTR}$-deficient female mice as compared with $p75^{NTR}$-deficient male mice, showing a gender-specific role of $p75^{NTR}$.[11] We might have detected that the Ser205Leu polymorphism of $p75^{NTR}$ have a sexually dimorphic effect on susceptibility to MDD.

As mentioned earlier, Gau *et al.* (2008) failed to replicate the finding and discussed that the inconsistency of these results could be attributed from the intrinsic difference in the MDD subgroups and different ethnic groups. With respect to Ser205Leu, individual studies contained 164–670 patients with MDD and 164–1130 controls, and the combined sample consisted of 1060 patients and 1688 controls (Table 2). There was no heterogeneity across studies (Q=2.86, d.f.=2, P=0.240). We performed the fixed effects meta-analyses. The meta-analysis revealed a significant association (OR 0.77, 95%CI 0.63–0.94; Z=−2.57, P=0.010; Figure 1). These meta-analytic procedures were carried out using Comprehensive Meta-Analysis v.2.0 (Biostat, Inc., Englewood, NJ, USA).

The PolyPhen program (http://genetics.bwh.harvard.edu/pph/), an automatic tool for prediction of possible impact of an amino-acid substitution on the structure and function of a protein, predicted 'possibly damaging' effect of the Ser205Leu. This polymorphic site may also be important in terms of glycosylation. $p75^{NTR}$ is a highly glycosylated protein. Using NetOglyc 3.1 program (http://www.cbs.dtu.dk/services/NetOGlyc/),[12] which predicts mucin type O-glycosylation sites, the Ser205 had a score of 0.616 indicating that it is a putative O-glycosylation site. These results suggest that the Ser205Leu polymorphism could modify roles in ligand binding and signaling,[13] although actual effects of this polymorphism are yet to be elucidated by cell biological approaches.

In conclusion, the present study replicated the association between the Ser205Leu polymorphism of the $p75^{NTR}$ gene and MDD in our previous study, suggesting that the Leu205 allele has a protective effect

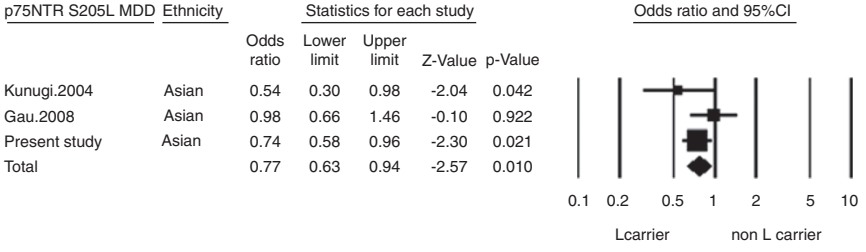

Figure 1 Forest plots of meta-analysis on the possible association of the Ser205Leu polymorphism of the $p75^{NTR}$ gene with MDD by Leu/Leu, Ser/Leu vs Ser/Ser model.

against the development of MDD. Further stratified analysis by sex suggested that the effect might be limited to females. Future reproductive studies to overcome the limitation by using larger sample size and cellular approaches to elucidate the effect of this polymorphism on gene functions are warranted.

ACKNOWLEDGEMENTS

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (HK, TF), Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health and Welfare) (HK), JST, CREST (HK), the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health), and Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (HK). These agencies had no role in study design, acquisition and interpretation of data or writing the report.

1 Reichardt, L. F. Neurotrophin-regulated signalling pathways. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361,** 1545–1564 (2006).
2 Duman, R. S. & Monteggia, L. M. A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry.* **59,** 1116–1127 (2006).
3 Verhagen, M., van der Meij, A., van Deurzen, P. A., Janzing, J. G., Arias-Vásquez, A., Buitelaar, J. K. *et al.* Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol. Psychiatry.* **15,** 260–271 (2010).
4 Kunugi, H., Hori, H., Adachi, N. & Numakawa, T. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. *Psychiatry. Clin. Neurosci.* **64,** 447–459 (2010).
5 Fujii, T. & Kunugi, H. p75NTR as a therapeutic target for neuropsychiatric diseases. *Curr. Mol. Pharmacol.* **2,** 70–76 (2009).
6 Kunugi, H., Hashimoto, R., Yoshida, M., Tatsumi, M. & Kamijima, K. A missense polymorphism (S205L) of the low-affinity neurotrophin receptor p75NTR gene is associated with depressive disorder and attempted suicide. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **129B,** 44–46 (2004).
7 Gau, Y. T., Liou, Y. J., Yu, Y. W., Chen, T. J., Lin, M., Tsai, S. J. *et al.* Evidence for association between genetic variants of p75 neurotrophin receptor (p75NTR) gene and antidepressant treatment response in Chinese major depressive disorder. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **147B,** 594–599 (2008).
8 American Psychiatric Association. in *DSM-IV: Diagn. Stat. Man. Ment. Disord.* 4th edn (American Psychiatric Press: Washington D.C.,, 1994).
9 Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry.* **59**(Suppl 20), 22–33; quiz 34–57 (1998).
10 Otsubo, T., Tanaka, K, Koda, R., Shinoda, J., Sano, N., Tanaka, S. *et al.* Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. *Psychiatry. Clin. Neurosci.* **59,** 517–526 (2005).
11 Griesmaier, E., Schlager, G., Wegleiter, K., Hermann, M., Urbanek, M., Simbruner, G. *et al.* Role of p75NTR in NMDAR-mediated excitotoxic brain injury in neonatal mice. *Brain. Res.* **1355,** 31–40 (2010).
12 Julenius, K., Molgaard, A., Gupta, R. & Brunak, S. Prediction, conservation analysis, and structural characterization of mammalian mucin-type O-glycosylation sites. *Glycobiology* **15,** 153–164 (2005).
13 Chapman, B. S., Eckart, M. R., Kaufman, S. E. & Lapointe, G. R. O-linked oligosaccharide on the 75-kDa neurotrophin receptor. *J. Neurochem.* **66,** 1707–1716 (1996).