was saturated. Finally, it is possible that females use a broader brain area outside the prefrontal area during the VFT-l than males do, so that the increase of the Oxy-Hb level seemed lower in females. To the best of our knowledge, no clear evidence for this latter explanation has been reported, but it has been reported that the brain activation during linguistic tasks in males might be more lateralized than in females [47], which implies that brain activation could be different between the genders according to the task.

4.3. Correlation of the Prefrontal Oxy-Hb Increase with BDNF

In the present study, the correlations of BDNF polymerphism, Val66Met, and serum BDNF level with the Oxy-Hb levels in the prefrontal lobe during cognitive tasks were negative in healthy subjects. Accumulating evidence suggests that the BDNF Val66Met polymerphism affects cognitive performance. In previous studies, subjects with Met carriers scored lower in the cognitive tasks than subjects with Val/Val genotypes, and relevance has been found in healthy subjects [14,15] as well as in patients with mental illness such as schizophrenia [15] and bipolar disorder [16]. One of the possible reasons for our negative result is that the performance of the episodic memory task associated with the BDNF genotypes in the previous studies with fMRI reflected hippocampal activation rather than prefrontal activation [15,48]. Thus the effect of the Val66Met polymorphism of BDNF might be difficult to detect by NIRS measurement. Another is that the relevance between the BDNF genotype and the prefrontal activation might be illness specific. Scores on the Wisconsin Card Sorting Test (WCST), which activates the dorsolateral prefrontal cortex, was reported to be associated with the BDNF variance in bipolar disorder but not in schizophrenia or in healthy controls [49]. In addition, it should be considered that no difference in cognitive function according to the BDNF genotype has yet been reported in a healthy Japanese population, and the distribution of the BDNF genotype shows clear ethnic differences [50]. Thus, our results might be different from those involving subjects of other ethnicities. To date, there is only one study that suggested a correlation between functional genotype variation and NIRS measurement. By using the 52-channel NIRS with the VFT-l, Takizawa *et al.* (2009) suggested that a functional SNP of catechol-O-methyltransferase (COMT), Met108/158Val, might affect the prefrontal Oxy-Hb level in subjects with schizophrenia, although relevance was not seen in healthy controls [51]. Serum BDNF level has been indicated to be correlated with the severity of major depression [30] and with treatment response [18-20], but there has been no report that investigated the correlation with prefrontal activity measured by NIRS. Our results suggest that there was no correlation between serum BDNF level and prefrontal activity in healthy individuals. Our results also suggest that, in healthy subjects, the BDNF level is neither affected by temporal depressed mood and/or anxiety nor correlated with the Oxy-Hb level of the prefrontal lobe measured by NIRS. Since neither of our negative results regarding the relevance of the Oxy-Hb levels measured by NIRS to BDNF genotype and serum BDNF level disproves that the relevance might be altered in those who suffer from mental illnesses such as mood disorders, anxiety disorders, or schizophrenia, further study with such patients would be of great interest. Moreover, it should be considered a limitation of our study that we could not collect the blood samples from all of the subjects recruited in the NIRS study. Thus, our results should be considered preliminary.

5. CONCLUSION

In applying NIRS measurement to basically healthy individuals, it was found that the increase of the prefrontal Oxy-Hb level during cognitive tasks (VFT-l and Stroop test) was significantly correlated with depressed mood only in males. In addition, the course of the Oxy-Hb increase in the prefrontal lobe was different depending on the cognitive task, *i.e.* the VFT-l or the Stroop test, in both genders. The correlations of the Oxy-Hb increase during the cognitive tasks with the BDNF genotype and serum BDNF level were negative. Our data suggest that the temporal mental status might affect the course of the prefrontal Oxy-Hb change during certain cognitive tasks and that NIRS measurement has the potential to be a useful tool for detecting early subclinical manifestations of depressed mood in males that are not correlated with the individual properties of BDNF.

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REFERENCES

[1] Adler, D.A., McLaughlin, T.J., Rogers, W.H., Chang, H., Lapitsky, L. and Lerner, D. (2006) Job performance deficits due to depression. *American Journal of Psychiatry*, **163**, 1569-1576. doi:/10.1176/appi.ajp.163.9.1569

[2] Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R. and Walters, E.E. (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, **62**, 593-602.

[3] Pignone, M.P., Gaynes, B.N., Rushton, J.L., Burchell, C. M., Orleans, C.T., Mulrow, C.D. and Lohr, K.N. (2002) Screening for depression in adults: A summary of the evidence for the U.S. Preventive Services Task Force. *Annals of Internal Medicine*, **136**, 765-776.

[4] Hammen, C., Kim, E.Y., Eberhart, N.K. and Brennan, P.A. (2009) Chronic and acute stress and the prediction of major depression in women. *Depression and Anxiety*, **26**, 718-723. doi:/10.1002/da.20571

[5] Mazure, C. (1998) Life Stressors as risk factors in depression. *Clinical Psychology: Science and Practice*, **5**, 291-313.

[6] Maki, A., Yamashita, Y., Ito, Y., Watanabe, E., Mayanagi, Y. and Koizumi, H. (1995) Spatial and temporal analysis of human motor activity using noninvasive NIR topography. *Medical Physics*, **22**, 1997-2005. doi:/10.1118/1.597496

[7] Irani, F., Platek, S.M., Bunce, S., Ruocco, A.C. and Chute, D. (2007) Functional near infrared spectroscopy (fNIRS): An emerging neuroimaging technology with important applications for the study of brain disorders. *Clinical Neuropsychologist*, **21**, 9-37. doi:/10.1080/13854040600910018

[8] Herrmann, M.J., Ehlis, A.C. and Fallgatter, A.J. (2004) Bilaterally reduced frontal activation during a verbal fluency task in depressed patients as measured by near-infrared spectroscopy. *Journal of Neuropsychiatry & Clinical Neurosciences*, **16**, 170-175. doi:/10.1176/appi.neuropsych.16.2.170,

[9] Kameyama, M., Fukuda, M., Yamagishi, Y., Sato, T., Uehara, T., Ito, M., Suto, T. and Mikuni, M. (2006) Frontal lobe function in bipolar disorder: A multichannel near-infrared spectroscopy study. *Neuroimage*, **29**, 172-184. doi:/10.1016/j.neuroimage.2005.07.025

[10] Suto, T., Fukuda, M., Ito, M., Uehara, T. and Mikuni, M. (2004) Multichannel near-infrared spectroscopy in depression and schizophrenia: Cognitive brain activation study. *Biological Psychiatry*, **55**, 501-511. doi:/10.1016/j.biopsych.2003.09.008

[11] Marcus, S.M., Young, E.A., Kerber, K.B., Kornstein, S., Farabaugh, A.H., Mitchell, J., Wisniewski, S.R., Balasubramani, G.K., Trivedi, M.H. and Rush, A.J. (2005) Gender differences in depression: Findings from the STAR*D study. *Journal of Affective Disorders*, **87**, 141-150. doi:/10.1016/j.jad.2004.09.008

[12] Binder, D.K. and Scharfman, H.E. (2004) Brain-derived neurotrophic factor. *Growth Factors*, **22**, 123-131. doi:/10.1080/08977190410001723308

[13] Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F. and Weinberger, D.R. (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience*, **23**, 6690-6694.

[14] Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B. and Weinberger, D.R. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, **112**, 257-269. doi:/10.1016/S0092-8674(03)00035-7

[15] Dempster, E., Toulopoulou, T., McDonald, C., Bramon, E., Walshe, M., Filbey, F., Wickham, H., Sham, P.C., Murray, R.M. and Collier, D.A. (2005) Association between BDNF val66 met genotype and episodic memory. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, **134B**, 73-75. doi:/10.1002/ajmg.b.30150

[16] Rybakowski, J.K., Borkowska, A., Czerski, P.M., Skibinska, M. and Hauser, J. (2003) Polymorphism of the brain-derived neurotrophic factor gene and performance on a cognitive prefrontal test in bipolar patients. *Bipolar Disorder*, **5**, 468-472. doi:/10.1046/j.1399-5618.2003.00071.x

[17] Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J.M. and Bertschy, G. (2005) Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biological Psychiatry*, **57**, 1068-1072. doi:/10.1016/j.biopsych.2005.01.008

[18] Rybakowski, J.K., Borkowska, A., Skibinska, M. and Hauser, J. (2006) Illness-specific association of val66met BDNF polymorphism with performance on Wisconsin Card Sorting Test in bipolar mood disorder. *Molecular Psychiatry*, **11**, 122-124. doi:/10.1038/sj.mp.4001765

[19] Gonul, A.S., Akdeniz, F., Taneli, F., Donat, O., Eker, C. and Vahip, S. (2005) Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *European Archives of Psychiatry and Clinical Neuroscience*, **255**, 381-386. doi:/10.1007/s00406-005-0578-6

[20] Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., Nakazato, M., Watanabe, H., Shinoda, N., Okada, S. and Iyo, M. (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biological Psychiatry*, **54**, 70-75. doi:/10.1016/S0006-3223(03)00181-1

[21] Lang, U.E., Hellweg, R. and Gallinat, J. (2004) BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. *Neuropsychopharmacology*, **29**, 795-798. doi:/10.1038/sj.npp.1300382

[22] Zigmond, A.S. and Snaith, R.P. (1983) The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica*, **67**, 361-370. doi:/10.1111/j.1600-0447.1983.tb09716.x

[23] Association, A.P. (1994) Diagnostic and statistical manual of mental disorders. 4th Edition, American Psychiatric Press, Washington DC.

[24] Watanabe, E., Maki, A., Kawaguchi, F., Takashiro, K., Yamashita, Y., Koizumi, H. and Mayanagi, Y. (1998) Non-invasive assessment of language dominance with near-infrared spectroscopic mapping. *Neuroscience Letters*, **256**, 49-52. doi:/10.1016/S0304-3940(98)00754-X

[25] MacLeod, C.M. (1991) Half a century of research on the Stroop effect: An integrative review. *Psychological Bulletin*, **109**, 163-203. doi:/10.1037/0033-2909.109.2.163

[26] Shimada, S. and Hiraki, K. (2006) Infant's brain re-

sponses to live and televised action. *Neuroimage*, **32**, 930-939. doi:/10.1016/j.neuroimage.2006.03.044

[27] Minagawa-Kawai, Y., Mori, K., Furuya, I., Hayashi, R. and Sato, Y. (2002) Assessing cerebral representations of short and long vowel categories by NIRS. *Neuroreport*, **13**, 581-584.

[28] Shimoda, N., Takeda, K., Imai, I., Kaneko, J. and Kato, H. (2008) Cerebral laterality differences in handedness: A mental rotation study with NIRS. *Neuroscience Letters*, **430**, 43-47. doi:/10.1016/j.neulet.2007.10.016

[29] Takeda, K., Gomi, Y., Imai, I., Shimoda, N., Hiwatari, M. and Kato, H. (2007) Shift of motor activation areas during recovery from hemiparesis after cerebral infarction: A longitudinal study with near-infrared spectroscopy. *Neuroscience Research*, **59**, 136-144. doi:/10.1016/j.neures.2007.06.1466,

[30] Itoh, K., Hashimoto, K., Kumakiri, C., Shimizu, E. and Iyo, M. (2004) Association between brain-derived neurotrophic factor 196 G/A polymorphism and personality traits in healthy subjects. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, **124B**, 61-63. doi:/10.1002/ajmg.b.20078

[31] Kakimoto, Y., Nishimura, Y., Hara, N., Okada, M., Tanii, H. and Okazaki, Y. (2009) Intrasubject reproducibility of prefrontal cortex activities during a verbal fluency task over two repeated sessions using multi-channel near-infrared spectroscopy. *Psychiatry and Clinical Neurosciences*, **63**, 491-499. doi:/10.1111/j.1440-1819.2009.01988.x

[32] Kono, T., Matsuo, K., Tsunashima, K., Kasai, K., Takizawa, R., Rogers, M.A., Yamasue, H., Yano, T., Taketani, Y. and Kato, N. (2007) Multiple-time replicability of near-infrared spectroscopy recording during prefrontal activation task in healthy men. *Neuroscience Research*, **57**, 504-512. doi:/10.1016/j.neures.2006.12.007

[33] Braver, T.S., Cohen, J.D., Nystrom, L.E., Jonides, J., Smith, E.E. and Noll, D.C. (1997) A parametric study of prefrontal cortex involvement in human working memory. *Neuroimage*, **5**, 49-62. doi:/10.1006/nimg.1996.0247

[34] Fridriksson, J. and Morrow, L. (2005) Cortical activation and language task difficulty in aphasia. *Aphasiology*, **19**, 239-250. doi:/10.1080/02687030444000714

[35] Drevets, W.C. (2000) Neuroimaging studies of mood disorders. *Biological Psychiatry*, **48**, 813-829. doi:/10.1016/S0006-3223(00)01020-9

[36] Galynker, II, Cai, J., Ongseng, F., Finestone, H., Dutta, E. and Serseni, D. (1998) Hypofrontality and negative symptoms in major depressive disorder. *Journal of Nuclear Medicine*, **39**, 608-612.

[37] Milak, M.S., Parsey, R.V., Keilp, J., Oquendo, M.A., Malone, K.M. and Mann, J.J. (2005) Neuroanatomic correlates of psychopathologic components of major depressive disorder. *Archives of General Psychiatry*, **62**, 397-408. doi:/10.1001/archpsyc.62.4.397

[38] Kameyama, M., Fukuda, M., Uehara, T. and Mikuni, M. (2004) Sex and age dependencies of cerebral blood volume changes during cognitive activation: A multichannel near-infrared spectroscopy study. *Neuroimage*, **22**, 1715-1721. doi:/10.1016/j.neuroimage.2004.03.050

[39] Herrmann, M.J., Walter, A., Ehlis, A.C. and Fallgatter, A.J. (2006) Cerebral oxygenation changes in the prefrontal cortex: Effects of age and gender. *Neurobiology of Aging*, **27**, 888-894. doi:/10.1016/j.neurobiolaging.2005.04.013

[40] Ehlis, A.C., Herrmann, M.J., Wagener, A. and Fallgatter, A.J. (2005) Multi-channel near-infrared spectroscopy detects specific inferior-frontal activation during incongruent Stroop trials. *Biological Psychology*, **69**, 315-331. doi:/10.1016/j.biopsycho.2004.09.003

[41] Schroeter, M.L., Zysset, S., Kruggel, F. and Von Cramon, D.Y. (2003) Age dependency of the hemodynamic response as measured by functional near-infrared spectroscopy. *Neuroimage*, **19**, 555-564. doi:/10.1016/S1053-8119(03)00155-1

[42] Yanagisawa, H., Dan, I., Tsuzuki, D., Kato, M., Okamoto, M., Kyutoku, Y. and Soya, H. (2010) Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test. *Neuroimage*, **50**, 1702-1710. doi:/10.1016/j.neuroimage.2009.12.023

[43] Kerns, J.G., Cohen, J.D., MacDonald, A.W., Johnson, M.K., Stenger, V.A., Aizenstein, H. and Carter, C.S. (2005) Decreased conflict- and error-related activity in the anterior cingulate cortex in subjects with schizophrenia. *American Journal of Psychiatry*, **162**, 1833-1839. doi:/10.1176/appi.ajp.162.10.1833

[44] Yucel, M., Pantelis, C., Stuart, G.W., Wood, S.J., Maruff, P., Velakoulis, D., Pipingas, A., Crowe, S.F., Tochon-Danguy, H.J. and Egan, G.F. (2002) Anterior cingulate activation during Stroop task performance: A PET to MRI coregistration study of individual patients with schizophrenia. *American Journal of Psychiatry*, **159**, 251-254. doi:/10.1176/appi.ajp.159.2.251

[45] Gur, R.C., Gur, R.E., Obrist, W.D., Hungerbuhler, J.P., Younkin, D., Rosen, A.D., Skolnick, B.E. and Reivich, M. (1982) Sex and handedness differences in cerebral blood flow during rest and cognitive activity. *Science*, **217**, 659-661. doi:/10.1126/science.7089587

[46] Rodriguez, G., Warkentin, S., Risberg, J. and Rosadini, G. (1988) Sex differences in regional cerebral blood flow. *Journal of Cerebral Blood Flow & Metabolism*, **8**, 783-789. doi:/10.1038/jcbfm.1988.133

[47] Kansaku, K., Yamaura, A. and Kitazawa, S. (2000) Sex differences in lateralization revealed in the posterior language areas. *Cerebral Cortex*, **10**, 866-872. doi:/10.1093/cercor/10.9.866

[48] Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F. and Weinberger, D.R. (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *Journal of Neuroscience*, **23**, 6690-6694.

[49] Rybakowski, J.K., Borkowska, A., Skibinska, M. and Hauser, J. (2006) Illness-specific association of val66met BDNF polymorphism with performance on Wisconsin Card Sorting Test in bipolar mood disorder. *Molecular Psychiatry*, **11**, 122-124. doi:/10.1038/sj.mp.4001765

[50] Shimizu, E., Hashimoto, K. and Iyo, M. (2004) Ethnic difference of the BDNF 196G/A (val66met) olymorphism frequencies: The possibility to explain ethnic mental traits. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, **126B**, 122-123. doi:/10.1002/ajmg.b.20118

[51] Takizawa, R., Tochigi, M., Kawakubo, Y., Marumo, K., Sasaki, T., Fukuda, M. and Kasai, K. (2009) Association between catechol-O-methyltrasferase Val108/158Met genotype and prefrontal hemodynamic response in schizophrenia. *PLoS One*, **4**, e5495. doi:/10.1371/journal.pone.0005495

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Critical Review

Possible Involvement of Brain-derived Neurotrophic Factor in Eating Disorders

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Summary

Eating disorders (EDs) manifest as abnormal patterns of eating behavior and weight regulation driven by low self-esteem due to weight preoccupation and perceptions toward body weight and shape. Two major groups of such disorders are anorexia nervosa (AN) and bulimia nervosa (BN). The etiology of EDs is complex and evidence indicates that both biological/genetic and psychosocial factors are involved. Several lines of evidence indicate that brain-derived neurotrophic factor (BDNF) plays a critical role in regulating eating behaviors and cognitive impairments in the EDs. BDNF is involved in neuronal proliferation, differentiation, and survival during development. BDNF and its tyrosine kinase receptor (TrkB) are expressed in hypothalamic nuclei associated with eating behaviors. A series of studies using BDNF knockout mice and the human BDNF gene indicate an association of BDNF and EDs with predisposition and vulnerability. In the previous studies, serum BDNF levels in subjects with EDs are reduced significantly compared with healthy controls, hence, we proposed that levels of serum BDNF would be a useful diagnostic indicator for EDs. © 2012 IUBMB

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family, which includes nerve growth factor, neurotrophin-3, and neurotrophin-4/5 (*1–3*). BDNF binds to a specific receptor TrkB, which is present on the surface of target cells, and regulates the growth of neuronal cells by mediating processes such as survival, development, and enhanced synaptic activity. Besides neuronal growth, differentiation, maintenance, and potentiation, BDNF also modulates neurotransmitters, such as dopamine, and neural plasticity and binding mechanisms underlying long-term potentiation and learning/memory (*1–4*). BDNF is widely distributed in the central nervous system (CNS), beginning early in development and extending throughout in the life span. In human brain, BDNF is most abundant in several regions, including the hippocampus and the cerebral cortex (*1–3*). BDNF readily crosses the blood–brain barrier in both directions, and therefore, a substantial part of circulating BDNF concentrations may originate from neurons and glia cells in the CNS. In the peripheral tissues, it is acquired from plasma membrane, partly produced in vascular endothelial cells or smooth muscle cells, then internalized and stored mainly in the platelets, and later, it is released into plasma through activation or clotting process (*5*).

The average serum levels of BDNF were about between 50- and 100-fold higher than plasma levels; the difference is due to degranulation of platelets during the clotting process. BNDF is initially synthesized as a precursor protein proBDNF, and then proBDNF is proteolytically cleaved to the mature BDNF. The BDNF gene produced precursor proteinBDNF (preproBDNF) in the endoplasmic reticulum. ProBDNF binds to intracellular sortilin in the Golgi to facilitate proper folding of the mature domain. ProBDNF preferentially binds $p75^{NTR}$ receptors. ProBDNF is cleaved by proteases at the synapses and concerted to mature BDNF. Mature BDNF preferentially binds

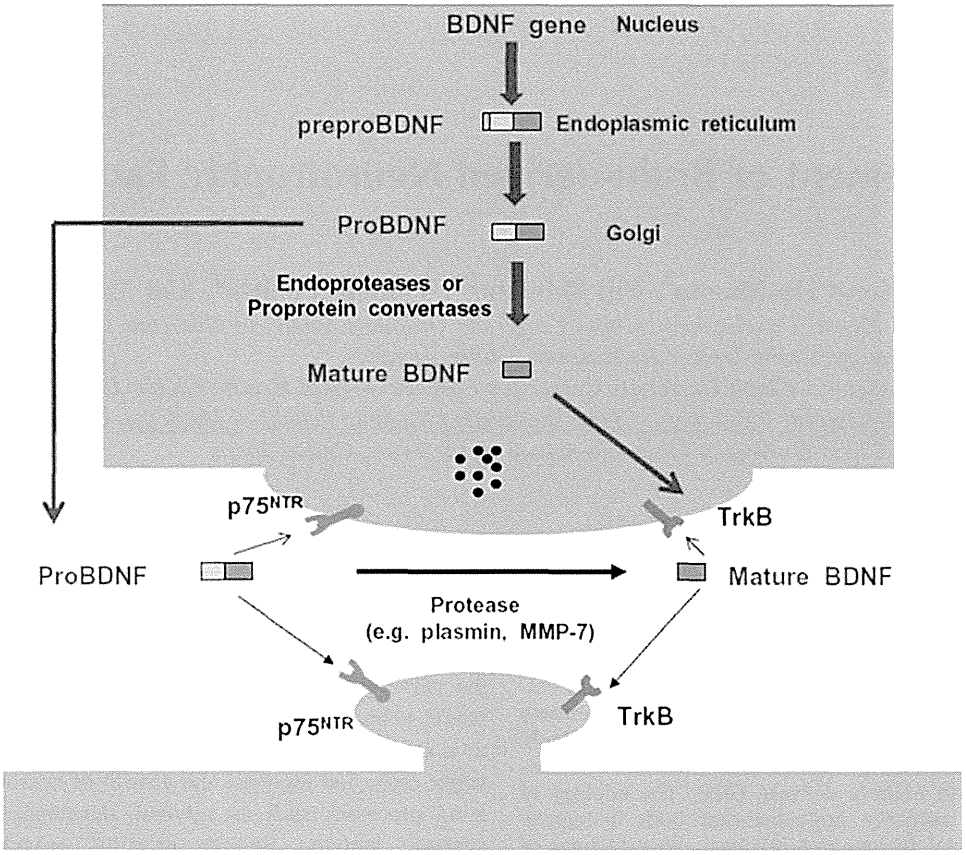

Figure 1. The synthesis of BDNF from proBDNF. This figure is referred from the article by Hashimoto (*6*).

tropomyosin receptor kinase (Trk) B receptor (ref. *6*; Fig. 1). BDNF and TrkB are expressed in various hypothalamic nuclei associated with eating behavior. In animal studies, mice heterozygous for the BDNF gene (reduced BDNF levels) develop enhanced intermale aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood, and these behavioral abnormalities are known to correlate with serotonergic dysfunction (*6*). BDNF gene of heterozygous knockout mice demonstrates increased levels of stress anxiety, locomotor activity, and abnormalities in eating behavior (*7, 8*).

Eating disorders (EDs) manifest as abnormal patterns of eating behavior and weight regulation driven by low self-esteem due to weight preoccupation and perceptions toward body weight and shape. Two major groups of such disorders are anorexia nervosa (AN) and bulimia nervosa (BN). Anorexic problems mainly involve low body weight, fear toward weight gain, persistent restriction of food intake, and pursuit of regulating weight and shape. BN is characterized by repetitive cycle of excessive uncontrolled binge eating followed by compensatory behavior such as purging. AN and BN are frequently observed among young women, the typical onset in adolescence to adulthood. In Europe and North America, the combined prevalence of AN (*9, 10*) among young women is reported to be 0.1–0.5% and that of BN (*11*) is 1.5–3.8%. They have been a major focus of attention for both the research community and sometimes life-threatening group of problems, predominantly affecting women.

The pathophysiology of EDs has been studied from various aspects, and a range of factors including biological and psychosocial factors are linked to the onset and persistence of symptoms. Some studies showed risk factors for EDs such as low self-esteem, anxiety, harm avoidance, impulsive control and obsessive–compulsive personality traits, and cognitive inflexibility (*12*); however, the details of biological markers for the pathophysiology of EDs are unknown. Identification of the biological markers of EDs is crucial for the development of new treatments as well as to aid both in the diagnosis and management. In addition, they could provide the basis for early intervention and prevention efforts targeting at-risk individuals.

Recently, there has been a series of findings on the involvement of BDNF in the pathophysiology of EDs (*11*). According to genetic studies on BDNF and EDs (*13, 14*), and a meta-analysis of the BDNF gene, a 30% higher incidence of EDs is seen among individuals with the *Val/Met* and the *Met/Met polymorphism* of the BDNF gene (*13*), indicating that the *BDNF Val66-Met* (*rs6265*) polymorphism is linked to EDs. On the other hand, an association of the BDNF gene with other mental disorders, such as mood disorders and substance abuse disorders,

have also been reported (*14, 15*). Thus, the special relevance of the BDNF gene to EDs is currently unclear.

In Japanese subjects, we previously revealed the associations of the *BDNF Val66Met polymorphism* with AN (restricting type) and BN (purging type) (*16*). In the previous studies, serum BDNF levels in subjects with EDs are reduced significantly when compared with healthy controls (HCs), and hence, we proposed that levels of serum BDNF would be a useful diagnostic indicator for EDs (*17–19*). Here, we introduce a number of studies on serum BDNF and EDs, describe the associations between BDNF and the pathophysiological features of EDs, and propose challenges and new perspectives for the future research.

BDNF AND EATING BEHAVIORS

BDNF is the most abundant neurotrophic factor in the brain and is a member of the nerve growth factor family that includes nerve growth factor, neurotrophin-3, and neurotrophin-4/5 (*1–3*). BDNF also modulates networks of neurotransmitters, such as dopaminergic (*20, 21*), serotoninergic (*22*), and glutamatergic neurotransmission (*23*), and plays a role in neural plasticity and binding mechanisms underlying long-term potentiation and learning/memory (*1*).

BDNF binds to a specific tyrosine kinase receptor, TrkB. BDNF is a 27-kDa polypeptide, which easily crosses through the blood–brain barrier (*24, 25*). BDNF functions as a neurotrophic factor and plays a role in the modulation and integration of signaling pathways in the nervous, immune, and endocrine systems (*26*). It also influences regulatory mechanisms of weight (*27*), eating behavior (*27*), and metabolism.

The human BDNF gene is located on chromosome 11p13 and comprises 11 exons. The gene encodes a precursor protein proBDNF (*6*), which mediates secretion and cellular transportation of BDNF. Synthesis of preproBDNF and its conversion to a single proBDNF peptide take place in the lumen of the endoplasmic reticulum, followed by transportation to the Golgi apparatus. ProBDNF preferentially binds p75NTR receptors. ProBDNF is cleaved by proteases at the synapses and is converted to mature BDNF, and it participates in a range of important physiological activities (*6*). Originally, proBDNF was considered biologically inactive; however, recent evidence shows them to be important factors that can bind to neurotrophin receptors and induce effects opposite to the mature BDNF (*22, 28, 29*).

ANIMAL STUDIES

Among BDNF transgenic mice, *BDNF*+/− knockout mice exhibit hyperactivity, high anxiety, and weight gain under stress (*8, 30*). *BDNF*+/− knockout mice also show aggressiveness and excessive appetite in early adulthood (*7*). *BDNF*−/− homozygote mutant mice die within a few weeks after birth (*31, 32*). In animal studies, appetite increases when the expression of TrkB in the hypothalamus is low, and BDNF regulates energy balance through melanocortin-4 receptor in the hypothalamus (*33*). Another study showed that dietary restriction (DR) may alter the behaviors in *BDNF*+/− mice and that the 5-HT system may be implicated in the beneficial effects of DR on these behaviors (*34*). Such findings indicate that the BDNF gene product is involved in the modulation of anxiety, eating behavior, and weight control.

HUMAN SERUM AND PLASMA BDNF

BDNF is widely distributed in the CNS (*33*) and is especially abundant in the hippocampus, prefrontal cortex, ventral tegmental area, and amygdala. High levels of BDNF and TrkB are found in the hypothalamus, which is one of the major regulatory centers for eating behavior (*30*). BDNF in the CNS crosses through the blood–brain barrier (*24, 25*). In the periphery, it is synthesized mainly in vascular endothelial cells and smooth muscle cells (*35*) and is stored in platelets (*5*). BDNF also circulates in plasma due to granulation of coagulation factors. In humans, the levels of plasma BDNF decrease with age, and in women, the levels of serum BDNF vary according to the phase of menstrual cycles (*27*).

When compared with serum BDNF, plasma BDNF is sensitive to environmental factors (*35*). Animal studies have shown a correlation between serum BDNF levels in the cortex and those in the hippocampus (*24, 36*). In rats, BDNF levels in serum and brain are highly correlated across all age groups (*37*).

GENETIC STUDIES

The frequency of the *BDNF196G/A* (*Val66Met*) polymorphism is higher among Japanese than Europeans and North Americans (*37*). The number of patients with EDs carrying the *BDNF196G/A* allele, especially of the AN restricting or BN purging type, is significantly high when compared with that of the HCs (*16*). Correlations were also observed between the energy intake and the body mass index (BMI) (*38*), AN restricting type, and minimum BMI value (*38, 39*). With regard to cognitive dysfunction and when compared with *BDNF Val/Val allele* carriers, individuals with the *BDNF Val/Met allele* demonstrate frontal lobe cognitive dysfunction (*40*), whereas those with the *BDNF Met66 allele* show episodic memory dysfunction (*41, 42*).

DECREASED LEVELS OF SERUM BDNF IN INDIVIDUALS WITH EDS

We hypothesized that serum BDNF levels would be a candidate biological markers in patients with EDs for both abnormal eating behavior and cognitive impairment related to body shape and weight. Accordingly, we compared serum BDNF levels of female patients with AN and BN with those of HCs. BDNF levels among patients with AN and BN were significantly reduced when compared with those of HCs ($P < 0.0001$). Furthermore, the BDNF levels were significantly lower in the patients with

Table 1
Data from studies measuring serum BDNF levels in patients with AN and healthy controls

Author	Ethnicity	Number of EDs	Number of HCs	AN age	HC age	AN BMI	HC BMI	AN BDNF (ng/mL) (+/−)	HC BDNF (ng/mL) (+/−)
Nakazato et al. (*17*)	Japanese	12 AN	21	19.6 (5.8)	20.4 (2)	14.2 (0.7)	20.4 (2)	24.9 (6.75)	61.4 (19.5)
Monteleone et al. (*43*)	Italian	12 AN	27	20.5 (5.4)	22.3 (3.4)	15.6 (1.8)	22 (1.9)	26.65 (12.46)	45.8 (29.1)
Monteleone et al. (*44*)	Italian	27 AN	24	20 (5.2)	22.4 (3.4)	15.9 (2.4)	21.8 (1.8)	28.87 (16.36)	50 (27.92)
Nakazato et al. (*18*)	Japanese	13 AN	17	19.6 (5.8)	20.4 (2)	14.2 (0.7)	20.4 (1.5)	14.5 (4.4)	22.1 (8.9)
Saito et al. (*45*)	Japanese	19 AN	24	25.3 (7.9)	24.5 (5.7)	14.0 (2.1)	20.4 (2.4)	20.0 (5.1)	26.0 (3.9)
Nakazato et al. (*19*)	British	29 AN	28	28.3 (11)	26.9 (5.8)	15.6 (1.6)	22.3 (2.5)	11.7 (4.9)	15.1 (5.5)
Ehrlich et al. (*46*)	German	33 AN	33	18.9 (3.9)	19 (3.1)	14.9 (1.4)	21.4 (2.1)	6.16 (2.88)	7.41 (3.22)

Abbreviations: AN, anorexia nervosa; HC, healthy controls; BDNF, brain-derived neurotrophic factor.

AN than in the patients with BN. In all subjects, there was a significant correlation between serum BDNF levels and BMI (*17*).

Following our study, other groups also reported reduced levels of serum BDNF in patients with EDs (*43–46*). In the two previous studies, serum BDNF levels (*45*) and BMI (*46*) were positively correlated with AN. In all the subjects, including subjects with AN and HC, the correlation between serum BDNF level and BMI has been established (*17, 18, 43, 44*). Although the present findings suggest correlations between BMI, one of the severity indexes of AN, and serum BDNF, a meta-analysis of circulating BDNF in AN (*47*) confirmed significant heterogeneity of effect size (Table 1).

Another group invested the correlation of the Eating Disorders Inventory (EDI) scales with BDNF plasma levels and suggested that BDNF levels of both of the AN and BN may influence the severity of EDs by modulating the associated psychopathology, in particular through the impairment of interoceptive awareness (*48*). Regarding hypothalamic-pituitary-adrenal (HPA) axis reactivity and BDNF polymorphism, *BDNF Val(66)Met* carriers are particularly sensitive in anticipating stressful events in healthy adults (*49*).

BDNF IN RECOVERED AN

In the previous pilot study, we longitudinally compared serum BDNF levels of patients with AN before and after partial recovery of their BMI (*18*). We did not observe a statistically significant difference. However, in the cross-sectional study, serum BDNF levels of AN group were significantly lower than those of not only the HCs but also the recovered AN group which had 1) a history of AN, 2) maintained BMI levels ranging from 18.5 to 25 kg/m^2 for at least 1 year, and 3) maintained a regular menstrual cycle for at least 1 year (*19*).

Ehrlich et al. (*46*) compared serum BDNF levels between 33 patients with AN, seven subjects in partial recovery after a longitudinal follow-up (>10% weight gain), 20 patients with AN in the recovery phase, and 33 HCs. The results showed that, when compared with current AN, serum BDNF levels in the partial recovery group were slightly higher; however, the difference between the two groups was not statistically significant. Although BDNF levels in the recovered AN group were increased when compared with those in the current AN and HC groups, again the differences did not attain statistical significance.

COMORBID MENTAL DISORDERS AND SERUM BDNF

EDs are often associated with mental disorders such as mood disorders and anxiety disorders. A high prevalence of concurrent mood disorders has been observed among patients with AN, and the involvement of genetic and environmental factors has been reported (*50*). Reduced levels of BDNF were found in the postmortem brains of individuals who suffered from depression (*51*). Studies using an animal model of depression have reported reduced BDNF levels in the CNS and the recovery of BDNF mRNA levels in the hippocampus after administration of antidepressants (*52, 53*). In individuals with depression, decreased levels of serum BDNF have been demonstrated (*50*). A meta-analysis also revealed that reduced serum BDNF levels are associated with depression, whereas antidepressant administration reverses such reduction of serum BDNF levels (*54, 55*). Recent study showed that classification based on the DNA methylation profiles of CpG I of the BDNF gene may be a valuable diagnostic biomarker for major depression (*56*). Obsessive–compulsive disorders often comorbid with AN (*57*), and serum BDNF levels in individuals with obsessive–compulsive disorders are also reduced when compared with HCs (*58*).

In our previous study (*17*), the Hamilton Depression Rating Scale (HDRS) of the participants with EDs was significantly lower than that of HCs. A significant positive correlation between HDRS and the Bulimic Investigatory Test, Edinburgh (BITE) symptom scale scores in all the patients was detected. Some lines of evidence suggest that altered 5-HT activity in patients with EDs could be a consequence of pathologic EDs. Furthermore, in the pharmacological treatment of EDs, antidepressants, including Selective Serotonin Reuptake Inhibitors (SSRI), have been shown to decrease symptoms of binge eating and purging behaviors, in addition to depressive symptoms. The

5-HT systems in the brain have been strongly associated with BDNF regulation of food intake, demonstrating that endogenous BDNF is critical for the normal development and functioning of central 5-HT neurons (*7*).

The differences were shown between AN and depression that pharmacotherapy with antidepressants results in normalization of BDNF levels in depressed patients and contributes to recovery or alleviation of symptoms, and on the other hand, little or no efficacy in AN treatment.

BDNF AND COGNITIVE FLEXIBILITY IN EDS

To study the relationship between BDNF and cognitive flexibility, we performed the Wisconsin Card Sorting Test as a set-shifting task. The results showed that patients with AN had a higher number of both incorrect answers and perseverative errors than HCs. However, there was no obvious correlation between serum BDNF levels and set-shifting dysfunction (*17*).

ASSOCIATION OF SERUM BDNF LEVEL AND MALNUTRITION

A connection between anorexia–cachexia syndrome and cytokines has also been suggested (*59*). Anorexia–cachexia syndrome associated with malignant tumor, chronic renal failure, cardiovascular disease, and chronic rheumatoid arthritis is marked by a reduction in muscle mass and fat tissue, and a series of changes in the metabolic, immune, and endocrine systems. Inflammatory cytokines in the circulation, such as tumor necrosis factor and interleukin-6, are elevated, while growth factors like BDNF are also affected by the syndrome. Peripheral cytokines are known to modulate neurotransmitter and neuropeptide functions through the hypothalamus (*59*). In AN, calorie deficit, severe malnutrition, reduced muscle mass and fat tissue, and stress-related biological responses are believed to cause a series of changes to peripheral cytokines. However, further study is needed to elucidate the mechanisms of such changes.

CONCLUDING REMARKS

ED, especially AN, is a severe mental illness for which an appropriate treatment has yet to be established. Various factors have been proposed as the link between the pathophysiology of EDs and the reduced level of serum BDNF.

A series of studies using BDNF knockout mice and the human BDNF gene indicate an association of BDNF and EDs with predisposition and vulnerability. Changes in brain BDNF were observed in a mouse model of depression and in mice suffering early maternal separation (*60*), in which stress and environmental factors during early developmental stages were suggested as causative factors. Epigenetic factors based on the DNA methylation of the BDNF gene might be a valuable biomarker for EDs, as well as depression (*61, 62*).

A variety of factors including influence of concurrent mental disorders and peripheral responses to starvation have also been considered. In addition, there is a question whether serum BDNF is a state marker or trait marker (Fig. 2).

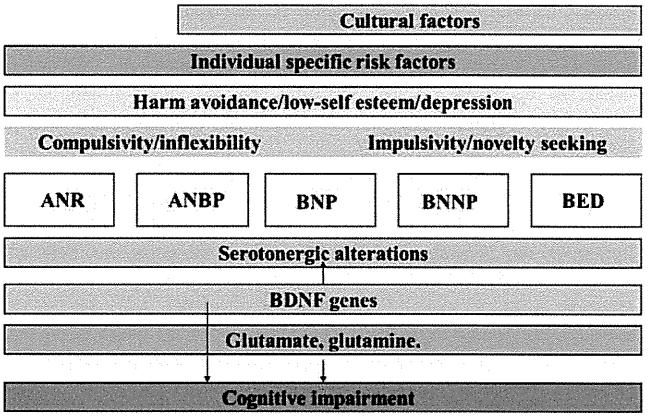

Figure 2. The hypothesis for etiology of eating disorders affected by BDNF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Further studies regarding BDNF and proBDNF are needed to elucidate the pathophysiology of EDs and to develop candidate biomarkers and their treatments. It is also important to perform animal studies of EDs to investigate changes in eating behaviors and anxiety disorders following BDNF administration. We would look forward to an innovative research field with high potential for developing treatments for EDs and discovering new drugs that work through BDNF and other nerve growth factors.

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REFERENCES

1. Lindsay, R., Wiegand, S., Altar, C., and DiStefano, P. S. (1994) Neurotrophic factors: from molecule to man. *Trends Neurosci.* **17**, 182–190.
2. Lindvall, O., Kokaia, Z., Bngzon, J., Elmér, E., and Kokaia, M. (1994) Neurotrophins and brain insults. *Trends Neurosci.* **17**, 490–496.
3. Snider, W. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77**, 627–638.
4. Thoenen, H. (1995) Neurotrophins and neuronal plasticity. *Science* **270**, 593–598.
5. Fujimura, H., Altar, C. A., Chen, R., Nakamura, T., Nakahashi, T., et al. (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb. Haemost.* **87**, 728–734.
6. Hashimoto, K. (2010) Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. *Psychiatry Clin. Neurosci.* **64**, 341–357.
7. Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., et al. (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl. Acad. Sci. USA* **96**, 15239–15244.
8. Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., et al. (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol. Endocrinol.* **15**, 1748–1757.

9. Rooney, B., McClelland, L., Crisp, A. H., and Sedgwick, P. M. (1995) The incidence and prevalence of anorexia nervosa in three suburban health districts in south London, UK. *Int. J. Eat. Disord.* **18**, 299–307.
10. Lucas, A. R., Beard, C. M., O'Fallon, W. M., and Kurland, L. T. (1991) 50-year trends in the incidence of anorexia nervosa in Rochester, Minn.: a population-based study. *Am. J. Psychiatry* **148**, 917–922.
11. Garfinkel, P. E., Lin, E., Goering, P., Spegg, C., Goldbloom, D. S., et al. (1995) Bulimia nervosa in a Canadian community sample: prevalence and comparison of subgroups. *Am. J. Psychiatry* **152**, 1052–1058.
12. Collier, D. and Treasure, J. (2004) The etiology of eating disorders. *Br. J. Psychiatry* **185**, 363–365.
13. Mercader, J. M., Ribasés, M., Gratacòs, M., González, J. R., Bayés, M., et al. (2007) Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes Brain Behav.* **6**, 706–716.
14. Gratacòs, M., González, J. R., Mercader, J. M., de Cid, R., Urretavizcaya, M., et al. (2007) Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol. Psychiatry* **61**, 911–922.
15. Verhagen, M., van der Meij, A., van Deurzen, P. A., Janzing, J. G., Arias-Vásquez, A., et al. (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol. Psychiatry* **15**, 260–271.
16. Koizumi, H., Hashimoto, K., Itoh, K., Nakazato, M., Shimizu, E., et al. (2004) Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* **127**, 125–127.
17. Nakazato, M., Hashimoto, K., Shimizu, E., Kumakiri, C., Koizumi, H., et al. (2003) Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. *Biol. Psychiatry* **54**, 485–490.
18. Nakazato, M., Hashimoto, K., Yoshimura, K., Hashimoto, T., Shimizu, E., et al. (2006) No change between the serum brain-derived neurotrophic factor in female patients with anorexia nervosa before and after partial weight recovery. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **30**, 1117–1121.
19. Nakazato, M., Tchanturia, K., Schmidt, U., Campbell, I. C., Treasure, J., et al. (2009) Brain-derived neurotrophic factor (BDNF) and set-shifting in currently ill and recovered anorexia nervosa (AN) patients. *Psychol. Med.* **39**, 1029–1035.
20. Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., et al. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350**, 230–232.
21. Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., et al. (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* **311**, 864–868.
22. Martinowich, K., Manji, H., and Lu, B. (2007) New insights into BDNF function in depression and anxiety. *Nat. Neurosci.* **10**, 1089–1093.
23. Carvalho, A. L., Caldeira, M. V., Santos, S. D., and Duarte, C. B. (2008) Role of the brain-derived neurotrophic factor at glutamatergic synapses. *Br. J. Pharmacol.* **153** (Suppl 1), S310–S324.
24. Sartorius, A., Hellweg, R., Litzke, J., Vogt, M., Dormann, C., et al. (2009) Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry* **42**, 270–276.
25. Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., and Kastin, A. J. (1998) Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* **37**, 1553–1561.
26. Nockher, W. A. and Renz, H. (2006) Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. *J. Allergy Clin. Immunol.* **117**, 583–589.
27. Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., et al. (2005) The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol. Aging* **26**, 115–123.
28. Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., et al. (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* **306**, 487–491.
29. Greenberg, M. E., Xu, B., Lu, B., and Hempstead, B. L. (2009) New insights in the biology of BDNF synthesis and release: implications in CNS function. *J. Neurosci.* **29**, 12764–12767.
30. Kernie, S. G., Liebl, D. J., and Parada, L. F. (2000) BDNF regulates eating behavior and locomotor activity in mice. *EMBO J.* **19**, 1290–1300.
31. Ernfors, P., Lee, K. F., and Jaenisch, R. (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* **368**, 147–150.
32. Jones, K. R., Fariñas, I., Backus, C., and Reichardt, L. F. (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell* **76**, 989–999.
33. Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., et al. (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat. Neurosci.* **6**, 736–742.
34. Koizumi, H., Hashimoto, K., and Iyo, M. (2006) Dietary restriction changes behaviours in brain-derived neurotrophic factor heterozygous mice: role of serotonergic system. *Eur. J. Neurosci.* **24**, 2335–2344.
35. Matthews, V. B., Aström, M. B., Chan, M. H., Bruce, C. R., Krabbe, K. S., et al. (2009) Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* **52**, 1409–1418.
36. Karege, F., Schwald, M., and Cisse, M. (2002) Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci. Lett.* **328**, 261–264.
37. Shimizu, E., Hashimoto, K., and Iyo, M. (2004) Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* **126**, 122–123.
38. Arija, V., Ferrer-Barcala, M., Aranda, N., and Canals, J. (2010) BDNF Val66Met polymorphism, energy intake and BMI: a follow-up study in schoolchildren at risk of eating disorders. *BMC Public Health.* **10**, 363.
39. Ribasés, M., Gratacòs, M., Armengol, L., de Cid, R., Badía, A., et al. (2003) Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol. Psychiatry* **8**, 745–751.
40. Ribases, M., Gratacos, M., Badia, A., Jimenez, L., Solano, R., et al. (2005) Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index. *Mol. Psychiatry* **10**, 851–860.
41. Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., et al. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269.
42. Dempster, E., Toulopoulou, T., McDonald, C., Bramon, E., Walshe, M., et al. (2005) Association between BDNF val66 met genotype and episodic memory. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* **134**, 73–75.
43. Monteleone, P., Tortorella, A., Martiadis, V., Serritella, C., Fuschino, A., et al. (2004) Opposite changes in the serum brain-derived neurotrophic factor in anorexia nervosa and obesity. *Psychosom Med.* **66**, 744–748.
44. Monteleone, P., Fabrazzo, M., Martiadis, V., Serritella, C., Pannuto, M., et al. (2005) Circulating brain-derived neurotrophic factor is decreased in women with anorexia and bulimia nervosa but not in women with binge-eating disorder: relationships to co-morbid depression, psychopathology and hormonal variables. *Psychol. Med.* **35**, 897–905.
45. Saito, S., Watanabe, K., Hashimoto, E., and Saito, T. (2009) Low serum BDNF and food intake regulation: a possible new explanation of the pathophysiology of eating disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* **33**, 312–316.

46. Ehrlich, S., Salbach-Andrae, H., Eckart, S., Merle, J. V., Burghardt, R., et al. (2009) Serum brain-derived neurotrophic factor and peripheral indicators of the serotonin system in underweight and weight-recovered adolescent girls and women with anorexia nervosa. *J. Psychiatry Neurosci.* **34**, 323–329.
47. Brandys, M. K., Kas, M. J., van Elburg, A. A., Campbell, I. C., and Adan, R. A. (2011) A meta-analysis of circulating BDNF concentrations in anorexia nervosa. *World J. Biol. Psychiatry* **12**, 444–454.
48. Mercader, J. M., Fernández-Aranda, F., Gratacòs, M., Aguera, Z., Forcano, L., et al. (2010) Correlation of BDNF blood levels with interoceptive awareness and maturity fears in anorexia and bulimia nervosa patients. *J. Neural. Transm.* **117**, 505–512.
49. Colzato, L. S., Van der Does, A. J., Kouwenhoven, C., Elzinga, B. M., and Hommel, B. (2011) BDNF Val66Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults. *Psychoneuroendocrinology* **36**, 1562–1569.
50. Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., et al. (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol. Psychiatry* **54**, 70–75.
51. Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., et al. (2005) Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol. Psychiatry* **57**, 1068–1072.
52. Russo-Neustadt, A., Ha, T., Ramirez, R., and Kesslak, J. P. (2001) Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behav. Brain Res.* **120**, 87–95.
53. Angelucci, F., Brenè, S., and Mathé, A. A. (2005) BDNF in schizophrenia, depression and corresponding animal models. *Mol. Psychiatry* **10**, 345–352.
54. Sen, S., Duman, R., and Sanacora, G. (2008) Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol. Psychiatry* **64**, 527–532.
55. Brunoni, A. R., Lopes, M., and Fregni, F. (2008) A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int. J. Neuropsychopharmacol.* **11**, 1169–1180.
56. Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., et al. (2011) DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS ONE* **6**, e23881.
57. Altman, S. E. and Shankman, S. A. (2009) What is the association between obsessive-compulsive disorder and eating disorders? *Clin. Psychol. Rev.* **7**, 638–646.
58. Maina, G., Rosso, G., Zanardini, R., Bogetto, F., Gennarelli, M., et al. (2010) Serum levels of brain-derived neurotrophic factor in drug-naïve obsessive-compulsive patients: a case-control study. *J. Affect. Disord.* **122**, 174–178.
59. Plata-Salamán, C. R. (2000) Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. *Nutrition* **16**, 1009–1012.
60. Gaszner, B., Jensen, K. O., Farkas, J., Reglodi, D., Csernus, V., et al. (2009) Effects of maternal separation on dynamics of urocortin 1 and brain-derived neurotrophic factor in the rat non-preganglionic Edinger-Westphal nucleus. *Int. J. Dev. Neurosci.* **27**, 439–451.
61. Hashimoto, T. K., Koizumi, H., Nakazato, M., Shimizu, E., and Iyo, M. (2005). Role of brain-derived neurotrophic factor in eating disorders: recent findings and its pathophysiological implications. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **29**, 499–504.
62. Hashimoto, K. (2007) BDNF variant linked to anxiety-related behaviors. *Bioessays* **29**, 116–119.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Functional Genetic Variation at the *NRGN* Gene and Schizophrenia: Evidence From a Gene-Based Case–Control Study and Gene Expression Analysis

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Genome-wide association and follow-up studies have reported an association between schizophrenia and rs12807809 of the *NRGN* gene on chromosome 11q24.2. We investigated the association of five linkage disequilibrium-tagging SNPs and haplotypes that cover the *NRGN* gene with schizophrenia in a Japanese sample of 2,019 schizophrenia patients and 2,574 controls to determine whether rs12807809 is the most strongly associated variant for schizophrenia in the vicinity of the *NRGN* gene. We found that the rs12807809–rs12278912 haplotype of the *NRGN* gene was associated with schizophrenia (global $P = 0.0042$). The frequencies of the TG and TA haplotypes of rs12807809–rs12278912 in patients were higher (OR = 1.14, $P = 0.0019$) and lower (OR = 0.85, $P = 0.0053$), respectively, than in the controls. We did not detect any evidence of association of schizophrenia with any SNPs; however, two nominal associations of rs12278912 (OR = 1.10, $P = 0.057$) and rs2075713 (OR = 1.10, $P = 0.057$) were observed. Furthermore, we detected an association between the rs12807809–rs12278912 haplotype and *NRGN* expression in immortalized lymphoblasts derived from 45 HapMap JPT subjects ($z = 2.69$, $P = 0.007$) and confirmed

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the association in immortalized lymphoblasts derived from 42 patients with schizophrenia and 44 healthy controls ($z = 3.09$, $P = 0.002$). The expression of the high-risk TG haplotype was significantly lower than the protective TA haplotype. The expression was lower in patients with schizophrenia than in controls; however, this difference was not statistically significant. This study provides further evidence of the association of the *NRGN* gene with schizophrenia, and our results suggest that there is a link between the TG haplotype of rs12807809–rs12278912, decreased expression of *NRGN* and risk of developing schizophrenia.

Key words: schizophrenia; *neurogranin* (*NRGN*); single nucleotide polymorphism (SNP); genome-wide association study (GWAS); gene expression

INTRODUCTION

Schizophrenia is a common and complex psychiatric disease with strong genetic components. Schizophrenia has an estimated heritability of approximately 80% [Cardno and Gottesman, 2000; Tsuang, 2000], and many genes have been implicated in the pathogenesis of schizophrenia [Sun et al., 2008].

Genome-wide association studies (GWAS) of single nucleotide polymorphisms (SNPs) investigate thousands of DNA samples from patients and controls, and these studies are a powerful tool for identifying common risk factors in complex diseases. Stefansson et al. [2009] combined the samples (from 12,945 patients with schizophrenia and 34,591 controls) from three large GWAS (the SGENE-plus, the International Schizophrenia Consortium and the Molecular Genetics of Schizophrenia GWAS) and conducted follow-up studies in 4,999 patients and 15,555 controls from four sets of samples from Europe, including from the Netherlands, Denmark, Germany, Hungary, Norway, Russia, Sweden, Finland, and Spain. The authors detected several significant association signals. Seven markers gave P values smaller than the genome-wide significance threshold of approximately 1.6×10^{-7} in the combined samples: five markers, rs6913660, rs13219354, rs6932590, rs13211507, and rs3131296, which spanned the major histocompatibility complex (MHC) region on chromosome 6p21.3-22.1; a marker, rs12807809, located 3,457 bases upstream of the *neurogranin* (*NRGN*) gene on chromosome 11q24.2; and a marker, rs9960767, in intron 4 of the transcription factor 4 (*TCF4*) gene on chromosome 18q21.2. Of the seven SNPs, four SNPs, rs6913660, rs13219354, rs13211507, and rs9960767, were not polymorphic in the HapMap Japanese in Tokyo (JPT) samples. The minor allele frequencies (MAFs) for two SNPs, rs6932590 and rs3131296, were less than 5%. Because only one marker, rs12807809, in the *NRGN* gene was a common SNP in the HapMap JPT samples (MAF greater than 5%), we focused on this SNP and the *NRGN* gene in the present study.

The *NRGN* gene is the human homolog of the neuron-specific rat RC3/*neurogranin* gene. *NRGN* encodes a postsynaptic protein kinase substrate that binds calmodulin (CaM) in the absence of calcium and has been implicated in dendritic spine formation and synaptic plasticity [Baudier et al., 1991]. NRGN plays an important role in the Ca^{2+}–CaM signaling pathway [Hayashi, 2009]. Ca^{2+} influx-induced oxidation of NRGN leads to the postsynaptic activation of CaM-dependent protein kinase II (CaMKII) by CaM, which is associated with strengthened *N*-methyl-D-aspartate (NMDA) receptor signaling [Li et al., 1999]. Reduced NRGN activity may mediate the effects of NMDA hypofunction implicated in the pathophysiology of schizophrenia.

The *NRGN* gene spans 7.3 kb of genomic DNA and contains four exons [Martinez de Arrieta et al., 1997]. Part of exon 1 and exon 2 encode a 78-amino-acid protein, and exons 3 and 4 contain untranslated sequences. A thyroid hormone response element (TRE) has been identified in intron 1 [Martinez de Arrieta et al., 1999]. An association between the *NRGN* gene and schizophrenia has previously been reported in a small population of male Portuguese and Brazilians [Ruano et al., 2008], although the associated SNP in the study, rs7113041, was not tightly correlated with the genome-wide supported SNP, rs12807809 (HapMap CEU $r^2 = 0.07$, JPT $r^2 = 0.01$). In addition, two separate studies reported no association between the genetic variants of *NRGN* and schizophrenia in Bulgarian [Betcheva et al., 2009] and Chinese populations [Li et al., 2010]. The genome-wide supported SNP and other SNPs in the *NRGN* gene were not genotyped in the GWAS of schizophrenia in Japanese populations because of a difference in the genotyping chips used among the separate GWAS, which the Illumina HumanHap 300 or 550 BeadChips, Affymetrix Genome-Wide Human SNP Array 5.0 and Affymetrix GeneChip Mapping 100 K microarrays [Stefansson et al., 2009; Ikeda et al., 2011; Yamada et al., 2011] were used. Here, we first investigated the association between the *NRGN* gene and schizophrenia in a Japanese population using a gene-based approach to determine whether rs12807809 is the most strongly associated variant for schizophrenia near the *NRGN* gene. Second, we examined whether the associated haplotype of *NRGN* influenced *NRGN* expression in immortalized lymphoblasts derived from the HapMap JPT samples and our Japanese case–control samples.

MATERIALS AND METHODS

Subjects

Subjects for the genetic association analysis included 2,019 unrelated patients with schizophrenia (54.5% males, with a mean age ± SD of 44.7 ± 15.1 years) and 2,579 unrelated healthy controls (49.4% males, 45.4 ± 19.4 years). The mean age did not differ significantly between cases and controls ($P = 0.24$); however, the male to female ratio of the patients was significantly higher than in the controls ($P < 0.05$). Age and sex-matched subjects for *NRGN* expression analysis consisted of 42 patients with schizophrenia (58.1%, 38.4 ± 11.2 years) and 44 healthy subjects (56.8% males, 38.0 ± 11.4 years). These subjects were included in the genetic association analysis. All subjects used in both analyses were biologically unrelated, of Japanese ethnicity and were recruited from four geographical regions in Japan: Osaka, Aichi, Tokushima, and Tokyo [Yamaguchi-Kabata et al., 2008; Ohi et al., 2009]. Cases were recruited from outpatient and inpatient facilities at university hospitals and psychiatric hospitals. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists based on an unstructured clinical interview; diagnoses were made based on the

criteria of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University, Tokushima University and Juntendo University.

SNP Selection and SNP Genotyping

This study was designed to examine the association between the *NRGN* gene and schizophrenia by selectively tagging SNPs in the *NRGN* gene and flanking regions (±5 kb). We selected five tagging SNPs using the TAGGER algorithm (Paul de Bakker, http://www.broad.mit.edu/mpg/tagger) with the criteria of r^2 greater than 0.80 in "pair-wise tagging only" mode and an MAF greater than 5%, which was implemented in Haploview 4.2 using HapMap data release 27 Phase II + III, Feb 2009, on NCBI B36 assembly, dbSNP b126 [Japanese in Tokyo (JPT), Chr 11: 124,109,952.124,127,307]. The five tagging SNPs were rs1939214, rs12807809, rs12278912, rs2075713, and rs11219769. Markers are shown in Table I; orientation and alleles are reported on the genomic plus strand (rs12807809 is reported as T/C, as has been reported in previous GWAS [Stefansson et al., 2009]). Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA) as previously described [Hashimoto et al., 2006, 2007]. Detailed information on the PCR conditions is available upon request. Genotyping call rates were 98.9% (rs1939214), 98.5% (rs12807809), 99.3% (rs12278912), 99.3% (rs2075713), and 99.5% (rs11219769). No deviation from Hardy–Weinberg equilibrium (HWE) in the examined SNPs was detected in the patients with schizophrenia or healthy controls ($P > 0.05$). The positions of the five SNPs analyzed in the present study are shown in Figure 1.

Quantitative Measurement of *NRGN* Gene Expression

Isolation and immortalization procedures of lymphocytes from blood using the Epstein–Barr virus (EBV) were performed by SRL of Tokyo, Japan. Immortalized, patient-derived lymphocytes were grown in culture media supplemented with 20% fetal bovine serum. Total RNA was extracted from cell pellets using the RNeasy Mini Kit (Qiagen K.K., Tokyo, Japan). The total yield of RNA was determined by absorbance at 260 nm, and the quality of the RNA was determined using agarose gel electrophoresis.

According to the manufacturer's protocol, total RNA was treated with DNase to remove contaminating genomic DNA using DNase Treatment and Removal Reagents (Ambion, Austin, TX). Total RNA (10 μg) treated with DNase was used in a 50-μl reverse transcriptase reaction to synthesize cDNA with the SuperScript

TABLE I. Genotype and Allele Distributions for SNPs in the *NRGN* Gene Between Patients With Schizophrenia and Controls in a Japanese Population

Marker			SCZ (n = 2019)			CON (n = 2579)			Genotypic	MAF		Allelic	
SNP IDs (M)	Position[a]	M/m[b]	Gene	M/M	M/m	m/m	M/M	M/m	m/m	*P*-value (χ^2)	SCZ	CON	*P*-value (χ^2)	OR (95% CI)
rs1939214 (A)	124110500	A/G	5′	0.67	0.30	0.04	0.66	0.30	0.04	0.51 (1.3)	0.19	0.19	0.29 (1.1)	1.06 (0.95–1.18)
rs12807809 (T)[c]	124111495	T/C	5′	0.58	0.35	0.07	0.56	0.37	0.07	0.44 (1.6)	0.25	0.26	0.25 (1.3)	1.06 (0.96–1.16)
rs12278912 (G)[d]	124117369	G/A	Intron1	0.61	0.34	0.05	0.59	0.35	0.06	0.13 (4.1)	0.22	0.23	0.057 (3.6)	1.10 (1.00–1.22)
rs2075713 (A)	124123149	A/C	3′	0.65	0.31	0.04	0.62	0.33	0.05	0.17 (3.5)	0.20	0.21	0.057 (3.6)	1.10 (1.00–1.22)
rs11219769 (G)	124125357	G/T	3′	0.57	0.37	0.06	0.55	0.38	0.07	0.24 (2.8)	0.25	0.26	0.09 (2.8)	1.09 (0.99–1.19)

SCZ, patients with schizophrenia; CON, healthy controls; M, major allele; m, minor allele; MAF, minor allele frequency; OR, odds ratio.
[a]db SNP build 129.
[b]The first alleles shown are major alleles. All the alleles are represented according to the plus strand DNA sequence.
[c]The genome-wide supported SNP for schizophrenia [Stefansson et al., 2009].
[d]Because a high linkage disequilibrium between rs12278912 and rs7113041 [Ruano et al., 2008] was found in the HapMap JPT samples ($r^2 = 0.93$), rs12278912 was selected as the tagging SNP by the TAGGER program.

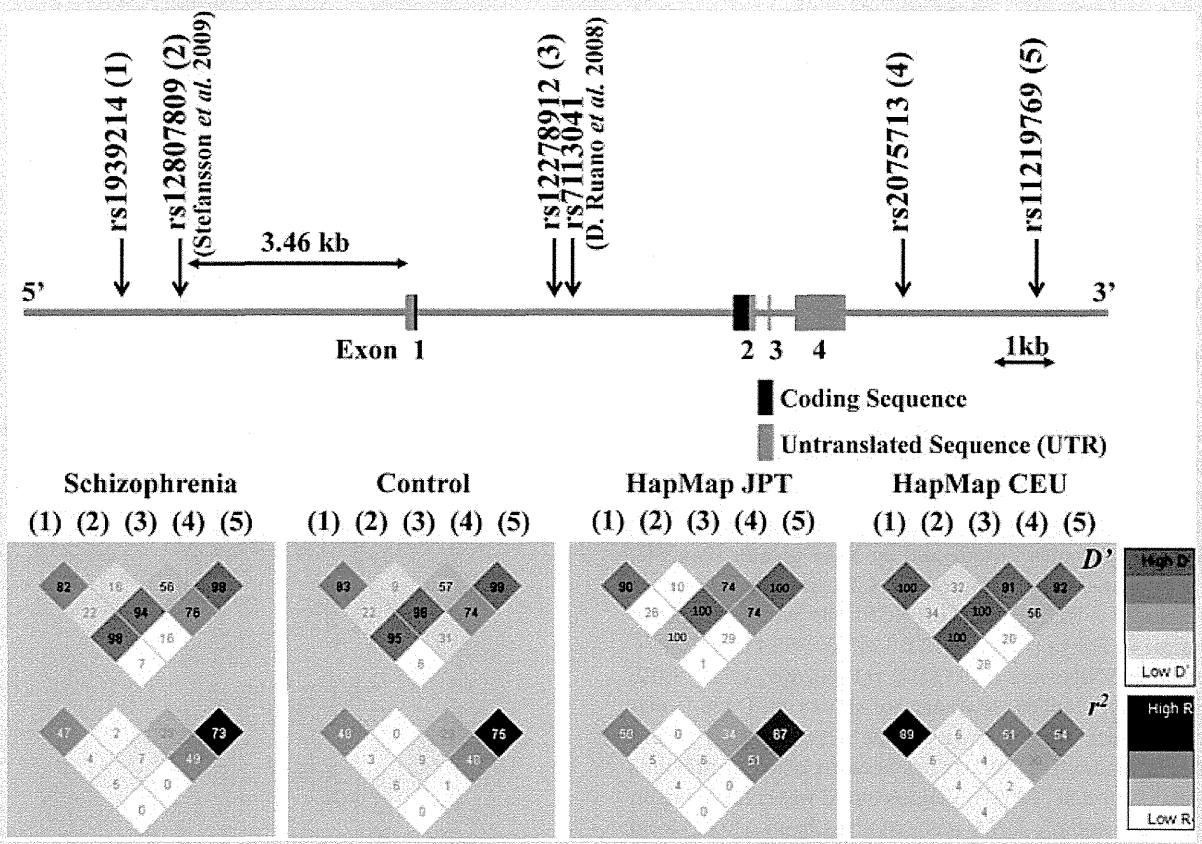

FIG. 1. The genomic structure of *NRGN*, including the locations of the five tagging SNPs studied and linkage disequilibrium of these SNPs in the patient, control, HapMap JPT, and CEU groups. Based on an entry in the Entrez Gene database (National Center for Biotechnology Information), the genomic structure of *NRGN* is shown above. The locations of the SNPs analyzed in this study are indicated by arrows, with numbers indicated in parentheses. The numbers indicated in parentheses refer to the numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of exons–introns and intermarkers are drawn to scale. The LDs between pairwise SNPs are shown using the D′ (upper) and r^2 (lower) values at the bottom of the map of the gene structure separately for cases, controls, the HapMap JPT samples and the HapMap CEU samples. High levels of LD are represented by black (D′ and r^2) coloring, with increasing color intensity from 0 to 100, as shown by color bars.

First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Detailed information on the PCR conditions is available upon request.

To measure mRNA expression levels of housekeeping (β-*actin*) and *NRGN* genes, we used the Pre-Developed TaqMan Assay Reagent kit (Applied Biosystems). Primer information (gene name: assay ID, transcript ID, target region) is as follows; *NRGN*: Hs00382922_m1, NM_001126181.1 and NM_006176.2, Exon1-2; β-actin: 4326315E, NM_001101, no region indicated (Applied Biosystems). Expression levels of these genes were measured by quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) using an ABI Prism 7900 Sequence Detection System (Applied Biosystems) with a 384-well format as previously described [Yamamori et al., 2011; Yasuda et al., 2011]. PCR data were obtained using Sequence Detector software (SDS version 2.1; Applied Biosystems) and quantified using a standard curve. This software plotted the real-time fluorescence intensity and selected the threshold within the linear phase of the amplicon profile. The software plotted a standard curve of the cycle at threshold C_t, which is where the fluorescence generated within a reaction crossed the threshold, versus the quantity of RNA. All samples were measured using a single plate per target gene, and their C_t values were in the linear range of the standard curve. The quantity of each sample was predicted by C_t values. The qRT-PCR reaction was performed in triplicate, and the expression level of the gene was taken as the average of three independent measurements. Standard curves were obtained using serial dilutions (1:4) of pooled complementary DNA prepared from 300 ng total RNA derived from immortalized lymphocytes. The standard curves of β-*actin* and *NRGN* showed that these genes were expressed in immortalized lymphocytes. In each experiment for β-*actin* and *NRGN*, the R^2 value of the standard curve was >0.99, and no-template control assays resulted in no detectable signal. The individual expression levels of the *NRGN* gene were normalized to the housekeeping gene (raw target gene expression level divided by raw housekeeping gene expression level) and were used for statistical analysis.

Haplotype Associated With *NRGN* Expression (eQTL)

To identify whether the haplotypes in *NRGN* associated with schizophrenia may be expression quantitative trait loci (eQTL), we analyzed *NRGN* expression in two datasets of lymphoblast-derived HapMap JPT samples and in the Japanese case–control samples. For the HapMap JPT samples, we extracted genotypes and *NRGN* lymphoblastoid expression data from the HapMap JPT samples (n = 45) deposited in GeneVar (http://www.sanger.ac.uk/humgen/genevar/ [Stranger et al., 2007]). For the Japanese case–control samples, we used our genotypes and *NRGN* lymphoblastoid expression data obtained using the method described above.

Statistical Analyses

We performed power calculations using the Power Calculator for Two-Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/ [Skol et al., 2006]). The power estimate was based on an allele frequency of 0.83 at rs12807809, an odds ratio of 1.19, which was indicated by Stefansson et al. [2009], a prevalence of 0.01, and an alpha level of 0.05 using a multiplicative model.

Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2 tests for sex and the Mann–Whitney *U*-test for age using PASW Statistics 18.0 software (SPSS Japan, Inc., Tokyo, Japan). Deviation from HWE was tested separately in test cases and controls using χ^2 tests for goodness of fit using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). The allelic and genotypic distributions of *NRGN* polymorphisms between patients and controls were analyzed using χ^2 tests with SNPAlyze V5.1.1 Pro software. The number of effective independent SNPs assayed was estimated to correct for multiple testing by the spectral decomposition method of Nyholt using the SNPSpD software [Nyholt, 2004]. The effective number of independent marker loci was 4.13 and corrected *P*-value for allelic and genotypic associations was set at $P < 0.012$. Pairwise linkage disequilibrium (LD) analyses expressed by D′ and r^2 were applied to detect the intermarker relationships in each group using Haploview 4.2 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated using the method of maximum likelihood with genotyping data using the expectation–maximization (EM) algorithm from SNPAlyze V5.1.1 Pro software. Rare haplotypes detected in less than 3% of patients and controls were excluded from the haplotypic association analysis, as previously described [Ohi et al., 2009, 2010]. We performed 10,000 permutations for significance tests to determine empirical significance using a 2×2 contingency table approach. We used a 2- to 5-window fashion analysis. Since Bonferroni correction for multiple testing is considered to be too conservative to apply to genetic association analyses [Nyholt, 2001], method of Nyholt [Nyholt, 2004] for allelic and genotypic associations and permutation tests [Dudbridge, 2003] for haplotypic associations are considered to be appropriate for these analyses.

The difference in expression levels between Japanese patients with schizophrenia and controls was analyzed using linear regression in PASW Statistics 18.0 software. Age and sex, which may influence gene expression, were corrected for in the expression analysis. HPlus (http://qge.fhcrc.org/hplus) is a software application for estimating haplotype frequencies and inferring individual haplotypes based on EM and progressive ligation (PL) algorithms [Li et al., 2003], and most significantly assessing haplotypic associations with various types of phenotypes using linear regression. Differences of expression levels among haplotypes were analyzed using linear regression in HPlus software. Each genotype was treated as the number of major alleles (0, 1, and 2) in the expression analysis. For the joint haplotype analysis in HPlus software, each haplotype was tested against the reference haplotype (equal to most frequent haplotype) using linear regression. As age and sex were not available for the HapMap samples, these confounding factors were not corrected for in the expression analysis. Expression levels in Japanese cases, control samples and in the combined samples were corrected for age and sex in the analyses. We applied a Bonferroni correction in expression analysis (three tests). The significance level for statistical tests was set at two-tailed $P < 0.05$.

RESULTS

Genetic Association Analysis

Our study size of 2,019 cases and 2,579 controls had sufficient power (>80%) to detect a genetic effect at ORs of 1.19 or greater for rs12807809 when the allele frequency was 0.83, as described in previous GWAS (SGENE-plus) [Stefansson et al., 2009].

The genotype and allele frequencies of five tagging SNPs located in the *NRGN* gene and flanking regions are summarized in Table I. There was no allelic or genotypic association with schizophrenia for any of the five SNPs (uncorrected $P > 0.05$). However, nominal differences in allele frequencies between patients and controls were observed in rs12278912 ($\chi^2 = 3.6$, $P = 0.057$, corrected $P = 0.24$) and rs2075713 ($\chi^2 = 3.6$, $P = 0.057$, corrected $P = 0.24$). The major allele frequencies of both SNPs were higher in patients than in controls. Consistent with previous GWAS [Stefansson et al., 2009], the frequency of the major T allele of rs12807809 was higher in patients (75.4%) than in controls (74.4%) in our Japanese population, although the results did not reach statistical significance [$\chi^2 = 1.3$, $P = 0.25$, OR (95% confidence interval (CI)) = 1.06 (0.96–1.16)].

We focused on haplotypic association between patients with schizophrenia and healthy subjects using a 2- to 5-window fashion analysis. Haplotype analysis showed a significant association with schizophrenia (rs12807809–rs12278912, $\chi^2 = 13.1$, global $P = 0.0042$) (Supplementary Table I). The frequency of the major TG haplotype of rs12807809–rs12278912 was higher in patients (62%) than in controls (58%) [$\chi^2 = 9.4$, $P = 0.0019$, OR (95% CI) = 1.14 (1.05–1.24)] (Table II). On the other hand, the frequency of the TA haplotype of rs12807809–rs12278912 was lower in patients (14%) than in controls (16%) [$\chi^2 = 7.3$, $P = 0.0053$, OR (95% CI) = 0.85 (0.76–0.96)] (Table II). There was no haplotypic association with schizophrenia for any other haplotypes. These findings suggest that the major TG haplotype of rs12807809–rs12278912 may be related to an increased risk of schizophrenia, and the TA haplotype may have a protective role against the susceptibility to schizophrenia. These results of allelic, genotypic, or haplotypic associations were not affected by excluding 86 samples used for expression analyses (data not shown).

TABLE II. Differences in the rs12807809–rs12278912 Haplotype Between Patients With Schizophrenia and Healthy Subjects

Haplotype rs12807809[a]–rs12278912[b]	Frequency: Patients	Frequency: Controls	Individual P (χ^2)	OR (95%CI)	Global P (χ^2)
					0.0042 (13.1)
TG	0.62	0.58	**0.0019 (9.4)**	1.14 (1.05–1.24)	
CG	0.17	0.18	0.07 (3.4)	0.90 (0.81–1.01)	
TA	0.14	0.16	**0.0053 (7.3)**	0.85 (0.76–0.96)	
CA	0.08	0.08	0.57 (0.3)	1.05 (0.90–1.22)	

Significant P values are shown as bold-faced and underlined type.
[a]The genome-wide supported SNP for schizophrenia [Stefansson et al., 2009].
[b]Because a high linkage disequilibrium between rs12278912 and rs7113041 [Ruano et al., 2008] was found in the HapMap JPT samples ($r^2 = 0.93$), rs12278912 was selected as the tagging SNP by the TAGGER program.

The LD relationships between the markers are provided in Figure 1. The LD pattern observed in our controls was similar to our patients and the JPT HapMap samples; however, it was different from that of the CEU HapMap samples. The strengths of the LD patterns of rs1939214–rs12807809 and rs12278912–rs2075713–rs11219769 were different between Japanese populations and the CEU HapMap samples. The low LD pattern of rs12807809–rs12278912 was similar among the groups ($D' < 0.50$, $r^2 < 0.10$).

NRGN Gene Expression Analysis

The *NRGN* expression level was lower in patients with schizophrenia (n = 42, mean ± SD, 0.86 ± 0.58) than in controls (n = 44, 1.00 ± 0.75). However, the results did not reach statistical significance (r = −0.14, beta = −0.11, SE = 0.14, t = −0.97, $P = 0.34$).

Based on the results from the genetic association analysis, we investigated whether the rs12807809–rs12278912 haplotype of the *NRGN* gene was an eQTL in two datasets. The rs12807809–rs12278912 haplotype related to schizophrenia was significantly associated with *NRGN* expression in healthy HapMap JPT samples. The *NRGN* gene expression of the high-risk TG haplotype of rs12807809–rs12278912 was significantly lower than that of the protective TA haplotype (z = 2.69, $P = 0.007$). We confirmed that the rs12807809–rs12278912 haplotype was significantly associated with *NRGN* expression normalized to the β-*actin* expression in the controls and combined samples (Fig. 2 and Table III, control samples: z = 2.30, $P = 0.021$, combined samples: z = 3.09, $P = 0.002$). The association occurred in the same direction among the HapMap JPT, control, and combined samples. In case samples, the expression level of rs12807809–rs12278912 was lower in samples with the high-risk TG haplotype than in those with the protective TA haplotype, although the result did not reach statistical significance (z = 1.49, $P = 0.14$). The association in the HapMap JPT and combined samples remained significant after correction for multiple tests (HapMap JPT samples: corrected $P = 0.021$, combined samples: corrected $P = 0.006$). However, there was no significant association after applying the correction in control samples (corrected $P = 0.063$).

DISCUSSION

In this study, we provided evidence that haplotypes, including the genome-wide-screen-supported SNP of the *NRGN* gene, were associated with an increased risk of schizophrenia. Our in silico analysis showed that the high-risk rs12807809–rs12278912 haplotype of the *NRGN* gene may be associated with a low expression level of the *NRGN* gene in lymphoblasts derived from the HapMap JPT samples. We confirmed the association between the haplotype and *NRGN* expression in the combined case–control samples. Our results suggest that the schizophrenia-associated haplotype at the

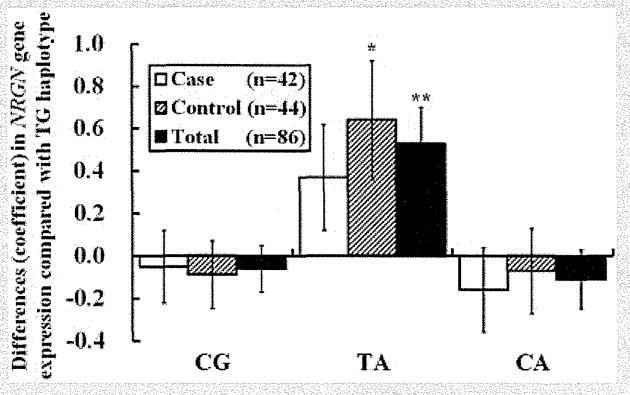

FIG. 2. The association between the rs12807809–rs12278912 haplotype of the *NRGN* gene and *NRGN* expression in lymphoblasts. Expression of the protective TA haplotype of rs12807809–rs12278912 was significantly higher than that of the high-risk TG haplotype in controls and combined case–control samples. The error bars represent standard errors of the coefficient. Estimated frequencies of each haplotype were as follows—TG haplotype: Case, 69%; Control, 61%; Total, 65%; CG haplotype: Case, 16%; Control, 20%; Total, 18%; TA haplotype: Case, 7%; Control, 11%; Total, 9%; CA haplotype: Case, 8%; Control, 9%; Total, 8%. *$P < 0.05$, **$P < 0.01$.

NRGN gene may be a functional variant, and the results support an association between the *NRGN* gene and schizophrenia.

This report is the first investigation of the association of tagging SNPs and haplotypes covering the *NRGN* gene with schizophrenia. To our knowledge, five genetic studies have investigated whether the *NRGN* gene is implicated in schizophrenia. A genome-wide linkage study has shown that the chromosomal region 11q23.3-24 including the *NRGN* gene is linked to schizophrenia in British and Icelandic populations [Gurling et al., 2001]. Subsequently, an association study determined that rs7113041, which displays high LD with rs12278912 and is located on intron 1 in the *NRGN* gene, is related to the risk of developing schizophrenia in male subjects of Portuguese origin [Ruano et al., 2008]. In addition, three GWAS and follow-up studies have shown that rs12807809 is associated with schizophrenia in large European samples [Stefansson et al., 2009]. However, two studies reported no association between *NRGN* and schizophrenia in Bulgarian or Chinese populations [Betcheva et al., 2009; Li et al., 2010]. In the present study, we determined that the rs12807809–rs12278912 haplotype is associated with an increased risk of schizophrenia in a Japanese population. However, there were no significant associations between any SNP, including rs12807809 and rs12278912, and schizophrenia in the population. The inconsistency of association among the previous studies and the present study might result from ethnic differences or type I or II errors for the different sample sizes: Portuguese, 315 cases, 295 controls and 73 trios [Ruano et al., 2008]; European Caucasian, 12,945 cases and 34,591 controls [Stefansson et al., 2009]; Japanese, 2,019 cases and 2,579 controls (present study); Bulgarian, 185 cases and 184 controls [Betcheva et al., 2009]; and Chinese, 2,496 cases and 5,184 controls [Li et al., 2010]. In addition, the SNPs investigated in each study were different. Ruano et al. [2008] and Betcheva et al. [2009] examined rs7113041, which has high LD with rs12278912 but not with rs12807809, whereas Stefansson et al. [2009] and Li et al. [2010] examined rs12807809 but not rs12278912. However, none of these studies examined haplotypes for the *NRGN* gene. Because the rs12807809–rs12278912 haplotype may be the most significant genetic variant in this region, further study is required to confirm the association between the rs12807809–rs12278912 haplotype and schizophrenia in other populations.

Differences in the relative *NRGN* expression levels between patients with schizophrenia and healthy subjects were not demonstrated. This result may be due to the small sample sizes in this study, which may have resulted in the failure to identify a modest difference in *NRGN* expression in this complex disease. We determined that the major TG haplotypic and the TA haplotypic frequencies of rs12807809–rs12278912 were higher and lower, respectively, in patients with schizophrenia than in healthy controls. In addition to these findings, we found that *NRGN* gene expression of the high-risk TG haplotype was significantly lower than that of the protective TA haplotype in lymphoblasts derived from our Japanese case–control subjects as well as the JPT HapMap sample. The low LD patterns of rs12807809–rs12278912 were similar across populations. This region may be vulnerable to recombination. Combinations of the TG and TA of rs12807809–rs12278912 could play an important role in the pathogenesis of schizophrenia. In this study, gene expression data derived from lymphoblasts raised the possibility that the rs12807809–rs12278912 haplotype may be a functional variant of *NRGN*. Further biological studies of the function of rs12807809–rs12278912 are required to verify the expression results.

Smith et al. [2011] analyzed *NRGN* expression in several brain tissues derived from a dataset of at least 130 individuals of European ancestry. However, they showed that neither the genome-wide supported SNP nor any individually correlated SNPs were associated with *NRGN* expression. They did not examine any association between haplotype and *NRGN* expression. There are several challenges in investigating expression findings in the postmortem

TABLE III. The Association Between the rs12807809–rs12278912 Haplotype and mRNA Expression

Haplotypes	Frequency	Coefficient	SE	CI	*P*-value (*Z*-score)
Schizophrenia ($n = 42$)					
TG	0.69	0 (ref)	—	—	—
CG	0.16	−0.05	0.17	(−0.39–0.29)	0.76 (−0.30)
TA	0.07	0.37	0.25	(−0.12–0.86)	0.14 (1.49)
CA	0.08	−0.16	0.20	(−0.55–0.24)	0.43 (−0.78)
Healthy control (n = 44)					
TG	0.61	0 (ref)	—	—	—
CG	0.20	−0.09	0.16	(−0.39–0.22)	0.58 (−0.55)
TA	0.11	0.64	0.28	(0.09–1.18)	**0.021 (2.30)**
CA	0.09	−0.07	0.20	(−0.46–0.32)	0.73 (−0.34)
Total subjects (n = 86)					
TG	0.65	0 (ref)	—	—	—
CG	0.18	−0.06	0.11	(−0.28–0.15)	0.57 (−0.57)
TA	0.09	0.53	0.17	(0.19–0.87)	**0.002 (3.09)**
CA	0.08	−0.11	0.14	(−0.39–0.17)	0.45 (−0.75)

Joint Association Analysis (the reference haplotype is the most frequent haplotype).
For the joint haplotype test, several haplotypes were tested against the reference haplotype.
Significant *P* values are shown as bold-faced and underlined type.

brain: (1) the choice of an appropriate brain region for investigation; (2) the heterogeneity of cell types within brain tissue; (3) the reliance on relatively small samples; and (4) the impact of cause of death and/or postdeath handling of the tissues on gene expression [Marcotte et al., 2003]. Thus, the use of postmortem brain tissue is compounded by a range of confounding factors (age, race, gender, different microarray platforms, and analysis methods) and may be the cause of the relative lack of gene/transcript-level consistency among expression studies. To overcome some of these problems, several groups have considered the use of lymphoblasts rather than the postmortem brain [Matigian et al., 2008; Slonimsky et al., 2010; Yamamori et al., 2011; Yasuda et al., 2011]. Lymphoblasts are useful for schizophrenia researchers because blood-based tissue (lymphoblasts) can be obtained with ease from living subjects, which allows larger case–control studies with optimal matching of key variables (age, sex, and race). In addition, immortalized lymphoblasts in culture are considered an effective tool for studying cells in the absence of the effect of antipsychotic treatments and duration of illness, both of which could mask the genetic differences in RNA expression. Thus, lymphoblasts could be good tool to investigate the impact of a gene in the absence of the impact of any confounding factors. On the other hand, there were some demerits of using lymphoblasts. In immortalized lymphocytes, it might be difficult to observe the effects of genes on their neuron-specific functions, for example, the effects of genes on glutamate and dopamine release and on the formation of synaptic vesicles. When isolation and immortalization procedures of lymphocytes from blood were performed or immortalized lymphocytes were grown in culture media, a genetic mutation might be inserted into genomic DNA in the cultured lymphoblasts and alter DNA sequences. It remains still controversial whether immortalized lymphocytes are an appropriate alternative to neuronal tissue, because there was a little evidence of analysis using immortalized lymphocytes from patients with schizophrenia. In this study, the difference in the association of gene expression with genetic variants between previous study and present study could be explained by the difference in the gene expression profile between immortalized lymphoblast and postmortem brain tissue. Other possible factors contributing to differences in association between studies could be a difference in the SNPs and haplotypes investigated or ethnic differences between Japanese and Caucasian populations.

Smith et al. [2011] performed mutation searches of all four exons of *NRGN* gene in 14 Caucasian subjects with schizophrenia and of the cording exons of *NRGN* gene in 1,113 Bulgarians individuals, 699 of whom had schizophrenia. However, they did not find any novel common polymorphism in the region. Thus, we did not perform a systematic mutation search in this study because there has been no novel common genetic variant in the region. If we perform sequencing and find a novel rare polymorphism, we cannot analyze association between the rare polymorphism and gene expression for only a small number of individuals with rare variant. A genetic variant, particularly a SNP not listed in the HapMap database, that is likely to be more strongly associated with schizophrenia may exist in the rs12807809–rs12278912 haplotype region. Sequencing the entire gene in individuals with risk haplotype in comparison with the protective haplotype carriers with larger sample sizes could provide further information underlying the genomic mechanism for this risk haplotype.

There are several limitations to interpreting our results. Because a number of statistical analyses supported the association of the *NRGN* gene and schizophrenia, such as genotypic and allelic associations for five SNPs (total 5×2), haplotype analysis using a window fashion analysis (total 10) and expression analysis for three individual haplotypes (total 3×4), a correction for multiple testing should be considered. In this study, the overall number of genetic association tests was 32; however, not all tests were independent, and several hypotheses were included. Thus, Bonferroni correction, a method to correct for multiple independent tests for one hypothesis, might not be appropriate. The consensus how to correct such multiple testing has not been reached in this research field. Thus, we applied SNPSpD correction for genotypic and allelic association analysis, permutation method for haplotype analysis and Bonferroni correction for expression analysis (three tests). However, even though we applied these methods of correcting such multiple testing, they might cause false positive results. We did not control for geographical variation of control origin because there is little possibility for ethnic/genetic difference among four geographical regions for feature of homogeneous race in Japan [Yamaguchi-Kabata et al., 2008]. Our significant results may be derived from sample bias owing to population stratification and non-sex-matched samples. In the present study, our results support an association between the *NRGN* gene and schizophrenia. We suggest that the functional haplotype of the *NRGN* gene, which is associated with *NRGN* expression, could be related to the pathogenesis of schizophrenia.

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REFERENCES

Baudier J, Deloulme JC, Van Dorsselaer A, Black D, Matthes HW. 1991. Purification and characterization of a brain-specific protein kinase C substrate, neurogranin (p17). Identification of a consensus amino acid sequence between neurogranin and neuromodulin (GAP43) that corresponds to the protein kinase C phosphorylation site and the calmodulin-binding domain. J Biol Chem 266(1):229–237.

Betcheva ET, Mushiroda T, Takahashi A, Kubo M, Karachanak SK, Zaharieva IT, Vazharova RV, Dimova II, Milanova VK, Tolev T, Kirov G, Owen MJ, O'Donovan MC, Kamatani N, Nakamura Y, Toncheva DI. 2009. Case-control association study of 59 candidate genes reveals the DRD2 SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. J Hum Genet 54(2):98–107.

Cardno AG, Gottesman II. 2000. Twin studies of schizophrenia: From bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17.

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS, Read T, Murphy P, Blaveri E, McQuillin A, Petursson H, Curtis D. 2001. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 68(3):661–673.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. 2006. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15(20):3024–3033.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, Nakajima M, Tanaka K, Kawagishi N, Nemoto K, Mori T, Ohnishi T, Noguchi H, Hori H, Suzuki T, Iwata N, Ozaki N, Nakabayashi T, Saitoh O, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Kunugi H, Baba A. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12(11):1026–1032.

Hayashi Y. 2009. Long-term potentiation: Two pathways meet at neurogranin. EMBO J 28(19):2859–2860.

Ikeda M, Aleksic B, Kinoshita Y, Okochi T, Kawashima K, Kushima I, Ito Y, Nakamura Y, Kishi T, Okumura T, Fukuo Y, Williams HJ, Hamshere ML, Ivanov D, Inada T, Suzuki M, Hashimoto R, Ujike H, Takeda M, Craddock N, Kaibuchi K, Owen MJ, Ozaki N, O'Donovan MC, Iwata N. 2011. Genome-wide association study of schizophrenia in a Japanese population. Biol Psychiatry 69(5):472–478.

Li J, Pak JH, Huang FL, Huang KP. 1999. N-methyl-D-aspartate induces neurogranin/RC3 oxidation in rat brain slices. J Biol Chem 274(3): 1294–1300.

Li SS, Khalid N, Carlson C, Zhao LP. 2003. Estimating haplotype frequencies and standard errors for multiple single nucleotide polymorphisms. Biostatistics 4(4):513–522.

Li T, Li Z, Chen P, Zhao Q, Wang T, Huang K, Li J, Li Y, Liu J, Zeng Z, Feng G, He L, Shi Y. 2010. Common variants in major histocompatibility complex region and TCF4 gene are significantly associated with schizophrenia in Han Chinese. Biol Psychiatry 68(7):671–673.

Marcotte ER, Srivastava LK, Quirion R. 2003. cDNA microarray and proteomic approaches in the study of brain diseases: Focus on schizophrenia and Alzheimer's disease. Pharmacol Ther 100(1):63–74.

Martinez de Arrieta C, Perez Jurado L, Bernal J, Coloma A. 1997. Structure, organization, and chromosomal mapping of the human neurogranin gene (NRGN). Genomics 41(2):243–249.

Martinez de Arrieta C, Morte B, Coloma A, Bernal J. 1999. The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron. Endocrinology 140(1):335–343.

Matigian NA, McCurdy RD, Feron F, Perry C, Smith H, Filippich C, McLean D, McGrath J, Mackay-Sim A, Mowry B, Hayward NK. 2008. Fibroblast and lymphoblast gene expression profiles in schizophrenia: Are non-neural cells informative? PLoS ONE 3(6):e2412.

Nyholt DR. 2001. Genetic case-control association studies—Correcting for multiple testing. Hum Genet 109(5):564–567.

Nyholt DR. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74(4):765–769.

Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, Fukumoto M, et al. 2009. Association study of the G72 gene with schizophrenia in a Japanese population: A multicenter study. Schizophr Res 109(1–3): 80–85.

Ohi K, Hashimoto R, Yasuda Y, Yoshida T, Takahashi H, Iike N, Iwase M, et al. 2010. The chitinase 3-like 1 gene and schizophrenia: Evidence from a multi-center case-control study and meta-analysis. Schizophr Res 116(2–3):126–132.

Ruano D, Aulchenko YS, Macedo A, Soares MJ, Valente J, Azevedo MH, Hutz MH, Gama CS, Lobato MI, Belmonte-de-Abreu P, Goodman AB, Pato C, Heutink P, Palha JA. 2008. Association of the gene encoding neurogranin with schizophrenia in males. J Psychiatr Res 42(2):125–133.

Skol AD, Scott LJ, Abecasis GR, Boehnke M. 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38(2):209–213.

Slonimsky A, Levy I, Kohn Y, Rigbi A, Ben-Asher E, Lancet D, Agam G, Lerer B. 2010. Lymphoblast and brain expression of AHI1 and the novel primate-specific gene, C6orf217, in schizophrenia and bipolar disorder. Schizophr Res 120(1–3):159–166.

Smith RL, Knight D, Williams H, Dwyer S, Richards A, Kirov G, O'Donovan MC, Owen MJ. 2011. Analysis of neurogranin (NRGN) in schizophrenia. Am J Med Genet Part B 156B(5):532–535.

Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, Werge T, et al. 2009. Common variants conferring risk of schizophrenia. Nature 460(7256):744–747.

Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, Beazley C, Ingle CE, Dunning M, Flicek P, Koller D, Montgomery S, Tavare S, Deloukas P, Dermitzakis ET. 2007. Population genomics of human gene expression. Nat Genet 39(10):1217–1224.

Sun J, Kuo PH, Riley BP, Kendler KS, Zhao Z. 2008. Candidate genes for schizophrenia: A survey of association studies and gene ranking. Am J Med Genet Part B 147B(7):1173–1181.

Tsuang M. 2000. Schizophrenia: Genes and environment. Biol Psychiatry 47(3):210–220.

Yamada K, Iwayama Y, Hattori E, Iwamoto K, Toyota T, Ohnishi T, Ohba H, Maekawa M, Kato T, Yoshikawa T. 2011. Genome-wide association study of schizophrenia in Japanese population. PLoS ONE 6(6):e20468.

Yamaguchi-Kabata Y, Nakazono K, Takahashi A, Saito S, Hosono N, Kubo M, Nakamura Y, Kamatani N. 2008. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: Effects on population-based association studies. Am J Hum Genet 83(4):445–456.

Yamamori H, Hashimoto R, Verrall L, Yasuda Y, Ohi K, Fukumoto M, Umeda-Yano S, Ito A, Takeda M. 2011. Dysbindin-1 and NRG-1 gene expression in immortalized lymphocytes from patients with schizophrenia. J Hum Genet 56(7):478–483.

Yasuda Y, Hashimoto R, Yamamori H, Ohi K, Fukumoto M, Umeda-Yano S, Mohri I, Ito A, Taniike M, Takeda M. 2011. Gene expression analysis in lymphoblasts derived from patients with autism spectrum disorder. Mol Autism 2(1):9.