Journal of Human Genetics (2009) 54, 437–439

www.nature.com/jhg

ORIGINAL ARTICLE

No association between the *brain-derived neurotrophic factor* gene and panic disorder in Japanese population

Takeshi Otowa[1,11], Takafumi Shimada[1,2,11], Yoshiya Kawamura[1], Xiaoxi Liu[3], Ken Inoue[4], Nagisa Sugaya[5], Takanobu Minato[1], Ryoichi Nakagami[1], Mamoru Tochigi[1], Tadashi Umekage[9], Kiyoto Kasai[1], Nobumasa Kato[1], Hisashi Tanii[6], Yuji Okazaki[7], Hisanobu Kaiya[5,8] and Tsukasa Sasaki[9,10]

Panic disorder (PD) is a severe and chronic psychiatric disorder, with significant genetic components in the etiology. *Brain-derived neurotrophic factor (BDNF)* gene, which has regulatory effects on neurotransmitter systems such as serotonin and dopamine, is a candidate for susceptibility locus of PD. This study investigated three single-nucleotide polymorphisms (SNPs) of *BDNF* (rs6265 (Val66Met), rs11030104 and rs7103411) in Japanese patients with PD and controls. No significant association was observed between the three SNPs and PD. No association of the Val66Met was consistent with two small studies in Japanese and Chinese populations. We therefore conclude that the *BDNF* polymorphism may not play a major role in PD in the East Asian populations.
Journal of Human Genetics (2009) **54,** 437–439; doi:10.1038/jhg.2009.46; published online 22 May 2009

Keywords: brain-derived neurotrophic factor; genetic association; Japanese population; panic disorder; Val66Met polymorphism

INTRODUCTION

Panic disorder (PD) is an anxiety disorder characterized by panic attacks and anticipatory anxiety, with a lifetime prevalence of 1–3% and a female/male ratio of 2:1.[1] PD frequently takes a chronic course, with many remissions and relapses, occasionally complicated by comorbidity with other psychiatric disorders, such as agoraphobia and major depression.[2] It is generally accepted that PD has genetic as well as environmental causes. A 2.6- to 20-fold relative risk in the first-degree relatives of proband with PD compared with the general population suggests a familial component in this disorder.[3,4] Twin studies show that about 40% of the liability toward PD consists of heritable factors.[5–7] Thus far however, the etiology of PD is currently unknown.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, promotes the survival, differentiation and maintenance of a broad variety of central nervous system neurons.[8,9] BDNF has regulatory effects on multiple neurotransmitter systems, including serotonin[10] and dopamine,[11] and maybe involved in the etiology of stress-related disorders, such as depression and PTSD. Chronic administration of antidepressants, including selective serotonin reuptake inhibitors, increases BDNF expression in the hippocampus.[12] On the other hand, animal studies support the fact that environmental stressors, such as immobilization, decreased central BDNF mRNA.[13,14] BDNF-deficient mice show behavioral abnormalities, such as aggressiveness and hyperphagia, consistent with serotonergic dysfunction, which are corrected through antidepressant treatment.[15]

A single-nucleotide polymorphism (SNP) of the *BDNF*, Val66Met (196C>T) or rs6265 in the coding region of exon XIII, has been reported to be associated with psychiatric disorders, such as Alzheimer's disease,[16] obsessive-compulsive disorder,[17] bipolar disorder[18] and eating disorders.[19] It has been shown that the T allele of the SNP was associated with poorer episodic memory, abnormal hippocampus activation, and that Val66Met substitution impacts activity-dependent secretion of *BDNF*.[20] In studies with respect to personality traits, the C allele was associated with anxiety-related personality traits, such as higher mean neuroticism scores in the NEO-FFI,[21] and higher levels of trait anxiety in the STAI.[22] Interestingly, the frequency of the T allele in the Japanese and other Asian populations (approximately 41%) was significantly higher than that (approximately 18%) in Caucasians.[23]

Thus far, two genetic association studies investigated this variant of the *BDNF* gene in PD, which observed no significant association in the Japanese or Chinese population.[24,25] Those studies are however of rather small size (approximately 100 cases and less than 200 controls). Further studies with larger sample size may be required, considering

[1]Department of Neuropsychiatry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [2]Department of Psychiatry, Tokyo metropolitan Fuchu Hospital, Tokyo, Japan; [3]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [4]Department of Public Health, Fujita Health University School of Medicine, Aichi, Japan; [5]Outpatient Clinic for Anxiety Disorders, Akasaka Mental Clinic, Tokyo, Japan; [6]Department of Neuropsychiatry, Graduate School of Medicine, Mie University, Mie, Japan; [7]Department of Neurology, Tokyo Metropolitan Matsuzawa Hospital, Tokyo, Japan; [8]Research Center for Panic Disorder, Nagoya Mental Clinic, Aichi, Japan; [9]Section of Mental Health, Health Service Center, The University of Tokyo, Tokyo, Japan and [10]Office for Mental Health Support and Graduate School of Education, The University of Tokyo, Tokyo, Japan
[11]These authors contributed equally to the study.
Correspondence: Professor T Sasaki, Office for Mental Health Support and Graduate School of Education, The University of Tokyo, Hongo 7-3-1, Bunkyo, Tokyo 113-8655, Japan.
E-mail: psytokyo@yahoo.co.jp
Received 23 November 2008; revised 16 April 2009; accepted 17 April 2009; published online 22 May 2009

polygenic- or oligogenic-multifactorial nature of the etiology in PD. Here we report our investigation of the association of the Val66Met of the *BDNF* gene and PD in the Japanese population. Two other SNPs of *BDNF* (rs11030104 and rs7103411) were also studied.

MATERIALS AND METHODS

Participants

All patients and control individuals were ethnically Japanese and were recruited in the vicinity of Tokyo and Nagoya, Japan. Participants comprised 686 unrelated Japanese patients with PD diagnosed according to DSM-IV criteria (216 men and 470 women; age=38.7+10.5 years (mean+s.d.), 398 from Tokyo and 288 from Nagoya). Controls consisted of 589 unrelated healthy volunteers (277 men and 312 women; age=35.6+11.6 years), who were recruited around Tokyo; for the genotyping of Val66Met. For the genotyping of other two SNPs, 555 unrelated healthy volunteers (264 men and 291 women; age=35.7+12.8 years, recruited around Tokyo (n=459) and Nagoya (n=96)) served as controls. The two sets of the control samples share 398 healthy participants from Tokyo. The diagnosis was confirmed using the Mini-International Neuropsychiatric Interview (MINI)[26] and clinical records were also reviewed. The controls received a short interview by one of the authors and filled out questionnaires to exclude the history of major psychiatric illness. The objective of this study was clearly explained and written informed consent was obtained from all participants. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

Genotyping and data analysis

Genomic DNA was extracted from leukocytes by using the standard phenol-chloroform method. Three SNPs including rs6265 (Val66Met), rs11030104 and rs7103411 were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA), according to the manufacture's protocol (Applied Biosystems), with ABI PRISM 7900 SDS2 Software (Applied Biosystems). The latter two SNPs were tag SNPs, which were selected according to the HapMap data (www.hapmap.org).

The χ^2-test was used to compare the allele or genotype frequencies between the patients and controls. Significance for the result was set at $P<0.05$.

Linkage disequilibrium (LD) and haplotype were analyzed in patients and controls who were studied for all three SNPs. D' was used to analyze pairwise LD.[27] Haplotype block analysis was conduced using the Gabriel method.[28] The HaploView 4.1 program (Broad Institute of Harvard and MIT, Cambridge, MA, USA)[29] was used to conduct the LD and haplotype block analyses.

RESULTS

The genotype and allele frequencies of the three SNPs are summarized in Table 1. The genotype distributions of three SNPs did not significantly deviate from the Hardy–Weinberg equilibrium in patients or controls. Minor allele frequencies of the three SNPs were not different between participants from Tokyo and those from Nagoya (40 and 42% for rs6265 (P=0.16), 41 and 45% for rs11030104 (P=0.45) and 41 and 43% for rs7103411 (P=0.22), in participants from Tokyo and Nagoya, respectively). No significant difference was observed in the genotype or allele frequencies of the three SNPs between patients and controls. When analyzed by sex (data not shown) or the patients with agoraphobia (Table 1) were studied, the difference was not significant either.

The three SNPs were at significantly high LD (D'=0.99 between rs6265 and rs11030104, 0.99 between rs6265 and rs7103411 and 0.99 between rs1103014 and rs7103411. In haplotype block analysis, two haplotypes were observed with estimated frequencies >1% (Table 2). Frequencies of haplotypes 'CAT' and 'TGC' were estimated 57 and 40–41%, respectively. These indicate that the three SNPs might almost be at the complete LD. Permutation test did not observe an association between the haplotypes and PD.

DISCUSSION

In this study, we investigated the association between the three SNPs of the *BDNF* gene, including Val66Met, and PD in Japanese populations. We did not observe significant associations between the *BDNF* polymorphisms and PD. The association was not significant when the patients were confined to those with agoraphobia (Table 1) or when the participants were studied by sex. The result of Val66Met may be consistent with the two earlier studies which found no association in the Japanese or Chinese population.[24,25] We therefore conclude that this polymorphism of *BDNF* may not play a major role in the development of PD. Two other SNPs might not likely play a major role either, considering the very high LD between those SNPs and the Val66Met.

Sample size of this study (630 patients and 540 controls) is larger than the earlier studies (approximately 100 patients and <200 controls).

Table 2 Estimated frequencies of the haplotypes consisting of the three SNPs and results of permutation test

SNP			*PD*	*Control*	*Permutation*
1	*2*	*3*			P-*value*
C	A	T	0.57	0.57	1.00
T	G	C	0.41	0.40	0.95

Abbreviations: PD, panic disorder; SNP, single-nucleotide polymorphism.
Haplotypes whose frequencies were estimated >1% were described.

Table 1 Genotype and allele frequencies of the three SNPs in the *BDNF* gene

				Minor allele frequency			*Genotype frequencies*[a]		
	db SNP ID (major/minor)	*Chromosome position (bp)*		*PD*[b]	*Control*[b]	P-*value*	*PD*	*Control*	P-*value*
SNP1	rs6265	27636492		0.42 (638)	0.42 (589)	0.97	0.35/0.46/0.19	0.34/0.48/0.18	0.74
	(C/T)		Agoraphobia	0.41 (337)		0.66	0.37/0.44/0.19		0.52
SNP2	rs11030104	27641093		0.42 (628)	0.42 (539)	0.99	0.35/0.47/0.18	0.34/0.49/0.17	0.78
	(A/G)		Agoraphobia	0.42 (352)		0.99	0.35/0.46/0.19		0.98
SNP3	rs7103411	27656701		0.41 (662)	0.41 (548)	0.98	0.36/0.47/0.17	0.35/0.49/0.16	0.93
	(T/C)		Agoraphobia	0.41 (360)		1.00	0.36/0.46/0.18		0.71

Abbreviations: PD, panic disorder; SNP, single-nucleotide polymorphism.
Statistical analysis was performed using χ^2-test.
Agoraphobia: patients with agoraphobia were compared with controls.
[a]Described as major homo/hetero/minor homo.
[b]Number of genotyped individuals for each SNP is given in parenthesis.

The present sample has statistical power of >0.92 ($\alpha=0.05$) for the detection of the role of the polymorphism with minor allele frequency of 0.4, when the genotype relative risk is >1.33. It may be noted that larger sample is requested for the detection of a smaller effect. Proportions of the participants from Tokyo and those from Nagoya were not same between patients and controls. This may be noted as a limitation of the study, while all participants were ethnically Japanese and minor allele frequencies were not different between participants from Tokyo and those from Nagoya.

In conclusion, this study did not provide evidence for the association of the *BDNF* polymorphisms with PD in the Japanese population.

1 Eaton, W. W., Kessler, R. C., Wittchen, H. U. & Magee, W. J. Panic and panic disorder in the United States. *Am. J. Psychiatry* **151,** 413–420 (1994).
2 Weissman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S. & Hwu, H. G. *et al.* The cross-national epidemiology of panic disorder. *Arch. Gen. Psychiatry* **54,** 305–309 (1997).
3 Crowe, R. R., Noyes, R., Pauls, D. L. & Slymen, D. A family study of panic disorder. *Arch. Gen. Psychiatry* **40,** 1065–1069 (1983).
4 Goldstein, R. B., Wickramaratne, P. J., Horwath, E. & Weissman, M. M. Familial aggregation and phenomenology of 'early'-onset (at or before age 20 years) panic disorder. *Arch. Gen. Psychiatry* **54,** 271–278 (1997).
5 Hettema, J. M., Neale, M. C. & Kendler, K. S. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am. J. Psychiatry* **158,** 1568–1578 (2001).
6 Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C. & Eaves, L. J. Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch. Gen. Psychiatry* **49,** 716–722 (1992).
7 Torgersen, S. Genetics of neurosis. The effects of sampling variation upon the twin concordance ratio. *Br. J. Psychiatry* **142,** 126–132 (1983).
8 Koliatsos, V. E., Clatterbuck, R. E., Winslow, J. W., Cayouette, M. H. & Price, D. L. Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons *in vivo*. *Neuron* **10,** 359–367 (1993).
9 Lindsay, R. M., Wiegand, S. J., Altar, C. A. & DiStefano, P. S. Neurotrophic factors: from molecule to man. *Trends Neurosci.* **17,** 182–190 (1994).
10 Mamounas, L. A., Blue, M. E., Siuciak, J. A. & Altar, C. A. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. *J. Neurosci.* **15,** 7929–7939 (1995).
11 Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J. C. & Sokoloff, P. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. *Nature* **411,** 86–89 (2001).
12 Nibuya, M., Morinobu, S. & Duman, R. S. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* **15,** 7539–7547 (1995).
13 Jonsson, E., Brene, S., Zhang, X. R., Nimgaonkar, V. L., Tylec, A., Schalling, M. *et al.* Schizophrenia and neurotrophin-3 alleles. *Acta. Psychiatr. Scand.* **95,** 414–419 (1997).
14 Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S. & Senba, E. Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci. Res.* **28,** 103–110 (1997).
15 Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H. *et al.* Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl Acad. Sci. USA* **96,** 15239–15244 (1999).
16 Ventriglia, M., Bocchio Chiavetto, L., Benussi, L., Binetti, G., Zanetti, O., Riva, M. A. *et al.* Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Mol. Psychiatry* **7,** 136–137 (2002).
17 Hall, D., Dhilla, A., Charalambous, A., Gogos, J. A. & Karayiorgou, M. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am. J. Hum. Genet.* **73,** 370–376 (2003).
18 Sklar, P., Gabriel, S. B., McInnis, M. G., Bennett, P., Lim, Y. M., Tsan, G. *et al.* Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol. Psychiatry* **7,** 579–593 (2002).
19 Koizumi, H., Hashimoto, K., Itoh, K., Nakazato, M., Shimizu, E., Ohgake, S. *et al.* Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **127B,** 125–127 (2004).
20 Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A. *et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112,** 257–269 (2003).
21 Sen, S., Nesse, R. M., Stoltenberg, S. F., Li, S., Gleiberman, L. Chakravarti, A. *et al.* A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* **28,** 397–401 (2003).
22 Lang, U. E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K. P., Sander, T. *et al.* Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berl)* **180,** 95–99 (2005).
23 Shimizu, E., Hashimoto, K. & Iyo, M. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **126B,** 122–123 (2004).
24 Shimizu, E., Hashimoto, K., Koizumi, H., Kobayashi, K., Itoh, K., Mitsumori, M. *et al.* No association of the brain-derived neurotrophic factor (BDNF) gene polymorphisms with panic disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **29,** 708–712 (2005).
25 Lam, P., Cheng, C. Y., Hong, C. J. & Tsai, S. J. Association study of a brain-derived neurotrophic factor (Val66Met) genetic polymorphism and panic disorder. *Neuropsychobiology* **49,** 178–181 (2004).
26 Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* **59** (Suppl 20), 22–33; quiz 34–57 (1998).
27 Lewontin, R. C. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics* **49,** 49–67 (1964).
28 Gabriel, S. B., Schaffner, S. F., Nguyen, H., Moore, J. M., Roy, J. Blumenstiel, B. *et al.* The structure of haplotype blocks in the human genome. *Science* **296,** 2225–2229 (2002).
29 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).