schizophrenia in Japanese subjects (4.8% in patients vs. 2.3% in control subjects). We observed a similar tendency for the T allele frequency (3.7% in patients vs. 2.2% in control subjects), although the difference was not statistically significant ($p = 0.090$). In contrast, other previous studies failed to find such an association in Finnish (Anttila et al., 2005), European (Galderisi et al., 2005) and Polish (Szczepankiewicz et al., 2005) populations. These conflicting results may be due to an ethnic heterogeneity in the C132T polymorphism. The control subjects in Anttila et al. (2005), Galderisi et al. (2005) and Szczepankiewicz et al. (2005) showed higher frequencies of the T allele (7.1%, 9.9% and 4.6%, respectively) than those in Szekeres et al. (2003), Nanko et al. (2003) and the present study (2.8%, 2.3% and 2.2%, respectively). Our sample size ($n = 792$) is similar to that in Szczepankiewicz et al. ($n = 777$) and larger than those of Szekeres et al. ($n = 169$), Nanko et al. ($n = 510$), Anttila et al. ($n = 192$) and Galderisi et al. ($n = 217$). However, we could not exclude the possibility of a type II error due to an insufficient statistical power, since our sample size only had a power of 0.25 to detect a significant association between the T allele and schizophrenia using the Genetic Power Calculation (Purcell et al., 2003). Thus, further studies with larger samples in

several ethnic populations will be required to draw any definitive conclusions. When we pooled our sample with that of Nanko et al. (2003), the C/T genotype and T allele were associated with schizophrenia ($p = 0.008$, OR = 1.9, 95% CI = 1.2-3.0 for the C/T genotype; $p = 0.009$, OR = 1.9, 95% CI = 1.3-2.6 for the T allele). However, even if there is a significant association, the majority of Japanese schizophrenia patients are not related to this polymorphism, since the risk allele is very rare.

The $(GT)_n$ dinucleotide repeat was one of the most extensively investigated polymorphisms in the *BDNF* gene for associations in schizophrenia, although we did not examine this repeat as described above. Muglia et al. (2003) showed biased transmission of $(GT)_n$ alleles from parents to schizophrenia probands, whereas other studies failed to find this association (Sasaki et al., 1997; Hawi et al., 1998; Wassink et al., 1999; Krebs et al., 2000; Virgos et al., 2001; Fanous et al., 2004; Neves-Pereira et al., 2005). Recently, Koizumi et al. (2005) reported that this repeat consists of three different and continuous dinucleotide repeats, rather than a simple $(GT)_n$ repeat. Accordingly, this polymorphism should be reanalyzed to confirm an association between the $(GT)_n$ repeats and schizophrenia.

In conclusion, the results of the present study suggest that three *BDNF* gene polymorphisms (rs988748, C132T and rs6265) do not play major roles in conferring susceptibility to schizophrenia in a Japanese population, although the possibility that the C132T polymorphism is associated with schizophrenia still remains to be elucidated. Further studies with larger samples for the *BDNF* gene polymorphisms, including the C132T polymorphism and $(GT)_n$ repeats, should be performed in several other ethnic populations.

Acknowledgements

The authors thank the patients, the patients' families and the healthy volunteers for their participation; and Hiroshi Kusano and Tomoko Yamada for excellent technical assistance. This work was supported by Health and Labour Sciences Research Grants for Research on the Human Genome, Tissue Engineering Food Biotechnology (to T.S.), Health and Labour Sciences Research Grants for Research on Psychiatric and Neurological Diseases and Mental Health (to T.S.), a Grant from the Research Group For Schizophrenia, Japan (to Y.W.), a Grant for Promotion of Niigata University Research Project (to Y.W.) and a Tsukada Memorial Grant for Niigata University Medical Research (to Y.W.).

References

Anttila, S., Illi, A., Kampman, O., Mattila, K.M., Lehtimäki, T., Leinonen, E., 2005. Lack of association between two polymorphisms of brain-derived neurotrophic factor and response to typical neuroleptics. J. Neural. Transm. 112, 885-890.

Chen, B., Dowlatshahi, D., MacQueen, G.M., Wang, J.-F., Young, L.T., 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol. Psychiatry 50, 260-265.

Chen, Q.-Y., Chen, Q., Feng, G.-Y., Wan, C.-L., Lindpaintner, K., Wang, L.-J., Chen, Z.-X., Gao, Z.-S., Tang, J.-S., Li, X.-W., He, L., 2006. Association between the brain-derived neurotrophic factor (*BDNF*) gene and schizophrenia in the Chinese population. Neurosci. Lett. (in press).

de Krom, M., Bakker, S.C., Hendriks, J., van Elburg, A., Hoogendoorn, M., Verduijn,

W., Sinke, R., Kahn, R., Adan, R.A.H., 2005. Polymorphisms in the brain-derived neurotrophic factor gene are not associated with either anorexia nervosa or schizophrenia in Dutch patients. Psychiatr. Genet. 15, 81.

Dempster, E., Toulopoulou, T., McDonald, C., Bramon, E., Walshe, M., Filbey, F., Wickham, H., Sham, P.C., Murray, R.M., Collier, D.A., 2005. Association between BDNF val^{66} met genotype and episodic memory. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 134B, 73-75.

Durany, N., Michel, T., Zöchling, R., Boissl, K.W., Cruz-Sánchez, F.F., Riederer, P., Thome, J., 2001. Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophr. Res. 52, 79-86.

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., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and

human memory and hippocampal function. Cell 112, 257-269.

Fanous, A.H., Neale, M.C., Straub, R.E., Webb, B.T., O'Neill, A.F., Walsh, D., 2004. Clinical features of psychotic disorders and polymorphisms in HT2A, DRD2, DRD4, SLC6A3 (DAT1), and BDNF: a family based association study. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 125B, 69-78.

Galderisi, S., Maj, M., Kirkpatrick, B., Piccardi, P., Mucci, A., Invernizzi, G., Rossi, A., Pini, S., Vita, A., Cassano, P., Stratta, P., Severino, G., Zompo, M.D., 2005. COMT $Val^{158}Met$ and BDNF $C^{270}T$ polymorphisms in schizophrenia: a case-control study. Schizophr. Res. 73, 27-30.

Gourion, D., Goldberger, C., Leroy, S., Bourdel, M.-C., Olié, J.-P., Krebs, M.-O., 2005. Age at onset of schizophrenia: interaction between brain-derived neurotrophic factor and dopamine D3 receptor gene variants. Neuroreport 16, 1407-1410.

Hashimoto, T., Bergen, S.E., Nguyen, Q.L., Xu, B., Monteggia, L.M., Pierri, J.N., Sun, Z., Sampson, A.R., Lewis, D.A., 2005. Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. J. Neurosci. 25, 372-383.

Hawi, Z., Straub, R.E., O'Neill, A., Kendler, K.S., Walsh, D., Gill, M., 1998. No linkage or linkage disequilibrium between brain-derived neurotrophic factor (BDNF) dinucleotide repeat polymorphism and schizophrenia in Irish families. Psychiatry Res. 81, 111-116.

Hong, C.-J., Yu, Y.W.-Y., Lin, C.-H., Tsai, S.-J., 2003. An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. Neurosci. Lett. 349, 206-208.

Iritani, S., Niizato, K., Nawa, H., Ikeda, K., Emson, P.C., 2003. Immunohistochemical study of brain-derived neurotrophic factor and its receptor, TrkB, in the hippocampal

formation of schizophrenic brains. Prog. Neuropsychopharmacol. Biol. Psychiatry 27, 801-807.

Itoh, K., Hashimoto, K., Shimizu, E., Sekine, Y., Ozaki, N., Inada, T., Harano, M., Iwata, N., Komiyama, T., Yamada, M., Sora, I., Nakata, K., Ujike, H., Iyo, M., 2005. Association study between brain-derived neurotrophic factor gene and methamphetamine abusers in Japan. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 132B, 70-73.

Jockers-Scherübl, M.C., Danker-Hopfe, H., Mahlberg, R., Selig, F., Rentzsh, J., Schürer, F., Lang, U.E., Hellweg, R., 2004. Brain-derived neurotrophic factor serum concentrations are increased in drug-naive schizophrenic patients with chronic cannabis abuse and multiple substance abuse. Neurosci. Lett. 371, 79-83.

Knable, M.B., Barci, B.M., Webster, M.J., Meador-Woodruff, J., Torrey, E.F., 2004. Molecular abnormalities of the hippocampus in severe psychotic illness: postmortem

findings from the Stanley Neuropathology Consortium. Mol. Psychiatry 9, 609-620.

Knüsel, B., Winslow, J.W., Rosenthal, A., Burton, L.E., Seid, D.P., Nikolics, K., Hefti, F., 1991. Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin 3. Proc. Natl. Acad. Sci. U. S. A. 88, 961-965.

Koizumi, H., Hashimoto, K., Shimizu, E., Iyo, M., Mashimo, Y., Hata, A., 2005. Further analysis of microsatellite marker in the *BDNF* gene. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 135B, 103.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., Bonhoeffer, T., 1995. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. U. S. A. 92, 8856-8860.

Krebs, M.O., Guillin, O., Bourdel, M.C., Schwartz, J.C., Olie, J.P., Poirier, M.F.,

Sokoloff, P., 2000. Brain Derived Neurotrophic Factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. Mol. Psychiatry 5, 558-562.

Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, N., Nabika, T., Kobayashi, S., Nanko, S., 2001. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. Mol. Psychiatry 6, 83-86.

Mizuno, K., Carnahan, J., Nawa, H., 1994. Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. Dev. Biol. 165, 243-256.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., Nabeshima, T., 2000. Involvement of brain-derived neurotrophic factor in spatial memeory formation and maintenance in a radial arm maze test in rats. J. Neurosci. 20, 7116-7121.

Muglia, P., Vicente, A.M., Verga, M., King, N., Macciardi, F., Kennedy, J.L., 2003. Association between the BDNF gene and schizophrenia. Mol. Psychiatry 8, 147-148.

Nanko, S., Kunigi, H., Hirasawa, H., Kato, N., Nabika, T., Kobayashi, S., 2003. Brain-derived neurotrophic factor gene and schizophrenia: polymorphism screening and association analysis. Schizophr. Res. 62, 281-283.

Nawa, H., Takahashi, M., Patterson, P.H., 2000. Cytokine and growth factor involvement in schizophrenia--support for the developmental model. Mol. Psychiatry 5, 594-603.

Neves-Pereira, M., Cheung, J.K., Pasdar, A., Zhang, F., Breen, G., Yates, P., Sinclair, M., Crombie, C., Walker, N., Clair, D.M.S., 2005. BDNF gene is a risk factor for schizophrenia in a Scottish population. Mol. Psychiatry 10, 208-212.

Pırıldar, Ş., Gönül, A.A., Taneli, F., Akdeniz, F., 2004. Low serum levels of

brain-derived neurotrophic factor in patients with schizophrenia do not elevate after antipsychotic treatment. Prog. Neuropsychopharmacol. Biol. Psychiatry 28, 709-713.

Purcell, S., Cherny, S.S., Sham, P.C., 2003. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19, 149-150.

Rosa, A., Cuesta, M.J., Fatjó-Vilas, M., Peralta, V., Zarzuela, A., Fañanás, L., 2006. The Val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with risk for psychosis: evidence from a family-based association study. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 141B, 135-138.

Sasaki, T., Dai, X.-Y., Kuwata, S., Fukuda, R., Kunugi, H., Hattori, M., Nanko, S., 1997. Brain-derived neurotrophic factor gene and schizophrenia in Japanese subjects. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 74, 443-444.

Schumacher, J., Jamra, R.A., Becker, T., Ohlraun, S., Klopp, N., Binder, E.B., Schulze,

T.G., Deschner, M., Schmäl, C., Höfels, S., Zobel, A., Illig, T., Propping, P., Holsboer, F., Rietschel, M., Nöthen, M.M., Cichon, S., 2005. Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. Biol. Psychiatry 58, 307-314.

Shimizu, E., Hashimoto, K., Watanabe, H., Komatsu, N., Okamura, N., Koike, K., Shinoda, N., Nakazato, M., Kumakiri, C., Okada, S., Iyo, M., 2003. Serum brain-derived neurotrophic factor (BDNF) levels in schizophrenia are indistinguishable from controls. Neurosci. Lett. 351, 111-114.

Szczepankiewicz, A., Skibinska, M., Czerski, P.M., Kapelski, P., Leszczynska-Rodziewicz, A., Slopien, A., Dmitrzak-Węglarz, M., Rybakowski, F., Rybakowski, J., Hauser, J., 2005. No association of the brain-derived neurotrophic factor (BDNF) gene C-270T polymorphism with schizophrenia. Schizophr. Res. 76, 187-193.

Szekeres, G., Juhász, A., Rimanóczy, Á., Kéri, S., Janka, Z., 2003. The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. Schizophr. Res. 65, 15-18.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D., Malhotra, A.K., 2005. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol. Psychiatry 10, 631-636.

Takahasi, M., Shirakawa, O., Toyooka, K., Kitamura, N., Hashimoto, T., Maeda, K., Koizumi, M., Wakabayashi, K., Takahashi, H., Someya, T., Nawa, H., 2000. Abnormal expression of brain-derived neurotrophic factor and its receptor in the corticolimbic system of schizophrenic patients. Mol. Psychiatry 5, 293-300.

Tan, Y.L., Zhou, D.F., Cao, L.Y., Zou, Y.Z., Wu, G.Y., Zhang, X.Y., 2005a. Effect of the BDNF Val66Met genotype on episodic memory in schizophrenia. Schizoph.r Res. 77,

355-356.

Tan, Y.L., Zhou, D.F., Cao, L.Y., Zou, Y.Z., Zhang, X.Y., 2005b. Decreased BDNF in serum of patients with chronic schizophrenia on long-term treatment with antipsychotics. Neurosci. Lett. 382, 27-32.

Tan, Y.L., Zhou, D.F., Zhang, X.Y., 2005c. Decreased plasma brain-derived neurotrophic factor levels in schizophrenic patients with tardive dyskinesia: association with dyskinetic movements. Schizophr. Res. 74, 263-270.

Toyooka, K., Asama, K., Watanabe, Y., Muratake, T., Takahashi, M., Someya, T., Nawa, H., 2002. Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. Psychiatry Res. 110, 249-257.

Virgos, C., Martorell, L., Valero, J., Figuera, L., Civeira, F., Joven, J., Labad, A., Vilella, E., 2001. Association study of schizophrenia with polymorphisms at six candidate genes.

Schizophr. Res. 49, 65-71.

Wassink, T.H., Nelson, J.J., Crowe, R.R., Andreasen, N.C., 1999. Heritability of BDNF alleles and their effect on brain morphology in schizophrenia. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 88, 724-728.

Weickert, C.S., Hyde, T.M., Lipska, B.K., Herman, M.M., Weinberger, D.R., Kleinman, J.E., 2003. Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. Mol. Psychiatry 8, 529-610.

Electronic Database Information

The URLs for the data presented herein are as follows:

Genetic Power Calculator, http://wbiomed.curtin.edu.au/genepop/

National Center for Biotechnology Information, Single Nucleotide Polymorphism Database, http://www.ncbi.nlm.nih.gov/SNP/ (for SNP reference identification numbers)

Table 1

Genotype and allele frequencies of three *BDNF* gene polymorphisms in the schizophrenia patients and control subjects.

SNP	Genotype			*p*	Allele		*p*
rs988748	C/C	C/G	G/G	0.891	C	G	0.959
Patients	122 (35.0%)	162 (46.4%)	65 (18.6%)		406 (58.2%)	292 (41.8%)	
Controls	143 (33.8%)	204 (48.2%)	76 (18.0%)		490 (57.9%)	356 (42.1%)	
C132T	C/C	C/T	T/T	0.090	C	T	0.095
Patients	323 (92.6%)	26 (7.4%)	0 (0.0%)		672 (96.3%)	26 (3.7%)	
Controls	404 (95.5%)	19 (4.5%)	0 (0.0%)		827 (97.8%)	19 (2.2%)	
rs6265	G/G	G/A	A/A	0.828	G	A	0.918
Patients	122 (35.0%)	163 (46.7%)	64 (18.3%)		407 (58.3%)	291 (41.7%)	
Controls	142 (33.6%)	207 (48.9%)	74 (17.5%)		491 (58.0%)	355 (42.0%)	

Table 2

Linkage disequilibrium (LD) indices (D' and r^2; above and below the diagonal line, respectively) in the control subjects.

SNP	rs988748	C132T	rs6265
rs988748	-	0.5828	0.9804
C132T	0.0057	-	0.7248
rs6265	0.9566	0.0087	-

Significant ($p < 0.05$) LD indices are underlined.

Table 3

Estimated haplotype frequencies in the schizophrenia patients and control subjects.

Haplotype	rs988748	C132T	rs6265	Patients	Controls	Permutation *p*
1	C	C	G	0.551	0.565	0.553
2	G	C	A	0.414	0.417	0.890
3	C	T	G	0.035	0.018	0.044

The global permutation *p* value of haplotypes 1-3 is 0.113.

No association between the *tumor necrosis factor-α* gene promoter polymorphisms and schizophrenia in a Japanese population

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Abstract

Tumor necrosis factor-α (TNF-α) is a pleiotrophic cytokine and exerts neuroprotective and neurodegenerative effects in brain. Several studies have indicated that TNF-α is likely related to the pathogenesis of schizophrenia. Recent genetic researches have revealed that a *TNF-α* gene promoter polymorphism (-G308A) is associated with schizophrenia, although contradictive negative findings have also been reported. To assess whether the *TNF-α* gene promoter variants including -G308A could be implicated in vulnerability to schizophrenia, we conducted a case-control association analysis (265 cases and 424 controls) and the transmission disequilibrium test (TDT) analysis (83 trios) for four polymorphisms (-G238A, -G308A, -C857T, and –T1031C) in Japanese subjects. In a case-control analysis, there was no significant association between the promoter polymorphisms or haplotypes in the *TNF-α* gene and schizophrenia. In the TDT analysis, we also did not observe transmission distortion. Our results suggest that the above four polymorphisms in the promoter region of the *TNF-α* gene might not confer increased susceptibility for schizophrenia in a Japanese population.