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1 Knox SS *et al. Am J Cardiol* 1998; 82: 1192–1196.
2 Knox SS *et al. Am J Cardiol* 2000; 86: 1089–1096.
3 Weidner G *et al. Psychosom Med* 2000; 62: 197–204.
4 Carmelli D *et al. J Soc Behav Pers* 1990; 5: 117–133.
5 Pederson NL *et al. Psychosom Med* 1989; 51: 428–440.
6 Rose RJ. *J Pers Soc Psychol* 1988; 55: 302–311.
7 Smith TW *et al. Psychosom Med* 1991; 53: 684–692.
8 Matthews KA *et al. Health Psychol* 1992; 11: 317–323.
9 Higgins M, *et al.*, for the NHLBI Family Heart Study Investigators. *Am J Epidemiol* 1996; 143: 1219–1228.
10 Hunt SC *et al. J Chronic Dis* 1986; 39: 809–821.
11 Barefoot JC *et al. Psychosom Med* 1989; 51: 46–57.
12 Cook W, Medley D. Proposed hostility and pharisaic-virtue scales for MMPI. *J Appl Psychol* 1954; 38: 414–418.
13 Kruglyak L *et al. Am J Hum Genet* 1996; 58: 1347–1363.
14 Pratt SC *et al. Am J Hum Genet* 2000; 66: 1153–1157.
15 Williams RB *et al. Psychosom Med* 2001; 63: 300–305.
16 Staner L, Mendlewicz J. Heredity and role of serotonin in aggressive impulsive behavior. *Encephale* 1998; 24: 355–364.

No association with the neuregulin 1 haplotype to Japanese schizophrenia

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SIR—Schizophrenia is a complex genetic disorder and has heritability of around 80%. The pathogenesis of the disease is hypothesized to be neurodevelopmental in nature based on reports of an excess of adverse events during the pre- and perinatal periods, the presence of cognitive and behavioral signs during childhood and adolescence, and the lack of evidence of a neurodegenerative process in most individuals with schizophrenia.[1]

To date, studies of the association between schizophrenia and genes coding for neurodevelopmental role has been published.[2] Recently, it has been reported that genetic variants around the gene neuregulin 1 (*NRG1*) are associated with schizophrenia in an Icelandic sample.[3] The replications of this finding have been reported independently from Scottish population[4] and Caucasians born in UK and Ireland.[5] The at-risk haplotype was found to be over-represented in schizophrenics compared to controls.

Here, we attempt to perform a replication of these results in our collection of Japanese schizophrenic patients and controls. Subjects consisted of 607 patients with schizophrenia and 515 controls. All subjects were unrelated Japanese. Most patients (93%) had more than 1-year duration of illness and 77% patients had history of hospitalization. Consensus diagnosis according to DSM-IV was made. After description of the study, written informed consent was obtained from each subject. This study was approved by the Fujita Health University and the National Center of Neurology and Psychiatry Hospital Ethics Committees.

SNP8NRG221132, SNP8NRG221533, SNP8NRG243177, and SNP8NRG433E1006 were genotyped using primer extension method by dHPLC. SNP8NRG241930 was genotyped using PCR-RFLP method. The microsatellites 478B14-848 and 420M9-1395 were genotyped following analysis on an ABI3100 capillary sequencer. Allele 0 for markers 478B14-848 and 420M9-1395 refers to PCR-product sizes of 219 and 274 bp, respectively.

Deviation from the genotype counts predicted by Hardy–Weinberg equilibrium expectations was tested using an exact test. D, D', and Δ^2 for pair-wise linkage disequilibrium (LD) were calculated. Estimation of the haplotype frequencies was performed by the expectation–maximization algorithm. Test for single-marker allelic association was performed by χ^2 analysis. Test for haplotypic association was performed using the software SAS/Genetics (Release 8.2 TS2M0, SAS Institute Japan) with a permutation test to obtain the empirical significance.[6]

We genotyped the five SNPs (SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, SNP8NRG433E1006) and two microsatellite markers (478B14-848, 420M9-1395) that constitute the core haplotype in 607 Japanese patients and 515 Japanese control individuals (Table 1). The genotypic distribution of each locus was not significantly different from the distribution expected according to Hardy–Weinberg equilibrium in this ethnic population. We found no association between each marker and schizophrenia, including the C allele of SNP8NRG221533 (P=0.42) that was significantly in excess in both Icelandic[3] and Scottish[4] schizophrenia (P=0.0028 and 0.000064, respectively). The estimated frequency of the core at-risk haplotype, which was enriched in the Scottish and Icelandic patients (10.2%, 14.4%) than in control individuals (5.9%, 7.6%) by Stefansson *et al*, had no difference between Japanese patients (4.5%) and Japanese controls (4.3%). The estimated Odds Ratio was 1.13 and the 95% confidence interval was (0.60–2.11). In order to be consistent with the literature by Williams and his colleagues, we performed three-marker 'at-risk' haplotype analysis with and without a family history of schizophrenia. However, there are no significant differences among each patent group and control.

Our results do not confirm an involvement of the NRG1 in conferring susceptibility to schizophrenia. One possible explanation for this result could stem from the clinical heterogeneity of schizophrenia. Although the disease is discussed as if it is a single disease, it probably comprises a group of disorders with heterogeneous etiologies. Indeed, the original study was based on individuals taken from large

Table 1 Replication study showing allele frequencies for markers and haplotypes of the NRG1 gene

Marker	*Allele*	*Frequency (%)* *Control (%)*	*(Number)*	*Schizophrenia (%)*	*(Number)*	*P (empirical)*	*OR (95% CI)*
a. Test of the seven- and five-marker haplotypes and each SNP							
Seven-marker haplotype		4.3	488	4.5	597	0.96	1.05 (0.59–1.89)
Five-marker SNP		45.1	514	45.2	603	0.98	1.01 (0.79–1.27)
SNP8NRG221132	G	100.0	515	100.0	607	NA[a]	NA[a]
SNP8NRG221533	C	51.5	515	53.1	606	0.42	1.07 (0.85–1.35)
SNP8NRG241930	G	87.9	515	89.4	607	0.26	1.16 (0.80–1.68)
SNP8NRG243177	T	52.7	515	54.4	607	0.43	1.07 (0.55–1.36)
SNP8NRG433E1006	G	98.9	514	98.9	604	0.99	1.01 (0.34–3.02)
478B14-848	0	42.2	507	49.1	603	0.50	1.32 (1.04–1.67)
420M9-1395	0	39.3	494	48.3	605	0.21	1.44 (1.13–1.84)

Test	*Frequency (%)*	*χ*	*P-value (one tailed)*	*OR (95% CI)*
b. Test of the 'at-risk' haplotype of markers SNP8NRG221533, 478B14-848, and 420M9-1395				
Case (n–600)	5.7	0.51	0.66	1.13 (0.67–1.92)
Control (n=490)	5.1			
Familial cases (n=69)	7.7	0.19	0.36	1.46 (0.54–3.95)
Nonfamilial cases (n=531)	5.0	0.86	0.93	0.98 (0.45–2.17)

[a]NA = not analysis.

multiply affected families from Iceland.[3] Subsequently, Williams *et al*[5] discussed sample stratification in which the genetic component might be different in their samples. Further investigations are needed by taking the effect of genetic load into consideration.

Another explanation is that population stratification may exist, since the initial findings started from linkage studies that point out initial evidence for schizophrenia to 8p22-21, where *NRG1* is located.[3] In contrast, from Japanese linkage study, no loci on chromosome 8 fulfiled the criteria for significant or suggestive evidence for linkage.[7] According to the common disease/common variants (CD/CV) hypothesis, if the at-risk haplotype is linked to real susceptibility variants, haplotype association test should detect the linkage disequilibrium to the disease variants in different ethnic populations.[8] However, the sample size was not enough such that the power of analysis was reached at 0.18 (when α=0.05). Additional studies of *NRG1* to evaluate across larger numbers of individuals and other ethnic populations should be needed to confirm the hypothesis.

However, it is unclear that single common variants will be the only relevant variants.[9] It is possible that the risk for some common diseases is due to a very large number of loci, with each having a low frequency of disease-predisposing alleles. Allelic heterogeneity may also be contributing to the association of the *NRG1* locus with schizophrenia.[10] In the study, we did not search for genetic variants at *NRG1* locus in our Japanese samples. Much greater depth of DNA resequencing should be conducted to confirm that unknown rare variants in linkage disequibrium might be a real disease gene.

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17 Lewis DA, Levitt P. *Annu Rev Neurosci* 2002; 25: 409–432.
18 Harrison PJ, Owen MJ. *Lancet* 2003; 361: 417–419.
19 Stefansson H *et al. Am J Hum Genet* 2002; 71: 877–892.
20 Stefansson H *et al. Am J Hum Genet* 2003; 72: 83–87.
21 Williams NM *et al. Mol Psychiatry* 2003; 8: 485–487.
22 Zhao JH *et al. Hum Hered* 2000; 50: 133–139.
23 JSSLG. *Am J Med Genet* 2003; 120B: 22–28.
24 Reich DE *et al. Nature* 2001; 411: 199–204.
25 Reich DE, Lander ES. *Trends Genet* 2001; 17: 502–510.
26 Grady DL *et al. Mol Psychiatry* 2003; 8: 536–545.

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Association Study Between Brain-Derived Neurotrophic Factor Gene Polymorphisms and Methamphetamine Abusers in Japan

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Several lines of evidence suggest that genetic factors might contribute to drug abuse vulnerability. Recent genomic scans for association demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene was associated with drug abuse vulnerability. In this study, we analyzed association of two *BDNF* gene single nucleotide polymorphisms (SNPs), 132C > T (C270T named formerly) in the noncoding region of exon V and 196G > A (val66met) in the coding region of exon XIIIA, with methamphetamine (MAP) abuse in Japan. No significant differences were found in the frequency of the genotype or allele in these two SNPs between MAP abusers and controls (132C > T in exon V: genotype, $P = 0.586$, allele, $P = 0.594$; 196G > A (val66met) in exon XIIIA: genotype, $P = 0.889$, allele, $P = 0.713$). Furthermore, there was no difference between clinical parameters (e.g., prognosis psychosis, spontaneous relapse, or poly-substance abuse) and the two SNPs of *BDNF* gene. These results suggest that the two SNPs (132C > T in exon V and 196G>A (val66met) in exon XIIIA) of the *BDNF* gene may not be associated with Japanese MAP abusers. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html.

KEY WORDS: **brain-derived neurotrophic factor; polymorphism; drug abuse; methamphetamine**

INTRODUCTION

Family, twin, and adoption studies suggest that genetic factors are implicated in vulnerability of substance abuse [Merikangas et al., 1998; Kendler, 2001; Tsuang et al., 2001]. The genome-scanning study of poly-substance abuse vulnerability demonstrated that the brain-derived neurotrophic factor (*BDNF*) gene might be one of the strong candidate genes to drug abuse [Uhl et al., 2001]. BDNF is a member of a neurotrophin superfamily mainly expressed within the brain. BDNF interacts with TrkB receptor tyrosine kinase, playing several important roles such as promotion of survival, differentiation, and maintenance of neurons in peripheral nervous system and central nervous system; influences to axonal growth and connectivity; participation in the local responses to various types of neuronal stress or insults [Manji et al., 2003; Mattson et al., 2003]. Furthermore, it also has been reported that the gene encoding BDNF might be an important candidate for susceptibility of neuropsychiatric disorders including bipolar disorder [Neves-Pereira et al., 2002; Sklar et al., 2002; Hashimoto et al., 2004] and schizophrenia [Krebs et al., 2000]. In the studies reporting possible association of BDNF and these disorders, two single nucleotide polymorphisms (SNPs) of *BDNF* gene has been reported. One is 196G > A (val66met) SNP in exon XIIIA (GENBANK: AF411339; at position 95422) located within the propeptide region of BDNF. The A of the ATG-translation initiation codon is denoted nucleotide +1 in exon XIIIA (GENBANK: AF411339; at position 95227). Sklar et al. [2002] reported that BDNF 196G > A (val66met) is significantly associated with bipolar disorder. Interestingly, it has been demonstrated that this SNP (val66met) is strongly suspected to influence human memory and hippocampal function [Egan et al., 2003]. Several lines of evidence demonstrated that methamphetamine (MAP) dependence may cause long-term neural damage in humans, with concomitant deleterious effects on cognitive processes such as memory and attention [Nordahl et al., 2003], suggesting the possible role of BDNF secretion in the memory deficits of MAP abusers. The other SNP frequently analyzed is 132C > T in the noncoding region of exon V (GENBANK: AF411339; at position 53620). This SNP at position 132 of exon V is numbered from the start of exon V (GENBANK: AF411339; at position 53488). It was detected and named C270T by Kunugi et al. [2001] after their searching for a novel nucleotide substitution in the

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noncoding region of the *BDNF* gene reported by Shintani et al. [1992]. It has been reported that the 132C > T in exon V of the *BDNF* gene was significantly associated with late-onset Alzheimer's disease [Kunugi et al., 2001], or schizophrenia [Szekeres et al., 2003]. In addition, it has been reported that the BDNF 196G > A (val66met) is associated with personality traits in healthy subjects [Sen et al., 2003; Itoh et al., 2004], suggesting the role of *BDNF* gene in personality traits and temperament. Considering the role of personality traits in substance use disorders [Howard et al., 1997], it is likely that the *BDNF* gene may be implicated in the vulnerability of drug abuse.

MAP is the most popular abused drug in Japan. Use of MAP induces a strong psychological dependence, and repeated usage frequently results in psychotic states, which symptoms are similar to those of paranoid-type schizophrenia [Sato et al., 1992; Ujike, 2002]. It has been demonstrated that BDNF plays a role in the survival and differentiation of midbrain dopaminergic neurons in vivo [Hyman et al., 1991] and in vitro [Spina et al., 1992], and that chronic BDNF treatment enhances locomotor activity and conditioned reward to cocaine [Hoger et al., 1999]. In addition, it is likely that BDNF could modulate the release of dopamine through the activation of TrkB receptors [Blochl and Sirrenberg, 1996]. Furthermore, it has been reported that locomotor behaviors by amphetamine was increased to a greater degree in the BDNF heterozygous (+/−) knock-out mice, and that striatal dopamine concentrations were significantly higher in the BDNF heterozygous (+/−) knock-out mice [Dluzen et al., 2001]. Moreover, it has been reported recently that pretreatment with intra-nucleus accumbens injection of BDNF antibody or TrkB antibody suppressed significantly the release of dopamine and dopamine-related behaviors induced by administration of MAP, suggesting the implication of BDNF in MAP-induced dopamine release and MAP-induced abnormal behaviors [Narita et al., 2003]. Taken together, it is of interest to study the influences of the *BDNF* gene SNPs in MAP abuse vulnerability. In this study, we analyzed the frequency of two known SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of *BDNF* gene between MAP abusers and healthy subjects in Japan.

MATERIALS AND METHODS

Subjects and Samples

This study was performed after obtaining the approval of the ethics committees of each affiliated institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA). All subjects provided written informed consent for the use of their DNA samples for this study. The subjects were 189 patients (150 males and 39 female; age, 36.6 ± 11.9 (mean ± SD)) with MAP dependence and a psychotic disorder meeting the ICD-10-DCR criteria (F15.2 and F15.5) who were outpatients or inpatients of psychiatric hospitals of the JGIDA. Two hundred and two volunteers were recruited as healthy controls. All controls have no significant lifetime history of use of any addictive substance (158 males and 44 females; 37.2 ± 10.6 (mean ± SD)), the majority of whom were medical staff with no past history and no family history of drug dependence or psychotic disorders. Diagnoses were made by two trained psychiatrists by interview and available information including hospital records. Detailed characteristics of patients were shown in the Supplement 1 (see the online Supplement 1 at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html).

Genotyping

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assay were performed to genotype the DNA sequence variants of the *BDNF* gene. Detailed methods for genotyping were shown in the Supplement 2 (see the online Supplement 2 at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html).

Statistical Analysis

Fisher's exact test was used for categorical comparisons, and Student's *t*-test was employed for age difference. Significance for the results was set at $P < 0.05$.

RESULTS

Both the genotype and allele frequencies for the patients and controls are shown in Tables I and II. The genotype distribution for patients groups and control groups did not deviate significantly from the Hardy–Weinberg equilibrium. No significant differences were found in the frequency of the genotype or allele in these two SNPs between patients and controls (132C > T in exon V: genotype, $P = 0.586$, allele, $P = 0.594$; 196G > A (val66met) in exon XIIIA: genotype, $P = 0.889$, allele, $P = 0.713$). As for the 132C > T substitution, there was no individual who was homozygous for the 132T allele in exon V. Within patients, we analyzed the effects of prognosis psychosis (transient or prolonged), spontaneous relapse (positive or negative), and poly-substance abuse (yes or no) on the *BDNF* gene SNPs (132C > T in exon V and 196G > A in exon XIIIA). The genotypic and allelic distribution of two SNPs was not

TABLE I. Genotype and Allele Frequencies of the Brain-Derived Neurotrophic Factor (BDNF) 132C > T (in Exon V) Gene Polymorphism of in Controls and Methamphetamine (MAP) Abusers

		Genotype				Allele		
132C > T	n	CC	CT	TT	*P*	C	T	*P*
Control	202	183 (90.6%)	19 (9.4%)	0 (0%)		385 (95.3%)	19 (4.7%)	
Abuser	189	175 (92.6%)	14 (7.4%)	0 (0%)	0.586	364 (96.3%)	14 (3.7%)	0.594
Prognosis of psychosis								
Transient	94	87 (92.6%)	7 (7.4%)	0 (0%)	0.664	181 (96.3%)	7 (3.7%)	0.671
Prolonged	66	62 (93.9%)	4 (6.1%)	0 (0%)	0.612	128 (97.0%)	4 (3.0%)	0.620
Spontaneous relapse								
Positive	64	60 (93.8%)	4 (6.3%)	0 (0%)	0.611	124 (96.9%)	4 (3.1%)	0.619
Negative	116	107 (92.2%)	9 (7.8%)	0 (0%)	0.685	223 (96.1%)	9 (3.9%)	0.692
Poly-substance abuse								
No	56	51 (91.1%)	5 (8.9%)	0 (0%)	1	107 (95.5%)	5 (4.5%)	1
Yes	122	114 (93.4%)	8 (6.6%)	0 (0%)	0.414	236 (96.7%)	8 (3.3%)	0.424

Statistical analysis was performed by a Fisher's exact test (vs. control).

TABLE II. Genotype and Allele Frequencies of the BDNF 196G > A (val66met) (in Exon XIIIA) Gene Polymorphism in Controls and MAP Abusers

		Genotype				Allele		
196G > A (val66met)	n	GG	GA	AA	*P*	G	A	*P*
Control	202	70 (34.7%)	107 (53.0%)	25 (12.4%)		247 (61.1%)	157 (38.9%)	
Abuser	189	70 (37.0%)	96 (50.8%)	23 (12.2%)	0.889	236 (62.4%)	142 (37.6%)	0.713
Prognosis of psychosis								
Transient	94	32 (34.0%)	53 (56.4%)	9 (9.6%)	0.778	117 (62.2%)	71 (37.8%)	0.856
Prolonged	66	25 (37.9%)	30 (45.5%)	11 (16.7%)	0.472	80 (60.6%)	52 (39.4%)	0.918
Spontaneous relapse								
Positive	64	27 (42.2%)	30 (46.9%)	7 (10.9%)	0.571	84 (65.6%)	44 (34.4%)	0.403
Negative	116	39 (33.6%)	62 (53.4%)	15 (12.9%)	0.972	140 (60.3%)	92 (39.7%)	0.866
Poly-substance abuse								
No	56	21 (37.5%)	27 (48.2%)	8 (14.3%)	0.791	69 (61.6%)	43 (38.4%)	1
Yes	122	47 (38.5%)	60 (49.2%)	15 (12.3%)	0.762	154 (63.1%)	90 (36.9%)	0.676

Statistical analysis was performed by a Fisher's exact test (vs. control).

significantly different between transient type of psychosis and prolonged type of psychosis (Tables I and II). Furthermore, the genotypic and allelic distribution of two SNPs was not significantly different between positive spontaneous relapse and negative spontaneous relapse (Tables I and II). Moreover, the genotypic and allelic distribution of two SNPs was not significantly different between poly-substance abuse and non-poly-substance abuse (Tables I and II). In addition, we found that two SNPs were not in linkage disequilibrium with each other.

DISCUSSION

The present study suggests that two SNPs (132C > T in exon V and 196G > A (val66met) in exon XIIIA) of the *BDNF* gene may not be susceptible to MAP abuse in Japanese samples. Using a European American sample and an African American sample, it has been reported that the *BDNF* gene could contribute to vulnerabilities to poly-substance abuse [Uhl et al., 2001]. It is possible that difference in ethnicity might contribute to discrepancy between our study and other study. Frequency of A allele of 196G > A (val66met) in Japanese population [Momose et al., 2002; Nakata et al., 2003; Itoh et al., 2004; this study] is higher than that of Caucasian population [Egan et al., 2003; Hakansson et al., 2003; Sen et al., 2003], suggesting the ethnic difference in this SNP (val66met) [Shimizu et al., 2004]. First, it has been reported that the 196G > A (val66met) of the *BDNF* gene is associated with Parkinson's disease in Japanese subjects [Momose et al., 2002]. However, lack of association between the BDNF 196G > A (val66met) and Parkinson's disease in a Swedish population was reported [Hakansson et al., 2003]. Second, it has been reported that the 196G > A (val66met) of the *BDNF* gene is associated with bipolar disorder in Caucasian [Neves-Pereira et al., 2002; Sklar et al., 2002]. However, no association between 196G > A (val66met) of the *BDNF* gene and bipolar disorder in Japanese population was detected [Nakata et al., 2003], suggesting that the *BDNF* gene may confer a susceptibility to bipolar disorder in Caucasian, but not in Japanese population. Thus, it is likely that ethnic differences may contribute to inconsistent findings between Caucasian sample and Japanese sample.

In this study, we investigated two SNPs; one (132C > T in exon V) in the noncoding region and the other (196G > A (val66met) in exon XIIIA) in the coding region. Whereas BDNF 196G > A (val66met) SNP does not affect the function of a mature BDNF protein, it has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF, and, thus, the regulated secretion of the mature BDNF protein [Egan et al., 2003]. At cellular levels, marked deficits were observed in the intracellular distribution, processing, and secretion of met-BDNF, suggesting that pro-BDNF may play a critical role in synaptic targeting and activity-dependent secretion at synapses [Egan et al., 2003]. Remarkably, healthy human subjects with the met allele exhibit impaired hippocampal activity and memory function [Egan et al., 2003]. However, it is currently unknown whether the BDNF 132C > T SNP could affect on the function, synthesis, or secretion of BDNF. There are still other known SNPs in the *BDNF* gene sequences, and it is possible that there are more unknown SNPs. Further studies of other SNPs and unknown SNPs should be done to clarify the involvement of the *BDNF* gene in substance abuse vulnerability.

In conclusion, we failed to detect evidence for a role of two SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of the *BDNF* gene in the pathogenesis of MAP abusers in our Japanese sample. Therefore, it is unlikely that the two SNPs (196G > A (val66met) in exon XIIIA and 132C > T in exon V) of *BDNF* gene are associated with Japanese MAP abusers.

REFERENCES

Blochl A, Sirrenberg C. 1996. Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and $p75^{Lntr}$ receptors. J Biol Chem 271:21100–21107.

Dluzen DE, Gao X, Story GM, Anderson LI, Kucera J, Walro JM. 2001. Evaluation of nigrostriatal dopaminergic function in adult +/+ and +/− BDNF mutant mice. Exp Neurol 170:121–128.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269.

Hakansson A, Melke J, Westberg L, Shahabi HN, Buervenich S, Carmine A, Klingborg K, Grundell MB, Schulhof B, Holmberg B, Ahlberg J, Eriksson E, Sydow O, Olson L, Johnels B, Nissbrandt H. 2003. Lack of association between the BDNF Val66Met polymorphism and Parkinson's disease in a Swedish population. Ann Neurol 53:823.

Hashimoto K, Shimizu E, Iyo M. 2004. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res Rev 45:104–114.

Hoger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. 1999. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 19:4110–4122.

Howard MO, Kivlahan D, Walker RD. 1997. Cloninger's tridimensional theory of personality and psychopathology: Applications to substance use disorders. J Stud Alcohol 58:48–66.

Hyman C, Hofer M, Barde YA, Jahasz M, Yancopoulos GD, Squinto SP, Lindsay RM. 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232.

Itoh K, Hashimoto K, Kumakiri C, Shimizu E, Iyo M. 2004. Association between brain-derived neurotrophic factor 196 G/A polymorphism and

personality traits in healthy subjects. Am J Med Genet (Neuropsychiatr Genet) 124B:61–63.

Kendler KS. 2001. Twin studies of psychiatric illness: An update. Arch Gen Psychiatry 58:1005–1014.

Krebs MO, Guillin O, Bourdell MC, Schwartz JC, Olie JP, Poirier MF, 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.

Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff KA, Gray N, Zarate CA Jr, Charney DS. 2003. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biol Psychiatry 53:707–842.

Mattson MP, Duan W, Guo Z. 2003. Meal size and frequency affect neuronal plasticity and vulnerability to disease: Cellular and molecular mechanisms. J Neurochem 84:417–431.

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ. 1998. Familial transmission of substance use disorders. Arch Gen Psychiatry 55:973–979.

Momose Y, Murata M, Kobayashi K, Tachikawa M, Nakabayashi Y, Kanazawa I, Toda T. 2002. Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. Ann Neurol 51:133–136.

Nakata K, Ujike H, Sakai A, Uchida N, Nomura A, Imamura T, Katsu T, Tanaka Y, Hamamura T, Kuroda S. 2003. Association study of the brain-derived neurotrophic factor (*BDNF*) gene with bipolar disorder. Neurosci Lett 337:17–20.

Narita M, Aoki K, Takagi M, Yajima Y, Suzuki T. 2003. Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. Neuroscience 119:767–775.

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: Evidence from a family-based association study. Am J Hum Genet 71:651–655.

Nordahl TE, Salo R, Leamon M. 2003. Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: A review. J Neuropsychiatry Clin Neurosci 15:317–325.

Sato M, Numachi Y, Hamamura T. 1992. Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. Schizophr Bull 18:115–122.

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M. 2003. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28:397–401.

Shimizu E, Hashimoto K, Iyo M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: The possibility to explain ethnic mental traits. Am J Med Genet (Neuropsychiatr Genet) 126B: 122–123.

Shintani A, Ono Y, Kaisho Y, Igarashi K. 1992. Characterization of the 5′-flanking region of the human brain-derived neurotrophic factor gene. Biochem Biophys Res Commun 182:325–332.

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES. 2002. 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.

Spina MB, Squinto SP, Miller J, Lindsay RM, Hyman C. 1992. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and *N*-methyl-4-phenyl-pyridinium ion toxicity: Involvement of the glutathione system. J Neurochem 59:99–106.

Szekeres G, Juhasz A, Rimanoczy A, Keri S, Janka Z. 2003. The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. Schizophr Res 65:15–18.

Tsuang MT, Bar JL, Harley RM, Lyons MJ. 2001. The Harvard twin study of substance abuse: What we have learned. Harv Rev Psychiatry 9:267–279.

Uhl GR, Liu QR, Walther D, Hess J, Naiman D. 2001. Polysubstance abuse-vulnerability genes: Genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 69:1290–1300.

Ujike H. 2002. Stimulant-induced psychosis and schizophrenia: The role of sensitization. Curr Psychiatry Rep 4:177–184.

BRIEF REPORT

Association of AKT1 with Schizophrenia Confirmed in a Japanese Population

Masashi Ikeda, Nakao Iwata, Tatsuyo Suzuki, Tsuyoshi Kitajima, Yoshio Yamanouchi, Yoko Kinoshita, Toshiya Inada, and Norio Ozaki

Background: *Abnormality of the V-akt murine thymoma viral oncogene homologue 1 (AKT1) may be a predisposing factor in schizophrenia. Recent evidence supporting this hypothesis showed decreased AKT1 protein levels in patients with schizophrenia and significant association of AKT1 haplotypes according to the transmission disequilibrium test.*

Methods: *We provide the first replication of this evidence using a relatively large case–control sample (507 Japanese schizophrenia and 437 control subjects). We genotyped five single nucleotide polymorphisms (SNPs) from the original study and one additional SNP.*

Results: *We found a positive association with an SNP (SNP5) different from the original study's findings (SNP3) and also significance in the haplotypes constructed from the combination of SNP5. Linkage disequilibrium around SNP5 was complex and may produce this positive association.*

Conclusions: *Our study provides support for the theory that AKT1 is a susceptibility gene for Japanese schizophrenia. Fine linkage disequilibrium mapping is required for a conclusive result.*

Key Words: Haplotypes, linkage disequilibrium, multiple testing, single nucleotide polymorphism

V-akt murine thymoma viral oncogene homologue 1 (AKT1)–glycogen synthase kinase 3β (GSK3β) signaling has a fundamental role in regulating cellular functions (Grimes and Jope 2001). Recently, several lines of evidence have suggested that AKT1 has important roles in neurodevelopment (Wang et al 2003) and in working memory formation (Mizuno et al 2003), abnormalities of which are hypothesized to be susceptibility factors in schizophrenia. A recent study reported decreased AKT1 protein levels and phosphorylation of GSK3β at Ser9 in peripheral lymphocytes and brains in schizophrenia, as well as a significant association between schizophrenia and AKT1 haplotypes according to the transmission disequilibrium test (Emamian et al 2004). We provide a replication of the evidence for AKT1 association with schizophrenia using a Japanese case–control sample.

Methods and Materials

Single Nucleotide Polymorphism (SNP) Genotyping

We obtained the SNP information described by Emamian et al (2004) through personal communication with the authors. We also obtained information for an additional SNP (SNP A: rs2498804), selected from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/; Figure 1). After polymerase chain reaction (PCR) amplification, restriction fragment length polymorphism (RFLP) assays were developed; BsaI for SNP1 (rs3803300), XcmI for SNP2 (rs1130214), HaeIII for SNP3 (rs3730358), HpyCH4IV for SNP4 (rs2498799), PstI for SNP5 (rs2494732), and BsiHKAI for SNP A. Information about primer sequences and PCR-RFLP conditions are available on request.

Subjects

Genotypes were determined for 507 patients with schizophrenia (265 men and 242 women; mean age 45.7 ± 14.9 years) and 437 controls (209 men and 228 women; mean age 34.2 ± 13.6 years). A general characterization and psychiatric assessment of the subjects is available elsewhere (Suzuki et al 2003). After description of the study, written informed consent was obtained from each subject. This study was approved by ethics committee at Fujita Health University and Nagoya University School of Medicine.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by conventional chi-square test. To evaluate pairwise linkage disequilibrium (LD) matrices between each SNP and to examine the LD block structure, version 2.05 of the HAPLOVIEW program was used (developed in Mark Daly's laboratory at the Massachusetts Institute of Technology; http://www.broad.mit.edu/personal/jcbarret/haploview/index.php; Gabriel et al 2002). Marker–trait association analysis was performed with a program COCAPHASE 2.403 (Dudbridge 2003). This program performs log-likelihood ratio tests under a log-linear model for a global p value. To estimate haplotype frequencies, the expectation-maximization (EM) algorithm was used. For haplotypic analyses, we calculated global p values in multi-SNP haplotype systems to replicate the original study. In both haplotypic and single-marker allelewise analysis, we performed the permutation procedure for each 1- to 5-marker in sliding-window fashion without the EM algorithm. We emphasized the permutation p values compared with each global p value (except complete 6-marker global p value) because this permutation procedure gives a significance corrected for the multiple haplotypes and markers tested. We also corrected these permutation p

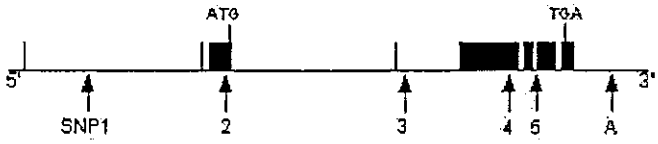

Figure 1. Genomic structure and single nucleotide polymorphisms (SNPs) of the human V-akt murine thymoma viral oncogene homologue 1 (AKT1) and positions of the SNPs.

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Table 1. Pairwise Linkage Disequilibrium in AKT1

	SNP1	SNP2	SNP3	SNP4	SNP5	SNPA	Distance to Next SNP (bp)
SNP1		.93 (.84–.98)[b]	.15 (.01–.38)	.33 (.23–.41)	.20 (.10–.29)	.28 (.19–.36)	—
SNP2	83 (.70–.91)[a]		.61 (.15–.85)	.09 (.00–.30)	.62 (.42–.77)	.05 (.00–.27)	10045
SNP3	.13 (.01–.46)	1.0 (.21–.99)[a]		.68 (.49–.81)	.70 (.43–.85)	.68 (.46–.82)	13327
SNP4	.40 (.31–.48)	.34 (.15–.50)	.85 (.68–.94)[a]		.86 (.79–.91)[a]	.93 (.89–.96)[a]	6513
SNP5	.20 (.08–.32)	.79 (.52–.92)[a]	.79 (.44–.93)[a]	.86 (.78–.92)[a]		.92 (.87–.96)[a]	702
SNPA	.35 (.27–.43)	.24 (.09–.39)	.82 (.61–.92)[a]	.99 (.96–1.0)[b]	.89 (.83–.94)[a]		6097

SNP, single nucleotide polymorphism. Numbers of upper diagonal are D' (95% confidence interval) of schizophrenia subjects; numbers of lower diagonal are D' of control subjects. Details are available on the HAPLOVIEW Web site: http://www.broad.mit.edu/personal/jcbarret/haploview/index:php

[a]Uninformative linkage disequilibrium.

[b]Strong evidence of linkage disequilibrium.

values by Bonferroni correction to obtain more robust results. We performed 10,000 permutations in each procedure. To detect risk haplotypes, we performed the individual procedure in this program. The significance level for all statistical tests was .05.

Results

The genotype frequencies of all SNPs were in Hardy–Weinberg equilibrium. Pairwise LD is shown in Table 1. By single marker association analyses and after adjustment by permutation procedure, we found significant association using the five original SNPs (p = .019). Assessing the component of this significance, SNP5 was associated with schizophrenia (p = .0037). Taking into account this positive result and the LD patterns, we tested an additional SNP (SNP A), located downstream of SNP5, for further assessment; a positive permutation p value was again obtained (p = .021; Table 2).

By haplotypic association analyses with additional SNP A, all permutation p values were significant. After Bonferroni correction among single-marker permutation (one) and haplotype combinations (five), the positive p values from haplotypic analyses of the 2- and 3-marker window remained significant (Table 2).

Individual haplotypic analyses of 2- and 3-markers with positive global p values are shown in Table 3. The most significant haplotype was more frequent in control subjects than in cases (SNP3-4-5 C-G-A; p = .00011).

Discussion

Our replication study using a relatively large Japanese sample supports a previous finding of a positive association between AKT1 and schizophrenia. Because we considered the correction of multiple markers by permutation and Bonferroni correction, the results of this study are robust. Aside from this permutation correction, in the worst case for multiple testing by Bonferroni correction alone, the lowest global p value of SNP4-5 remains significant even after 94 corrections. On the other hand, the criteria for the correction among global and individual haplotypic analysis is not well established. Considering that individual haplotypic analysis is another hypothesis from multi-SNP haplotype systems (Van Den Bogaert et al 2003), we did not perform correction in individual haplotypic analysis. Although we applied Bonferroni correction independently from global haplotypic analyses, the lowest p value (SNP3-4-5 C-G-A) could withstand 450 corrections.

Through individual haplotypic analyses, we observed negatively associated haplotypes (e.g., SNP4-5 G-A, SNP3-4-5 C-G-A) and positively associated haplotypes (e.g., SNP4-5 G-G, SNP3-4-5

Table 2. Association Analyses of the AKT1 gene

	Multi-SNP Haplotype Systems[a]						Genotypic Distribution					
							M/M		M/m		m/m	
SNP ID	1SNP	2SNP	3SNP	4SNP	5SNP	6SNP	SCZ	CON	SCZ	CON	SCZ	CON
SNP1(G > A)	.61						163	124	245	234	99	79
		.35										
SNP2(G > T)	.48		.19				372	315	124	108	11	14
		.39		.14								
SNP3(C > T)	.26		.56		.0083		408	364	92	68	7	5
		.58		.037		.053						
SNP4(G > A)	.59		.0017		.032		124	121	265	211	118	105
		.00053		.0062								
SNP5(A > G)	.0037		.00091				208	212	235	192	64	33
		.0010										
SNPA(T > G)	.29						136	142	272	206	99	89
Permutation p value[b]	.021 (.13)	.004 (.024)	.0048 (.029)	.023 (.14)	.025 (.15)							

M, major allele; m, minor allele; SCH, schizophrenia patients; CON, control subjects.

[a]p values were calculated by log-likelihood ratio test (1SNP; allelewise association; SNP2-6; global haplotypic association).

[b]Parenthetic numbers represent p-values after Bonferroni correction.

Table 3. Haplotype Frequencies from Positive Permutation Analyses

SNPs	Marker Haplotype	SCZ	CON	*p* Values
SNP4-5	A-A	.47	.46	ns
	G-G	.33	.28	.0076
	G-A	.17	.24	.00020
	A-G	.025	.020	ns
SNP5-A	A-T	.52	.54	ns
	G-G	.34	.28	.0024
	A-G	.12	.16	.0091
	G-T	.015	.018	ns
SNP3-4-5	C-A-A	.38	.38	ns
	C-G-G	.32	.27	.014
	C-G-A	.17	.24	.00011
	T-A-A	.084	.079	ns
	C-A-G	.022	.017	ns
SNP4-5-A	A-A-T	.47	.46	ns
	G-G-G	.33	.28	.0012
	G-A-G	.12	.16	.0056
	G-A-T	.055	.079	.047
	A-G-T	.011	.018	ns

CON, control subjects; SCZ, schizophrenia subjects; SNPs, single nucleotide polymorphisms.

C-G-G). These distributions of protective or risk haplotypes might be derived from the accompaniment of SNP5 because the negatively associated haplotypes always involved the A allele of SNP5 and the positively associated haplotypes always involved the G allele. Here, a trend for significance for SNP5 and for haplotypes including SNP5 was key for interpreting a positive association. Interestingly, LD patterns around SNP5 were complex. The LD matrices among SNP4, SNP5, and SNP A of schizophrenia subjects were slightly different from those of control subjects. In patients, each matrix among SNP4, SNP5, and SNP A revealed an uninformative LD that did not meet the HAPLOVIEW program criteria for strong evidence of LD or recombination; in control subjects, however, SNP5 fit the criterion for a "hole" as defined by Wall and Pritchard (2003a). In other words, an LD block was not defined around SNP5 in cases. On the other hand, although the LD block between SNP4 and SNP A seemed to be detected, SNP5 disturbed this virtual LD block structure. Therefore, we consider the possibility that neither SNP4 nor SNP A is perfectly representative of SNP5. These complex LD patterns might be one of the reasons we obtained a positive result from SNP5 but not from the other SNPs in tight LD with SNP5.

Another explanation could be the unsatisfactory features of D' by which we evaluated the LD (North et al 2004; Wall and Pritchard 2003b). Van der Walt et al (2004) reported that the potential for allelic heterogeneity might result in similar fashion. Considering these factors, SNP5 could be a predisposing factor related to the splicing process (Mukai et al 2004) or in perfect LD with a "true" predisposing SNP, and haplotypes from the combination of SNP5 would be in allelic heterogeneity (Schork et al 2001) in Japanese people.

Our positive associations from SNP5, as opposed to SNP3 as found by Emamian et al (2004) might be explained by the different study populations. A recent study suggests that predisposing SNPs or risk haplotypes were different among various populations (Panguluri et al 2004). Thus, it is possible that there are different significant associations with different SNPs among various populations.

Three cautions must be exercised in interpreting our results. First, we did not perform a systematic mutation search using large Japanese schizophrenia samples. Second, other candidate genes of the AKT1-GSK3β signaling cascade must be studied in considering locus heterogeneity (Schork et al 2001). Third, our positive results might be derived from sample bias because of population stratification and non-age-matched samples. Further biological study for the function of SNP5 is required, as is very fine LD mapping to arrive at a conclusive result and avoid overlooking associations.

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

Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA (2004): Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet* 36:131–137.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al (2002): The structure of haplotype blocks in the human genome. *Science* 296:2225–2229.

Grimes CA, Jope RS (2001): The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* 65:391–426.

Mizuno M, Yamada K, Takei N, Tran MH, He J, Nakajima A, et al (2003): Phosphatidylinositol 3-kinase: A molecule mediating BDNF-dependent spatial memory formation. *Mol Psychiatry* 8:217–224.

Mukai J, Liu H, Burt RA, Swor DE, Lai WS, Karayiorgou M, et al (2004): Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. *Nat Genet* 36:725–731.

North BV, Curtis D, Martin ER, Lai EH, Roses AD, Sham PC (2004): Further investigation of linkage disequilibrium SNPs and their ability to identify associated susceptibility loci. *Ann Hum Genet* 68:240–248.

Panguluri RC, Long LO, Chen W, Wang S, Coulibaly A, Ukoli F, et al (2004): COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis* 25:961–966.

Schork NJ, Fallin D, Thiel B, Xu X, Broeckel U, Jacob HJ, et al (2001): The future of genetic case–control studies. *Adv Genet* 42:191–212.

Suzuki T, Iwata N, Kitamura Y, Kitajima T, Yamanouchi Y, Ikeda M, et al (2003): Association of a haplotype in the serotonin 5-HT4 receptor gene (HTR4) with Japanese schizophrenia. *Am J Med Genet* 121B:7–13.

van Den Bogaert A, Schumacher J, Schulze TG, Otte AC, Ohlruan S, Kovalenko S, et al (2003): The DTNBP1 (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. *Am J Hum Genet* 73:1438–1443.

van der Walt JM, Noureddine MA, Kittappa R, Hauser MA, Scott WK, McKay R, et al (2004): Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. *Am J Hum Genet* 74:1121–1127.

Wall JD, Pritchard JK (2003a): Assessing the performance of the haplotype block model of linkage disequilibrium. *Am J Hum Genet* 73:502–515.

Wall JD, Pritchard JK (2003b): Haplotype blocks and linkage disequilibrium in the human genome. *Nat Rev Genet* 4:587–597.

Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, et al (2003): Control of synaptic strength, a novel function of Akt. *Neuron* 38:915–928.

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Journal of Neural Transmission

Association analysis of the −308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-α) gene in Japanese patients with schizophrenia

Short Communication

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Summary. Two research groups have thus far reported a significant association between schizophrenia and a promoter polymorphism (−308G>A) of the gene encoding tumor necrosis factor alpha (TNF-α), while contradictive negative results have also been reported. We examined the possible association in a Japanese sample of 297 schizophrenia cases and 458 controls. Allele frequencies of both the patients and controls were very low (1.5% and 0.8%, respectively), and the difference was not statistically significant. We conclude that the effect of the −308G>A polymorphism on the development of schizophrenia is, if any, weak and the majority of Japanese schizophrenics are unrelated to the −308G>A polymorphism of the TNF-α gene.

Keywords: Schizophrenia, tumor necrosis factor α, association study, genetics, single nucleotide polymorphism (SNP), promoter.

Introduction

Schizophrenia is a complex disorder characterized by profound disturbances of perception, thought, cognition, emotion and social functioning. It affects

approximately 0.5–1% of the general population worldwide. The pathogenesis of schizophrenia is unclear; however, immune alterations in schizophrenia have been described for decades (for review; Muller et al., 2000). Tumor necrosis factor alpha (TNF-α) is one of the major factors playing a central role in immune systems. As TNF-α serum levels were significantly higher in schizophrenic patients compared with healthy controls (Naudin et al., 1997; Erbagci et al., 2001; Kowalski et al., 2001; Theodoropoulou et al., 2001), abnormal production of TNF-α could increase the risk of developing schizophrenia. The level of TNF-α production is under genetic control and determined in part by alleles of a single nucleotide polymorphism (SNP), −308G>A, in the promoter region of the TNF-α gene (Wilson et al., 1997). Furthermore, the TNF-α gene is located on chromosome 6p21.1–21.3 where linkage and association studies have suggested a locus of susceptibility to schizophrenia (Wright et al., 2001).

Recently, Boin et al. (2001) found a significant association between the −308G>A polymorphism of the TNF-α gene and schizophrenia in an Italian population. Subsequently, Meira-Lima et al. (2003) obtained evidence supporting this association in a Brazilian sample; whilst other research groups from Germany, Australia, and Korea failed to find such an association (Riedel et al., 2002; Handoko et al., 2003; Pae et al., 2003). This inconsistency requires further investigations. Here we examined the −308G>A polymorphism of the TNF-α gene for allelic association with schizophrenia in a Japanese sample.

Materials and methods

Subjects

Subjects were 297 patients (164 males, mean age: 43.7 years [SD 14.1]) with schizophrenia and 458 healthy controls (235 males, 36.4 years [12.6]). All the subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria. The controls were not assessed with any structured psychiatric interviews; however, they showed good social functioning (i.e. no history of long-term absence from school or work) and reported themselves to be in good health. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees (Fujita Health University School of Medicine, Showa University School of Medicine, and National Center of Neurology and Psychiatry).

Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Genotypes for the −308G>A polymorphism were determined using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2003). Two fluorescent dyes, a reporter and a quencher, are attached to the probes used with the TaqMan PCR Reagent Kit. When both dyes are attached to the probe, reporter dye emission is quenched. During each extension cycle, Taq DNA polymerase cleaves the reporter dye from the probe via its 5′–3′ exonuclease. Once separated from the quencher, the reporter dye emits its characteristic fluorescence which can then be measured by the ABI PRISM7000 (Applied Biosystems, Japan). The amount of fluorescence measured is proportional to the amount of PCR product made.

Primers and probes for detection of the SNP are: 5′-GGACCCTGGAGGCTGAAC-3′ (forward primer), 5′-CCAAAAGAAATGGAGGCAATAGGTT-3′ (reverse primer), 5′-VIC-CCCGTCCCCATGCC-MGB-3′ (probe 1), and 5′-FAM-CCCGTCCTCATGCC-MGB-3′ (probe 2). Thermal cycling conditions for polymerase chain reaction were: at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute.

Statistical analysis

The presence of Hardy-Weinberg equilibrium was examined by the χ^2 test for goodness of fit. Genotype and allele distributions between patients and controls were analyzed by the χ^2 test for independence. The critical p-value was set at 0.05 (two-tail).

Results

Genotype distributions and allele frequencies of the −308G>A polymorphism of the TNF-α gene among the patients and controls are shown in Table 1. The genotype distributions for the two groups were both in Hardy-Weinberg equilibrium (patients: $\chi^2=0.07$, df=1, p=0.79; controls: $\chi^2=0.03$, df=1, p=0.87). We found that the minor allele (−308A) was rare in our Japanese sample (0.8% in the controls), which was substantially different from that (6∼7%) reported in a Korean sample (Pae et al., 2003). There was no individual homozygous for the minor allele among the patients and controls. Although the frequency of the minor allele was a bit increased in the schizophrenics compared to the controls, the difference in genotype distribution (proportion of heterozygous individuals) or allele frequency between the two groups was not statistically significant (genotype: $\chi^2=2.0$, df=1, p=0.16; allele: $\chi^2=1.9$, df=1, p=0.16).

Discussion

We failed to find a statistically significant association between the −308G>A polymorphism of the TNF-α gene and schizophrenia in our Japanese sample, suggesting that the examined polymorphism has no major role in the pathogenesis of schizophrenia. However, a possibility remains that we may have yielded a false negative result due to an inadequate statistical power resulting from the very low frequency of the minor allele (−308A) in our Japanese

Table 1. Genotype and allele frequencies of the −308G>A polymorphism of the tumor necrosis factor alpha gene among the patients with schizophrenia and controls

	Genotype distribution				Allele frequency			Odds ratio	95% CI
	N	G/G	G/A	A/A	N	G	A		
Patients	297	288 (97.0%)	9 (3.0%)	0 (0%)	594	585 (98.5%)	9 (1.5%)	2.03	0.74–6.54
Controls	458	451 (98.5%)	7 (1.5%)	0 (0%)	916	909 (99.2%)	7 (0.8%)		

sample (approximately 1%), since the frequency of the −308A allele was somewhat increased in the schizophrenics (1.5%) than in the controls (0.8%). The obtained odds ratio (2.03) in the current study was similar to that reported by Boin et al. (2001) (2.24). When power analysis was performed, an extraordinarily large sample (approximately 1500 cases and 1500 controls) is required for Japanese to detect a significant association between the risk allele (−308A) and schizophrenia with a power of 90% when odds ratio was assumed to be 2.0 and the critical p-value was set at 0.05. Thus multi-center study or meta-analysis of multiple data would be required to draw any conclusion. However, even if such an association exists, the minor allele was very rare, and accordingly the population attributable risk for the possible association was estimated to be less than 0.01. We conclude that the effect of the −308G>A polymorphism on the development of schizophrenia is, if any, weak and the majority of Japanese schizophrenics are unrelated to the polymorphism of the TNF-α gene.

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References

Boin F, Zanardini R, Pioli R, Altamura CA, Maes M, Gennarelli M (2001) Association between −G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. Mol Psychiatry 6: 79–82

Erbagci AB, Herken H, Koyluoglu O, Yilmaz N, Tarakcioglu M (2001) Serum IL-1beta, sIL-2R, IL-6, IL-8 and TNF-alpha in schizophrenic patients, relation with symptomatology and responsiveness to risperidone treatment. Mediators Inflamm 10: 109–115

Handoko HY, Nancarrow DJ, Hayward NK, Ohaeri JU, Aghanwa H, McGrath JJ, Levinson DF, Johns C, Walters MK, Nertney DA, Srinivasan TN, Thara R, Mowry BJ (2003) Tumor necrosis factor haplotype analysis amongst schizophrenia probands from four distinct populations in the Asia-Pacific region. Am J Med Genet 121B: 1–6

Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR (2003) Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatry (in press)

Kowalski J, Blada P, Kucia K, Madej A, Herman ZS (2001) Neuroleptics normalize increased release of interleukin-1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. Schizophr Res 50: 169–175

Meira-Lima IV, Pereira AC, Mota GF, Floriano M, Araujo F, Mansur AJ, Krieger JE, Vallada H (2003) Analysis of a polymorphism in the promoter region of the tumor necrosis factor alpha gene in schizophrenia and bipolar disorder: further support for an association with schizophrenia. Mol Psychiatry 8: 718–720

Muller N, Riedel M, Gruber R, Ackenheil M, Schwarz MJ (2000) The immune system and schizophrenia. An integrative view. Ann NY Acad Sci 917: 456–467

Naudin J, Capo C, Giusano B, Mege JL, Azorin JM (1997) A differential role for interleukin-6 and tumor necrosis factor-alpha in schizophrenia? Schizophr Res 26: 227–233

Pae CU, Chae JH, Bahk WM, Han H, Jun TY, Kim KS, Kwon YS, Serretti A (2003) Tumor necrosis factor-alpha gene polymorphism at position −308 and schizophrenia in the Korean population. Psychiatry Clin Neurosci 57: 399–403

Riedel M, Kronig H, Schwarz MJ, Engel RR, Kuhn KU, Sikorski C, Sokullu S, Ackenheil M, Moller HJ, Muller N (2002) No association between the G308A polymorphism of the tumor necrosis factor-alpha gene and schizophrenia. Eur Arch Psychiatry Clin Neurosci 252: 232–234

Theodoropoulou S, Spanakos G, Baxevanis CN, Economou M, Gritzapis AD, Papamichail MP, Stefanis CN (2001) Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr Res 47: 13–25

Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94: 3195–3199

Wright P, Nimgaonkar VL, Donaldson PT, Murray RM (2001) Schizophrenia and HLA: a review. Schizophr Res 47: 1–12

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Association study of polymorphisms in the excitatory amino acid transporter 2 gene (*SLC1A2*) with schizophrenia

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Abstract

Background: The glutamatergic dysfunction hypothesis of schizophrenia suggests that genes involved in glutametergic transmission are candidates for schizophrenic susceptibility genes. We have been performing systematic association studies of schizophrenia with the glutamate receptor and transporter genes. In this study we report an association study of the excitatory amino acid transporter 2 gene, *SLC1A2* with schizophrenia.

Methods: We genotyped 100 Japanese schizophrenics and 100 controls recruited from the Kyushu area for 11 single nucleotide polymorphism (SNP) markers distributed in the *SLC1A2* region using the direct sequencing and pyrosequencing methods, and examined allele, genotype and haplotype association with schizophrenia.The positive finding observed in the Kyushu samples was re-examined using 100 Japanese schizophrenics and 100 controls recruited from the Aichi area.

Results: We found significant differences in genotype and allele frequencies of SNP2 between cases and controls (P = 0.013 and 0.008, respectively). After Bonferroni corrections, the two significant differences disappeared. We tested haplotype associations for all possible combinations of SNP pairs. SNP2 showed significant haplotype associations with the disease ($P = 9.4 \times 10^{-5}$, P = 0.0052 with Bonferroni correction, at the lowest) in 8 combinations. Moreover, the significant haplotype association of SNP2-SNP7 was replicated in the cumulative analysis of our two sample sets.

Conclusion: We concluded that at least one susceptibility locus for schizophrenia is probably located within or nearby *SLC1A2* in the Japanese population.

Background

Schizophrenia is a severe mental disorder characterized by hallucinations, delusions, disorganized thoughts, and various cognitive impairments. The life-time prevalence is about 1%, and genetic factors were known to play a critical role in its pathogenesis [1]. Based on the fact that

phencyclidine (PCP) induces schizophreniform psychosis, a glutamatergic dysfunction hypothesis has been proposed for the pathogenesis of schizophrenia [2-4]. This hypothesis has been supported by recent multiple reports of association of schizophrenia with glutamate receptor genes and with the genes related to glutamatergic transmission, such as *G72* and *NRG1* [5-10].

In addition, other synaptic elements related to glutamate, such as excitatory amino acid transporters (EAATs), also potentially affect glutamatergic neurotransmission. EAATs maintain extracellular glutamate concentrations within physiological levels by reuptaking the synaptically released glutamate. A deficient uptake has been implicated in the pathogenesis of ischemic brain damage [11] and may be involved in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) [12]. Recently significant increases of mRNA expression of EAAT1 and EAAT2 have been reported in the thalamus of schizophrenics, suggesting the possibility that an excessive glutamate uptake is involved in schizophrenia [13]. On the other hand, a significant decrease of EAAT2 mRNA expression was observed in the parahippocampal gyrus of schizophrenics [14]. Therefore the EAAT genes are reasonable candidates for schizophrenia, as well as glutamate receptor genes.

The EAATs family consists of five members (EAAT1-EAAT5). Their cellular localizations are different: EAAT1 and EAAT2 are astroglial, whereas EAAT3 EAAT4 and EAAT5 are neuronal [25]. Since EAAT2 accounts for approximately 90% of glutamate reuptake in the rodent forebrain [16,17], we focused on the EAAT2 gene (*SLC1A2*) in association studies of schizophrenia. *SLC1A2* has been mapped to 11p13-p12 [18] and consists of 11 exons spanning over 165 kb. In this study we tested associations of schizophrenia with 11 SNPs distributed in *SLC1A2* with an average interval of 15.9 kb. To enhance the detection power of the study, we also examined the haplotype associations of the SNPs with the disease.

Methods

Human subjects

Blood samples were obtained from unrelated Japanese individuals who had provided written informed consent. We used two Japanese sample sets in this study. In the first one, Kyushu samples, 100 schizophrenia patients (mean age 49.5; 44.0% female) were recruited from hospital in the Fukuoka and Oita areas and 100 healthy unrelated controls (mean age 51.2; 44.0% female) were recruited from the Fukuoka area. In the initial SNP selection process, we used another 16 Japanese samples which are recruited in the Fukuoka area and informed in the same way. In the second one, Aichi samples, 100 schizophrenia patients (mean age 34.4; 44% female) and 100 healthy unrelated controls (mean age 39.9; 45% female) were collected in the Aichi area about 600 km east of Fukuoka. All patients fulfilled the DSM-IV criteria for schizophrenia [19]. All of the case and control samples are ethnically Japanese. DNA samples were purified from whole peripheral blood by the method previously described [20]. This study was approved by the Ethics Committee of Kyushu University, Faculty of Medicine and the Fujita Health University Ethics Committee.

SNP selection in the SLC1A2 region

We retrieved the primary SNP information from the dbSNP database http://www.ncbi.nlm.nih.gov/SNP/. Assuming the same size of the half length of linkage disequilibrium (LD) (60 kb) as reported in Caucasians [21], we initially intended to select common SNPs every 50 kb in *SLC1A2*. We tested 22 candidate SNPs including all of the exonic SNPs, in the 16 healthy Japanese samples by the direct sequencing method. Out of the 22 SNPs we selected the following 7 common SNPs with minor allele frequencies over 10% for further analyses: SNP1, rs1923295; SNP3, rs4534557; SNP6, rs1885343; SNP8, rs752949; SNP9, rs1042113; SNP10, rs3838796; SNP11, rs1570216. We also identified a novel SNP, SNP7, in intron 1 (conting location: 34105026). After the LD analyses described below, we noticed LD gaps ($D' < 0.3$) of the initial SNP set and examined additional 20 candidate SNPs. Out of the 20 SNPs, we selected the following 3 SNPs to fill the LD gaps: SNP2, rs4755404; SNP4, rs4756224; SNP5, rs1923298. The locations of the total 11 SNPs are shown in Figure 1.

Genotyping

Eleven SNPs were amplified as 11 individual fragments by PCR using the primers shown in Table 1 - additional file 1. The reaction mixture for PCR was prepared in a total volume 10 μl with 5 ng of genomic DNA, 10 pmol of each primer (4 pmol of SNP3), 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP and 0.25 U of *Taq* DNA polymerase. An initial denaturing step of 1 min at 94°C was followed by 30, 35 or 40 cycles of 94°C for 30 sec, appropriate annealing temperature for 30 sec and 72°C for 30 sec. A final extension step was carried out at 72°C for 7 min. The nucleotide sequences of each primer, PCR conditions and genotyping methods for each SNP are shown in Table 1 - additional file 1. We genotyped SNP3 by pyrosequencing analysis on a PSQ™96MA Pyrosequencer according to the manufacturer's specifications with a biotinylated reverse primer (5'-CGCCTACTCCTGGTGACTTC-3'), and the sequencing primer (5'-CGCCCCCATGTGT-3'). The other 10 SNPs were genotyped by direct sequencing, as previously described [7]. The raw data of direct sequencing were compiled on PolyPhred [22].

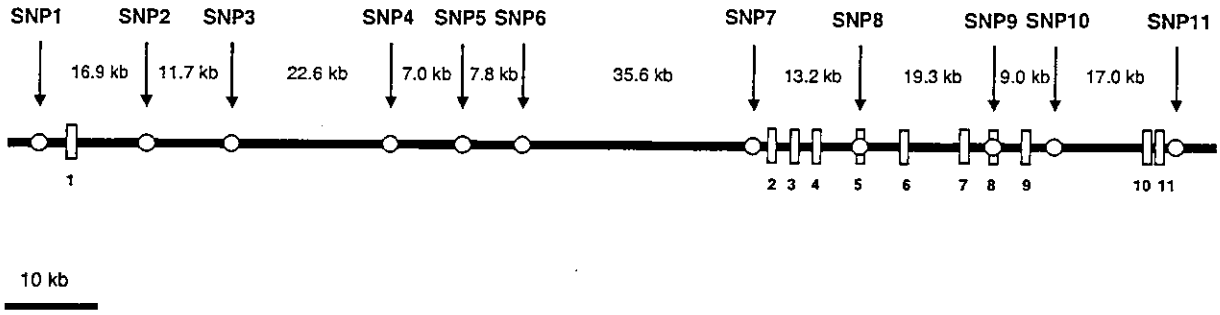

Figure 1
Genomic organization of *SLC1A2* and locations of the SNPs. Exons are shown as vertical bars with exon numbers. Eleven SNPs are indicated by circles. Distances between the SNPs are indicated above with kb.

Statistical analyses

To control genotyping errors, Hardy-Weinberg equilibrium (HWE) was checked in the control samples by the χ^2-test (d.f. = 1). We evaluated the statistical differences in genotype and allele frequencies between cases and controls by the χ^2-test (d.f. = 2) and the Fisher's exact probability test (d.f. = 1), respectively. The magnitude of LD was evaluated in D' and r^2 using the haplotype frequencies estimated by the EH program, version 1.14 [23]. Statistical analysis of the haplotype association was carried out as previously described [24]. The significance level for all statistical tests was 0.05.

Results

Genotyping and SNP association analysis

We selected 11 SNPs at average interval of 15.9 kb to cover the entire *SLC1A2* region with LD as described in Materials and Methods. Table 2 - additional file 2. shows the results of genotype and allele frequencies of SNPs in case and control samples. No significant deviation from HWE in control samples was observed (data not shown). SNP2 showed significant differences in genotype (P = 0.013) and allele (P = 0.008) frequencies between cases and controls. After Bonferroni corrections, these two P values became non-significance levels (P_{corr} = 0.143, P_{corr} = 0.088, respectively).

Pairwise linkage disequilibrium and haplotype association analyses

We compared the magnitude of LD for all possible pairs of the 11 SNPs in controls by calculating D' and r^2 (Table 3 - additional file 3. , upper diagonal), because LD around common alleles can be measured with a modest sample size of 40–50 individuals to a precision equal to 10–20% of the asymptotic limit [19]. We observed relatively strong LD (D' > 0.8) in the seven combinations: SNP4-SNP5 (D' = 0.800), SNP7-SNP8 (D' = 0.877), SNP8-SNP9 (D' = 0.925), SNP4-SNP11 (D' = 0.838), SNP5-SNP11(D' = 0.999), SNP7-SNP11 (D' = 0.816), SNP9-SNP11 (D' = 0.819). Modest LD (D' > 0.4) was observed in the combinations of adjacent SNPs except for SNP5-SNP6 (D' = 0.286) in the control samples. However, modest LD was detected in cases in the SNP5-SNP6 combination (D' = 0.497).

We constructed pairwise haplotypes for all of the 55 possible SNP pairs (Table 3 - additional file 3. , lower diagonal). We observed significant associations with schizophrenia in eight combinations: SNP2-SNP3 (P = 0.0021), SNP2-SNP4 (P = 0.0274), SNP2-SNP5 (P = 0.0054), SNP2-SNP6 (P = 0.0178), SNP2-SNP7 ($P = 9.4 \times 10^{-5}$), SNP2-SNP9 (P = 0.0354), SNP2-SNP10 (P = 0.0089) and SNP2-SNP11 (P = 0.0216). The combination of SNP2-SNP7 was the only one remained significant after Bonferroni correction (P_{corr} = 0.0052).

Cumulative analysis using the second sample set

In this study, we detected significant associations of one haplotype in the *SLC1A2* region with schizophrenia in the Kyushu samples. To confirm the positive finding, we investigated the second Japanese sample set recruited from the Aichi area. Although significant association of the disease was observed with neither genotype, allele frequencies of SNP2 (P = 0.195, P = 0.178, respectively), nor haplotypes of SNP2-SNP7 (P = 0.084) in the second sample set, the significant haplotype association of SNP2-

SNP7 was replicated in the cumulative analysis including the two sample sets ($P = 5.0 \times 10^{-4}$) (Table 4 - additional file 4.).

Discussion

SLC1A2 is located on the chromosomal region of 11p13-p12, to which no evidence has been reported for linkage of schizophrenia, [25,26]. However, there is still a possibility that *SLC1A2* is a candidate for schizophrenia susceptibility genes, because linkage studies could only detect genes with the large genotype relative risk [27]. We carried out the genotyping of 100 cases and 100 controls for 11 SNPs, which were selected to cover the entire *SLC1A2* region with LD. Since minor allele frequencies of each SNP we tested ranges from 0.220 to 0.485, the expected detection power of our case-control study is from 0.89 to 0.94 for the susceptibility gene assuming 2 for genotype relative risk [28].

Modest LD ($D' = 0.925 \sim 0.409$) was observed in the combinations of neighboring SNPs except for SNP5-SNP6 ($D' = 0.286$) in the control samples, suggesting that there may be a recombination hot spot present in the small region (7.8 kb) between the two SNPs (Table 3 - additional file 3.). We plotted the magnitude of LD with the physical distance for each pair of the SNPs, and estimated the average half-length of LD to be 31.8 kb by assuming a linear regression (Fig. 2). This is approximately half of the previously estimated size 60 kb in a United States population of north-European descent [21].

Significant associations of schizophrenia with genotype ($P = 0.013$) and allele ($P = 0.008$) frequencies of SNP2 (rs4755404) were detected (Table 2 - additional file 2.). However, none of these "single-marker" associations survived after Bonferroni corrections. An A-G transition in codon 206, causing a substitution of serine for asparagine, was identified in the exon 5 of *SLC1A2* in a heterozygous sporadic ALS patient [29]. Since located in a putative glycosylation site, the nonsynonymous SNP is potentially involved in the pathophysiology of schizophrenia through affecting the glycosylation status and the transport activity of *SLC1A2* [30]. No occurrence of the G allele of the SNP in 124 Italian schizophrenic and 50 control subjects has been reported [30]. We found also only A allele of the SNP in the 100 controls and 100 cases of the Kyushu samples (data not shown).

In pairwise haplotype association analyses, SNP2 consistently showed significant haplotype associations. The *P* value of the combination SNP2-SNP7 was still significant even after Bonferroni correction ($P = 9.4 \times 10^{-5}$, $P_{corr} = 0.0052$). In our second sample set, the Aichi sample, no significant association of SNP2 was observed in any of the analyses of genotypes, alleles and haplotypes. Cumulative analyses of the two sample sets, however, provide the replication of the significant haplotype association of SNP2-SNP7 with schizophrenia ($P = 5.0 \times 10^{-4}$). The frequency of the G-C haplotype in schizophrenics (26.6%) was notably higher than in controls (5.6%), suggesting that the G-C haplotype may be a risk haplotype for schizophrenia. We observed that the G-C haplotype frequency of schizophrenics (20.0%) was only slightly higher than controls (14.2%) in the Aichi sample, suggesting a less contribution of this locus on schizophrenia pathogenesis in the Aichi sample, although no apparent difference in clinical subtypes between both sample sets studied in this paper. The positive association reported here needs to be validated in larger sample sets, and it would also be worthwhile to search for functional SNPs in the region spanning SNP2-SNP7.

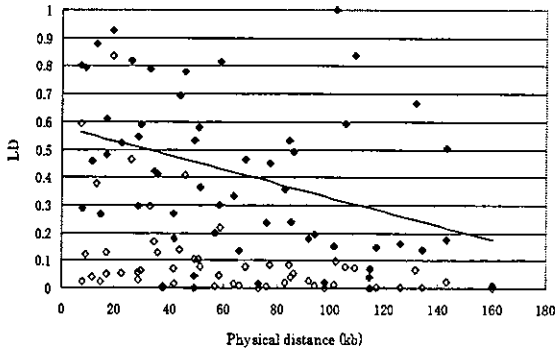

Figure 2
A plot of pairwise linkage disequilibrium (LD) vs. physical distance between the SNPs in the *SLC1A2* region. *D'* were plotted with filled diamonds, and r^2 with open diamonds. From the regression line, the half-length of LD was estimated to be 31.8 kb in the *SLC1A2* region.

Conclusion

We concluded that at least one susceptibility locus for schizophrenia is probably located within or nearby *SLC1A2* in the Japanese population.

Competing interests

None declared.

List of abbreviations used

SNP; single nucleotide polymorphism

DSM-IV; dianostic and statistical manual of mental disorders, 4th edn

PCR; polymerase chain reaction

HWE; Hardy-Weinberg equilibrium

LD; linkage disequilibrium

EAAT; excitatory amino acid transporter

Authors' contributions

XD carried out genotyping, statistical analyses and drafted the manuscript: HS participated in design of this study and statistical analyses: HN, NT, NI and NO participated in collecting specimens and clinical data: YF conceived of the study and participated in its design and coordination.

Additional material

Additional file 1

Table 1

PCR primers for genotyping of SNPs in SLC1A2.

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Additional file 2

Table 2

Genotype and allele frequencies of SNPs in SLC1A2 in Kyushu samples.

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Additional file 3

Table 3

Pairwise linkage disequilibrium and haplotype association in SLC1A2.

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Additional file 4

Table 4

Association analysis of the SNP2-SNP7 haplotype using two sample sets.

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References

1. McGuffin P, Owen MJ, Farmer AE: **Genetic basis of schizophrenia.** *Lancet* 1995, **346**:678-682.
2. Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R: **Study of a new schizophrenomimetic drug, sernyl.** *Arch Neurol Psychiatr* 1959, **81**:363-369.
3. Javitt DC, Zukin SR: **Recent advances in the phencyclidine model of schizophrenia.** *Am J Psychiat* 1991, **148**:1301-1308.
4. Mohn AR, Gainetdinov RR, Caron MG, Koller BH: **Mice with reduced NMDA receptor expression display behaviors related to schizophrenia.** *Cell* 1999, **98**:427-436.
5. Begni S, Popoli M, Moraschi S, Bignotti S, Tura GB, Gennarelli M: **Association between the ionotropic glutamate receptor kainate 3 (*GRIK3*) ser310ala polymorphism and schizophrenia.** *Mol Psychiatr* 2002, **7**:416-418.
6. Begni S, Moraschi S, Bignotti S, Fumagalli F, Rillosi L, Perez J, Gennarelli M: **Association between the G1001C polymorphism in the *GRIN1* gene promoter region and schizophrenia.** *Biol Psychiatr* 2003, **53**:617-619.
7. Makino C, Fujii Y, Kikuta R, Hirata N, Tani A, Shibata A, Ninomiya H, Tashiro N, Shibata H, Fukumaki Y: **Positive association of the AMPA receptor subunit GluR4 gene (*GRIA4*) haplotype with schizophrenia.** *Am J Med Genet* 2003, **116B**:17-22.
8. Fujii Y, Shibata H, Kikuta R, Makino C, Tani A, Hirata N, Shibata A, Ninomiya H, Tashiro N, Fukumaki Y: **Positive associations of polymorphisms in the metabotropic glutamate receptor type 3 gene (*GRM3*) with schizophrenia.** *Psychiatr Genet* 2003, **13**:71-76.
9. Takaki H, Kikuta R, Shibata H, Ninomiya H, Tashiro N, Fukumaki Y: **Positive associations of polymorphisms in the metabotropic glutamate receptor type 8 gene (*GRM8*) with schizophrenia.** *Am J Med Genet* 2004, **128**:6-14.
10. Owen MJ, Williams NM, O'Donovan MC: **The molecular genetics of schizophrenia: new findings promise new insights.** *Mol Psychiatr* 2004, **9**:14-27.
11. Kuwahara O, Mitsumoto Y, Chiba K, Mohri T: **Characterization of D-aspartic acid uptake by rat hippocampal slices and effect of ischemic conditions.** *J Neurochem* 1992, **59**:616-621.
12. Rothstein JD, Kykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF: **Knockout of glutamate transporters reveals a major role for astroglial transport in excitoxicity and clearance of a glutamate.** *Neuron* 1996, **16**:675-686.
13. Smith RE, Haroutunian V, Davis KL, Meador-Woodruff JH: **Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia.** *Am J Psychiat* 2001, **158**:1393-1399.
14. Ohnuma T, Tessler S, Arai H, Faull RL, McKenna PJ, Emson PC: **Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus.** *Mol Brain Res* 2000, **85**:24-31.
15. Gegelashvili G, Schousboe A: **Cellular distribution and kinetic properties of high-affinity glutamate transporters.** *Brain Res Bull* 1998, **45**:233-238.
16. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW: **Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis.** *Ann Neurol* 1995, **38**:73-84.
17. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K: **Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1.** *Science* 1997, **276**:1699-1702.
18. Li X, Francke U: **Assignment of the gene *SLC1A2* coding for the human glutamate transporter EAAT2 to human chromosome 11 bands p13-p12.** *Cytogenet Cell Genet* 1995, **71**:212-213.
19. American Psychiatric Association: *DSM-IV: Diagnostic and Statistical Manual of Mental Disorders* American Psychiatric Press, Washington; 1994.
20. Lahiri DK, Nurnberger JI Jr: **A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies.** *Nucleic Acids Res* 1991, **19**:5444.
21. Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES: **Linkage disquilibrium in the human genome.** *Nature* 2001, **411**:199-204.
22. Nickerson DA, Tobe VO, Taylor SL: **Polyphred: substitutions using fluorescence-based resequencing.** *Nucleic Acids Res* 1997, **25**:2745-2751.

23. Xie X, Ott J: **Testing linkage disequilibrium between a disease gene and marker loci.** *Am J Hum Genet* 1993, **53**:1107.
24. Sham P: *Statistics in Human Genetics* Oxford University Press, New York; 1998.
25. Berry N, Jobanputra V, Pal H: **Molecular genetics of schizophrenia: a critical review.** *J Psychiatr Neurosci* 2003, **28**:415-429.
26. Japanese Schizophrenia Sib-Pair Linkage Group: **Initial genome-wide scan for linkage with schizophrenia in the Japanese Schizophrenia Sib-Pair Linkage Group (JSSLG) families.** *Am J Med Genet* 2003, **120B**:22-28.
27. Risch NJ: **Searching for genetic determinants in the new millennium.** *Nature* 2000, **405**:847-856.
28. Ohashi J, Yamamoto S, Tsuchiya N, Hatta Y, Komata T, Matsushita M, Tokunaga K: **Comparison of statistical power between 2 × 2 allele frequency and positivity tables in case-control studies of complex disease genes.** *Ann Hum Genet* 2001, **65**:197-206.
29. Aoki M, Lin CL, Rothstein JD, Geller BA, Hosler BA, Munsat TL, Horvitz HR, Brown RH: **Mutations in the glutamate transporter EAAT2 gene do not cause abnormal EAAT2 transcripts in amyotrophic lateral sclerosis.** *Ann Neurol* 1998, **43**:645-653.
30. Catalano M, Lorenzi C, Bocchio L, Racagni G: **No occurrence of the glutamate transporter EAAT2 A206G polymorphism in schizophrenic subjects.** *Mol Psychiatr* 2002, **7**:671-672.

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