male; 47.0 ± 14.9) and 529 controls (270 female; 39.7 ± 15.4 years: 259 male; 34.9 ± 12.4 years) were genotyped for association analysis of rs175174. Moreover, in additional linkage disequilibrium (LD) mapping around this SNP, 95 schizophrenic patients (50 female and 45 male) and 96 controls (44 female and 52 male), part of each sample used in association analysis, were genotyped for three SNPs. The general characterization of these subjects and a description of their psychiatric assessment according to identical criteria were published elsewhere [13]. After explaining the study to all subjects, written informed consent was obtained from each. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University.

Genomic DNA was extracted from peripheral blood of all subjects. For rapid genotyping of SNPs, rs175174 and additional three SNPs for LD mapping (rs175169, rs175175 and rs2292570), polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays were developed. The information of PCR primers is available on request. The PCR reactions of all SNPs were carried out in a 10 μl volume containing 10 ng genomic DNA, 0.4 M of each primer, 200 μM of dNTP, 1× PCR Gold Buffer, 1.5 mM $MgCl_2$ and 0.25 U of Amplitaq Gold™ (Applied Biosystems Japan Ltd., Tokyo, Japan), using the GeneAmp™ PCR system 9700 (Applied Biosystems Japan Ltd.). PCR cycling conditions consisted of an initial denaturation step at 95 °C for 9 min, followed by 45 cycles of 95 °C for 15 s, 56 °C for 20 s, 72 °C for 30 s, and ending with a final extension step at 72 °C for 7 min. PCR product was digested using appropriate restriction enzymes according to the manufacture's recommendation (New England Biolabs, England, UK) (Table 1). DNA fragments were resolved by electrophoresis in a 6% acrylamide gel stained with ethidium bromide.

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by χ^2 test. Marker-trait association analysis was also evaluated by χ^2 test (SPSS 10.0J, SPSS Japan Inc., Japan). To evaluate pairwise LD matrices among SNPs (by D' and r^2), we used the software HAPLOVIEW version 2.05 (developed in Mark Daly's lab., URL; http://www.broad.mit.edu/personal/jcbarret/haploview/index.php). This software also defined "LD blocks" by reasonable criteria based on 95% confidential bounds on D' values [4]. Power calculation was performed using a statistical program prepared by Ohashi et al. [10]. The significance level for all statistical tests was 0.05.

In view of the gender differences in gene effects, we included analyses of samples divided according to the gender. Both in cases and controls, genotype frequencies of total, female and male samples were not significantly different from HWE.

In association analysis, we could not find associations of rs175174 with schizophrenia in either male or female (Table 2).

Next, to test whether rs175174 is representative for ZDHHC8 or not, we performed LD mapping using three additional SNPs around ZDHHC8 (Fig. 1). LD matrices between each pair of SNPs showed strong LD both in cases and controls (Table 1). Even after dividing samples according to the gender, all LD patterns showed the same trends (data not shown). These findings may suggest that the LD pattern of ZDHHC8 is a block-like pattern and that rs175174 is the "representative SNP" of this gene.

The power based on genotype relative risk (GRR) was calculated to evaluate the non-significant results due to type II error. When we set the GRR at 1.28, 1.42 and 1.40 in all, female and male samples, respectively (multiplicative model), our sample size provided powers of more than 80%.

We could not replicate an original positive association using TDT of ZDHHC8 with schizophrenia by the present case–control association analysis among Japanese. Nor could we replicate the gender-specific effect of the risk SNP. In this association analysis, our sample sizes provide enough power to deny the hypothesis. We also performed the fine LD mapping of Japanese samples and showed that the LD pattern of ZDHHC8 was the same block-like pattern as one of the samples from the United States and South Africa. The results provide evidence that not only rs175174 but also ZDHHC8 would not be a susceptibility factor for schizophrenia in either Japanese females or males. The discrepancy between Japanese and the samples from the United States and South Africa may derive from ethnic differences.

A couple of limitation should be addressed to discuss the present results. Initially, the mean age of controls is much younger than that of patients in the present study. This means that a number of young controls, although not more than five subjects given a lifetime morbidity risk of 0.8–1.0%, may go on to develop schizophrenia. This confounding factor might weaken the power of the present study. Another limitation

Table 1
SNPs in LD mapping and pairwise LD matrices

SNP ID	D'				Restriction enzyme
	rs175169	rs175174	rs175175	rs2292570	
rs175169		0.97 (0.78)	1.0 (0.29)	1.0 (0.67)	*Bsl*I
rs175174	0.97 (0.80)		1.0 (0.36)	1.0 (0.58)	*Bse*RI
rs175175	1.0 (0.26)	1.0 (0.31)		1.0 (0.21)	*Alw*NI
rs2292570	0.93 (0.76)	0.97 (0.70)	1.0 (0.23)		*Tsp*RI

Upper diagonal figures are D' (r^2) of controls and lower diagonal figures are D' (r^2) of schizophrenia.

Table 2
Association analysis of rs175174

Samples	Number	G/G	G/A	A/A	*P* value (genotype)	MAF[a]	*P* value (allele)
Total							
SCZ	561	238	245	78		0.357	
CON	529	205	259	65	0.213	0.368	0.618
Female							
SCZ	259	114	106	39		0.355	
CON	270	112	130	28	0.133	0.344	0.714
Male							
SCZ	302	124	139	39		0.359	
CON	259	93	129	37	0.457	0.392	0.260

SCZ: schizophrenia; CON: control.
[a] Minor allele frequency.

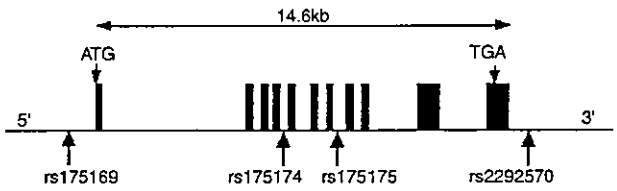

Fig. 1. Genomic structure of ZDHHC8 and SNPs used in association analysis and LD mapping. Vertical bars represent exons of ZDHHC8, and each number under arrows represents SNP ID.

which must be exercised is that the other candidates related to the neurodevelopmental and neuroprotective effect of ZDHHC8 would be in locus heterogeneity [11]. For example, ZDHHC8 encodes a putative transmembrane palmitoyltransferase modulating numerous classes of neuronal proteins including proteins important for neuronal development, neurotransmitter receptors such as NMDA [3]. Thus, the combined effect between ZDHHC8 and the other genes might be a stronger predisposing factor. Further genetic analysis including related candidate genes would definitely be required for a conclusive result.

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ORIGINAL INVESTIGATION

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Fluvoxamine, a selective serotonin reuptake inhibitor, suppresses tetrahydrobiopterin levels and dopamine as well as serotonin turnover in the mesoprefrontal system of mice

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Abstract *Rationale:* Tetrahydrobiopterin (BH_4) is a coenzyme of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), rate-limiting enzymes of monoamine biosynthesis. According to the monoamine hypothesis of depression, antidepressants will restore the function of the brain monoaminergic system, and BH_4 concentration. *Objective:* To investigate the effects of fluvoxamine on BH_4 levels and dopamine (DA) and serotonin (5-HT) turnover in the mesoprefrontal system, incorporating two risk factors of depression, social isolation and acute environmental change. *Methods:* Male ddY mice (6W) were divided into two housing groups, i.e. group-housing (eight animals per cage; 35 days), and isolation-housing (one per cage; 35 days), SC injected with fluvoxamine (20 or 40 mg/kg; days 29–35), and exposed to 20-min novelty stress (day 35). The levels of BH_4, DA, homovanilic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the prefrontal cortex and midbrain. *Results:* Under the group-housing condition, novelty stress significantly increased BH_4 levels in both regions, and the HVA/DA ratio in the midbrain, whereas it did not change any parameters in either region under the isolation-housing condition. In the prefrontal cortex, fluvoxamine significantly decreased the 5-HIAA/5-HT ratio under the group-housing condition, and BH_4 levels and the HVA/DA ratio under the isolation-housing condition. In the midbrain, fluvoxamine significantly decreased all parameters, except for an increasing in the 5-HIAA/5-HT ratio under the isolation-housing condition. *Conclusion:* Isolation-housing suppressed the increase of BH_4 levels and DA turnover elicited by novelty stress. Fluvoxamine suppressed BH_4 levels, and DA and 5-HT turnover. Fluvoxamine may have altered DA turnover by suppressing BH_4 levels.

Keywords Social isolation · Novelty stress · Animal model · Tetrahydrobiopterin · Dopamine turnover · Serotonin turnover

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Introduction

(6R)-5,6,7,8-tetrahydrobiopterin (BH_4) is a coenzyme of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), which are the rate-limiting enzymes of monoamine biosynthesis. BH_4 is also a coenzyme of NO synthase (NOS). NO is known to act as a signaling molecule in the central nervous system (CNS) (Barañano et al. 2001; Kiss and Vizi 2001; Ohkuma and Katsura 2001; Prast and Philippu 2001; Esplugues 2002). Thus, BH_4 plays an important role in CNS activity.

With regard to the cause of human depression, the monoamine hypothesis focuses on impaired function of the monoaminergic system of the brain (Smith et al. 1997; Delgado 2000; Hirschfeld 2000; Leonard 2000). Pharmacological studies of almost all clinically effective antidepressants have supported this hypothesis. Antidepressant-induced suppression in the activity of TH (Nestler et al. 1990) and TPH (Lapierre et al. 1983) may be related to BH_4 levels. Furthermore, recent studies suggest the possibility that alteration of NOS activity may be related to the antidepressant-like effects of NOS inhibitors in animal models (Harkin et al. 1999; Karolewicz et al. 1999; Da Silva et al. 2000). Investigation into the relationship between changes in the activities of these enzymes (TH, TPH, and NOS) and BH_4 levels induced by antidepressants should therefore help to clarify the pathophysiology of human depression.

Concerning environmental risk factors of depression, most patients become ill after adverse life events, such as interpersonal loss (separation, etc.) (Paykel 1994). Further, absence of social support appears to be associated with an

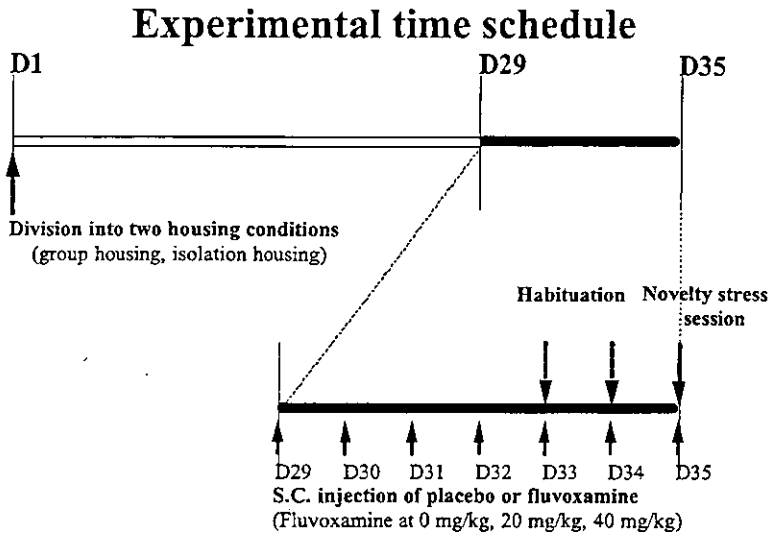

Fig. 1 Experimental time schedule. Mice were divided into 12 groups as described in Materials and methods

onset and relapse of depression (Paykel 1994). Kendler showed that genetic factors cooperate with environmental factors to induce the onset of depression in humans (Kendler et al. 1993). Thus, we proposed an animal model that simulated two of the major environmental risk factors of human depression (Miura et al. 2002a,b; Miura et al. 2004), i.e. social isolation and acute environmental change (Kendler et al. 1993; Paykel 1994).

We propose that changes in brain BH_4 levels play an important role in the pathophysiology of depression, and that antidepressants modulate these changes. Our recent study suggested that fluvoxamine, an SSRI, suppressed BH_4 levels as well as 5-HT turnover in the hippocampus of mice (Miura et al. 2004). In the present study, we further investigated other regions that we suspect are involved in the pathophysiology of human depression, and we measured BH_4, dopamine (DA), and serotonin (5-HT) levels simultaneously. Mesocorticolimbic DA projections (A8, A10) originating from the ventral tegmental area (VTA) of the midbrain (Cooper et al. 2003) have been shown to play an important role in a reward system, i.e. in motivating behavior (Kupferman and Schwartz 1995). We therefore selected two regions of focus, the prefrontal cortex and the midbrain. The aim of the present study was to investigate the effects of fluvoxamine on BH_4 levels and DA and 5-HT turnover in the mesoprefrontal regions, and to clarify the role of BH_4 in our novel animal model simulating two of the major environmental risk factors of human depression (Miura et al. 2002a,b, 2004).

Materials and methods

Animals

A total of 96 male ddY mice were used in the present experiments. The mice were transported from a breeding company at 5 weeks of age to our experimental animal center. After a 1-week habituation period, the mice, all of which had previously been housed in groups (eight per cage), were divided into two different groups according to housing conditions, i.e. a group-housing group (eight per cage; n=48) and an isolation-housing (one per cage; n=48; Fig. 1) group. The cages used for group-housing were 21×31×13 cm, and the cages used for isolation-housing were 17×29×13 cm. After being assigned to one of the two housing conditions, the mice were reared for 35 days (Fig. 1). Cage exchange was performed twice a week. Food and water were provided ad libitum. A 12-h light/dark cycle was maintained and room temperature was maintained at 21–23°C. All efforts were made to minimize both the number of animals used and the degree of their suffering. All of the experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experiments also comply with the current laws of Japan.

Fluvoxamine injection

In week 5 (days 29–35) after being assigned to one of the two housing conditions, the mice were SC injected with placebo (distilled water) or low-dose (20 mg/kg) or high-dose (40 mg/kg) fluvoxamine once per day (Fig. 1). The fluvoxamine was kindly donated by Solvay Pharmaceuticals (Brussels, Belgium). The mice were then further divided into three groups: a control (0 mg/kg, n=32), a low-dose (20 mg/kg, n=32), and a high-dose (40 mg/kg, n=32; Fig. 1) group.

Novelty stress test

After being assigned to one of the two housing conditions, the animals were further separated into two groups: a stress group (n=48) in which the animals were exposed to a 20-min novelty stress on day 35 [i.e. the animals were placed into a transparent plastic box (28×35×30 cm) that they had not yet experienced]; and a non-stress group (n=48) in which the animals experienced a habituation

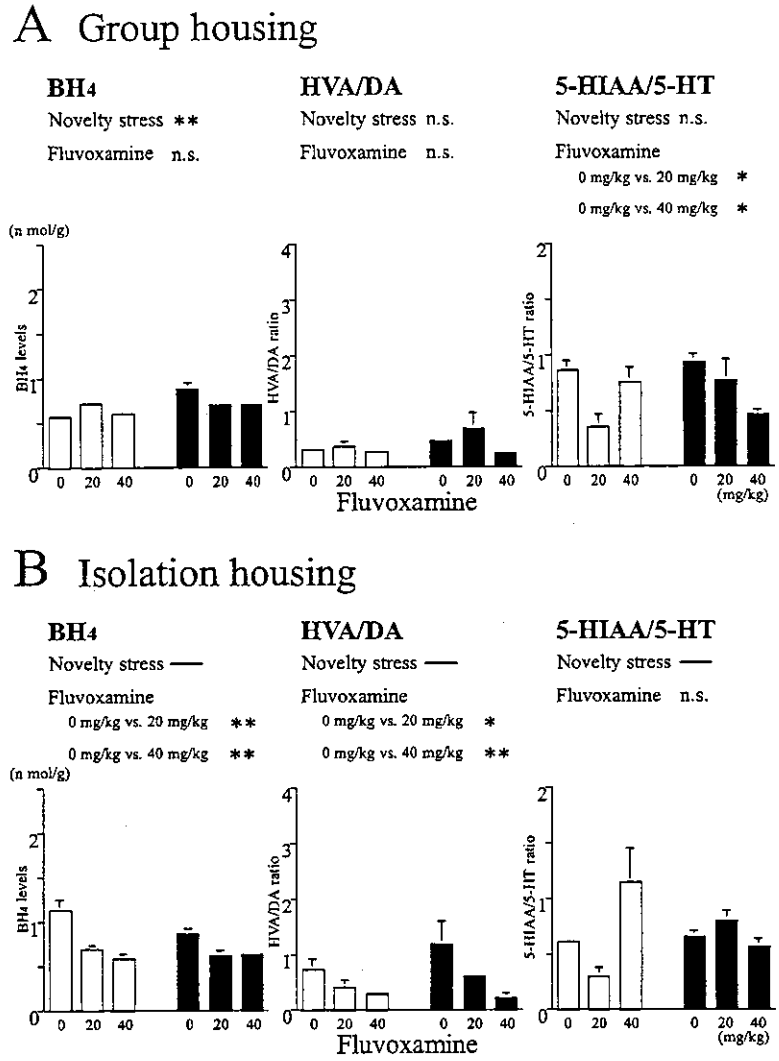

Fig. 2 Changes in BH_4 levels, and HVA/DA and 5-HIAA/5-HT ratios in the prefrontal cortex elicited by novelty stress and by fluvoxamine. **A** group-housing condition (n=48); **B** isolation-housing condition (n=47). *White bars*, non-stress (n=48); *black bars*, novelty stress (n=47, n=95 total). Fluvoxamine: 0.0 mg/kg (n=31); 20.20 mg/kg (n=32); 40.40 mg/kg (n=32, n=95 total). Each *bar* indicates the final group division. The number of animals used for each group was eight, except in the case of the isolation-housed, stress, 0 mg/kg group (n=7). Values are shown as the mean ±SEM. *Asterisks* indicate the results of the Tukey-Kramer test for novelty stress and fluvoxamine in each housing and stress condition: $*P<0.05$, $**P<0.01$, *n.s.* not significant. In the isolation-housing condition, the post hoc test for novelty stress was not performed because the MANOVA result was not significant

session (i.e. the animals were placed into the transparent plastic box for 10 min on days 33 and 34 before the 20-min session on day 35; Fig. 1). The habituation session was performed in the room in which the mice had been reared, whereas the novelty stress test was performed in a dark room that was separated from the breeding room. By combining the above conditions, the mice were divided into 12 groups.

Sample preparation

Mice were killed by decapitation immediately after the 20-min stress session. The brains were removed and, as quickly as possible, the prefrontal cortex and midbrain were dissected out on glass plates over ice. The samples were weighed and treated with 1000 µl of an ice-cold 0.2 M perchloric acid (PCA) solution containing 0.2 mM sodium pyrosulfite, 0.01% EDTA-2Na, and 0.5 µM isoproterenol (ISO) as an internal standard per 100 mg wet tissue. The solution was sonicated and then centrifuged at 10,000 *g* for 20 min at 4°C. The supernatant was filtered through a Millipore HV filter (0.45 µm pore size) and then subjected to both high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) of monoamines (DA, 5-HT) and their metabolites (homovanilic acid, HVA; 5-hydroxyindoleacetic acid, 5-HIAA), and HPLC with fluorimetric detection (FD) of BH_4.

HPLC-ECD determination of brain levels of monoamines and their metabolites

The levels of DA, HVA, 5-HT, and 5-HIAA in the brain extracts were measured by HPLC with ECD. The system employed for HPLC-ECD consisted of a CMA/200 autosampler (CMA/Microdialysis AB, Stockholm, Sweden), a micro LC pump (BAS, West Lafayette, Ind., USA), an LC-4C ECD (BAS), a Bio-Phase ODS-4 51-6034 column (4.0×110 mm; BAS), a CR-6A recorder (Shimadzu, Kyoto, Japan), an LC-26A vacuum degasser (BAS),

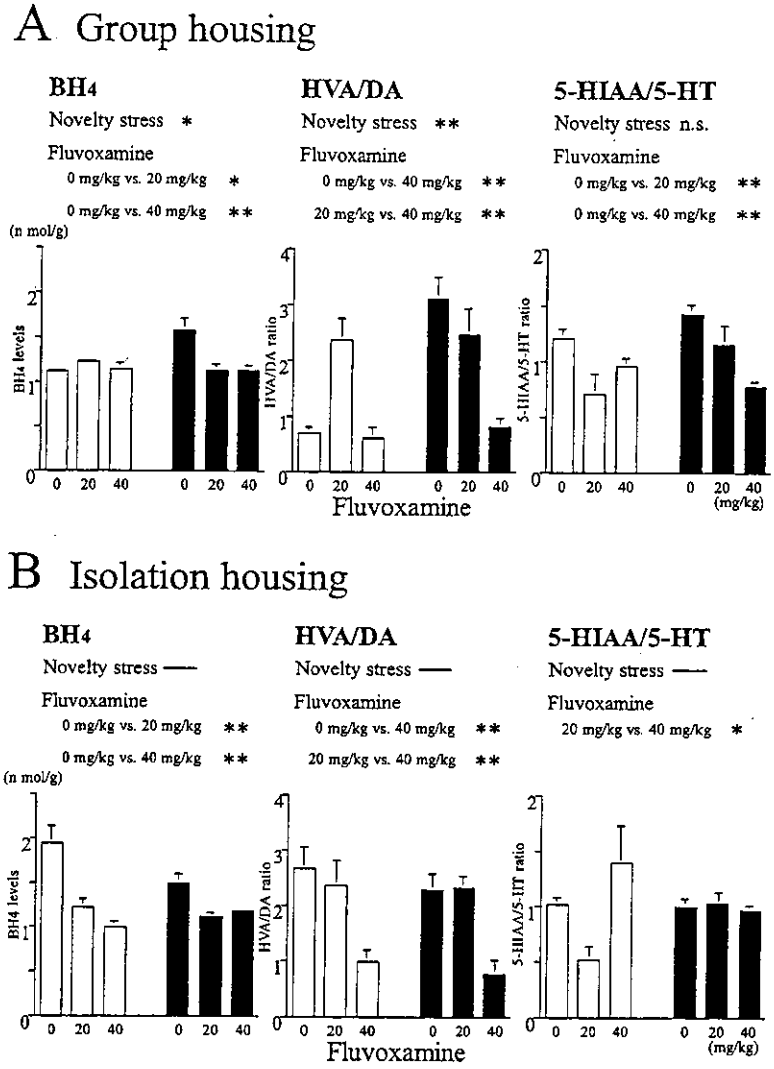

Fig. 3 Changes in BH_4 levels, and HVA/DA and 5-HIAA/5-HT ratios in the midbrain elicited by novelty stress and by fluvoxamine. **A** group-housing condition (n=48); **B** isolation-housing condition (n=45). *White bars*, non-stress (n=46); *black bars*, novelty stress (n=47, n=93 total). Fluvoxamine: 0:0 mg/kg (n=29); 20:20 mg/kg (n=32); 40:40 mg/kg (n=32, n=93 total). Each *bar* indicates the final group division. The number of animals used for each group was eight, except in the case of the isolation-housed, stress, 0 mg/kg group (n=7), and isolation-housed, non-stress, 0 mg/kg group (n=6). Values are shown as the mean±SEM. *Asterisks* indicate the results of the Tukey-Kramer test for novelty stress and fluvoxamine in each housing and stress condition: $*P<0.05$, $**P<0.01$, n.s. not significant. In the isolation-housing condition, the post hoc test for novelty stress was not performed because the MANOVA result was not significant

and a CTO-10A column heater set at 35°C (Shimadzu). The mobile-phase solution consisted of 0.1 M tartaric acid–0.1 M sodium acetate buffer, pH 3.2, containing 0.5 mM EDTA-2Na, 555 μM sodium 1-octane sulfonate, and 5% acetonitrile. The flow rate was 700 μl/min. The concentration of each compound was calculated by comparison with both the internal and the external standards.

HPLC-FD by post-column sodium nitrite oxidation for the determination of brain levels of BH_4

Tani and Ohno developed a method for the direct measurement of BH_4, the active form of biopterin (Tani and Ohno 1993), and we used this method to measure BH_4 levels in the present study. BH_4 (SIGMA) was stored in 0.1 M HCl (20 mM), and was prepared in 0.01 M HCl as an external standard (0.25 μM) immediately before sample injection. This system consisted of two LC-10AD pumps (Shimadzu), a CMA/200 autosampler, a Cosmosil 5C18 column (4.6×250 mm), a CR-6A recorder (Shimadzu), an LC-26A vacuum degasser, and a PF-10A FD (Shimadzu). The excitation wavelength was 350 nm, and the emission wavelength was 440 nm. The temperature of the reaction coil was set at 80°C using a column heater. The concentration of BH_4 was calculated by comparison with an external standard. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 5% methanol, 3 mM sodium 1-octane sulfonate, 0.1 mM EDTA-2Na, and 0.1 mM ascorbic acid (to prevent oxidation). The flow rate was 1.0 ml/min. Reduced pterins were oxidized by $NaNO_2$ (5 mM; flow rate: 1.0 ml/min) in the reaction coil (80°C).

Statistical analyses

To examine differences in the levels of BH_4, and in the ratios of HVA/DA and 5-HIAA/5-HT, three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted on dependent measures in each brain region. Further analyses were performed to

consider the interactions. In each housing condition, i.e. group-housing and isolation-housing, two-way MANOVA (Wilks's lambda) for novelty stress and fluvoxamine was conducted on dependent measures in each brain region, followed by the Tukey-Kramer test. There were some missing values: in both regions, isolation-housing/stress/0 mg/kg (n=7, due to undetected DA in one animal), and in the midbrain, isolation-housing/non-stress/0 mg/kg (n=6, due to undetected DA in two animals).

Results

Prefrontal cortex

Three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted for BH_4 levels, and to determine the HVA/DA and 5-HIAA/5-HT ratios. Housing condition [F(3, 81)=3.630, P=0.0163)]and fluvoxamine [F(6, 162)=12.013, P<0.0001] significantly altered the dependent measures, whereas novelty stress [F(3, 81)=1.663, P=0.1814] did not. The interactions between housing condition and novelty stress [F(3, 81)=4.932, P=0.0034], housing condition and fluvoxamine [F(6, 162)=9.153, P<0.0001], and novelty stress and fluvoxamine [F(6, 162)=4.527, P=0.0003] were significant. The interaction among housing condition, novelty stress, and fluvoxamine [F(6, 162)=2.749, P=0.0143] was also significant.

In the group-housing condition, two-way MANOVA for novelty-stress and fluvoxamine was conducted on dependent measures. Novelty stress [F(3, 40)=7.011, P=0.007] and fluvoxamine [F(6, 80)=4.722, P=0.0004] significantly altered the dependent measures. The interaction between novelty stress and fluvoxamine was significant [F(6, 80) =4.526, P=0.0005]. The post hoc test revealed that novelty stress significantly increased BH_4 levels (P<0.01, Fig. 2A), and fluvoxamine significantly decreased the 5-HIAA/5-HT ratio (0 mg/kg versus 20 mg/kg, P<0.05; 0 mg/kg versus 40 mg/kg, P<0.05; Fig. 2A). In the isolation-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [F(3, 39)=1.363, P=0.2683] did not alter these measures, whereas fluvoxamine [F(6, 78)=11.442, P<0.0001] significantly altered them. The interaction between novelty stress and fluvoxamine was significant [F(6, 78)=3.419, P=0.0048]. The post hoc test revealed that fluvoxamine significantly decreased BH_4 levels (0 mg/kg versus 20 mg/kg, P<0.01; 0 mg/kg versus 40 mg/kg, P<0.01; Fig. 2B) and HVA/DA ratio (0 mg/kg versus 20 mg/kg, P<0.05; 0 mg/kg versus 40 mg/kg, P<0.01; Fig. 2B).

Thus, in the group-housing condition, novelty stress was found to increase BH_4 levels and fluvoxamine to decrease 5-HT turnover. In the isolation-housing condition, novelty stress did not alter dependent measuresand fluvoxamine decreased BH_4 levels and DA turnover.

Midbrain

Three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted for BH_4 levels, and to determine the HVA/DA and 5-HIAA/5-HT ratios. Neither housing condition [F(3, 79) =2.251, P=0.0889] nor novelty stress [F(3, 79)=1.646, P=0.1854] altered dependent measures, whereas fluvoxamine [F(6, 158)=22.222, P<0.0001] significantly altered them. The interactions between housing condition and novelty stress [F(3, 79)=4.513, P=0.0057], housing condition and fluvoxamine [F(6, 158)=4.790, P=0.0002], and novelty stress and fluvoxamine [F(6, 158)=4.470, P=0.0003] were significant. The interaction among housing condition, novelty stress, and fluvoxamine [F(6, 158)=4.807, P=0.0002] was also significant.

In the group-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [F(3, 40)=5.011, P=0.0048] and fluvoxamine [F(6, 80)=9.868, P<0.0001] significantly altered the dependent measures. The interaction between novelty stress and fluvoxamine was significant [F(6, 80) =6.807, P<0.0001]. The post hoc test revealed that novelty stress significantly increased BH_4 levels (P<0.05, Fig. 3A) and the HVA/DA ratio (P<0.01, Fig. 3A), and fluvoxamine significantly decreased BH_4 levels (0 mg/kg versus 20 mg/kg, P<0.05; 0 mg/kg versus 40 mg/kg, P<0.01; Fig. 3A), and decreased the HVA/DA (0 mg/kg versus 40 mg/kg, P<0.01; 20 mg/kg versus 40 mg/kg, P<0.01; Fig. 3A) and 5-HIAA/5-HT (0 mg/kg versus 20 mg/kg, P<0.05; 0 mg/kg versus 40 mg/kg, P<0.05; Fig. 3A) ratios. In the isolation-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [F(3, 37)=1.044, P=0.3845] did not significantly alter the dependent measures, whereas fluvoxamine [F(6, 74) =13.336, P<0.0001] did significantly alter them. The interaction between novelty stress and fluvoxamine was significant [F(6, 74)=3.264, P=0.0067]. The post hoc test revealed that fluvoxamine significantly decreased BH_4 levels (0 mg/kg versus 20 mg/kg, P<0.01; 0 mg/kg versus 40 mg/kg, P<0.01; Fig. 3B), decreased the HVA/DA ratio (0 mg/kg versus 40 mg/kg, P<0.01; 20 mg/kg versus 40 mg/kg, P<0.01; Fig. 3B), and significantly increased 5-HIAA/5-HT ratio (20 mg/kg versus 40 mg/kg, P<0.05; Fig. 3B).

Thus, in the group-housing condition, novelty stress increased BH_4 levels and DA turnover, and fluvoxamine decreased BH_4 levels, as well as the DA and 5-HT turnover. In the isolation-housing condition, novelty stress did not alter dependent measures and fluvoxamine decreased BH_4 levels and DA turnover, whereas fluvoxamine increased 5-HT turnover.

Discussion

Human depression has a number of etiological risk factors. Both environmental and genetic factors have been

associated with the pathogenesis of the disease (Kendler et al. 1993). Among environmental factors, adverse life events such as interpersonal loss (e.g. separation) or an absence of social support (e.g. that occurring with a loss of social contact) appear to play important roles in the onset and relapse of depression (Paykel 1994). In the present study, we investigated the effects of fluvoxamine on BH_4 levels and on the DA and 5-HT turnover ratio in the mesoprefrontal system of mice using an animal model incorporating these two environmental risk factors. Isolation-housing is known to change the activities in the mesoprefrontal monoaminergic system. Isolation-housing suppresses TPH activity in the midbrain (Yanai and Sze 1983), whereas it increases the accumulation of the monoamine precursor, dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) in the cerebral cortex (Miachon et al. 1993). Isolation has also been shown to increase the DA level and decrease the 5-HIAA/5-HT ratio in the prefrontal cortex (Jones et al. 1992), and to increase the KCl-induced release of 5-HT and DA from slices of prefrontal cortex (Jaffe 1998). Finally, Crepsi et al. (1992) showed by in vivo voltammetric analysis of the prefrontal cortex that isolation-housing prolonged 5-HT release and increased DA release following KCl or fenfluramine treatment.

In the prefrontal cortex and midbrain in the present study, novelty stress increased BH_4 levels under the group-housing condition (Figs 2A, 3A), whereas these levels were not altered under the isolation-housing condition (Figs 2B, 3B). Our recent study also suggested that novelty stress increases BH_4 levels in the hippocampus, although they did not change under the isolation-housing condition (Miura et al. 2004). According to these results, it appears likely that BH_4 plays a role in stress response mechanisms, and that isolation-housing attenuates these changes. A recent review demonstrated that intracellular concentrations of BH_4, which are mainly determined by GTP cyclohydrolase 1(GCH), probably regulate the activity of TH, TPH, and also NOS (Nagatsu and Ichinose 1999). Thus, the increase in BH_4 levels elicited by novelty stress may have been related to the activities of TH, TPH, and NOS. Previous studies have shown elevations in brain TH and TPH activities elicited by physiological stress (Boadle-Biber et al. 1989; Serova et al. 1998; Chamas et al. 1999). Physiological stress has also been shown to increase NOS I mRNA levels in the hypothalamic paraventricular nucleus (Kishimoto et al. 1996). Thus, novelty stress may have elevated GCH activity, increased the BH_4 concentration, and differentially regulated TH, TPH, and NOS I activities in each brain region. Although the mechanisms of the BH_4 elevation elicited by novelty stress and the effect of isolation-housing on this type of elevation remain unknown, our results suggest that isolation-housing suppressed the elevation of BH_4 levels elicited by novelty stress. Further in vivo study using GCH inhibitors will help to clarify the mechanisms of these stress responses of BH_4.

In the midbrain, novelty stress enhanced DA turnover under the group-housing condition (Fig. 3A), whereas it did not alter DA turnover under the isolation-housing condition (Fig. 3B). Novelty stress did not alter DA turnover in the prefrontal cortex (Fig. 2A,B). Thus, isolation-housing may have attenuated the increase in DA turnover elicited by novelty stress in the midbrain. Our previous study suggested that isolation-housing suppressed the elevation of monoamine (DA and 5-HT) turnover elicited by novelty stress (Miura et al. 2002a), although the regions in which such suppression was evident in that study, i.e. the prefrontal cortex and the nucleus accumbens (Miura et al. 2002a), differed from the regions of the present study. Although in our previous studies we reported that novelty stress significantly changed the levels of monoamines and their metabolites (Miura et al. 2002a,b), novelty stress was not found to significantly alter either of these levels in the present study. These differences in the stress response between our present and previous studies may be attributable, at least in part, to differences in the stress-session procedures. Here, we employed a non-stress condition in which animals were habituated twice to the novel environment before final exposure, and a stress condition in which they experienced the novel environment without prior habituation. In our previous studies, animals in the non-stress group were killed without exposure to the novel environment. We cannot rule out the possibility that these habituation sessions influenced CNS activity in the non-stress group. The differences between housing cages may also have had an influence. In the previous studies we used hanging-type cages with wire-mesh bottoms to minimize the influence of handling and social experience (Holson et al. 1991; Krebs-Thomson et al. 2001). However, the use of these cages may itself have constituted a chronic stressor. Finally, the difference in species used (rats versus mice) may also have contributed to the difference in results. Despite these differences, however, isolation-housing was clearly shown to attenuate the elevation of DA turnover elicited by novelty stress.

Fluvoxamine was also shown to suppress BH_4 levels in this study (Figs 2B, 3A,B), with the exception of the group-housing condition in the prefrontal cortex (Fig. 2A). In both regions, two-way MANOVA revealed significant interactions between novelty stress and fluvoxamine in each housing condition; thus fluvoxamine attenuated the elevation of BH_4 levels elicited by novelty stress. Our recent study demonstrated that fluvoxamine decreased BH_4 levels in the hippocampus of mice (Miura et al. 2004). Because chronic antidepressant treatment has been shown to suppress TH (Nestler et al. 1990) as well as TPH activity (Lapierre et al. 1983), the decrease in BH_4 levels elicited by fluvoxamine would seem to have attenuated the activities of these enzymes. Recently, NOS inhibitors have been shown to exhibit antidepressant-like effects in animal models (Harkin et al. 1999; Karolewicz et al. 1999; Da Silva et al. 2000). Paroxetine, a selective serotonin reuptake inhibitor (SSRI), is known to act as an NOS inhibitor (Finkel et al. 1996). Thus, antidepressants may possess clinical potency by inhibiting NOS activities. Fluvoxamine, an inhibitor of cytochrome P450 isozymes

that are structurally homologous to NOS (Richelson 1997), may influence NOS activity by decreasing BH_4 levels, although the mechanism of BH_4 suppression remains unknown. However, further research into the relation between alterations in BH_4 levels and GCH activity, and that between changes in BH_4 levels and NOS I activity elicited by fluvoxamine will help to clarify the role of NOS I in the clinical efficacy of the drug.

Fluvoxamine was found to inhibit DA turnover (Figs 2B, 3A,B), with the exception that there was no change in DA turnovers in the prefrontal cortex under the group-housing condition (Fig. 2A). In both regions, two-way MANOVA revealed significant interactions between novelty stress and fluvoxamine in each housing condition, and thus fluvoxamine attenuated the elevation of DA turnover elicited by novelty stress. To our knowledge, this is the first study showing a decrease in BH_4 levels and a simultaneous inhibition of DA turnover elicited by fluvoxamine. The mechanism of the effects of fluvoxamine on the DA system remains unknown. We suspect that the inhibition of 5-HT transporter (SERT) activity elicited by fluvoxamine cannot solely account for the inhibition of DA turnover. It is likely that other pharmacological effects related to the changes in BH_4 levels also played a role in inducing these changes. We propose here two possible explanations for these findings, although these are only speculation at present. The first explanation involves the regulation of DA neuron activity by the innervation of 5-HT neurons. The 5-HT innervations of the DA system are thought to attenuate the activity of DA neurons, and thus fluvoxamine may have potentiated the attenuation by increasing 5-HT levels (Di Mascio et al. 1998; Dong et al. 1999). The second possibility is that fluvoxamine may have suppressed TH activity via the decrease in BH_4 levels, and thereby suppressed DA biosynthesis. A study using 6-pyruvoyltetrahydropterin synthase-knockout mice (i.e. mice in which the second step of BH_4 biosynthesis is blocked) showed that the suppression of TH and NOS activities in the brain did not affect TPH activity (Sumi-Ichinose et al. 2001). In a study by Flatmark (2000), TH activity was highly dependent on the intracellular concentration of BH_4.

Fluvoxamine decreased the 5-HIAA/5-HT ratio under the group-housing condition in both regions (Figs 2A, 3A). Under the isolation-housing condition fluvoxamine did not alter the 5-HIAA/5-HT ratio in the prefrontal cortex (Fig. 2B), whereas it increased this ratio in the midbrain (Fig. 3B).

Although the original monoamine hypothesis has advanced our understanding of the etiology and pathophysiology of human depression, it does not address several major issues. The hypothesis has evolved to include adaptive changes in receptors to explain why there should be only a gradual clinical response to antidepressant treatment when the increase in availability of monoamine is rapid (Hirschfeld 2000). On the other hand, the dysfunction of SERT is the target of some of the newest forms of antidepressant pharmacotherapy, including the SSRIs (Leonard 2000). Activity of the SERT in platelets is reduced in patients with depression (Owens and Nemeroff 1994), and changes in the SERT in platelets have been found to correlate with response to treatment (Leonard 2000). A study using single photon emission computed tomography with [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane, the radiolabeled tracer binding with high affinity to SERT in the midbrain, revealed a reduction in the activity of the transporter in patients with depression (Malison et al. 1998). In the present study, fluvoxamine may have modified the activity and/or expression of SERT. The discrepancy of fluvoxamine-induced changes in 5-HT turnover between isolation and group-housing might be attributable to the difference in the responses of SERT activities to fluvoxamine between the two housing conditions, although the underlying mechanism remains to be clarified. Further, impaired activity of enzymes essential for monoamine synthesis may play a role in depression, although reports on this subject are few (Leonard 2000). Our results suggest the possibility that the decreased BH_4 levels elicited by fluvoxamine suppressed TH and/or TPH activity.

In the present study, both novelty stress and fluvoxamine induced changes in BH_4 levels, DA turnover, and 5-HT turnover in the mesoprefrontal system. In the group-housing animals, novelty stress significantly increased BH_4 levels. The suppression of BH_4 levels by fluvoxamine may have in turn been related to the suppression of DA turnover. As mentioned above, our results suggest the possibility that the clinical efficacy of fluvoxamine may be due to its influence on BH_4 levels as well as due to its effect on SERT. Further investigation of these potential mechanisms will help clarify the pathophysiology and pathogenesis of human depression.

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Association study of the frizzled-3 (FZD3) gene with schizophrenia and mood disorders

Short Communication

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Summary. Two research groups have recently reported a significant association between schizophrenia and genetic variants of Frizzled-3 (FZD3) gene. We examined a possible association in a Japanese sample of schizophrenia, bipolar disorder, unipolar depression and controls with four single nucleotide polymorphisms (SNPs), tested in previous reports. We failed to find significant association in the four SNPs or haplotype analysis. The FZD3 gene might not play a role in conferring susceptibility to major psychosis in our sample.

Keywords: FZD3, schizophrenia, mood disorder, association study, single nucleotide polymorphism (SNP).

Introduction

Schizophrenia is a complex genetic disorder characterized by disturbances of cognition, emotion and social functioning. This disease is believed to involve genetic abnormalities in developmental/plasticity related processes during a critical period in neuronal growth (Weinberger et al., 2001). Wnt signal transduction cascades have been implicated in a variety of neurodevelopmental processes, e.g. segmentation, central nervous system patterning, and cell divisions (Wodarz and Nusse, 1998). Wnt proteins signal via cell surface transmembrane receptors, termed frizzleds, which display many properties

characteristic of members of the superfamily of G-protein-coupled receptors (Wang and Malbon, 2004). The frizzled-3 (FZD3) gene, a member of frizzles, is located on chromosome 8p21, repeatedly suggested as a positive linkage locus for schizophrenia (Lewis et al., 2003; McGuffin et al., 2003). The FDZ3 gene consists of 8 exons and 7 introns, spanning approximately 70 Kb (Kirikoshi et al., 2000). In accordance with this, two research groups have recently reported a significant association between schizophrenia and the FZD3 gene in Japanese and Chinese samples (Katsu et al., 2003; Yang et al., 2003). We tried to replicate these findings in an independent Asian sample. Furthermore, we also examined the possible association between the FZD3 gene with mood disorders, since schizophrenia and mood disorders might share the genetic vulnerability (Berrettini, 2003).

Methods and materials

Subjects

Subjects were 427 patients with schizophrenia (221 males and 206 females with mean age of 44.2 years [SD 14.5]), 91 with bipolar disorder (40 and 51; 53.6 years [SD 14.8]), and 396 with major depression (155 and 241; 53.4 years [SD 16.1]) and 473 healthy controls (228 and 245; 36.1 years [SD 12.5]). All the subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria. Controls were healthy volunteers who had no current or past contact to psychiatric services. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees.

SNP genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. We genotyped four SNPs (single nucleotide polymorphisms; dbSNP accession: rs960914 in intron3, rs2241802 in exon5: A435G, L145L, rs2323019 in intron5 and rs352203 in intron5) in the FZD3 gene, which were examined in the previous two studies (Katsu et al., 2003; Yang et al., 2003). Genotyping was performed with the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004a, b). Briefly, primers and probes for detection of the SNPs are: rs960914: forward primer 5′-CTTTTATAAAGAAATTTGAAACAT CAGAACATGGGA-3′, reverse primer 5′-ACTTTTTCACTGCTTGGGAGTTATTCT-3′, probe 1 5′-VIC-CTGAATGGCTGCTATC-MGB-3′, and probe 2 5′-FAM-TCTGAATGGCTACTATC-MGB-3′; rs2241802: forward primer 5′-ATGAGCCATATCCTCGACTTGTG-3′, reverse primer 5′-GGACACCAAAAACCATAGTCTCTCT-3′, probe 1 5′-VIC-TCCAGCTAAATTCAG-MGB-3′, and probe 2 5′-FAM-CAGCCAAATTCAG-MGB-3′; rs2323019: forward primer 5′-GAAT TACTTTGTTTTTCTAGATTCTTGAATTGAAAGC-3′, reverse primer 5′-CCAACCTGGTTAA TAATGGTCTTTTGG-3′, probe 1 5′-VIC-TCATTTATTGTCAATGTTTTAA-MGB-3′, and probe 2 5′-TCATTTATTGTCAATATTTTAA-MGB-3′; rs352203: forward primer 5′-CCTGAAAAAA TATTCTATATCTCTTGTTTTGCCA-3′, reverse primer 5′-CAACCAGGACATAACAGTATTA CAGTTTCTAT-3′, probe 1 5′-VIC-TCCTTCATGTCGTATTC-MGB-3′, and probe 2 5′-FAM-TTTCCTTCATATCGTATTC-MGB-3′. PCR cycling conditions were: at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute.

Statistical analysis

Statistical analysis of association studies was performed using SNPAlyse software (DYNACOM, Yokohama, Japan). The presence of Hardy-Weinberg equilibrium was examined using the $\chi\chi^2$ test for goodness of fit. Allele distributions between patients and controls were analyzed by the

Table 1. Allele distribution for SNPs in the FZD3 gene between major psychoses and controls

dbSNP ID	SNP	Controls	Schizophrenia		Bipolar		Unipolar	
		n = 473	n = 427	*P* value	n = 91	*P* value	n = 397	*P* value
rs960914	T/C	.398	.396	.91	.352	.23	.386	.61
rs2241802	G/A	.453	.458	.85	.451	.94	.455	.96
rs2323019	A/G	.407	.420	.57	.357	.21	.409	.91
rs352203	T/C	.397	.396	.97	.352	.24	.386	.64

χ^2 test for independence. The measures of linkage disequilibrium (LD), denoted as D', was calculated from the haplotype frequency using Expectation-Maximization algorithm. Case-control haplotype analysis was performed by the permutation method to obtain empirical significance (Good, 2000). The global p-values represent the overall significance using the χ^2 test when the observed versus expected frequencies of all the haplotypes are considered together. The individual haplotype was tested for association by grouping all others together and applying the χ^2 test with 1 df. P-values were calculated based on 10,000 replications. All p-values reported are two tailed. Statistical significance was defined at $p < 0.05$.

Results

The obtained allele frequencies for the patients and controls are shown in Table 1. The genotype distributions for all the diagnostic groups were in Hardy-Weinberg equilibrium (data not shown). There was no significant difference in genotype distributions or allele frequencies of the four SNPs in the FZD3 gene between the controls and any patient group, although previous studies reported positive associations between schizophrenia and several SNPs (Katsu et al., 2003; Yang et al., 2003). We computed the LD between the SNPs using D', which ranged between 0.8 and 1.0, indicating strong to intermediate LD between the markers. Adjacent combinations of up to four markers were tested, however, any haplotype combination was not significantly associated with any diagnostic group (all global p-values > 0.2).

Discussion

This study examined the possible association of the FZD gene with schizophrenia and mood disorders in our Japanese sample. We obtained no evidence for a significant association of the genetic variations of the FZD gene with any diagnostic group, suggesting that the examined polymorphisms play no major role in the pathogenesis of major psychoses in our sample. Our results are thus inconsistent with the results of the previous case-control study which reported a significantly higher frequency of the T allele of rs960914 in patients with schizophrenia than in controls (Katsu et al., 2003). The frequencies of the T allele were 0.62 and 0.51 in schizophrenics (n = 209) and controls (n = 200) in their Japanese sample, while the frequencies of the T allele were 0.60 and 0.60 in patients with schizophrenia (n = 427) and controls (n = 473) in ours. A highly significant association of the other three SNPs (rs2241802, rs2323019 and rs352203) and their three marker haplotypes with schizophrenia patients was reported in a family-based association study in a Chinese population

(Yang et al., 2003), while no evidence of such an association was obtained in our results (Chinese: global p-value < 0.000001, GAT haplotype p value $<$ 0.000001; vs our results: global p-value $= 0.31$, GAT haplotype p-value $=$ 0.97). Recently, the positive association between schizophrenia and the FZD3 gene has been reported in case-control study in a Chinese population (Zhang et al., 2004). This study presented that a new marker, rs880481, created the most positive results. Further analysis using this new marker should be examined in other ethnic populations.

A possible explanation for the discrepancy between the previous results and ours is a type II error in our sample. The odds ratio of the T allele of rs960914 was 1.54 in Japanese study (Katsu et al., 2003). However, power analysis revealed that our sample size could detect a significant association between the examined risk alleles (frequency of 0.4–0.6) and schizophrenia with a power of 90% when odds ratio was assumed to be 1.4 or more and the critical p-value was set at 0.05. There is only a small chance that a clinically meaningful difference would have been missed with the data. Secondly, it is possible that LD with other unknown polymorphisms, that is truly responsible for giving susceptibility to schizophrenia, may explain the discrepancy. Alternatively, the significant association observed by the previous two groups may have arisen by chance. The case-control association study is subject to the effect of population stratification, although the patients and controls were ethnically matched.

More recently, an extensive family-transmission and case-control analysis in a Japanese population with additional post-mortem mRNA expression data and family trio analysis in a British population yielded negative results for association between the FZD3 gene and schizophrenia (Ide et al., 2004; Wei and Hemmings, 2004), which is consistent with our results.

In conclusion, we obtained no evidence for an association between the FZD3 gene and schizophrenia or mood disorders, suggesting that this gene has no major role in conferring susceptibility to major psychoses in our sample.

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Letter to the Editors

A missense polymorphism (H204R) of a Rho GTPase-activating protein, the chimerin 2 gene, is associated with schizophrenia in men

Dear Editors:

Schizophrenia is a complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. This disease is believed to involve genetic abnormalities in developmental/plasticity related processes (DeLisi, 2000). The pathophysiology of schizophrenia is still unclear; however, the high incidence developing schizophrenia was observed in mental retardation, suggesting common pathophysiological basis of these two diseases including genetic basis. As several X-linked mental retardation genes are involved in Rho signaling pathways (Ramakers, 2002), Rho GTPase related genes could be strong candidate genes for schizophrenia. The Rho family of GTP binding proteins act as a key regulator for developing neuronal network, e.g. neurite and growth cone formation (Negishi and Katoh, 2002). Chimerin 2 gene, CHN2, is one of the GTPase-activating proteins expressed in a variety of human tissues with the highest expression levels in brain (Yuan et al., 1995). Therefore, genetic variability of the chimerin 2 gene is of considerable interest in the evaluation of risk of schizophrenia. To our knowledge, however, there is no study examining the possible association between the CHN2 gene and schizophrenia.

The CHN2 maps to chromosome 7p15.3 and consisted of 13 exons and 12 introns, spanning 318 Kb (Yuan et al., 1995). We searched for polymorphisms in the CHN2 gene in silico and detected a common single nucleotide substitution (A611G; NCBI SNP ID: rs3750103) (Haga et al., 2002) in exon7 giving rise to an amino acid change of histidine to arginine at codon 204 (H204R) (amino acid numbering is according to NCBI protein data base accession NP_004058). In our search there was no other missense polymorphism reported in the CHN2 gene. Since this polymorphism may alter functions of the CHN2, we performed an association analysis between this polymorphism and schizophrenia in a Japanese sample of 293 patients (162 males and 131 females with mean age of 43.7 years [S.D. 14.2]) with schizophrenia and 450 healthy controls (222 males and 228 females with mean age of 36.5 years [S.D. 12.6]). 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. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees.

The H204R polymorphism genotypes were determined using the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004). Briefly, probes and primers for detection of the SNP are: forward primer, 5′-CAGATCTCCTCCCTGGTTCGA-3′, reverse primer, 5′-TGCTTACCTTAAAGTTGTGTGTCTTCT-3′, probe 1, 5′-VIC-CCCTCACACACAACGA-MGB-3′, and probe 2, 5′-FAM-CCTCACACGCAACGA-MGB-3′.

Genotype distributions and allele frequencies of the H204R missense polymorphism of the chimerin 2 gene among the patients and controls are shown in Table 1. The genotype distributions for the two groups and those of male and female patients as well as controls were in Hardy–Weinberg equilibrium (data not shown). There was a trend towards an increased frequency of the R204 allele in the patients than in the controls ($\chi^2=3.74$, $df=1$, $p=0.053$, odds ratio=1.29, 95%CI 1.00–1.66). The individuals homozygous for the R204 allele were significantly

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Table 1
Genotype and allele distributions for the H204R polymorphism of the chimerin 2 gene between the patients with schizophrenia and controls

Group	N	Genotype distribution				Mantel Haenszel	Allele frequency			odds ratio (95%CI)
		His/His	His/Arg	Arg/Arg	P value	P value	His	Arg	P value	
Male										
Patients	162	95 (58.6%)	57 (35.2%)	10 (6.2%)	0.047	0.018	247 (76.2%)	77 (23.8%)	0.018	1.53 (1.07–2.19)
Controls	222	152 (68.4%)	65 (29.3%)	5 (2.3%)			369 (83.1%)	75 (16.9%)		
Female										
Patients	131	80 (61.2%)	44 (33.6%)	7 (5.3%)	0.703	0.678	204 (77.9%)	58 (22.1%)	0.681	1.08 (0.75–1.56)
Controls	228	141 (61.9%)	79 (34.6%)	8 (3.5%)			361 (23.8%)	95 (20.8%)		
Total										
Patients	293	175 (59.7%)	101 (34.5%)	17 (5.8%)	0.087	0.052	451 (77.0%)	135 (23.0%)	0.053	1.29 (1.00–1.66)
Controls	450	293 (65.1%)	144 (32.0%)	13 (2.9%)			730 (81.1%)	170 (18.9%)		

more common in the cases than in the controls ($\chi^2 = 3.94$, $df = 1$, $p = 0.049$, odds ratio = 2.07, 95%CI 0.99–4.33). The observed frequency for the minor allele (R204) in our control group (19%) was quite similar to that reported by Haga et al. (2002) (18%) estimated from 48 Japanese chromosomes. Thus, the observed significant difference in the allele frequency between the cases and controls cannot be ascribed to an unusually lower frequency of the R204 allele in our control subjects.

As gender differences occur in various aspects of the disease, including earlier age of onset, poorer course and medication response in men, we examined males and females separately. The R204 allele was excess in our cases when compared to controls among males ($\chi^2 = 5.57$, $df = 1$, $p = 0.018$, odds ratio = 1.53, 95%CI 1.07–2.19). Genotype distributions also revealed significant difference between male controls and male patients with schizophrenia ($\chi^2 = 6.12$, $df = 2$, $p = 0.047$; $\chi^2 = 5.56$, $df = 1$, $p = 0.018$ by Mantel Haenszel test). However, there was significant difference in neither allele frequency nor genotype distribution between the schizophrenics and controls in females.

CHN2 protein acts as a receptor of diacylglycerol/phorbol esters and regulates the activity of the Rac GTPase, one of the Rho GTPase family proteins (Caloca et al., 2003). The CHN2 inhibits Rac-GTP activation by the stimulation of epidermal growth factor (EGF). EGF protein levels were decreased in the prefrontal cortex of schizophrenic patients, and conversely, EGF receptor expression was elevated in the prefrontal cortex (Futamura et al., 2002). Serum EGF levels were markedly reduced in schizophrenic patients, even in young, drug-free patients (Futamura et al., 2002). Neonatal perturbation of EGF in rats resulted in abnormal sensorimotor gating and social interaction in adults (Futamura et al., 2003). In addition, neuregulin-1, one of the EGF family proteins, was reported as a schizophrenia susceptibility gene (Harrison and Owen, 2003) and the abnormal expression of neuregulin-1 has been observed in schizophrenic brain (Hashimoto et al., 2003). Therefore, the CHN2 H204R polymorphism might lead to the abnormality of neuregulin signaling pathways. As the location of H204R is close to diacylglycerol/phorbol ester binding domain (214–264 amino acid), this polymorphism could alter the protein structure of the region, which may change the second messenger signaling. H204R polymorphism, next to a casein kinase II phosphorylation site, might also play a potential role in the CHN2 phosphorylation state, although the physiological phosphorylation status is unclear.

We demonstrated, for the first time, the possible association between a missense polymorphism (H204R) of the CHN2 gene and schizophrenia in a Japanese population. A false-positive association due to population stratification could not be excluded in our case control designed study, despite the precaution of ethnic matching of this study. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples. If our results are replicated, functional analysis of the CHN2 H204R polymorphism might contribute to understanding the molecular mechanisms of schizophrenia.

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ARTICLE

Expression of Ndrg2 in the rat frontal cortex after antidepressant and electroconvulsive treatment

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Abstract

Although the therapeutic action of antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction, no consensus has been reached concerning their precise molecular or cellular mechanisms of action. In the present study, we demonstrated that chronic treatment with a tricyclic antidepressant (imipramine) and a selective serotonin reuptake inhibitor (sertraline) reduced the expression of Ndrg2 mRNA and protein in the rat frontal cortex. Ndrg2 is a member of the N-Myc downstream-regulated genes. Interestingly, repeated ECT also significantly decreased Ndrg2 expression in this region of the brain. These data suggest that Ndrg2 may be a common functional molecule that is decreased after antidepressant treatment and ECT. Although, the functional role of Ndrg2 in the central nervous system remains unclear, our findings suggest that Ndrg2 may be associated with treatment-induced adaptive neural plasticity in the brain, a chronic target of antidepressant action. In conclusion, we have identified Ndrg2 as a candidate target molecule of antidepressants and ECT.

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Key words: Microarray, neural plasticity, pharmacogenomics, selective serotonin reuptake inhibitor.

Introduction

Antidepressants are very effective agents for preventing and treating depression and have been used clinically for more than 50 yr. Typical antidepressants significantly increase the synaptic concentration of norepinephrine and/or serotonin. However, a latency period of several weeks generally elapses before the therapeutic effects of antidepressants are observed. This delayed therapeutic action could result from either the indirect regulation of other neuronal signal transduction systems or the regulation of gene transcription following chronic treatment. Indeed, antidepressants have been shown to affect the expression of immediate early genes and transcription factors, including c-fos, FosB, junB, NGF1-A, and CREB (see review by Yamada and Higuchi, 2002). These regulatory proteins activate or repress genes that encode specific proteins, and may be involved in critical steps that mediate treatment-induced alterations of central nervous system function. We recently performed expressed-sequence tag (EST) analyses to identify some biological changes observed in rat brain after chronic treatment with antidepressants (Yamada et al., 2001). We developed our original ADRG microarray for high-throughput secondary screening of these candidate genes (Yamada et al., 2000). To date, we have cloned several cDNA candidates as ESTs from the rat brain and have named these antidepressant-related genes (ADRGs).

While antidepressant pharmaceuticals have been shown to be an effective treatment, another important therapy that is widely used for treating depression is repeated electroconvulsive treatment (ECT). Because of its safety, high efficacy, and rapid onset of action, ECT is well-suited for treating patients with severe psychotic depression, severe depression with suicidal ideation, drug-resistant depression, and for treating

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