Neuroimaging analysis

Brain MR procedure is described in Supplementary Material. The basic principle of TBM is to analyze the local deformations of an image and to infer local differences in the brain structure. The method was described in detail previously (46) (Supplementary Material). Diffusion tensor imaging (DTI) analysis was performed using FA maps by a voxel-by-voxel analysis (Supplementary Material). The statistical parametric maps of Jacobian determinants and FA values were analyzed using statistical parametric mapping (SPM) 2, which implements a 'general linear model'. To test hypotheses about regional population effects, data were analyzed by a two-sample *t*-test without global normalization. We used $P < 0.001$ without a correction for multiple comparisons to avoid type-II error to explore whole brain and then applied small-volume correction ($P < 0.01$) to each cluster. Since there has been no a priori hypothesis for FA changes associated with DISC1 polymorphism, we applied conservative statistical threshold ($P < 0.001$) for the analysis of FA values. The resulting sets of *t*-values constituted the statistical parametric maps {SPM (t)}.

Molecular biology

Primary cultures were prepared from the cerebral cortex of postnatal 2-day-old rats (Wister ST; SLC, Shizuoka, Japan) as reported previously (47,48).

The siRNA transfection was performed as reported previously (49). We used 21 nt siRNA duplexes with two nucleotides of the rat DISC1 mRNA coding region (113–131, GACCAGGCTACATGAGAAG, NM_175596). Sense (GAC CAGGCUACAUGAGAAGtt) and antisense (CUUCUCAU GUAGCCUGGUCtc) strands were chemically synthesized by Ambion Ltd (Cambridge, UK). The siRNA (GCGCGC UUUGUAGGAUUCG) named ScrambleII from Dharmacon Research Inc. was used as a scramble control. The plasmid for viral construction of the DISC1 gene was derived from pSinRep5 (Invitrogen, USA) and had two subgenomic promoters followed by a multiple cloning site for an arbitrary gene insertion and an enhanced GFP open-reading frame, thus the virus can produce both arbitrary protein and enhanced GFP independently in the infected cell (50). The control virus produces GFP only, whereas DISC1 virus produces both DISC1 and GFP independently. Detail procedure for viral construction is in Supplementary Material.

Immunocytochemistry was performed, as described previously (51). We used anti-MAP2 (1:1000; Sigma) or anti-DISC1 (1:100) (17) antibodies as a primary antibody, respectively. Alexa Fluor (1:1000, Molecular Probes) was applied as a secondary antibody. Fluorescent images were observed by an inverted microscope (Axiovert 200, ZEISS) with a CCD (cool SNAPfx, ZEISS).

Immunoblotting was carried out as described previously (47). Primary antibodies for immunoblotting were used at the following dilutions: anti-Akt (1:1000, Cell Signaling), anti-phospho-Akt (1:1000, Cell Signaling), anti-ERK (1:1000, Cell Signaling), anti-phospho-ERK (1:1000, Cell Signaling), anti-TUJ1 (1:5000, Berkeley antibody company), anti-GFP (1:1000, Medical & Biological Laboratories) and anti-DISC1 antibodies (1:1000) (17). To quantify the amount of proteins after immunoblotting, we measured the density of immunoblots with an image-analysis software (Science Lab 98 Image Gauge; Fuji Photo Film Co. Ltd, Tokyo, Japan). The level of protein expression was indicated as a ratio that was normalized to control the condition (none, sole GFP-infected, or scramble-transfection, respectively) in each experiment. Statistical analysis was performed with unpaired *t*-test or ANOVA, followed by the Tukey *post hoc* comparisons when applicable.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement. The authors declare that they have no conflict of interests.

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Possible association between nonsynonymous polymorphisms of the anaplastic lymphoma kinase (ALK) gene and schizophrenia in a Japanese population

Short Communication

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Summary. We examined, for the first time, the possible association between schizophrenia and the anaplastic lymphoma kinase (ALK) gene which plays an important role in neurodevelopment. When two nonsynonymous polymorphisms (Arg1491Lys and Glu1529Asp) were examined, there were significant differences in genotype and allele distributions between patients and controls. Individuals homozygous for the minor allele (1491Lys–1529Asp) were more common in patients than in controls ($p = 0.0064$, odds ratio 2.4, 95% CI 1.3–4.6). These results suggest that genetic variations of the ALK gene might confer susceptibility to schizophrenia.

Keywords: Schizophrenia, anaplastic lymphoma kinase (ALK), single nucleotide polymorphism (SNP), association, susceptibility.

Introduction

Growing evidence has suggested that alterations of neurotrophic factors may be involved in the morphological, cytoarchitectural and neurobiochemical abnormalities in the brain of schizophrenic patients (Thome et al., 1998; Durany and Thome, 2004). Anaplastic lymphoma kinase (ALK) was originally identified as an oncogene activated in anaplastic large cell lymphomas with chromosomal translocation t (2;5) (Morris et al., 1994; Shiota et al., 1994). Subsequent cloning of the ALK gene revealed that it encodes a receptor-type protein tyrosine kinase (RTK) of the insulin receptor family (Iwahara et al., 1997; Morris et al., 1997). Neurotrophic factors exert their effects through binding to RTKs and play an important role in neurodevelopment such as

differentiation, proliferation, survival, and synaptic formation. Indeed, ALK was found to be a receptor for heparin-binding growth factors, midkine (Stoica et al., 2002) and pleiotrophin (Stoica et al., 2001). Midkine and pleiotrophin show approximately 50% identity in amino acid sequence and share the same genomic organization. These proteins play an important role in early neurogenesis, neurite outgrowth, nerve cell migration, and neuroprotection (reviewed by Kadomatsu and Muramatsu, 2004). Of note, a recent study reported alterations in serum midkine levels in patients with schizophrenia (Shimizu et al., 2003).

ALK is expressed almost exclusively in perinatal neural cells. In the central nervous system, it is highly expressed in diencephalons, midbrain, and the ventral half of the spinal cord. After birth, its expression decreases; however, it persists to be expressed in some regions such as the thalamus, olfactory bulb, and midbrain (Iwahara et al., 1997). These brain regions have been implicated in the pathophysiology of schizophrenia (e.g., Moberg and Turetsky, 2003; Clinton and Meador-Woodruff, 2004). The ALK gene is, therefore, a good candidate gene for association analysis with schizophrenia. To our knowledge, however, there is no study examining the possible association between the ALK gene and schizophrenia. The ALK gene maps to chromosome 2p23 (Morris et al., 1994). We searched for nonsynonymous single nucleotide polymorphisms (SNPs) in the ALK gene *in silico* and found only 2 common SNPs which have been well validated: a nucleotide substitution (G>A: NCBI SNP ID rs1881420) resulting in an amino acid change of Arg1491Lys (amino acid numbering is according to NCBI protein data base accession NP_004295) and G>C (rs1881421) resulting in Glu1529Asp. Since these polymorphisms may alter functions of ALK protein, we performed an association study between these polymorphisms and schizophrenia.

Materials and methods

Subjects

Subjects were 300 patients with schizophrenia (154 males, mean age of 45.3 years [SD 14.3]) and 308 healthy controls (140 males, 39.8 years [SD 11.5]). All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from hospital staffs and their associates. They were interviewed and those individuals who had current or past history of psychiatric treatment were not enrolled in the study.

The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). After description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committees at the Showa University School of Medicine and the National Center of Neurology and Psychiatry, Japan.

Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. The index SNPs (rs1881420 and rs1881421) were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay, as described previously (Hashimoto et al., 2004, 2005). Primers and probes for detection of the SNPs were as follows: 5′-TTCTCTCAGTCCAACCCTCCTT-3′ (forward primer), 5′-CTGGTGGGCTTGTTTCTGGAT-3′ (reverse primer), 5′-VIC-TTGCACAAGGTCCAC-MGB-3′ (probe 1), and 5′-FAM-TGCACAGGGTCCAC-MGB-3′ (probe 2) for rs1881420; 5′-AGAGAAACCC ACCAAAAAGAATAATCCT-3′ (forward primer), 5′-GTTAGGTGGGACAGTACAGCTT-3′ (reverse primer), 5′-VIC-CAGGTTACCCCTGTCGTGT-MGB-3′ (probe 1), and 5′-FAM-CAGGTTACCCCTCTCGTGT-MGB-3′ (probe 2) for rs1881421. Thermal cycling for polymerase chain reaction (PCR) were 1 cycle at 95°C for 10 minutes followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 minute. Genotype data were read blind to the case-control status.

Statistical analysis

The presence of Hardy-Weinberg equilibrium was examined by using the χ^2 test for goodness of fit.

Table 1. Genotype distributions and allele frequencies of the Glu1529Asp polymorphism of the ALK gene (rs1881421) in patients with schizophrenia and controls

	Genotype distribution				Allele frequency		
	N	Glu/Glu	Glu/Asp	Asp/Asp	N	Glu	Asp
Patients	300	141 (47%)	128 (43%)	31 (10%)	600	410 (68%)	190 (32%)
Controls	308	171 (55%)	123 (40%)	14 (5%)	616	465 (75%)	151 (25%)

Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. All p-values reported are two-tailed.

Results

Nearly all the subjects except for three (99.5%) had the same genotype for the two SNPs of rs1881420 and rs1881421, i.e., genotypes of G/G, G/A, and A/A in the former corresponded to those of G/G, G/C, and C/C in the latter. Thus, we show results of statistical analyses for the SNP rs1881421 (Glu1529Asp) only. Genotype distributions and allele frequencies in patients and controls are shown in Table 1. The genotype distribution was not significantly deviated from Hardy-Weinberg equilibrium for patients and controls (patients: $\chi^2 = 0.1$, df = 1, p = 0.81; controls: $\chi^2 = 1.9$, df = 1, p = 0.16). There was a significant difference in the overall genotype distribution between patients and controls ($\chi^2 = 9.3$, df = 2, p = 0.0095). Individuals homozygous for the minor allele (1529Asp) was significantly more common in patients than in controls ($\chi^2 = 7.4$, df = 1, p = 0.0064, odds ratio 2.4, 95% CI 1.3–4.6). When allele frequencies were compared, the 1529Asp allele was significantly more frequent in patients than in controls ($\chi^2 = 7.7$, df = 1, p = 0.0055, odds ratio 1.4, 95% CI 1.1–1.8).

Discussion

We examined, for the first time, the possible association between schizophrenia and the anaplastic lymphoma kinase (ALK) gene which plays an important role in neurodevelopment such as early neurogenesis, neurite outgrowth, nerve cell migration, and neuroprotection. We found that the minor allele (1529Asp) of the Glu1529Asp polymorphism (rs1881421) and homozygosity for this allele were significantly more common in patients with schizophrenia than in controls. Since nearly all the subjects had the same genotype for the other SNP, Arg1491Lys (rs1881420), the risk alleles constitute a haplotype 1491Lys–1529Asp. Thus, our results suggest that the 1491Lys–1529Asp haplotype or its homozygosity may confer susceptibility to schizophrenia. However, we do not know whether these nonsynonymous polymorphisms do alter functions of the ALK protein to give susceptibility to schizophrenia. Accordingly, there remains a possibility that other polymorphisms, which are in linkage disequilibrium to these polymorphisms, are truly responsible for giving susceptibility.

The ALK gene encodes a 1620 amino acid protein containing a putative 26 amino acid signal peptide, an extracellular domain of 1004 amino acid after signal peptide cleavage, a transmembrane domain of 28 hydrophobic amino acids, a juxtamembrane segment of 64 amino acids, a catalytic domain (protein tyrosine kinase domain) of 254 amino acids, followed by the carboxyl-terminal tail of 244 amino acids (Morris et al., 1997). The Arg1491Lys and Glu1529Asp residues lie close to a NPTY motif (residue 1504–1507) in the carboxyl-terminal tail (Morris et al., 1997). Such motifs mediate the interaction of RTKs with signaling substrates such as the insulin receptor substrate-1 and Src homology

and collagen proteins through the substrates's phosphotyrosine binding (PTB) domain (van der Geer and Pawson, 1995). It is possible that amino acid changes of Arg1491Lys and Glu1529Asp may alter protein structure and affect functions (e.g., binding to these substrates).

ALK is a receptor-type protein kinase (RTK) that is expressed preferentially in neurons of the central and peripheral nervous systems at late embryonic stages (Iwahara et al., 1997; Morris et al., 1997). Neurotrophic factors exert their effects through binding to RTKs, and ALK is a receptor for heparin-binding growth factors, midkine and pleiotrophin (Stoica et al., 2001, 2002). Thus it is likely that ALK play an important role in neurodevelopment such as differentiation, proliferation, survival, neurite outgrowth and synaptic formation, and alterations of ALK functions may result in vulnerability to developing schizophrenia, which accords with the neurotrophic factor theory of schizophrenia (Thome et al., 1998; Durany and Thome, 2004). Indeed, alterations in other neurotrophic factors such as brain-derived neurotrophic factors (BDNF) and neurotrophin-3 have been implicated in schizophrenia (e.g., Durany et al., 2001; Nanko et al., 2003; Hattori et al., 2002).

A limitation in the present study might be that the obtained evidence for association was not very strong (p-values of <0.01 level in a single sample). Replication studies in independent samples are required. If our results are replicated, experiments elucidating the possible effects of the amino acid substitutions (Arg1491Lys and Glu1529Asp) on the ALK protein functions may serve to advance our understanding of the molecular mechanisms of schizophrenia and may provide clues to production of new treatment of the illness.

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Antipsychotic medication and cognitive function in schizophrenia

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Abstract

Antipsychotic polypharmacy and excessive dosing still prevail worldwide in the treatment of schizophrenia, while their possible association with cognitive function has not well been examined. We examined whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive function. Furthermore, we compared cognitive function between patients taking only atypical antipsychotics and those taking only conventionals. Neurocognitive functions were assessed in 67 patients with chronic schizophrenia and 92 controls using the Wechsler Memory Scale-Revised (WMS-R), the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the Wisconsin Card Sorting Test (WCST), and the Advanced Trail Making Test (ATMT). Patients showed markedly poorer performance than controls on all these tests. Patients on non-standard antipsychotic medication demonstrated poorer performance than those on standard medication on visual memory, delayed recall, performance IQ, and executive function. Patients taking atypical antipsychotics showed better performance than those taking conventionals on visual memory, delayed recall, and executive function. Clinical characteristics such as duration of medication, number of hospitalizations, and concomitant antiparkinsonian drugs were different between the treatment groups (both dichotomies of standard/non-standard and conventional/atypical). These results provide evidence for an association between antipsychotic medication and cognitive function. This association between antipsychotic medication and cognitive function may be due to differential illness severity (e.g., non-standard treatment for severely ill patients who have severe cognitive impairment). Alternatively, poorer cognitive function may be due in part to polypharmacy or excessive dosing. Further investigations are required to draw any conclusions.

Keywords: Schizophrenia; Cognitive function; Atypical antipsychotics; Conventional antipsychotics; Polypharmacy

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1. Introduction

Schizophrenia is associated with wide-ranging deficits in neurocognitive function, including memory, attention, executive function, and working memory (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004), and these deficits are considered to be core to the pathophysiology of the illness. Reports of cognitive impairments in schizophrenia date back to the pioneering efforts of Kraepelin (1919) and Bleuler (1950) and, more recently, the characteristics of these deficits have been clarified with increasing sophistication and precision. School children who will later develop schizophrenia are more likely than their classmates to under-perform in school (Erlenmeyer-Kimling et al., 2000; Fuller et al., 2002; Kremen et al., 1998; Reichenberg et al., 2005), and cognitive deficits become widely present at the onset of psychosis (Bilder et al., 1992; Hoff et al., 1992). Growing evidence has suggested that cognitive deficits in schizophrenia are not byproducts of positive symptoms (Addington et al., 1991; Davidson et al., 1995) or negative symptoms (Bell and Mishara, 2006; Harvey et al., 1996; Harvey et al., 2005).

As impaired performance on measures of neurocognition is more closely linked to functional outcome than symptoms (Green, 1996; Green et al., 2000), enhancement of cognitive functioning is considered an important component of treatment for schizophrenia (Green et al., 2005; Hofer et al., 2005). Investigators have focused on the cognitive pathology of schizophrenia and have sought to assess the effects of treatment on this dimension. A large number of treatment studies have demonstrated that therapeutic effects of conventional drugs are limited to the positive symptoms of the illness and they have substantially less impact on cognitive impairments (Medalia et al., 1988; Spohn and Strauss, 1989), whereas atypical antipsychotics may ameliorate cognitive deficits (Bender et al., 2006; Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 1999, 2006; Kern et al., 1999; Meltzer and McGurk, 1999; Muller et al., 2005; Purdon et al., 2000; Rossi et al., 1997; Sumiyoshi et al., 2005; Thornton et al., 2006).

In spite of extensive research and recommendations as to the optimal prescription of antipsychotics, antipsychotic polypharmacy and excessive dosing are still highly prevalent worldwide, especially in Japan (Bitter et al., 2003; Chong et al., 2004; Faries et al., 2005; Ganguly et al., 2004; Procyshyn et al., 2001; Sim et al., 2004a; Weissman, 2002). This may be due in part to the scarcity of evidence for the possible association between antipsychotic medication in terms of dosage or type and cognitive function in these countries. In this context, the present study was aimed (1) to examine whether the "non-standard" use of antipsychotics (defined as antipsychotic polypharmacy or dosage >1000 mg/day of chlorpromazine equivalents) is associated with cognitive functions and (2) to compare cognitive deficits between patients treated with atypical antipsychotics and those with conventional drugs, using a comprehensive set of neurocognitive tests and by examining extensive clinical characteristics of patients.

2. Methods

2.1. Subjects

Patients with schizophrenia ($n=67$) who were under treatment at the National Center of Neurology and Psychiatry Musashi Hospital, Tokyo, Japan were recruited. All patients met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia. Consensus diagnosis was made by treating and research clinicians who were all senior psychiatrists, based on clinical interviews, observations, and case notes. Patients were chronic schizophrenia and were prescribed a stable dose of antipsychotic medication for at least 3 months prior to neuropsychological test sessions. Schizophrenic symptoms were rated by using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Healthy volunteers ($n=92$) who had no history of current or past contact to psychiatric services were recruited from the hospital staffs and their associates through fliers and by word of mouth. Those individuals who had a history of regular use of psychotropic agents were not enrolled in the control group. Participants were excluded from both the patient and control groups if they had prior medical histories of central nervous system disease or severe head injury, or if they met criteria for alcohol/drug dependence or mental retardation. All subjects were biologically unrelated Japanese who resided in the same geographical area (Western part of Tokyo Metropolitan). Written informed consent was obtained from all subjects and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Neuropsychological test measures

A comprehensive battery of neurocognitive tests was administered to all subjects in a random order that took at least 4 h to complete. The battery included the Wechsler Memory Scale-Revised (WMS-R, Sugishita,

2001; Wechsler, 1987), the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Shinagawa et al., 1990; Wechsler, 1981), the Wisconsin Card Sorting Test (WCST, Heaton, 1981; Kashima et al., 1987), and the Advanced Trail Making Test (ATMT, Nakahachi et al., 2006; Takahashi et al., 2005).

2.2.1. Wechsler Memory Scale-Revised

A full version of the WMS-R (Wechsler, 1987) was administered. The average score and standard deviation (S.D.) of WMS-R in the general population are 100 and 15, respectively. This test mainly measures memory functions, while it can also assess attention. Its four main outcome measures were verbal memory, visual memory, attention, and delayed recall.

2.2.2. Wechsler Adult Intelligence Scale-Revised

A full version of the WAIS-R (Wechsler, 1981) was administered, yielding scores of verbal IQ, performance IQ, and full-scale IQ.

2.2.3. Wisconsin Card Sorting Test

The WCST (Heaton, 1981) mainly assesses executive function including cognitive flexibility in response to feedback. We used a modified and computerized version of the test (Kashima et al., 1987; Kobayashi, 1999). Outcome measures were numbers of categories achieved, total errors, and perseverative errors of Milner and Nelson types.

2.2.4. Advanced Trail Making Test

The ATMT (Takahashi et al., 2005) is a computerized task modified from the original Trail Making Test (Reitan and Wolfson, 1993), and is considered to measure subjects' abilities of spatial working memory and psychomotor speed. In the present study, only spatial working memory was rated in all subjects.

2.3. Grouping procedures

Daily doses of antipsychotics, including depot antipsychotics, were converted to approximate chlorpromazine equivalents (CPZeq) using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999). The patient group was subdivided into two different types of subgroups by medication patterns. One grouping criterion was a "standard" or "non-standard" use of antipsychotics. The "standard" was defined as receiving antipsychotic monotherapy with a CPZeq dose of 1000 mg/day or less, and "non-standard" as polypharmacy (the use of more than one antipsychotic) or a CPZeq dose of more than 1000 mg/day. This classification was according to several precedent studies (Diaz and De Leon, 2002; Edlinger et al., 2005; Ito et al., 2005; Lehman and Steinwachs, 1998; Sim et al., 2004b; Waddington et al., 1998; Weissman, 2002). The other grouping criterion was whether patients were treated only with conventional or only with atypical antipsychotics, and those who were treated with both types of antipsychotics were excluded from this grouping criterion.

2.4. Statistical analyses

Averages are reported as means±S.D. Demographic characteristics and test results were compared between groups. We used the *t*-test to compare mean scores. Categorical variables were compared with χ^2 test or Fisher's exact test where appropriate. The analysis of covariance (ANCOVA) was used to compare neuropsychological test results of patients and those of controls, controlling for a confounding variable. All comparisons were made between two groups, namely between patients and controls, the "standard" and "non-standard" groups, or conventional and atypical groups. Statistical significance was set at two-tailed $p<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

3.1. Sample characteristics

Demographic and clinical characteristics are presented in Table 1. There were no differences between patients and controls in sex, age, or handedness. Patients with schizophrenia demonstrated significantly shorter education years and a higher rate of cigarette smoking compared to controls. The "non-standard" group, as expected, showed significantly greater CPZeq and more frequent use of conventional antipsychotics and antiparkinsonian drugs than the "standard" group. The number of hospitalizations was significantly larger in "non-standard" than in "standard" group. The conventional group showed significantly longer duration of medication, more frequent use of antiparkinsonian drugs, and larger number of hospitalizations than the atypical group.

3.2. Neuropsychological test scores in patients vs. controls

Patients with schizophrenia showed significantly poorer performance than healthy controls on all the neuropsychological tests (Table 2). Although control

Table 1
Demographic and clinical characteristics of schizophrenia patients and controls (including comparisons of "standard" vs. "non-standard" and conventional vs. atypical group)

Variable	Schizophrenia patients (N=67)	Healthy controls (N=92)	p value (patients vs. controls)	Standard group (N=26)	Non-standard group (N=41)	p value (standard vs. non-standard)	Conventional group (N=23)	Atypical group (N=22)	p value (conventional vs. atypical)
Sex (male/female)	40/27	54/38	0.90	14/12	26/15	0.44	16/7	11/11	0.18
Age (years)	42.7±11.9	43.0±14.5	0.90	42.4±14.5	42.9±10.1	0.87	46.2±11.7	39.8±13.6	0.095
Education (years)	13.4±2.7	16.4±3.1	<0.001	13.8±2.1	13.0±3.0	0.24	13.5±3.3	13.6±2.2	0.93
Handedness (right/left)	64/3	84/8	0.50	26/0	38/3	0.28	22/1	22/0	1.0
Smoking (yes/no)	32/35	23/69	0.0029	12/14	20/21	0.83	12/11	10/12	0.65
Family history of schizophrenia (yes/no)	15/52			7/19	8/33	0.48	17/6	17/5	0.79
Age at onset (years)	25.0±8.3			26.2±9.4	24.2±7.5	0.33	24.2±7.2	26.7±10.1	0.34
Duration of medication (years)	13.3±10.4			10.6±12.8	15.1±8.3	0.090	17.9±11.5	8.2±9.9	0.0045
CPZeq of total antipsychotic drugs (mg/day)	877±749			473±268	1134±840	<0.001	804±634	521±322	0.065
Antipsychotics (conventional/ both/atypical)	23/22/22			5/0/21	18/22/1	<0.001	–	–	
Antiparkinsonian drug use (yes/no)	48/19			14/12	34/7	0.010	2/21	12/10	0.0012
Age at first hospitalization (years)	29.1±12.2			29.0±15.2	29.1±10.7	0.98	30.3±10.7	27.5±14.9	0.52
Number of hospitalizations	2.1±2.1			1.2±1.7	2.7±2.1	0.0043	2.4±2.1	1.2±1.7	0.042
Duration of total hospitalizations (months)	35.0±78.4			27.0±68.8	40.1±84.4	0.51	46.3±107.2	20.0±53.1	0.31
Outpatients/inpatients	45/22			19/7	26/15	0.41	17/6	16/6	0.93
PANSS total score	63.1±17.4			59.7±17.9	65.1±17.1	0.36	63.3±17.0	57.6±16.4	0.39

Underlined figures represent significant differences.

Table 2
Test results of patients with schizophrenia and control subjects (including comparisons of standard vs. non-standard and conventional vs. atypical group)

Variable	Patients with schizophrenia (N=67)	Control subjects (N=92)	p value (patients vs. controls)	Standard group (N=26)	Non-standard group (N=41)	p value (standard vs. non-standard)	Conventional group (N=23)	Atypical group (N=22)	p value (conventional vs. atypical)
WMS-R									
Verbal memory	81.9±18.3	112.2±14.2	<0.001	86.0±19.9	79.2±16.8	0.14	85.3±16.0	83.6±20.1	0.75
Visual memory	83.8±21.0	110.1±10.4	<0.001	92.2±20.1	78.4±20.0	0.008	79.6±21.0	94.7±19.1	0.016
Attention	90.8±14.0	107.9±14.5	<0.001	92.3±14.6	89.8±13.7	0.46	92.0±12.5	92.5±14.6	0.89
Delayed recall	80.4±19.8	113.2±12.8	<0.001	88.8±20.0	75.1±17.8	0.005	78.2±14.7	90.8±20.6	0.023
WAIS-R									
Information	8.7±3.7	11.3±3.0	<0.001	9.4±3.2	8.2±3.9	0.16	9.5±3.5	8.6±3.5	0.38
Digit span	8.5±2.8	11.4±3.1	<0.001	8.7±2.5	8.4±3.0	0.74	8.5±3.3	8.8±2.0	0.72
Vocabulary	8.3±3.3	12.1±2.9	<0.001	9.5±2.9	7.6±3.3	0.015	8.4±3.4	9.2±3.2	0.40
Arithmetic	8.0±3.2	12.6±3.0	<0.001	8.3±2.6	7.8±3.5	0.47	8.7±3.4	8.3±3.0	0.66
Comprehension	7.6±3.3	11.7±2.8	<0.001	8.4±3.3	7.2±3.3	0.15	7.1±3.0	8.3±3.4	0.22
Similarities	9.6±3.3	12.7±2.0	<0.001	10.2±3.4	9.2±3.2	0.22	9.5±3.3	10.2±3.5	0.49
Picture completion	8.5±3.0	10.6±2.4	<0.001	9.3±2.3	8.0±3.3	0.056	8.8±3.1	9.1±2.2	0.70
Picture arrangement	8.1±3.3	11.7±2.4	<0.001	8.3±3.0	7.9±3.4	0.59	7.9±2.8	8.5±3.2	0.48
Block design	9.3±3.5	13.1±2.6	<0.001	10.6±3.3	8.4±3.4	0.012	9.1±3.4	10.6±3.0	0.12
Object assembly	8.3±3.4	11.5±3.0	<0.001	9.4±3.0	7.7±3.5	0.036	7.6±3.3	9.4±3.0	0.062
Digit symbol	7.0±2.8	12.6±2.7	<0.001	7.2±2.6	6.8±3.0	0.58	7.6±2.1	7.1±2.8	0.52
Verbal IQ	90.2±16.7	112.7±13.7	<0.001	94.4±14.2	87.6±17.8	0.090	91.3±16.2	93.0±15.3	0.72
Performance IQ	86.8±16.6	112.1±11.5	<0.001	92.1±13.9	83.4±17.4	0.036	86.4±14.0	91.7±14.2	0.21
Full-scale IQ	87.7±17.0	113.5±12.4	<0.001	92.7±13.9	84.4±18.0	0.0502	88.3±15.0	91.7±14.6	0.44
WCST									
Categories achieved	2.0±2.0	3.7±2.0	<0.001	2.6±2.1	1.7±1.8	0.080	1.6±1.9	2.4±2.2	0.18
Total errors	26.1±10.3	18.4±8.5	<0.001	22.1±9.8	28.7±9.9	0.010	30.6±11.3	21.9±10.0	0.0089
Perseverative errors of Milner	7.5±9.2	2.7±3.7	<0.001	5.8±8.2	8.5±9.7	0.26	11.3±12.5	5.3±7.1	0.051
Perseverative errors of Nelson	9.9±8.9	4.9±5.2	<0.001	7.2±8.1	11.5±9.1	0.055	13.5±11.3	6.0±7.0	0.0098
ATMT (spatial working memory)	27.8±8.9	35.8±8.6	<0.001	30.3±8.7	26.3±8.8	0.068	27.3±8.4	30.4±9.1	0.25

Underlined figures represent significant differences.

subjects in the present study performed rather better than general population on the standardized WMS-R and WAIS-R, performance on all indices of the two tests in patients were poorer than that in general population. Since the difference in education years between the two diagnostic groups had a possibility of confounding the difference in the test results, we performed ANCOVA, controlling for education years. It revealed that all the performance on the tests were significantly poorer in patients than in controls (all $p<0.01$).

3.3. Test scores in standard vs. non-standard group

As presented in Table 2, mean scores on all indices of the cognitive tests were better in patients treated with the standard use of antipsychotics than in those treated with the non-standard use; seven measures reached statistical significance, i.e., visual memory, delayed recall (WMS-R), vocabulary, block design, object assembly, performance IQ (WAIS-R), and number of total errors (WCST).

3.4. Test scores in conventional vs. atypical antipsychotics group

Test results in patients treated with conventional drugs and those with atypical drugs are presented in Table 2. All mean scores except verbal memory (WMS-R), information, arithmetic, and digit symbol (WAIS-R) were favorable to the atypical antipsychotic group. The atypical group performed significantly better than the conventional group on visual memory, delayed recall, WCST total errors, and perseverative errors of Nelson.

4. Discussion

Our results confirmed that a wide range of cognitive functions including memory, attention, working memory, executive function, and general intellectual function are substantially impaired in patients with chronic schizophrenia, which is consistent with an abundance of studies (Bozikas et al., 2006; Fioravanti et al., 2005; Gold et al., 1992; Joyce and Huddy, 2004; Keefe et al., 2005; Reed et al., 2002; Silver et al., 2003; Suwa et al., 2004).

4.1. Standard vs. non-standard medication

Congruent with recent reports (Bitter et al., 2003; Chong et al., 2004; Sim et al., 2004a), non-standard use of antipsychotics (i.e., excessive use of antipsychotics or polypharmacy) was frequent in our Japanese patients with schizophrenia (standard 39% vs. non-standard 61%). Patients in non-standard group showed significantly poorer performance than those in standard group on visual memory, delayed recall, performance IQ, and executive function. Since the symptom severity (assessed with PANSS) of the two groups was similar, the difference in cognitive performance cannot be ascribed to difference in symptom severity at the time of neurocognitive tests. However, other clinical characteristics such as number of hospitalizations (with statistical significance) and duration of medication (with statistical trend), from which the original illness severity would be presumed, were different between the two treatment groups. In this situation, the illness of the non-standard group might be severer than that of the standard group at the outset, thus requiring the additional medication to reach the same level of improvement. Moreover, Joyce et al. (2005) reported that cognitive heterogeneity was present in patients with schizophrenia at illness onset. In this context, primary explanation for the association between differences of antipsychotic medication (standard/non-standard) and of cognitive function could be that both of them are due to the same cause, namely the difference of original illness severity. Alternatively, the other plausible explanation for the difference of cognitive performance between the two medication groups might be that polypharmacy and excessive dosing of antipsychotics have detrimental effects on brain and cause poorer cognitive function. This raises the possibility that cognitive deficits could be reduced by changing non-standard to standard prescription if symptoms of patients permit. Since the non-standard treatment group was more likely to be on concomitant antiparkinsonian medication, such drugs could also play a causal role in the poorer cognitive function, which was in line with prior reports (McGurk et al., 2004; Minzenberg et al., 2004; Strauss et al., 1990). To draw any conclusion, longitudinal studies that investigate from illness onset to chronic phase are necessary.

4.2. Conventional vs. atypical

When patients were divided into conventional and atypical antipsychotic groups, the latter demonstrated significantly better performance than the former on visual memory, delayed recall, and executive function. In our subjects, most patients in the atypical group were medicated with either risperidone or olanzapine. Indeed, these drugs have been reported to be superior to conventional drugs or even to other atypical antipsychotics (Bilder et al., 2002; Cuesta et al., 2001; Kern et al., 1999; McGurk et al., 2005; Mori et al., 2004;

Thornton et al., 2006). Although findings to date on specific effects of these two agents on cognition have been somewhat inconsistent, beneficial effects of risperidone on several cognitive domains including memory (Bilder et al., 2002; Keefe et al., 2006; Kern et al., 1999), executive function (Keefe et al., 2006; Meltzer and McGurk, 1999; Rossi et al., 1997), and working memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004), and olanzapine on memory (Keefe et al., 2006; Meltzer and McGurk, 1999; Mori et al., 2004; Sumiyoshi et al., 2005; Thornton et al., 2006) and executive function (Bender et al., 2006; Bilder et al., 2002; Keefe et al., 2006; Meltzer and McGurk, 1999) have been reported. Precedent studies have demonstrated that atypical antipsychotics had favorable effects especially on verbal memory out of memory function (Meltzer and McGurk, 1999), which was not in harmony with our results that suggested favorable effects of atypical agents on visual memory instead of verbal memory. In general, our results might be consistent with prior studies reporting superiority of atypical to conventional antipsychotics in terms of cognitive function. In the present study, however, clinical characteristics such as duration of medication, number of hospitalizations, and antiparkinsonian drug use were significantly different between the two medication groups. Moreover, CPZeq reached nearly significant difference between the two groups. In this study, therefore, causal relationship between medication type (conventional/atypical) and cognitive function is quite difficult to argue due to these confounders.

4.3. Limitations

There were several limitations to the current study. The cross-sectional nature of the study did not allow drawing any definite conclusions regarding causality between antipsychotic medication and its correlates. Since control subjects in the present study performed better than the general population on the standardized WMS-R and WAIS-R, it was possible that the differences between patients and controls were exaggerated. As the patients involved in this study suffered from chronic schizophrenia, the findings can not be generalized to recent-onset schizophrenia. The sample size was not very large, which may have resulted in type II errors.

4.4. Conclusion

In conclusion, this study confirms that patients with chronic schizophrenia have wide-ranging cognitive impairments and provides evidence for an association between antipsychotic medication (both standard/non-standard and conventional/atypical) and cognitive function. The differences of medication and of cognitive function are associated but both could be due to original illness severity. Alternatively, cognitive deficits in schizophrenia could be reduced in part by specific medication pattern, especially by atypical antipsychotic monotherapy at ordinary dosage.

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The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology

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Abstract

We investigated the effects of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism on age-associated changes of brain morphology in 109 Japanese healthy subjects using MRI with optimized voxel-based morphometry technique. A significant age-related volume reduction was found in the dorsolateral prefrontal cortices (DLPFC), anterior cingulate cortices, and temporal and parietal cortices in all subjects. Further analysis revealed a significantly negative correlation between age and the volume of the bilateral DLPFC only in the Met-BDNF carriers, and a significant interaction between the polymorphism and age-associated volume changes in the bilateral DLPFC. Furthermore, Met-carriers showed a significant interaction ($p < 0.0001$) between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Our data suggest that the Val66Met polymorphism may impact on age-related changes of the brain, which might be associated with the functional variance of neuroprotective effects of the BDNF. Furthermore, we suggest that genotype effects of the BDNF gene on brain morphology might differ in female from in male.

Keywords: Brain-derived neurotrophic factor; Val66Met polymorphism; Magnetic resonance imaging; Voxel-based morphometry; Dorsolateral prefrontal cortex; Aging

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, has important roles in hippocampal plasticity and hippocampal-related learning and memory through long-term potentiation [15]. It also plays an important role in preventing death of neurons during development and protecting cholinergic neurons of the basal forebrain and the hippocampus from induced death in the adult brain [21].

A common missense polymorphism of the BDNF gene producing a valine to methionine amino acid substitution (Val66Met) affects the activity dependent secretion of BDNF in neurons and affects memory function [6,8]. Neuroimaging studies revealed that this polymorphism affected memory-related neuronal activities measured by functional magnetic resonance imaging (MRI) and macroscopic morphology of the hippocampus [8,12,23,28]. Regarding the brain morphology in normal individuals, Pezawas et al. [23] reported that Met-BDNF carriers had smaller volumes of the hippocampi and the prefrontal cortices as compared to individuals with homozygous Val-BDNF. This result was recently replicated in another mixed study of healthy and schizophrenic subjects [28]. Although several neuroimaging studies have indicated that environmental factors considerably impact on human brain structures even in normal adult brains [18], these data suggest that genetic factors such as polymorphism of BDNF might also strongly affect human brain morphology, and contribute to individual differences of brain morphology.

Aging is another factor which strongly affects brain morphology in human. There are several studies that demon-

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strated morphological changes associated with normal aging in vivo [10,24]. A general trend in the in vivo volumetric studies of healthy volunteers points to the prefrontal cortex as the cortical region in which the largest age-related volume reduction is observed. Considering the previous findings that BDNF is expressed abundantly in the prefrontal cortex [25] and that BDNF has a neuroprotective effect, Val66Met polymorphism might have some impacts on age-related morphological changes. However, there is no datum whether this polymorphism is associated with age-related morphological changes.

To clarify whether the BDNF polymorphism impacts on morphological changes associated with aging, we analyzed structural MR images in 109 normal individuals using optimized voxel-based morphometry (VBM) technique.

One hundred and thirty healthy subjects participated in the study. Written informed consent was obtained from all subjects in accord with ethical guidelines in place at local ethical committee. All of the subjects were recruited from local advertisements and underwent a Japanese version of National Adult Reading Test (JART) that is essentially the same as National Adult Reading Test [22] and MRI scanning. We employed JART as a convenient tool to measure IQ for each participant because previous study reported that it showed high correlation with IQ in healthy subjects [20]. All subjects were screened by a questionnaire regarding medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2 weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. Template creation for the optimized VBM was based on a sample of the 120 subjects, aged 36.2 ± 12.1 years (range 20–72). All subjects were Japanese. Since single nucleotide polymorphism (SNP) genotyping, described in the next section, was done successfully in 109 subjects, the MR images of these 109 subjects were used for subsequent analyses. According to the polymorphism, subjects were categorized into the following three groups: a homozygous Val-BDNF group ($n=41$), a Val/Met-BDNF group ($n=51$), or a homozygous Met-BDNF group ($n=17$). The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium (chi-square = 0.03, $p=0.86$). Because of the small number of subjects with homozygous Met-BDNF, the Val/Met-BDNF group and homozygous Met-BDNF group were treated as one group, the Met-BDNF carriers ($n=68$). The demographic data of these groups are the following; the homozygous Val-BDNF comprised 26 females and 15 males, two were left-handed, aged 36.9 ± 13.0 years (range 21–68), and the mean education period and JART score were 16.2 ± 2.8 years (range 12–24) and 75.5 ± 13.3 (equivalent to 108.8 ± 9.55 for full scale IQ (range 50–96; equivalent to 90.5–123.6 for full scale IQ), respectively. The Met-BDNF carriers comprised 45 females and 23 males, three were left-handed, aged 35.8 ± 11.6 years (range 20–72), and their mean education period and JART score were 16.9 ± 3.0 years (range 12–28) and 78.0 ± 11.6 (equivalent to 110.7 ± 8.3) for full scale IQ (range 45–99; equivalent to 86.9–125.8 for full scale IQ), respectively. The mean age, gender ratio, handedness, education period, or JART score did not differ between the two groups (two sample t-test, data not shown).

The detail process of genotyping of BDNF Val66Met SNP (dbSNP accession: rs6265) was described previously [13]. Primers and probes for detection of the SNP (TaqMan SNP Genotyping assays on demand) were purchased from Applied Biosystems (ABI, Foster City, CA, USA). PCR cycling conditions were: at 95 °C for 10 min, 50 cycles of 92 °C for 15 s and 60 °C for 1 min.

All MR studies were performed on a 1.5 T Siemens Magnetom Vision plus system. A three dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 144 sagittal sections using an MPRage sequence (TE/TR, 4.4/11.4 ms; flip angle, 15°; acquisition matrix, 256×256; 1NEX, field of view, 31.5 cm; slice thickness, 1.23 mm).

Data were analyzed with Statistical Parametric Mapping 2 (SPM2) (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Department of Imaging Neuroscience, London, UK) running on MATLAB 6.5 R1 (MathWorks, Natick, MA). Before analyses, each image was confirmed by a neuroradiologist to eliminate images with artifacts, and then anterior commissure–posterior commissure line was adjusted. First, we made a customized anatomical T1 template and prior probability images from the sample of 120 brains [10]. Then, images were processed using an optimized VBM script (dbm.neuro.uni-jena.de/vbm.html). The detail of this process is described elsewhere [2,10]. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue density changes in the normal space. Finally, images were smoothed using a 12 mm full width half maximum of isotropic Gaussian kernel. Statistical analyses were performed with SPM2, which implemented a General Linear Model. Proportional scaling was used to achieve global normalization of voxel values between images. First, we used a two-sample t-test to test regional population effect on gray matter volume. For this analysis, we set $p<0.005$ without a correction for multiple comparisons, followed by applying small volume correction to each cluster with a false discovery rate (FDR) < 0.05. For the small volume correction, spheres with radius 10 mm around the peak were set as regions of interest (ROIs). The resulting sets of t-values constituted the statistical parametric maps {SPM (t)}. Anatomic localization was according to both MNI coordinates and Talairach coordinates, obtained from M. Brett's transformations (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates. Since a previous study with Caucasians demonstrated a significant reduction of volumes in the hippocampi and the frontal cortices in Met-BDNF carriers, we applied an additional hypothesis-driven ROI method to test regional population effects in these regions by using the Wake Forest University PickAtlas [19].

The genotype effects on age-related morphological changes were tested using a single subject condition and covariate model. Since several studies reported gender different age-related mor-

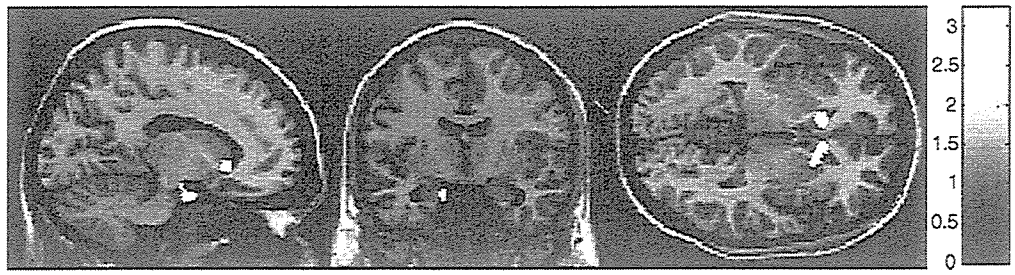

Fig. 1. The volume reduction of Met-BDNF carriers compared to that of individuals with homozygous Val-BDNF ($p < 0.05$, small volume correction with FDR). A significant reduction of volumes of the left parahippocampal gyrus (t-value: 2.92, Talairach coordinates (TAL): −12, −3, −19) and the bilateral heads of caudate nucleus (left: t-value; 3.23, TAL: −9, 22, −3, right: t-value; 3.02, TAL: 10, 21, −4) in the Met-BDNF carriers was noted.

phological changes in the brain [7], we additionally examined genotype effects on age-related morphological changes in each gender, separately. Orthogonalized first order polynomial expansion of age was treated as a covariate of interest to determine the linear effects of age [5]. Since second- and third-order polynomial expansions did not contribute to the age effect model of our sample, we removed them from a design matrix. Considering the possible association between IQ and brain morphology, we treated JART score as a nuisance variable. For this analysis, we applied $p < 0.001$, corrected for multiple comparisons with FDR < 0.05 as a statistical threshold [9]. MarsBar program (marsbar.sourceforge.net/) was also used to extract data from the regions of interest.

Fig. 1 shows a significant reduction of gray matter volumes of the left parahippocampal gyrus (Brodmann area (BA) 34), and bilateral heads of the caudate nucleus in Met-BDNF carriers when compared to homozygous Val-BDNF individuals. Even in hypothesis-driven ROI approach with a lenient statistical threshold (uncorrected $p = 0.05$), we could not find any significant differences of hippocampal nor prefrontal cortical volumes between the two groups. The results were essentially unchanged even when the restricted samples of subjects (female group, male group, or young group aged under 40 years old) were analyzed (data not shown).

Fig. 2 shows morphological changes related to normal aging. A significant negative correlation between age and the gray matter volumes was noted in the bilateral dorsolateral prefrontal cortices (DLPFC; BA9, 46), right superior temporal gyrus (STG; BA22), bilateral insulae (BA13), bilateral caudate nuclei, left anterior cingulate gyrus (BA24), bilateral inferior parietal lobules (BA40), bilateral precunei (BA7), and bilateral fusiform gyri (BA37) in all subjects. In homozygous Val-BDNF individuals, a significant age-related volume reduction was found in the bilateral insulae (BA13) and right STG (BA22). On the other hand, Met-BDNF carrier showed an additional negative correlation of the gray matter volumes in the bilateral DLPFC (BA9, 46) and right dorsal premotor area (BA6) with age. Additional analyses in each gender revealed a significant interaction ($p < 0.0001$) in Met-carriers between the gender and the genotype on the gray matter volume in the DLPFC, and female Met-carriers showed more widespread age-associated volume reduction in DLPFC than male Met-carriers. Male Met-carrier also showed volume

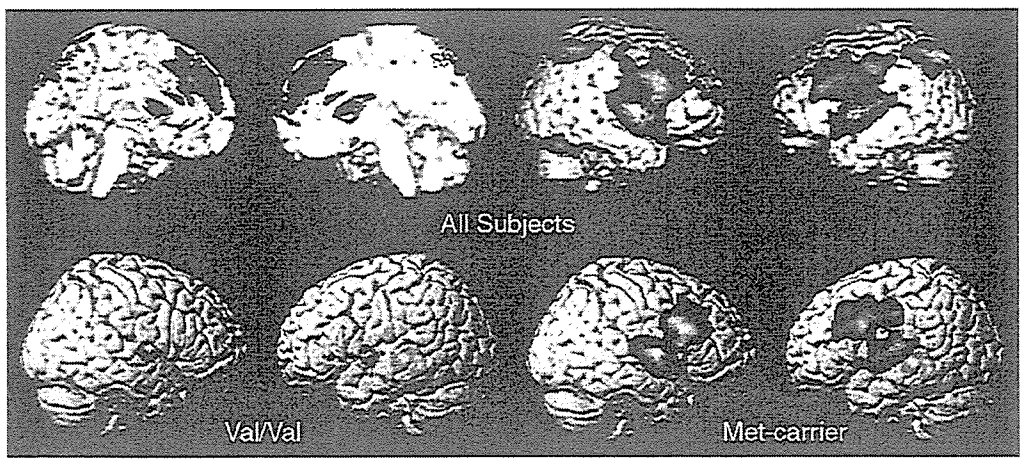

Fig. 2. (Top) The volume reduction associated with normal aging in all subjects ($p < 0.05$, FDR corrected). All subjects showed negative correlation with age in the bilateral DLPFC, right STG, bilateral insulae, bilateral caudate nuclei, left anterior cingulate gyrus, bilateral inferior parietal lobules, bilateral precunei, and bilateral fusiform gyri. (Bottom) The volume reduction associated with normal aging in each genotypic group ($p < 0.05$, FDR corrected). (Left) Results of individuals with homozygous Val-BDNF. Individuals with homozygous Val-BDNF showed negative correlation with age in the bilateral insulae (right: t-value: 4.36, TAL: 42, −2, 4; left: t-value: 4.52, TAL: −43, −2, 4) and the right superior temporal gyrus (t-value: 4.57, TAL: 47, 9, −4). (Right) Results of Met-BDNF carriers. The Met-BDNF carriers showed negative correlation with age in the bilateral dorsolateral prefrontal cortices (right: t-value: 6.5, TAL: 52, 21, 26; left: t-value: 6.12, TAL: −48, 19, 32) as well as the bilateral insulae and the superior temporal gyri.