Akita Hospital, Fujii Hospital, Hosogi Unity Hospital, Jounan Hospital, Jousei Hospital, Kawauchi Hospital, Nankai hospital, Sea Gull hospital, Taoka East Hospital, Tokushima University Hospital, Tokushima Prefectural Central Hospital and Yu-ai Hospital). The authors would like to thank Mrs. Akemi Okada and Mrs. Kumiko Kikuchi for their technical assistance. This work was supported by a Health and Labor Science Research Grant from the Japanese Ministry of Health, Labor and Welfare, a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology and a Grant-in-Aid for Scientific Research from the 21st Century COE program, Human Nutritional Science on Stress Control, Tokushima, Japan.

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Progress in Neuro-Psychopharmacology & Biological Psychiatry 31 (2007) 873–877
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Failure to confirm an association between the *PLXNA2* gene and schizophrenia in a Japanese population

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Received 27 October 2006; received in revised form 5 January 2007; accepted 29 January 2007
Available online 7 February 2007

Abstract

Plexins are receptors for multiple classes of semaphorins, either alone or in combination with neuropilins. Plexins participate in many cellular events that include axonal repulsion, axonal attraction, cell migration, axon pruning, and synaptic plasticity. *PLXNA2* maps to chromosome 1q32. Several linkage studies reported schizophrenia susceptibility loci in the 1q22–42 region. A recent study reported that intronic single nucleotide polymorphisms (SNPs) of *PLXNA2* were associated with schizophrenia in a European American population. We attempted to replicate this finding in a Japanese sample of 336 patients with schizophrenia and 304 controls. In addition, we examined 3 non-synonymous SNPs (Arg5Gln, GLn57Arg, and Ala267Thr) in *PLXNA2*. Genotyping was performed by the TaqMan allelic discrimination assay. There was no significant difference in genotype or allele distribution of either the 4 intronic SNPs or the 3 non-synonymous SNPs between patients and controls. Furthermore, haplotype-based analyses did not provide evidence for an association. These results suggest that *PLXNA2* may not play a major role in the development of schizophrenia in our Japanese sample.

Keywords: Association; Haplotype; Plexin A2; Schizophrenia; Single nucleotide polymorphism (SNP)

1. Introduction

Plexin was originally identified as a neuronal cell surface molecule that has been identified in *Xenopus* (Ohta et al., 1995; Takagi et al., 1987). To date, plexins have been identified in both invertebrate and vertebrate (Fujii et al., 2002; Kameyama et al., 1996a,b; Maestrini et al., 1996; Suto et al., 2003; Tamagnone et al., 1999; Winberg et al., 1998). Plexins were shown to act as receptors for multiple classes of semaphorins either alone or in a complex together with neuropilins (reviewed in Yazdani and Terman, 2006). Plexin A families and neuropilins form a stable complex as functional receptors for class 3 semaphorins (Takahashi et al., 1999). For example, semaphorin 3A (Sema3A) binds to neuropilin-1 (Nrp1) and activates plexin A1 (Plxna1) or plexin A2 (Plxna2) to transduce a repulsive axon guidance signal (Takahashi and Strittmatter, 2001). Many studies of plexins have concentrated on its roles in nervous development (reviewed in Kruger et al., 2005; Waimey and Cheng, 2006; Halloran and Wolman, 2006). In addition to their roles in axon guidance, semaphorin–plexin signaling has been known to play important roles in axon pruning (Liu et al., 2005; Bagri et al., 2003). Moreover, recent studies suggest that some semaphorins and their receptors might be involved in modulation of synaptic structure (Godenschwege et al., 2002; Morita et al., 2006; Bouzioukh et al., 2006; Waimey and Cheng, 2006).

Growing evidence has suggested that schizophrenia has neurodevelopmental abnormalities that might occur early in life

Abbreviations: SNP; single nucleotide polymorphism; DSM-IV; 4th edition of the Diagnostic and Statistical Manual of Mental Disorders.

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0278-5846/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2007.01.027

(Conrad and Scheibel, 1987; Weinberger, 1987; Murray, 1994; Waddington et al., 1998). A recent study reported that SEMA3A was increased in the cerebellum in the postmortem brains of schizophrenia patients (Eastwood et al., 2003). More recently, a genome-wide association study using 25494 single nucleotide polymorphisms (SNPs) pointed out that an intronic SNP of the *PLXNA2* gene were most consistently associated with schizophrenia in a European American population (Mah et al., 2006). These findings suggested the possibility that genetic variations of the *PLXNA2* gene confer susceptibility to schizophrenia through its effects on neurodevelopment and synaptic plasticity. To our knowledge, however, the association between the *PLXNA2* gene and schizophrenia has not yet been supported or refuted by any other study. The present study was thus aimed to examine whether there is such an association in a Japanese sample. In addition, we examined 3 additional non-synonymous polymorphisms of the *PLXNA2* gene (Arg5Gln, GLn57Arg, and Ala267Thr) for association with schizophrenia.

2. Materials and methods

2.1. Subjects

Subjects were 336 patients with schizophrenia (204 males, mean age of 44.2 years [SD 13.7]) and 304 healthy controls (99 males, mean age of 38.8 years [SD 13.4]). 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. Control individuals were interviewed and those who had a current or past history of psychiatric treatment were not enrolled in the study. The study protocol was

Table 1
Genotype and allelic distribution of the *PLXNA2* SNPs in Japanese patients with schizophrenia and controls

dbSNP ID	Position[a]	Inter-SNP distance (bp)	Group	*N*	Genotype distribution (frequency)			Allele distribution (frequency)		Odds ratio (95% CI)	Chi-square test[b]		
											HWE ($df=1$)	GF ($df=2$)	AF ($df=1$)
rs2782948	206457877				CC	CT	TT	C	T				
	Exon 2	–	Schizophrenia	334	89 (0.27)	160 (0.48)	85 (0.25)	338 (0.51)	330 (0.49)	1.15	$\chi^2=0.58$, $P=0.45$	$P=0.31$	$P=0.23$
	Arg5Gln		Control	303	65 (0.21)	156 (0.51)	82 (0.27)	286 (0.47)	320 (0.53)	(0.92–1.43)	$\chi^2=0.33$, $P=0.57$	$\chi^2=2.34$	$\chi^2=1.47$
rs11119014	206457721				AA	AG	GG	A	G				
	Exon 2	156	Schizophrenia	334	265 (0.79)	61 (0.18)	8 (0.02)	591 (0.88)	77 (0.12)	0.82	$\chi^2=3.65$, $P=0.056$	$P=0.37$	$P=0.28$
	Gln57Arg		Control	301	246 (0.82)	52 (0.17)	3 (0.01)	544 (0.90)	58 (0.10)	(0.57–1.17)	$\chi^2=0.02$, $P=0.90$	$\chi^2=1.99$	$\chi^2=1.19$
rs3748735	206457092				CC	CT	TT	C	T				
	Exon 2	629	Schizophrenia	334	249 (0.75)	78 (0.23)	7 (0.02)	579 (0.86)	92 (0.14)	1.06	$\chi^2=0.09$, $P=0.76$	$P=0.31$	$P=0.77$
	Ala267Thr		Control	303	228 (0.75)	63 (0.21)	12 (0.04)	519 (0.86)	87 (0.14)	(0.77–1.45)	$\chi^2=7.23$, $P=0.0072$	$\chi^2=2.33$	$\chi^2=0.09$
rs2498028	206321936				GG	GA	AA	G	A				
	Intron11	135156	Schizophrenia	335	147 (0.44)	147 (0.44)	41 (0.12)	441 (0.66)	229 (0.34)	1.00	$\chi^2=0.21$, $P=0.65$	$P=0.65$	$P=0.99$
	–		Control	303	128 (0.42)	143 (0.47)	32 (0.11)	399 (0.66)	207 (0.34)	(0.79–1.26)	$\chi^2=0.73$, $P=0.39$	$\chi^2=0.87$	$\chi^2=0.00$
rs1327175	206313757				GG	GC	CC	G	C				
	Intron12	8179	Schizophrenia	334	266 (0.80)	63 (0.19)	5 (0.01)	595 (0.89)	73 (0.11)	0.92	$\chi^2=0.32$, $P=0.57$	$P=0.83$	$P=0.63$
	–		Control	302	244 (0.81)	55 (0.18)	3 (0.01)	543 (0.90)	61 (0.10)	(0.64–1.31)	$\chi^2=0.00$, $P=0.96$	$\chi^2=0.38$	$\chi^2=0.23$
rs752016	206304300				AA	AG	GG	A	G				
	Intron12	9457	Schizophrenia	334	106 (0.32)	153 (0.46)	75 (0.22)	365 (0.55)	303 (0.45)	0.96	$\chi^2=1.92$, $P=0.17$	$P=0.56$	$P=0.68$
	–		Control	303	94 (0.31)	150 (0.50)	59 (0.19)	338 (0.56)	268 (0.44)	(0.77–1.19)	$\chi^2=0.00$, $P=0.95$	$\chi^2=1.15$	$\chi^2=0.17$
rs841865	206292532				GG	GA	AA	G	A				
	Intron14	11768	Schizophrenia	335	171 (0.51)	132 (0.39)	32 (0.10)	474 (0.71)	196 (0.29)	1.11	$\chi^2=0.77$, $P=0.38$	$P=0.35$	$P=0.39$
			Control	302	139 (0.46)	136 (0.45)	27 (0.09)	414 (0.69)	190 (0.31)	(0.87–1.41)	$\chi^2=0.59$, $P=0.44$	$\chi^2=2.08$	$\chi^2=0.73$

[a] Chromosome position was referred to dbSNP database.
[b] Without correction.

Table 2
Pair-wise linkage disequilibrium and association with schizophrenia of the 7 SNPs and haplotypes in *PLXNA2*

dbSNP ID	Inter-SNP distance (bp)	LD		Haprotype P^*					
		D'	r^2	2 Locus	3 Locus	4 Locus	5 Locus	6 Locus	7 Locus
rs2782948				0.44					
	156	1.00	0.13						
rs11119014					0.48				
	629	1.00	0.02	0.49		0.67			
rs3748735					0.58		0.32		
	135156	0.30	0.03	0.70		0.53		0.11	
rs2498028					0.78		0.19		0.13
	8179	1.00	0.06	0.90		0.63		0.20	
rs1327175					0.73		0.38		
	9457	0.90	0.12	0.59		0.13			
rs752016					0.64				
	11768	0.88	0.42	0.53					
rs841865									

* Global P-value (without correction).

approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

2.2. SNP selection

In the recent genome-wide association study for schizophrenia (Mah et al., 2006), the most consistent effect was observed for rs752016, a SNP within intron 12 of *PLXNA2*. For the initial discovery sample, the minor allele frequency was 0.22 in the control group and 0.16 in the case group (OR=1.49; P=0.006). To fine map the region of association, they analyzed additional 67 SNPs in a 100-kb window surrounding the marker SNP (rs752016) in the discovery case and control pools. Based on the association fine-mapping and genotyping results obtained in the case-control studies, 4 SNPs that best represented the region (rs752016, rs841865, rs1327175 and rs2498028) were genotyped in their collection. All the 4 SNPs showed a significant association with schizophrenia in their European American sample. Therefore, we chose these SNPs for replication study in our Japanese sample.

Since genetic variations that result in an amino acid change are most likely to alter functions, we searched for non-synonymous polymorphisms of *PLXNA2 in silico* based on the NCBI dbSNP database and found 3 well-validated SNPs with a heterozygosity value of >0.10. The selected ones are described in Table 1. They were rs2782948 (223G>A, Arg5Gln), rs11119014 (379A>G, Gln57Arg), and rs3748735 (1008G>A, Ala267Thr). The numbers of base and amino acid positions were according to NM_025179 and NP_079455, respectively.

2.3. Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay; the assay ID (Applied Biosystems, Foster City, CA) of each SNP was C_26000091_10 for rs2782948, C_3245269_10 for rs11119014, C_3245268_20 for rs3748735, C_11673239_10 for rs2498028, rs1327175-CG for rs1327175, C_1489466_10 for rs752016, and C_8886901_1 for rs841865. Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. Genotype data were read blind to the case-control status. Ambiguous genotype data were not included in the analysis.

2.4. Statistical analysis

Deviations of genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. These tests were performed with the SPSS software ver 11 (SPSS Japan, Tokyo, Japan). Haplotype-based association analyses were examined with the COCAPHASE software ver 2.4 (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) (Dudbridge et al., 2000). The expectation-maximization (EM) and "droprare" options were used. Haplotypes with frequencies less than 5% were considered to be rare. All P-values reported are two-tailed.

3. Results

Genotype and allele distributions of the examined SNPs of *PLXNA2* in patients and controls are shown in Table 1. The genotype distributions did not significantly deviate from the HWE in patients and controls for all SNPs except for rs3748735 (Ala267Thr) in controls. When the P-value (0.0072) of the HWE for rs3748735 in controls was corrected by Yates and Bonferroni corrections due to the very small cell counts of TT genotype and multiple testing (*i.e.*, 7 SNPs examined), respectively, the corrected P-value (0.091) was not significant anymore.

With respect to the 4 intronic SNPs (rs752016, rs841865, rs1327175, and rs2498028), there was no significant difference in genotype or allele distributions between patients and controls. Both genotype and allele distributions were approximately the same in the two groups; therefore, we failed to replicate the finding of Mah et al. (2006). For the non-synonymous polymorphisms of the *PLXNA2* gene (rs2782948, rs11119014, and rs3748735), there was again no significant difference in genotype or allele distributions between patients and controls (Table 1). We further performed stratified analysis of the data by sex. However no significant associations were observed (data not shown) for any SNP.

Results of haplotype-based analyses and Pair-wise linkage disequilibrium between neighboring SNPs are shown in Table 2. We obtained no significant difference in haplotype frequencies for any of two-, three-, four-, five-, six-, or seven-marker analyses between patients and controls.

4. Discussion

In the present study, we tried to replicate the finding of Mah et al. (2006) who reported a significant association between

PLXNA2 and schizophrenia. Furthermore, we examined 3 additional non-synonymous polymorphisms of *PLXNA2*. However, we failed to obtain any evidence for association between variants of *PLXNA2* and schizophrenia in our Japanese sample. Our sample size had a power of 90% with a 5% significance level to detect an odds ratio of 1.45 for allelic association with the SNP rs752016. There is only a small chance that a clinically meaningful difference would have been missed with the data.

Our failure to replicate the finding of Mah et al. (2006) may be attributable to ethnic differences between European Americans and Japanese. Indeed, Mah et al. (2006) obtained no evidence for significant association in their Asian American subjects with respect to the 4 intronic SNPs (rs752016, rs841865, rs1327175, and rs2498028), although this may have been attributable in part to the small number of their Asian subjects (57 patients and 71 controls). This ethnic difference might have resulted from differential linkage disequilibrium between the 4 intronic markers and functional variants responsible for giving susceptibility to schizophrenia. Indeed, based on the HapMap database (http://www.hapmap.org/) around the *PLXNA2* gene, linkage disequilibrium data between SNPs are somewhat different between Europeans and Japanese (data not shown). It is also possible that there may be differential effect of *PLXNA2* on the development of schizophrenia between the ethnic groups. Alternatively, the significant association between *PLXNA2* and schizophrenia in European Americans reported by Mah et al. (2006) may have arisen by chance. To draw any conclusion, further studies in other samples are required.

Since polymorphisms that result in an amino acid change are most likely to alter functions, we searched for non-synonymous SNPs in *PLXNA2* and examined 3 additional SNPs for association with schizophrenia. We again obtained no evidence of association for any SNP, which consolidated our failure to find evidence for association. A limitation might be the possible departure from the HWE in the control subjects for rs3748735 (uncorrected $P=0.0072$). However, the corrected P-value (0.091) was not significant any more. Furthermore, since we excluded ambiguous genotype data from the analysis, genotyping errors were unlikely. Another limitation is the differential sex distribution between the patients and controls in our sample. To resolve this issue, we compared the genotype and allelic distribution for each sex separately; however, we obtained no evidence for association with respect to any SNP in men and women. Furthermore, there were no significant difference in genotype or allele distribution for any SNP between males and females within both patients and control groups (data not shown). These ensure that the possible effect of the differential sex distribution were, if any, minimal.

In the present study, we found no evidence for association between the seven SNPs of *PLXNA2* and schizophrenia, suggesting that *PLXNA2* may not play a major role in our Japanese sample. However, we did not examine the entire region of *PLXNA2*; thus the possibility remains that other unknown SNPs in the gene may be associated in Asian schizophrenia. Furthermore, given the neurodevelopmental hypothesis of schizophrenia and the neurodevelopmental function of plexins, genes encoding plexins other than *PLXNA2* could also be candidate genes for schizophrenia.

5. Conclusion

The present study failed to confirm the findings of Mah et al. (2006), who reported a significant association between *PLXNA2* and schizophrenia. Furthermore, analysis of additional 3 non-synonymous SNPs of the gene also failed to find such an association. These results suggest that *PLXNA2* may not play a major role in the development of schizophrenia at least in the Japanese population.

Acknowledgements

This study was supported by Japan Society for Promotion of Science (Y.I.), Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health) (H.K.), Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug innovation) (H.K.), and Grant-in-Aid for Scientific Research on Priority Areas of Applied Genomics from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K.).

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ScienceDirect
Psychiatry Research: Neuroimaging 154 (2007) 133–145
PSYCHIATRY RESEARCH NEUROIMAGING
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Progressive changes of white matter integrity in schizophrenia revealed by diffusion tensor imaging

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Received 16 March 2006; received in revised form 6 July 2006; accepted 11 September 2006

Abstract

Recent magnetic resonance imaging (MRI) studies using diffusion tensor imaging (DTI) have suggested reduced fractional anisotropy (FA) in the white matter (WM) of the brain in patients with schizophrenia. We tried to examine whether such reduction in FA exists and whether such changes in FA progress in an age-dependent manner in a Japanese sample of chronic schizophrenia. FA values were compared between 42 patients with chronic schizophrenia and 42 controls matched for age and gender, by using DTI with voxel-by-voxel and region-of-interest analyses. Correlations of FA values with age and duration of illness were examined. Patients with schizophrenia showed lower FA values, compared to controls, in the widespread WM areas including the uncinate fasciculi and cingulum bundles. A significant group-by-age interaction was found for FA in the WM, i.e., age-related reduction of FA was more pronounced in schizophrenics than in controls. A significant negative correlation between FA and duration of illness was also found in the WM. Our data confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas. Such decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes after the onset of the illness.

Keywords: Magnetic resonance imaging (MRI); DTI; Fractional anisotropy (FA); Aging

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doi:10.1016/j.pscychresns.2006.09.004

1. Introduction

Diffusion tensor imaging (DTI) (Basser et al., 1994), a newly developed method to estimate the white matter (WM) integrity, provides information about the diffusion of water in biological tissues. In the WM, water diffusion is highly anisotropic, with greater diffusion in the direction parallel to axonal tracts. Thus, diminished anisotropy of water diffusion has been proposed to reflect compromised WM integrity (Lim et al., 1999). Fractional anisotropy (FA) (Basser, 1995) is a quantitative measure of diffusion anisotropy acquired from DTI.

The normally aging brain exhibits an assortment of micro- and macroscopic changes in the WM as well as the cerebral cortex. Histological studies demonstrate a decrease in myelin density and in the number of myelinated fibers (Meier-Ruge et al., 1992). Postmortem brain (Meier-Ruge et al., 1992) and volumetric neuroimaging studies (Christiansen et al., 1994; Salat et al., 1999) have suggested that WM changes are more prominent than cortical changes with aging, at least during certain segments of the age span and in certain regions of the brain. For example, volume loss in prefrontal WM is disproportionately greater than that in prefrontal cortex with late aging {comparison of elderly adults aged 60–75 with those aged >85 years (Salat et al., 1999)}. Several DTI studies have demonstrated age-related reductions of WM anisotropy in the genu of the corpus callosum (Pfefferbaum et al., 2000b), anterior WM (Pfefferbaum et al., 2000a; O'Sullivan et al., 2001), periventricular WM (Nusbaum et al., 2001), and the prefrontal WM (Nusbaum et al., 2001; Pfefferbaum et al., 2005; Salat et al., 2005).

Regarding schizophrenia, impairments of the neural connectivity between certain cortical areas, such as frontal and temporal areas, have been implicated in the pathophysiology of the disease (Frith and Dolan, 1996; Andreasen et al., 1997; Bullmore et al., 1997). Indeed, volumetric magnetic resonance (MR) studies and pathological studies demonstrated abnormalities of the WM in schizophrenia (Miyakawa et al., 1972; Cannon et al., 1998; Davis et al., 2003; Ho et al., 2003; Uranova et al., 2004). Changes in WM integrity in schizophrenia has relevance to the neural disconnection model of the disorder and may provide a basis for focal abnormalities as well. Several previous DTI studies in chronic schizophrenia showed decrease of FA in schizophrenics mainly in the front-temporal white matter and corpus callosum (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003). Furthermore, FA decrease in patients with first episode schizophrenia might be less pronounced compared to chronic patients (Price et al., 2005; Szeszko et al., 2005), suggesting that the decreased FA in schizophrenics might be attributed, at least in part, to progressive and exaggerated age-dependent changes in schizophrenics rather than neurodevelopmental abnormalities in the WM. To date, there is only one cross-sectional study with a small sample size investigating age-related FA changes in schizophrenia that demonstrated an age-related FA increase in schizophrenics (Jones et al., 2006).

The present study was aimed to examine whether patients with chronic schizophrenia do have reduced FA values compared to controls and whether such changes in FA progress in an age-dependent manner.

2. Methods

2.1. Subjects

Table 1 shows the characteristics of participants of this study. Forty-two patients with chronic schizophrenia were recruited at the National Center of Neurology and Psychiatry, Tokyo, Japan. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria (American Psychiatric Association, 1994), based on all available information, including clinical interviews, medical records and other research assessments. All patients were stable and/or partially remitted and had been taking antipsychotic medication at the time of MR measurement and neuropsychological tests. Forty-two healthy volunteers who had no current or past contact to any psychiatric services served as controls. All the subjects were biologically unrelated Japanese. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committee of the National Center of Neurology and Psychiatry, Tokyo, Japan. Exclusion criteria for all the participants included asymptomatic or symptomatic cerebral infarctions detected by T2 weighted MRI, serious neurological or endocrine disorder, any medical condition that could potentially affect the central nervous system, or mental retardation according to DSM-IV criteria.

2.2. Image acquisition

MR studies were performed on a 1.5 tesla Magnetom Vision Plus system (Siemens, Erlangen, Germany). Axial DTI scans aligned to the plane containing anterior and posterior commissures were acquired with a pulsed-

Table 1
Characteristics of participants

	Controls	Schizophrenics	Two sample *t*-test	(Two-tailed; df=82)
			t	*P*
Number of subjects	42	42		
Gender (male/female)	26/16	26/16		
Handedness (right/left)	41/1	41/1		
Age (years)	39.2 (9.0)	40.0 (9.3)	−0.42	0.68
Range of age (years)	22–59	22–59		
Education (years)	17.1 (3.5)	13.0 (2.9)	8.1	<0.001
Full-scale IQ (WAIS-R)	114.3 (11.6)	86.0 (21.3)	6.0	<0.001
Age of onset		23.3 (7.0)		
Duration of illness (years)		16.8 (9.0)		
Duration of hospitalization (months)		31.2 (61.3)		
Dose of total antipsychotic drugs (mg/day, chlorpromazine equivalent)		1005.1 (735.3)		
Dose of typical antipsychotic drugs (mg/day, chlorpromazine equivalent)		694.8 (748.3)		
Dose of atypical antipsychotic drugs (mg/day, chlorpromazine equivalent)		310.3 (464.2)		

Mean (S.D.).
WAIS-R: Wechsler Adult Intelligence Scale-Revised.

gradient, spin-echo, single-shot echo planar imaging (EPI) sequence (TR/TE=4000/100 ms; acquisition matrix, 256×256; NEX=4, FOV 240 mm; b=1000 s/mm^2; 20 slices, slice thickness 5 mm, gap 1.5 mm). Diffusion was measured along six non-collinear directions. For each of six gradient directions, four acquisitions were averaged. Four acquisitions without diffusion weighting (b=0) were also averaged. Additionally, a three dimensional volumetric acquisition of a T1-weighted gradient echo sequence with a gapless series of thin sagittal sections using an MPRage sequence (TR/TE=11.4/4.4 ms; flip angle, 15 degree; acquisition matrix, 256×256; NEX=1, FOV 315 mm; slice thickness 1.23 mm) was acquired for evaluating the volume of grey matter (GM), WM and cerebrospinal fluid (CSF) space.

2.3. Image processing

FA images for each subject were computed from seven diffusion images acquired as above by an in-house script on Matlab 6.5 software (Mathworks, Inc., MA, USA). Then, the FA images were spatially-normalized using high-dimensional-warping algorithm (Ashburner et al., 1999) and were matched to the FA template image. To make the FA template image, we warped FA images of 4 normal subjects (other than 42 control subjects) to the single-subject T1 template (skull stripped image) using spatial normalization function of SPM2 and averaged the 4 warped FA images. The transformed FA images were smoothed with a Gaussian kernel. The filter size (full-width at half-maximum: FWHM) was varied from zero to 16 mm in steps of 2 mm to validate the consistency of results of SPM analyses, because a previous study (Jones et al., 2005) reported that the statistical results of SPM analyses were differed depending on filter size. For measuring the volume of GM, WM and CSF space, an additional function of an optimized VBM script (http://dbm.neuro.uni-jena.de/vbm) was used (Good et al., 2001).

2.4. Statistical analysis

2.4.1. Voxel-by-voxel analysis

The resultant FA images were analyzed using statistical parametric mapping with the framework of the General Linear Model in SPM2 (Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995). We constituted following three

Table 2
The relationship between smoothing kernel sizes (FWHM) and number of resels in our sample

FWHM (mm)	Number of resels
None	12460.4
2×2×2	5131.1
4×4×4	1720.2
6×6×6	706.0
8×8×8	289.4
10×10×10	119.7
12×12×12	52.1
14×14×14	24.4
16×16×16	12.4

statistical analyses: 1) a two-sample *t*-test for estimating group differences (controls versus schizophrenics), 2) a correlational analysis between age and FA values in both controls and the schizophrenics and 3) a correlational analysis of FA values with duration of illness, age of onset, duration of hospitalization, and daily dose of

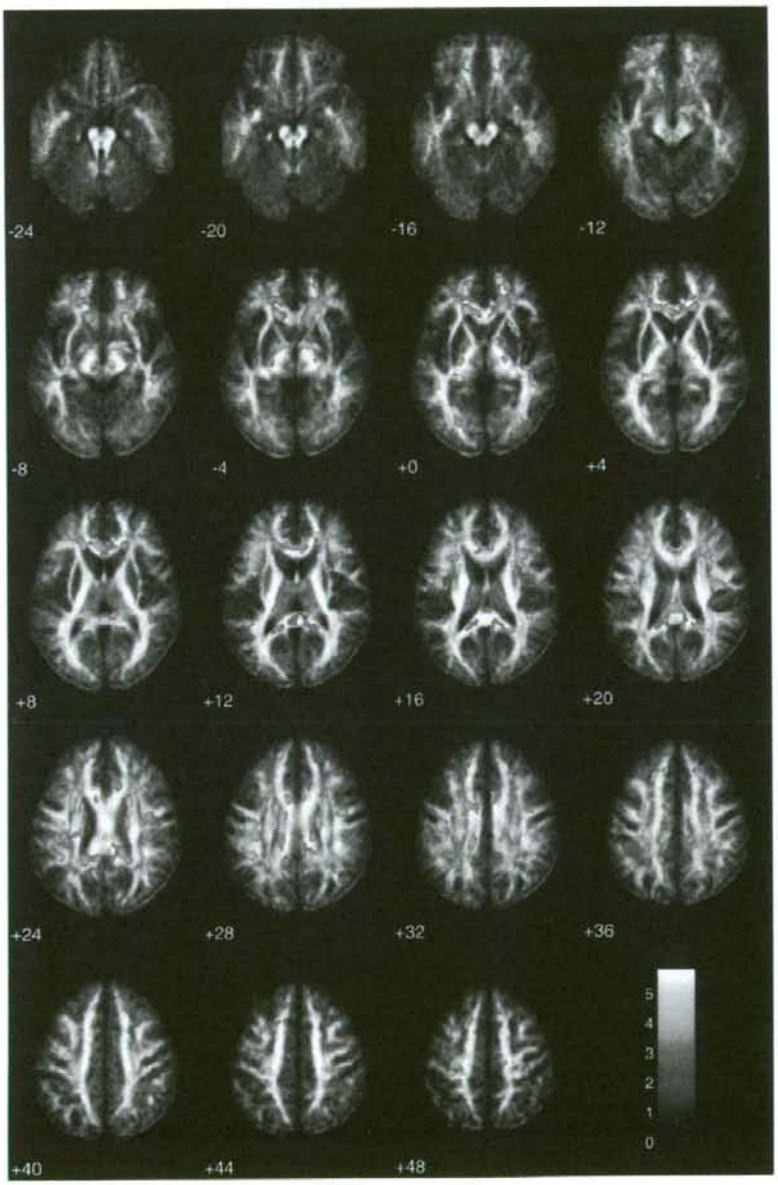

Fig. 1. Comparison in FA values between patients with schizophrenia and controls. The SPM {t} is displayed onto axial FA template images. The WM areas in which significantly lower FA values in patients compared with controls were observed, including the bilateral frontal and temporal WM, uncinate fasciculi, cingulum bundles, and genu and splenium of the corpus callosum ($P<0.001$, uncorrected).

antipsychotic drugs in the schizophrenics. In all the three analyses, relative WM volume (WM volume divided by the summation of GM, WM and CSF volumes) and WAIS-R (Wechsler Adult Intelligence Scale-Revised) full-scale IQ score were treated as nuisance variables. The former was included for eliminating the possible effect of WM volume change associated with aging on the FA values through partial voluming from non-WM voxels. The latter was included to allow for the effects of IQ, because there was some evidence which suggested DTI measures were correlated with cognitive decline in elderly (O'Sullivan et al., 2004). We additionally conducted the analyses without these two nuisance variables to check whether there were any differences in the results with or without nuisance variables in the statistical models. To estimate population effects (diagnostic effects), we used a single-subject, condition (controls or schizophrenics) and covariate (no covariate of interest) model for the SPM analysis. In the second analysis, we applied the single subject condition (controls or schizophrenics) and covariate (interaction with condition, covariate of interest; age) model. A single-subject, covariate only model was applied in the third analysis. For these three analyses, we set masking threshold for FA values of 0.2 for excluding voxels containing partial volume of WM and other tissues. Since the previous study demonstrating a positive correlation between FA values and age in schizophrenics reported mean FA values of around 0.4 (Jones et al., 2006), we additionally set masking threshold for FA values of 0.35 for examining correlation between age and FA values of more anisotropic WM structure in the second analysis. For the evaluation of the statistical models, we used Wake Forest University Pickatlas (Maldjian et al., 2003) to pick up cerebral WM in the Montreal Neurological Institute (MNI) space. We used uncorrected $P<0.001$ as a statistical threshold to search significant differences. As demonstrated in Table 2, the number of resels differed profoundly depending on smoothing kernel sizes (FWHM) and the statistical results with correction for multiple comparisons could change dramatically relying on number of resels. On the other hand, SPM results without correction for multiple comparisons were essentially unchanged regardless of smoothing kernel size (data not shown). Therefore, we did not perform correction for multiple comparisons. The resultant set of t values constituted statistical parametric map (SPM {t}). We employed the filter size of 6 mm for presentation of the results considering for the original voxel dimensions of acquired data {0.94 mm × 0.94 mm × (5.00+1.50) mm}.

2.4.2. ROI analysis

To ensure the robustness of the results of the voxel-by-voxel analyses, we additionally performed ROI analyses. We used MarsBar (http://marsbar.sourceforge.net/) for extracting region of interest (ROI) containing all the voxels classified as WM with Wake Forest University Pickatlas from spatially normalized and smoothed FA images and calculated mean FA values of the ROI. Then, we performed correlational analyses of mean FA values with the same variables in voxel-by-voxel analysis using Statistical Package for Social Science (SPSS), 1) in both controls and schizophrenics, 2) in controls and 3) in schizophrenics. We constituted a General Linear Model for the first analysis and entered diagnosis-by-age interaction effects into the statistical model to examine if there were any diagnosis-by-age interaction effects. For the second and third analyses, Pearson's correlation coefficients between mean WM FA values and covariates were calculated.

3. Results

3.1. Voxel-by-voxel analyses

3.1.1. Comparison between schizophrenics and controls

Schizophrenics demonstrated significantly lower FA values in widespread WM areas, compared with controls. These WM areas included bilateral frontal and temporal lobes, uncinate fasciculi (external capsules), cingulum bundles, and genu and splenium of corpus

Table 3
The summary of the WM areas in which significantly lower FA values in patients compared with controls were observed

Anatomical regions	t-value	MNI coordinates		
	(Voxel level)	x	y	z
Rt frontal lobe white matter	4.34	22.5	52.5	−4.5
Lt frontal lobe white matter	5.43	−13.5	49.5	−6
Rt temporal lobe white matter	4.25	48	−33	−7.5
Lt temporal lobe white matter	4.19	−45	−31.5	−10.5
Rt uncinate fasciculus (external capsule)	4.00	33	12	−1.5
Lt uncinate fasciculus (external capsule)	3.84	−33	12	−1.5
Rt cingulate bundle	4.23	6	6	33
Lt cingulate bundle	4.32	−7.5	6	30
genu of corpus callosum	3.79	6	24	10.5
splenium of corpus callosum	4.18	−3	−33	19.5

callosum (Fig. 1, Table 3). There would be increased possibility of alpha errors because we did not perform correction for multiple comparisons. However, our results were in well concordance with the results of the previous studies (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003; Kubicki

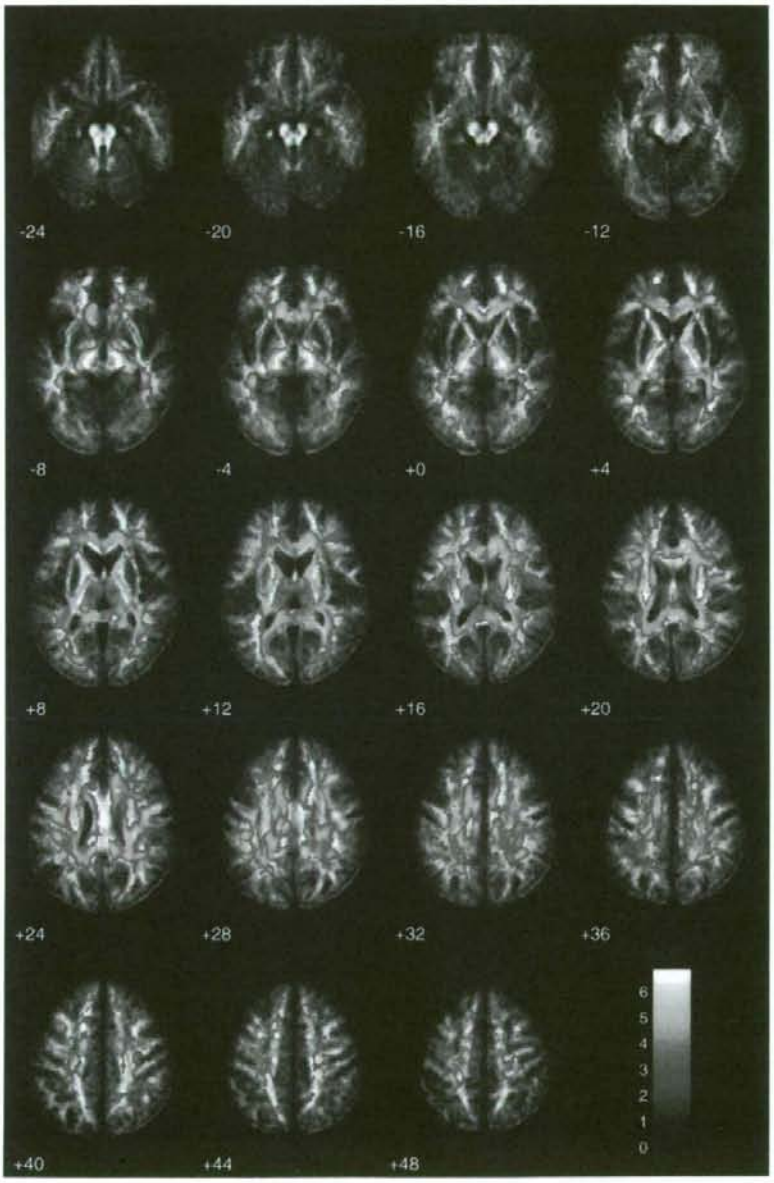

Fig. 2. Correlational analysis between FA values and age with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and age in schizophrenics ($P<0.001$, uncorrected).

et al., 2003). Therefore, we might be able to regard the results of these previous studies as a priori hypotheses. There were no areas of significantly higher FA values in patients compared with controls even at a lenient threshold ($P<0.05$, uncorrected). In these results of the analysis without nuisance variables in the statistical

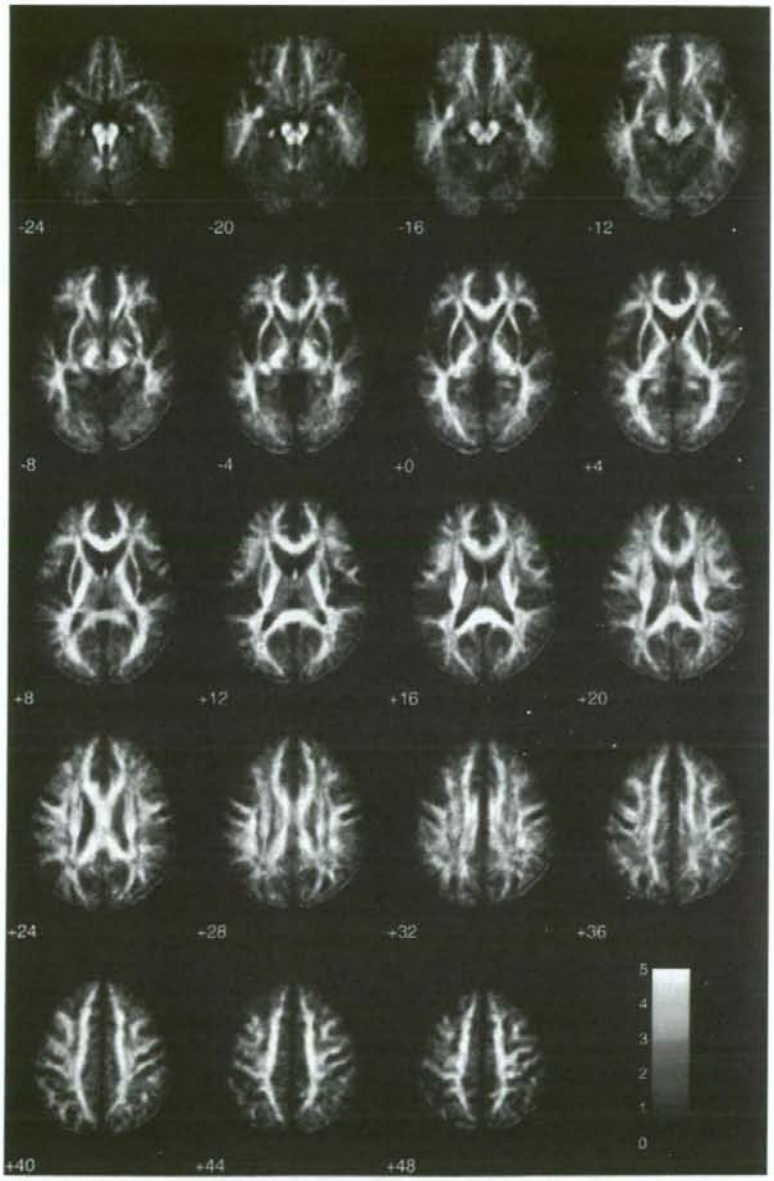

Fig. 3. Correlational analysis between FA values and age with 0.2 as a masking threshold in controls. The SPM {t} is displayed onto axial FA template images. The WM areas showed a significant negative correlation between FA values and age in controls ($P<0.001$, uncorrected), including right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$; (−30.0, −60.0, 15.0), $t=4.47$}.

models, the distributions of the statistically significant areas were essentially unchanged compared to the results with nuisance variables although the spatial extents of the statistically significant areas were slightly larger (data not shown), which was the case with the results of other two analyses.

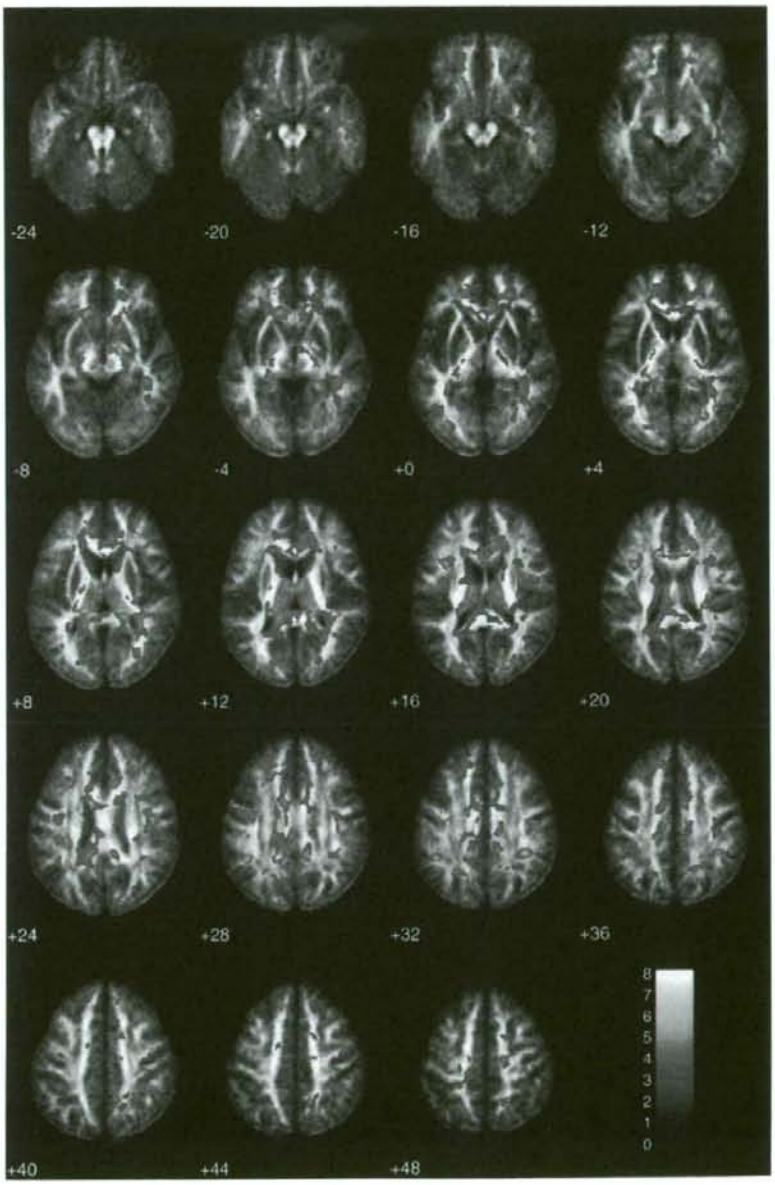

Fig. 4. Correlational analysis between FA values and duration of illness with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and duration of illness in schizophrenics ($P<0.001$, uncorrected).

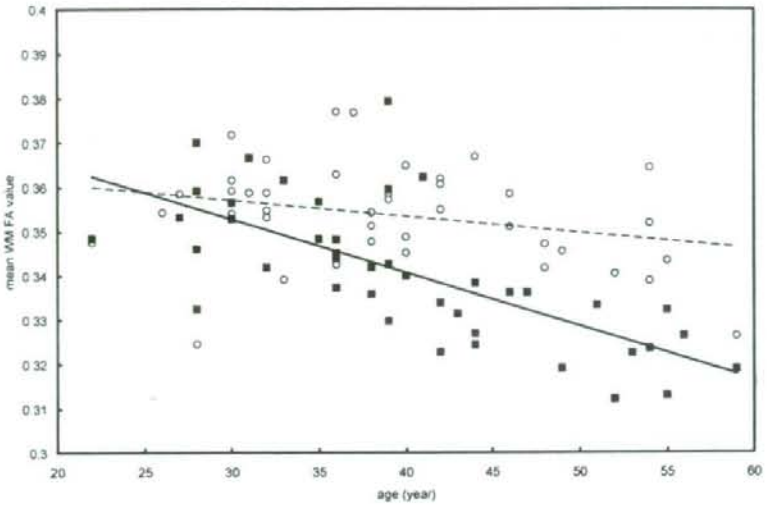

Fig. 5. A scatter plot between age and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics and open circles represent controls. The solid line indicates a regression line for schizophrenics ($y=-0.0012\times+0.3888$, $R^2=0.49$, test for regression slope: df=40; $t=-6.24$; $P<0.0001$). The dashed line indicates a regression line for controls ($y=-0.0004\times+0.3679$, $R^2=0.083$, test f or regression slope: df=40; $t=-1.90$; $P=0.065$). A significant diagnosis-by-age interaction effect (general linear model: $P=0.009$) was noted.

3.1.2. *Correlational analysis in schizophrenic and control groups*

As the results of the second analysis considering aging effects, a significant negative correlation with age was observed in the FA values of widespread, almost diffuse WM areas in the schizophrenic group (Fig. 2), while in the control group, only FA values in right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$;

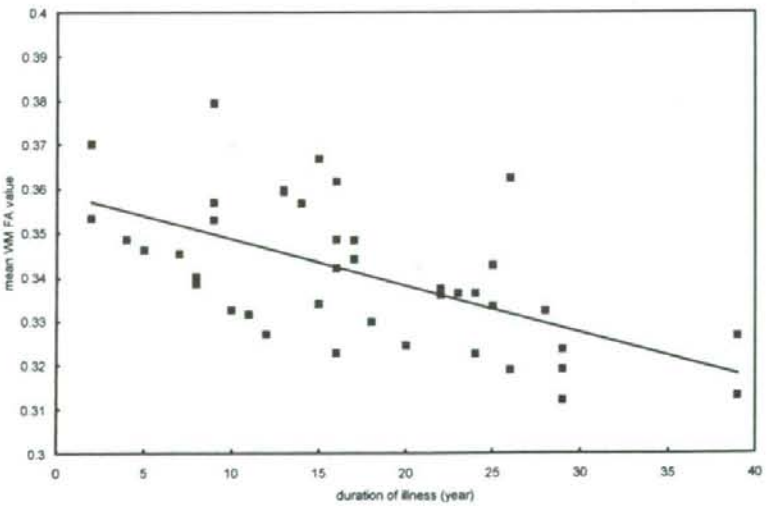

Fig. 6. A scatter plot between duration of illness and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics. The solid line indicates a regression line for schizophrenics ($y=-0.0011\times+0.3590$, $R^2=0.36$, test for regression slope: df=40; $t=-4.78$; $P<0.0001$).

(−30.0, −60.0, 15.0), t=4.47} demonstrated a significant negative correlation with age (Fig. 3). Even if the analysis was done on voxels with FA values higher than 0.35, to examine more anisotropic WM areas, the results were essentially unchanged (data not shown).

3.1.3. Correlational analysis between FA values and clinical factors in schizophrenics

There was a significant negative correlation between FA values and duration of illness in widespread WM areas (Fig. 4), while there was no significant correlation of FA values with age of onset, duration of hospitalization or daily dose of antipsychotic drugs (data not shown).

3.2. ROI analyses

3.2.1. ROI-based correlational analysis in both schizophrenics and controls

First, we constituted a General Linear Model putting diagnosis as a fixed factor and age, IQ and relative WM volume as covariates. F values (significance probabilities) were as follows; diagnosis: 10.8 (P=0.001), age: 26.1 (P<0.001), IQ: 0.029 (P=0.865) and relative WM volume: 16.6 (P<0.001). Then, we added diagnosis-by-age interaction into the model. F values (significance probabilities) changed as follows; diagnosis: 2.34 (P=0.130), age: 27.8 (P<0.001), IQ: 0.059 (P=0.809), relative WM volume: 14.1(P<0.001) and diagnosis-by-age interaction: 7.08 (P=0.009). Effect of IQ was not significant in both models. There was a significant diagnosis-by-age interaction effect.

3.2.2. ROI-based correlational analysis in controls

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with age, IQ and relative WM volume in controls were as follows; FA vs. age: −0.287 (P=0.065), FA vs. IQ: −0.108 (P=0.496) and FA vs. mean WM volume: 0.481 (P=0.001). Only positive correlation between mean WM FA value and relative WM volume was statistically significant.

3.2.3. ROI-based correlational analysis in schizophrenics

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with clinical factors in schizophrenics were as follows; FA vs. age: −0.702 (P< 0.001), FA vs. duration of illness: −0.603 (P<0.001), FA vs. age of onset: −0.305 (P=0.049), FA vs. total daily dose of antipsychotics: 0.110 (P=0.489), FA vs. duration of hospitalization: −0.172 (P=0.277), FA vs. IQ: −0.064 (P=0.686), FA vs. relative WM volume: 0.421 (P=0.006). Significant positive correlation was observed between mean WM FA value and relative WM volume. Fig. 5 shows a scatter plot between age and mean WM FA value in controls and schizophrenics. Fig. 6 shows a scatter plot between duration of illness and mean WM FA value in schizophrenics. Significant negative correlations were observed between mean WM FA value and age (or duration of illness).

4. Discussion

In this study, we obtained three main findings; 1) lower FA values in schizophrenic patients compared with controls in WM areas including frontal and temporal WM, bilateral uncinate fasciculi (external capsules) and cingulum bundles and genu and splenium of corpus callosum, 2) age-related reductions of FA value in the widespread WM were more prominent in schizophrenics than in controls, and 3) a negative correlation between FA value in the widespread WM and duration of illness in schizophrenics.

Recent studies demonstrated age-related FA decline in normal individuals occurred in the prefrontal WM, while temporal WM were relatively preserved (Pfefferbaum et al., 2005; Salat et al., 2005). However, in this study, negative age-dependent effects were observed only in the lenient statistical threshold in the FA values of restricted areas of the WM in controls. This could be explained by the fact that all our subjects were under the age of 60, relatively less old compared to the participants of normal aging studies.

We replicated the results of the most of the previous studies, decreased FA values in the WM of schizophrenics. In the earlier studies concerning FA values in WM of patients with schizophrenia, an inherent abnormality in WM was expected to be detected since the decrease of FA values in the WM of the schizophrenic brain was assumed to occur as neurodevelopmental impairments before onset of the illness. Several studies demonstrated that schizophrenics had reduced FA value in the prefrontal WM (Buchsbaum et al., 1998), prefrontal and parieto-occipital WM (Lim et al., 1999), splenium of the corpus callosum (Agartz et al., 2001) and adjacent occipital WM (forceps major) (Agartz et al., 2001), left uncinate fasciculus and bilateral arcuate fasciculus (Burns et al., 2003), bilateral cingulum bundles (Kubicki et al., 2003). Some of them indicated that the reduction of FA values in schizophrenics might occur independently of reduction of the white matter volume. Although some studies reported no significant FA changes in schizophrenics (Steel et al., 2001; Foong et al., 2002), most studies with chronic

schizophrenia demonstrated lower FA values in schizophrenia (Kanaan et al., 2005). A few DTI studies have examined first episode patients (Price et al., 2005; Szeszko et al., 2005). Szeszko et al. found FA decrease in the left internal capsule and left-hemisphere WM of the middle frontal gyrus and posterior superior temporal gyrus of first-episode schizophrenics and schizoaffective disorder patients, however, the decrease was less pronounced compared with results of the majority of the studies in chronic schizophrenics. On the other hand, Price et al. reported that there was no FA decrease in the corpus callosum of patients with first-episode schizophrenia. They suggested that FA reduction in schizophrenia might reflect neuropathological abnormalities, which may occur after the onset of the disease and could be progressive. Our results, 1) age related FA reduction was more prominent in schizophrenics than controls, and 2) duration of illness was related to FA reduction in schizophrenics, suggest that changes of FA value in schizophrenia are attributable, at least in part, to progressive neuropathological changes after onset of the illness.

Contrary to our results, a previous DTI study demonstrated 'positive' correlation between age and FA in schizophrenics (Jones et al., 2006). They measured FA values of WM tracts captured from tractography, and they set seedpoints of the tracts manually from one slice of FA images. Such methods might overlook general decline of FA values in the WM. Their mean FA values (average of 8 WM tracts in each subjects) were around 0.4, which was relatively higher than those of our study {our mean FA value of entire WM was 0.35 + 0.01 (mean + S.D.)}. To simulate the analysis of the previous study, we additionally performed an analysis setting masking threshold for FA values of 0.35. As a result, the significant negative correlation remained to be present even in more anisotropic WM areas.

Previous pathological studies demonstrated microscopic abnormalities of the WM in schizophrenia such as decreased expression of myelin and oligodendrocyte-related genes, the decrease in density of oligodendrocytes (Hof et al., 2002), damage of myelin sheath lamellae (Uranova et al., 2001) and maldistribution of interstitial neurons (Akbarian et al., 1996) in prefrontal WM of the brains of schizophrenic patients. Further, a previous longitudinal MR study demonstrated progressive atrophy of the white matter in schizophrenics (Ho et al., 2003). Given these previous findings and ours, it seems likely that age-dependent FA decrease, but not increase, occurs in schizophrenic brains.

As well as a negative correlation with age, FA values of schizophrenics showed negative correlation with duration of illness but not with age of onset or daily dose of antipsychotics. The facts seem to support the hypothesis that FA reduction in schizophrenia might be associated with neuropathological abnormalities which may emerge, at least in part, after the onset of the disease and could be progressive. Further, the spatial distribution of age-related FA reduction in schizophrenics was different from those of normal individuals in previous studies that demonstrated preserved temporal white matter (Pfefferbaum et al., 2005; Salat et al., 2005). Such different distributions suggest that FA changes in schizophrenics might be associated with disease progression rather than merely exaggerated aging effects. However, it is difficult for neuroimaging studies, even for longitudinal studies, to discriminate disease progression from aging effects. The correlational study between DTI findings and pathological findings should be conducted to clarify whether reduction of FA values in schizophrenics reflect pure disease progression or merely exaggerated aging effects.

Several limitations should be considered in our study. First, our study is a cross-sectional study. To confirm progressive pathological process in the WM of the patients of schizophrenia, longitudinal studies should be conducted. Second, IQ score was not matched between groups, i.e., mean IQ score was significantly lower in schizophrenics in our samples. O'Sullivan et al. (2004) reported DTI measures were correlated strongly with cognitive decline in elderly. Thus, it could be problematic whether age-related FA decrease in our study was reflected by cognitive decline. However, no significant correlation was observed between mean WM FA values and IQ in our sample. Also, regarding schizophrenia, it has been hypothesized that most cognitive change takes place early in their psychotic episodes and it remains relatively stable through long term in the illness (Hoff et al., 2005). Hence, at least from our data, we cannot attribute age-related FA decline in schizophrenia to IQ changes. Third, the issue of partial volume effect should be addressed. In schizophrenia, progressive WM atrophy has been reported in the previous studies (Ho et al., 2003). Due to the atrophy, it is possible that the voxels located in the border of the WM and other tissues in schizophrenics were estimated as having lower FA values. However, we minimized the problem by using the high dimensional warping algorithm, threshold masking for FA values and adopting relative WM volume as a nuisance variable. Another issue is the possible effect of long-term medication with antipsychotics. Although daily dose of antipsychotics was not correlated with FA values in schizophrenics, we could not estimate accurate cumulative doses of antipsychotics

throughout the duration of illness. Several morphological MR studies and animal studies suggested that the administration of antipsychotics could affect brain morphology (Wang et al., 2004; Lieberman et al., 2005). It is possible that long-term medication with antipsychotics also affects microstructure of the WM in schizophrenics. The longitudinal animal studies may clarify this issue.

In conclusion, we confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas in a Japanese sample. We found that age-dependent FA decline was more pronounced in chronic schizophrenics compared to controls, and that such FA decline was significantly correlated with duration of illness in patients. These observations suggest that decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes in the WM after the onset of the illness.

Acknowledgment

We appreciate Osamu Takizawa (Siemens-Asahi Medical Technologies, Tokyo, Japan) for supporting to develop a program for calculation of FA values. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare (H17-kokoro-007 and H16-kokoro-002, the Research Grant for Nervous and Mental Disorders 17B-2), the Japanese Ministry of Education, Culture, Sports, Science and Technology, Core research for Evolutional Science and Technology of Japan Science and Technology Agency and Japan Foundation for Neuroscience and Mental Health.

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Available online at www.sciencedirect.com
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Schizophrenia Research 93 (2007) 58–65
SCHIZOPHRENIA RESEARCH
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Association analysis of AKT1 and schizophrenia in a UK case control sample

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Received 8 January 2007; received in revised form 13 February 2007; accepted 16 February 2007
Available online 26 March 2007

Abstract

AKT1 (V-akt murine thyoma viral oncogene homolog 1) is involved in intracellular signalling pathways postulated as of aetiological importance in schizophrenia. Markers in the *AKT1* gene have also recently been associated with schizophrenia in two samples of European origin and in Japanese and Iranian samples. Aiming to replicate these findings, we examined ten SNPs spanning *AKT1* in a UK case-control sample (schizophrenia cases $n=673$, controls $n=716$). These included all SNPs previously reported to be associated in European, Japanese and Iranian samples, alone or in haplotypes, as well as additional markers defined by the Haploview Tagger program (pair-wise tagging, minimum $r^2=0.8$, minor allele frequency=0.02). We found no association with single markers (min $p=0.17$). We found weak evidence for association ($p=0.04$) with a four marker haplotype reported as significant in the original positive European sample of Emamian et al. [Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat. Genet. 36, 131–137] and also an overlapping three marker haplotype ($p=0.016$) that had previously been reported as significant in a Japanese sample. Nominal p-values for these haplotypes did not survive correction for multiple testing. Our study provides at best weak support for the hypothesis that *AKT1* is a susceptibility gene for schizophrenia. Examination of our own data and those of other groups leads us to conclude that overall, the evidence for association of *AKT1* as a susceptibility gene for schizophrenia is weakly positive, but not yet convincing.

Keywords: AKT1; Schizophrenia; Association; Candidate gene

1. Introduction

Emamian et al. (2004) proposed that alterations in brain protein kinase activity contribute to the aetiology of schizophrenia. In pursuit of this hypothesis, they examined the abundance of seven protein kinases in lymphoblast cell lines. Reduced AKT1 expression was found in cell lines derived from schizophrenic patients compared to controls, a finding subsequently confirmed in *post-mortem* frontal cortex and hippocampus. Moreover, they also found reduced phosphorylation of GSKβ3, a substrate of AKT1. These data provide a

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doi:10.1016/j.schres.2007.02.006