TABLE 3. Decreases in the FA Values of Patients With Delirium

Anatomical Regions	MNI Coordinates			*t*	
	x	*y*	*z*	Model 1[a] (*df* = 114)	Model 2[b] (*df* = 113)
Thalamus					
Left ventral anterior nucleus	−8	−6	6	4.96	3.37
Left ventral lateral nucleus	−10	−12	8	4.42	
Left pulvinar	−20	−28	12	4.31	
Left ventral posterior lateral nucleus	−16	−16	8	3.62	
Left lateral posterior nucleus	−20	−20	12	3.76	
Right anterior nucleus	6	−4	8	4.04	
Right ventral anterior nucleus	12	−6	8	3.39	
Right pulvinar	16	−32	12	4.08	
Right ventral lateral nucleus	16	−16	12	3.28	
Temporal lobe					
Left superior temporal gyrus WM	−36	−40	8	4.44	
Left middle temporal gyrus WM	−56	−36	10	3.21	
Left subgyral WM	−44	−36	−4	3.85	
Right superior temporal gyrus WM	38	−38	8	3.50	
Right subgyral WM	42	−36	−4	3.51	
Frontal lobe					
Left inferior frontal gyrus WM	−36	32	8	3.46	
Left precentral gyrus WM	−36	−4	36	3.41	
Left subgyral WM	−22	−32	34	4.86	3.46
Right subgyral WM	40	0	22	3.53	
Parietal lobe					
Left supramarginal gyrus WM	−48	−42	34	3.42	
Left precuneus WM	−20	−56	36	3.61	
Left subgyral WM	−22	−52	38	3.53	
Right subgyral WM	34	−52	32	3.23	
Limbic lobe					
Left cingulate gyrus WM	−20	−10	42	3.86	
Right cingulate gyrus WM	20	−24	36	4.75	3.47
Left anterior cingulate WM	−12	24	22	3.60	
Corpus callosum (splenium)	−6	−34	20	4.58	3.18

Notes: The FA values were compared between the two groups (patients with or without postoperative delirium) by a voxel-by-voxel analysis using the SPM2. MNI: Montreal Neurological Institute.

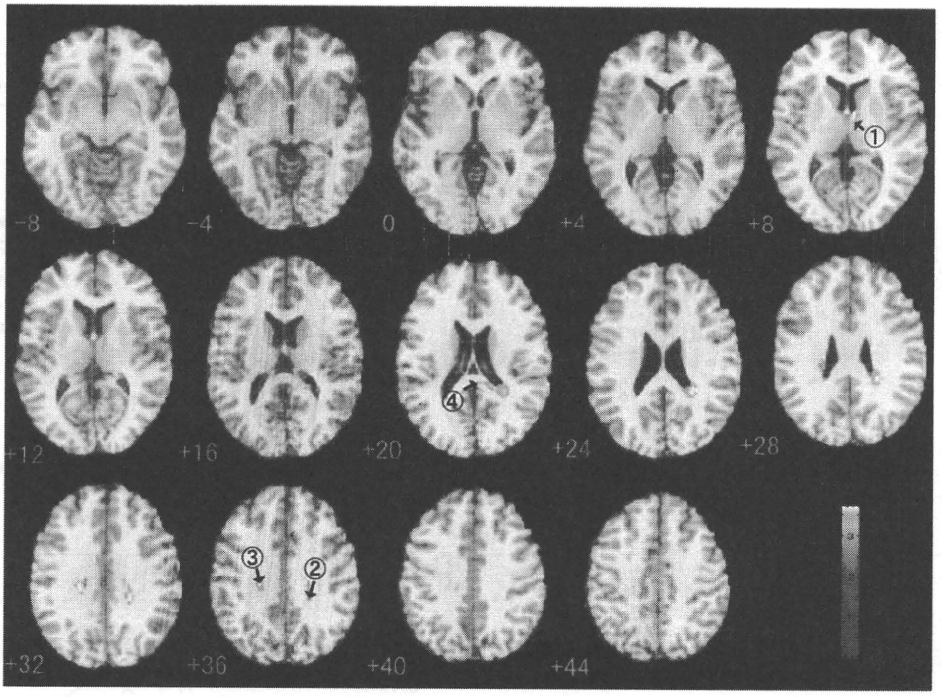
[a]In Model 1, to estimate population effects (diagnostic effects), we used a single subject condition (nondelirium [n = 97] or delirium [n = 19]) and covariate (no covariate of interest) model for the SPM analysis.

[b]In Model 2, we applied the single subject condition (nondelirium [n = 97] or delirium [n = 19]) and covariate (covariate of interest; age). For the analyses, we set the masking threshold for the FA values of 0.2 for excluding voxels containing partial volume of WM and other tissues. We used the one-tailed $p < 0.001$ (uncorrected) as a statistical threshold to search for significant differences between the groups.

brainstem and projects through synaptic relays in the thalamus to the cerebral cortex,[47,48] and neural networks composed of a number of brain areas including the cerebral cortices (e.g., posterior parietal cortex, frontal eye fields, and cingulate cortex) and subcortical areas (e.g., thalamus, striatum, and the reticular activating system) play an important role in visuospatial attention.[49–51] It is also suggested that there is widespread disruption of higher cortical function involving the several brain areas in delirium.[52] Consequently, it is likely that the alterations in the microstructure of the WM underlay a vulnerability of the patients to develop postoperative delirium in this study.

All the subjects of this study had a minimal impairment of cognitive function. A sensitive decline in the delirium group was observed in the executive functions such as the Trail Making tests and the word fluency tests, in accordance with a recent study reporting that the mildly impaired cognitive performance can be an independent risk factor for postoperative delirium.[13] The decrease in the functions with normal aging is supposed to be paralleled with the anatomical changes of the frontal lobe and its connection with other brain areas.[23,53] Moreover, the multivariate stepwise logistic analysis indicated that the lower WFTA score was an important predisposed risk factor for the postoperative delirium. It has been reported that the semantic fluency

FIGURE 3. Comparisons of FA Values Between the Delirium and Nondelirium Group by a Single Subject Condition With the Covariate (Age) Model for the SPM2



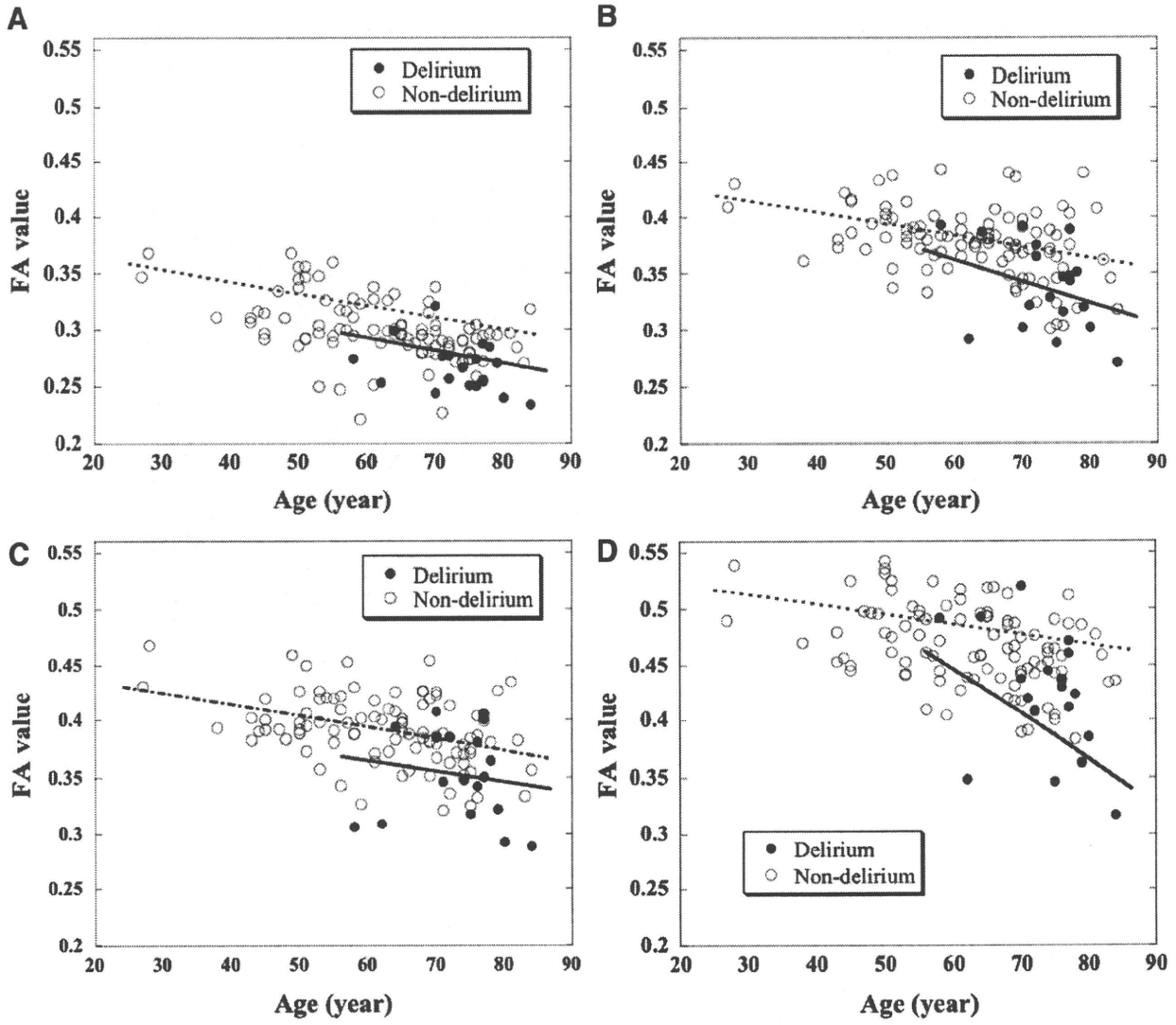
The SPM{t} values (one-tailed $p < 0.001$, uncorrected) are displayed on the axial FA template images. A significant decrease in the FA values was observed in four clusters of the brain area. The area of maximum change in each cluster was 1 the left thalamus (nucleus ventralis anterior)(the Montreal Neurological Institute (MNI) coordinates; $x = -8, y = -6, z = 6$), 2 the left frontal lobe (subgyral white matter) (MNI coordinates; $x = 22. y = -32, z = 34$), 3 the right limbic lobe (cingulate gyrus white matter) (MNI coordinates; $x = 20, y = -24, z = 36$) and 4 the corpus callosum (splenium) (MNI coordinates; $x = -6, y = -34, z = 20$) (Table 3). The brain area is marked by an arrow with the number.

task such as WFTA was more useful than the letter fluency task for discriminating between healthy aging and mild dementia of the Alzheimer type.[33,54] Thus, this study suggests a similar usefulness of the word fluency tests for predicting a risk for postoperative delirium.

A number of limitations of our study ought to be mentioned. Because this study was carried out in a limited number of patients who underwent the planned cardiac operation, further examinations carried out in a larger group of subjects or with any modification to the design of the study, such as a noncardiac operation, are required to confirm and generalize the observation of this study. Secondary, although the assessment by the T1 and T2-weighed images could not find any quantitative difference between the two groups in this study (data not shown), it is likely that small and silent insults of cerebrovascular arteriosclerosis observed in the older persons interfere the FA values of the WM. Thus, the relationship between the fiber integrity and the pathological changes needs to be explored further. Another limitation is the spatial normalization of WM regions in the voxel-based analysis of FA data,[14,41] whereas many studies using the analysis have already provided an important insight into microstructural WM abnormalities in neuropsychiatric disorders.[39–41,55,56] Large variations of brain shape in older subjects might limit an ability to achieve the normalization across the subjects. The FA values in some regions surrounding the ventricles (Figs. 2 and 3) could be distorted by the misregistrations of image and underlying variation of brain shapes.

In conclusion, this study revealed that the advanced age and the cognitive decline were important predictive indicators of the postoperative delirium and suggests that the abnormalities of the microstructure in the

FIGURE 4. Scatter Plots of the FA Values With Age in Four Brain Areas



[A] The left thalamus (nucleus ventralis Anterior), [B] the left frontal lobe (subgyral white matter), [C] the right limbic lobe (cingulated gyrus white matter), and [D] the corpus callosum (splenium). The FA values of the delirium group were significantly decreased in the four brain areas (left thalamus [nucleus ventralis anterior]: $F = 11.13$, $df = 1,113$, $p < 0.01$, left frontal lobe [subgyral]: $F = 11.87$, $df = 1,113$, $p < 0.001$, right cingulate gyrus: $F = 11.94$, $df = 1,113$, $p < 0.001$, corpus callosum: $F = 10.00$, $df = 1,113$, $p < 0.01$) compared with those of the nondelirium group. The analysis was performed by ANCOVA with the age of the patients treated with as a nuisance covariate. The linear regression lines between the age (x) and the FA values (y) of each brain area for the nondelirium group (the thalamus [$y = -0.001 \times x + 0.383$, $r = 0.488$, $df = 95$, $p < 0.0001$], the frontal lobe [$y = -0.001 \times x + 0.352$, $r = 0.394$, $df = 95$, $p < 0.0001$], the cingulate gyrus [$y = -0.001 \times x + 0.465$, $r = 0.376$, $df = 95$, $p < 0.0001$] and the corpus callosum [$y = -0.002 \times x + 0.465$, $r = 0.398$, $df = 95$, $p < 0.0001$]) and the delirium group (the thalamus [$y = -0.001 \times x + 0.352$, $r = 0.346$, $df = 17$, $p = 0.147$], the frontal lobe [$y = -0.002 \times x + 0.51$, $r = 0.396$, $df = 17$, $p = 0.0936$], the cingulate gyrus [$y = -0.001 \times x + 0.394$, $r = 0.089$, $df = 17$, $p = 0.716$], and the corpus callosum [$y = -0.004 \times x + 0.685$, $r = 0.431$, $df = 17$, $p = 0.0656$]) are indicated by the dotted lines and solid lines, respectively.

deep WMs and thalamus are predisposed in the patients with delirium and putatively account for the underlying mechanism of age-related vulnerability to delirium. On the other hand, the FA values in the four brain areas such as left ventral anterior nucleus of the thalamus may be affected by factors other than aging,

supported by the additional analysis in the subgroup of the subjects older than 60 years indicating that the decreased FA values of the brain areas in the delirious patients were also statistically significant. Thus, further DTI studies to clarify factors other than aging affecting the microstructure of the WM, e.g., arteriosclerosis, can provide a new insight into the brain condition vulnerable to delirium.

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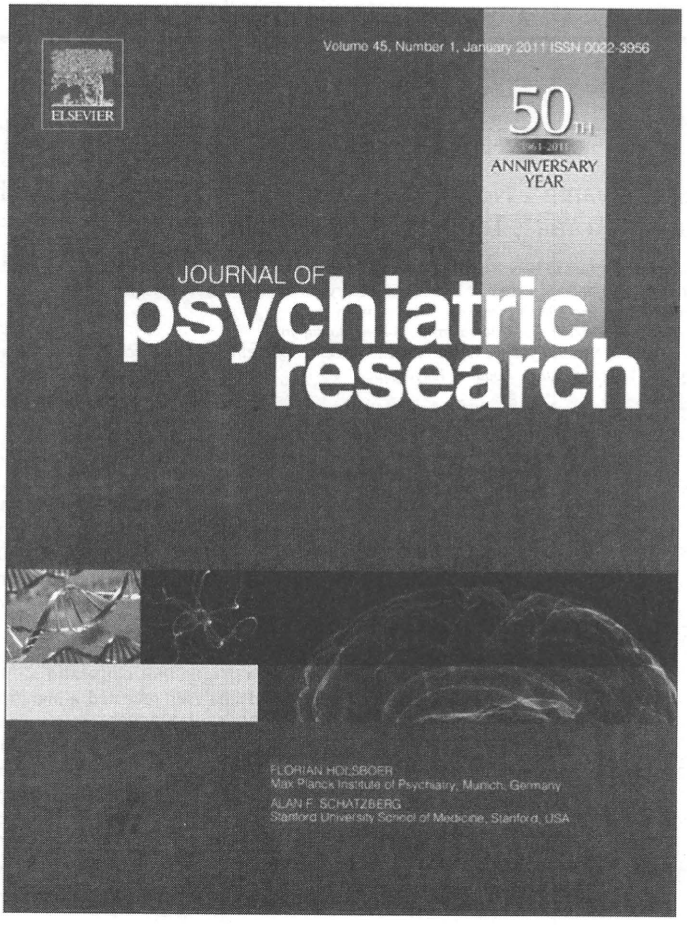
References

1. American Psychiatric Association. Diagnostic and statistical manual, 4th ed., text revision. Washington, DC, American Psychiatric Association, 2000
2. Bhat R, Rockwood K: Delirium as a disorder of consciousness. J Neurol Neurosurg Psychiatry 2007; 78:1167–1170
3. Cole MG: Delirium in elderly patients. Am J Geriatr Psychiatry 2004; 12:7–21
4. Meagher DJ, Moran M, Raju B, et al: Phenomenology of delirium: assessment of 100 adult cases using standardized measures. Br J Psychiatry 2007; 190:135–141
5. Inouye SK: Delirium in older persons. N Engl J Med 2006; 354: 1157–1165
6. Tan MC, Felde A, Kuskowski M, et al: Incidence and predictors of post-cardiotomy delirium. Am J Geriatr Psychiatry 2008; 16:575–583
7. Litaler D, Locala J, Franco K, et al: Preoperative risk factors for postoperative delirium. Gen Hosp Psychiatry 2001; 23:84–89
8. Schneider F, Böhner H, Habel U, et al: Risk factors for postoperative delirium in vascular surgery. Gen Hosp Psychiatry 2002; 24:28–34
9. Minden SL, Carbone LA, Barsky A, et al: Predictors and outcomes of delirium. Gen Hosp Psychiatry 2005; 27:209–214
10. Kazmierski J, Kowman M, Banach M, et al: Preoperative predictors of delirium after cardiac surgery: a preliminary study. Gen Hosp Psychiatry 2006; 28:536–538
11. Chang Y-L, Tsai Y-F, Lin P-J, et al: Prevalence and risk factors for post-operative delirium in a cardiovascular intensive care unit. Am J Critical Care 2008; 17:567–575
12. Waker P, Nunes PV, Cabrita H, et al: Post-operative delirium is associated with poor cognitive outcome and dementia. Dement Geriatr Cogn Disord 2006; 21:221–227
13. Rudolph JL, Jones RN, Rasmussen LS, et al: Independent vascular and cognitive risk factors for postoperative delirium. Am J Med 2007; 120:807–813
14. Nucifora pp, Verma R, Lee S-K, et al: Diffusion-tensor MR imaging and tractography: exploring brain microstructure and connectivity. Radiology 2007; 245:367–384
15. Basser PJ: Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed 1995; 8:333–344
16. Rovaris M, Iannucci G, Sormani MP, et al: Age-related changes in conventional, magnetization transfer, and diffusion-tensor MR imaging findings: study with whole-brain tissue histogram analysis. Radiology 2003; 227:731–738
17. Salat DH, Tuch DS, Greve DN, et al: Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiol Aging 2005; 26:1215–1227
18. Pfefferbaum A, Adalsteinsson E, Sullivan EV: Frontal circuitry degradation marks healthy adult aging: Evidence from diffusion tensor imaging. Neuroimage 2005; 26:891–899
19. Sullivan EV, Pfefferbaum A: Diffusion tensor imaging and aging. Neurosci Biobehav Rev 2006; 30:749–761
20. Wozniak JR, Lim KO: Advances in white matter imaging: a review of in vivo magnetic resonance methodologies and their applicability to the study of development and aging. Neurosci Biobehav Rev 2006; 30:762–774
21. Yoon B, Shim Y-S, Lee K-S, et al: Region-specific changes of cerebral white matter during normal aging: a diffusion-tensor analysis. Arch Gerontol Geriatr 2008; 47:129–138
22. Grieve SM, Williams LM, Paul RH, et al: Cognitive aging, executive function, and fractional anisotropy: a diffusion tensor MR imaging study. Am J Neuroradiol 2007; 28:226–235
23. O'Sullivan M, Jones DK, Sunners PE, et al: Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline. Neurology 2001; 57:632–638
24. Sullivan EV, Adalsteinsson E, Pfefferbaum A: Selective age-related degradation of anterior callosal fiber bundles quantified in vivo with fiber tracking. Cereb Cortex 2005; 16:1030–1039
25. Esposito G, Kirkby BS, Van Horn JD, et al: Context-dependent, neural system-specific neurophysiological concomitants of aging: mapping PET correlates during cognitive activation. Brain 1999; 122:963–979
26. Duncan J, Owen AM: Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends Neurosci 2000; 23:475–483
27. Takeuchi T, Matsushima E, Moriya H, et al: Delirium in inpatients with respiratory diseases. Psychiatry Clin Neurosci 2005; 59:253–258
28. Takeuchi T, Furuta K, Hirasawa T, et al: Perospirone in the treatment of patients with delirium. Psychiatry Clin Neurosci 2007; 61:67–70
29. Trzepacz PT, Mittal D, Torres R, et al: Validation of the Delirium Rating Scale-revised-98: comparison with the delirium rating scale and the cognition test for delirium. J Neuropsychiatry Clin Neurosci 2001; 13:229–242
30. Folstein MF, Folstein SE, McHugh PR: "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12:189–198
31. Reitan RM, Wolfgan D: Category test and trail making test as measures of frontal lobe function. Clin Neuropsychol 1995; 9:50–56
32. Stroop JR: Studies of interference in serial verbal reactions. J Exp Psychol 1935; 12:643–662
33. Gomez RG, White DA: Using verbal fluency to detect very mild dementia of the Alzheimer type. Arch Clinical Neurol 2006; 21:771–775

34. Beck AT, Ward CH, Mendelson M, et al: An inventory for measuring depression. Arch Gen Psychiatry 1961; 4:561-571
35. Riker RR, Shehabi Y, Bokesch PM, et al: Dexmedetomidine vs midazolam for sedation of critically ill patients: a randomized study. JAMA 2009; 301:489-499
36. Pierpaoli C, Basser PJ: Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 1996; 36:893-906
37. Pierpaoli C, Jezzard P, Basser PJ, et al: Diffusion tensor MR imaging of the human brain. Radiology 1996; 201:637-648
38. Friston KJ, Holmes AP, Poline JB, et al: Analysis of fMRI time-series revisited. Neuroimage 1995; 2:45-53
39. Hirata Y, Matsuda H, Nemoto K, et al: Voxel-based morphometry to discriminate early Alzheimer's disease from controls. Neurosci Lett 2005; 382:269-274
40. Mori T, Ohnishi T, Hashimoto R, et al: Progressive changes of white matter integrity in schizophrenia revealed by diffusion tensor imaging. Psychiatry Res 2007; 154:133-145
41. Zhang Y, Schuff N, Du A-T, et al. White matter damage in frontotemporal dementia and Alzheimer's disease measured by diffusion MRI. Brain 2009; 132:2579-2592.
42. Meier-Ruge W, Ulrich J, Brühlmann M, et al: Age-related white matter atrophy in the human brain. Ann N Y Acad Sci 1992; 673:260-269
43. Tang Y, Nuengard JR, Pakkenberg B, et al: Age-induced white matter changes in the human brain: a stereological investigation. Neurobiol Aging 1997; 18:609-615
44. Marner L, Nyengaard JR, Tang Y, et al: Marked loss of myelinated nerve fibers in the human brain with aging. J Com Neurol 2003; 462:144-152
45. Bartzokis G, Beckson M, Lu PH, et al: Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. Arch Gen Psychiatry 2001; 58:461-465
46. Sowell ER, Peterson BS, Thompson PM, et al: Mapping cortical change across the human life span. Nat Neurosci 2003; 6:309-315
47. Steriade M: Awakeing the brain. Nature 1996; 383:24-25
48. Barth DS, MacDonald KD: Thalamic modulation of high-frequency oscillating potentials in auditory cortex. Nature 1996; 383:78-81
49. Mesulam MM: Large-scale neurocognitive network and distributed processing for atttnetion, language, and memory. Ann Neurol 1990; 28:597-613
50. Corbetta M, Miezin FM, Shulman GL, et al: A PET study of visuospatial attention. J Neursci 1993; 13:1202-1226
51. Nobre AC, Sebestyen GN, Gitelman DR, et al: Functional localization of the system for visuospatial attention using positron emission tomography. Brain 1997; 120:515-533
52. Trzepacz P, van der Mast R: The neuropathophysiology of delirium, in Delirium in Old Age. Edited by Lindesay J, Rockwood K, Macdonald A. Oxford, England, Oxford University Press, 2002, pp 51-90
53. Jurado MB, Rosselli M: The elusive nature of executive functions: a review of our current understanding. Neuropsychol Rev 2007; 17:213-233
54. Monsch AU, Bondi MW, Butters N, et al: Comparison of verbal fluency tasks in the detection of dementia of Alzheimer type. Arch Neurology 1992; 49:1253-1258
55. Kubicki M, McCarley R, Westin C-F, et al: A review of diffusion tensor imaging studies in schizophrenia. J Psychiatric Res 2007; 41:15-30
56. Gunning-Dixon FA, Hoptman MJ, Lim KO, et al: Macromolecular white matter abnormalities in geriatric depression: a magnetization transfer imaging study. Am J Geriatr Psychiatry 2008; 16: 255-262



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Possible association of the semaphorin 3D gene (*SEMA3D*) with schizophrenia

Takashi Fujii [a,b,c], Hirofumi Uchiyama [a], Noriko Yamamoto [a], Hiroaki Hori [a], Masahiko Tatsumi [d], Masanori Ishikawa [e], Kunimasa Arima [e], Teruhiko Higuchi [f], Hiroshi Kunugi [a,b,*]

[a] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo, Japan*
[b] *Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi-shi, Saitama, Japan*
[c] *Japan Human Sciences Foundation, 13-4 Kodenma-cho Nihonbashi, Chuo-ku, Tokyo, Japan*
[d] *Yokohama Shinryo Clinic, Yamamoto Bldg. 2F, 3-28-5 Tsuruyacho, Kanagawa-ku, Yokohama, Japan*
[e] *Department of Psychiatry, National Center Hospital of Neurology and Psychiatry, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo, Japan*
[f] *National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo, Japan*

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ABSTRACT

Semaphorins are ligands of plexins, and the plexin–semaphorin signaling system is widely involved in many neuronal events including axon guidance, cell migration, axon pruning, and synaptic plasticity. The plexin A2 gene (*PLXNA2*) has been reported to be associated with schizophrenia. This finding prompted us to examine the possible association between the semaphorin 3D gene (*SEMA3D*) and schizophrenia in a Japanese population. We genotyped 9 tagging single nucleotide polymorphisms (SNPs) of *SEMA3D* including a non-synonymous variation, Lys701Gln (rs7800072), in a sample of 506 patients with schizophrenia and 941 healthy control subjects. The Gln701 allele showed a significant protective effect against the development of schizophrenia ($p = 0.0069$, odds ratio = 0.76, 95% confidence interval 0.63 to 0.93). Furthermore, the haplotype-based analyses revealed a significant association. The four-marker analysis (rs2190208–rs1029564–rs17159614–rs12176601), in particular, not including the Lys701Gln, revealed a highly significant association ($p = 0.00001$, global permutation), suggesting that there may be other functional polymorphisms within *SEMA3D*. Our findings provide strong evidence that *SEMA3D* confers susceptibility to schizophrenia, which could contribute to the neurodevelopmental impairments in the disorder.

1. Introduction

The first discovered semaphorin, collapsing-1 (now Sema3A), was originally reported as a repulsive cue in axon guidance (Luo et al., 1993). To date, more than 20 semaphorins of secreted or membrane forms have been identified in various species ranging from nematodes to humans (Luo et al., 1993; Fujii et al., 2002; Yazdani and Terman, 2006). Semaphorins act as ligands for plexins, and the plexin–semaphorin signaling system has been widely investigated in nervous systems (Mann et al., 2007). Class 3 semaphorins (SEMA3A-G) have been well-studied and generally act as secreted ligands for the heterodimerized complex of the plexin A family members and neuropilins (Fujisawa, 2004). For example, Sema3A binds to neuropilin-1 and activates plexin A1 or plexin A2 to transduce a repulsive axon guidance signal (Takahashi and Strittmatter, 2001). Many studies of the plexin–semaphorin signaling system have concentrated on their roles in neuronal development and plasticity (reviewed in (Kruger et al., 2005; Halloran and Wolman, 2006; Waimey and Cheng, 2006; Mann et al., 2007)).

Recently, the relationship between schizophrenia and molecules in the plexin–semaphorin signaling system has begun to receive much attention, for several reasons (Mann et al., 2007). An increase in levels of SEMA3A was noted in the cerebellum in postmortem brains of schizophrenia patients, as measured by immunoreactivity in the inner molecular layer and by the enzyme-linked immunosorbent assay (ELISA) in cerebellar protein extract (Eastwood et al., 2003). A genome-wide association study using 25,494 single nucleotide polymorphisms (SNPs) revealed that an intronic SNP of *PLXNA2* was most consistently associated with schizophrenia in European–American populations (Mah et al., 2006). Our replication study in a Japanese sample failed to confirm such an association (Fujii et al., 2007); however, a meta-analysis combining data from previous studies of *PLXNA2* yielded a positive association with schizophrenia (Allen et al., 2008), in which it was reported that the C allele of the SNP rs752016 of *PLXNA2* showed a nominally significant protective effect (odds ratios (OR) = 0.82, 95%

* Corresponding author. Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan. Tel./fax: +81 42 346 1714.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

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confidence interval (CI) = 0.69–0.99), and association of the SNP rs841865 approached statistical significance (OR = 0.84, 95% CI = 0.69–1.01) when samples of Mah et al. and Fujii et al. were combined (Mah et al., 2006; Fujii et al., 2007). Furthermore, in the updated online database, "SchizophreniaGene (http://www.schizophreniaforum.org/)," association of the SNP rs1327175 approached statistical significance (OR = 0.76, 95% CI = 0.57–1.00) (Mah et al., 2006; Fujii et al., 2007; Takeshita et al., 2008; Budel et al., 2008). Therefore, genes of the plexin family, the semaphorin family, and neuropilins, are intriguing candidates for schizophrenia susceptibility genes. We then focused on *SEMA3D* as a candidate gene for schizophrenia. *SEMA3D* was mapped to chromosome 7q21 (Clark et al., 2003); interestingly, a previous genome-wide scan suggested that this chromosomal region contains a susceptibility locus for schizophrenia (Ekelund et al., 2000) and recent studies have provided additional support for this possibility (Tastemir et al., 2006; Wedenoja et al., 2008, 2009; Idol et al., 2008).

The aim of the present study was to examine the possible association between *SEMA3D* and schizophrenia. *SEMA3D* has a common variant in the coding region due to an A to C base substitution (rs7800072), which results in an amino acid change (701 Lys to Gln). This SNP has previously been examined with regard to brain morphology (assessed with magnetic resonance imaging) in patients with schizophrenia (Gregorio et al., 2009). Although this study failed to find significant alterations in brain morphology, it is still unclear whether this SNP confers susceptibility to schizophrenia. We examined the possible association of schizophrenia with this non-synonymous SNP, plus 8 tagging SNPs encompassing the entire *SEMA3D* gene.

2. Subjects and methods

2.1. Subjects

Subjects were 506 patients with schizophrenia (278 males [54.9%], mean age 44.3 years [SD 14.1]) and 941 healthy controls (334 males [35.5%], mean age 44.8 years [SD 16.3]). All subjects were Japanese, biologically unrelated, 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 the same geographical area. 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 approved by the ethics committee of 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

The tagging SNPs were selected using the phase III version of HapMap (http://www.hapmap.org/cgi-perl/gbrowse/). SNP genotype data for the JPT (Japanese in Tokyo, Japan) were downloaded for the genomic region of *SEMA3D* plus 2 kb 5′ and 2 kb 3′ of this region (chr7q21.11). The most centromeric and telomeric HapMap markers downloaded were rs6944966 and rs11762367, respectively. HapMap markers were analyzed using the Haploview 4.1 system (http://www.broad.mit.edu/mpg/haploview) with the following criteria of marker selection: Hardy–Weinberg (HW) p value cutoff: 0.05; minimum genotypes: 90%; maximum number of Mendelian errors: 1; minimum minor allele frequency: 0.1; minimum distance between tags: 10 kb. Tagging SNPs were selected using the Tagger function implemented in Haploview with the following criteria: pairwise tagging only and r^2 threshold 0.8. We preselected rs7800072 and rs6966472 as markers and used the Tagger function implemented in Haploview to select other markers. As a result, 9 markers were selected as suitable for analysis for *SEMA3D*. SNP rs7800072 is non-synonymous (2141A > C, Lys701Gln). The numbers of base and amino acid positions were according to NM_152754.2 and NP_689967.2, respectively.

2.3. Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to 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__15937080_10 for rs2190208, C___7585979_10 for rs1029564, C__33462384_10 for rs17159614, C__31373903_10 for rs12176601, C___2635874_10 for rs6966472, C___2635864_10 for rs17559978, C__33462432_10 for rs17159577, C__33462438_10 for rs17159556, and C__25994972_10 for rs7800072. 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. Haplotype and statistical analysis

Deviations of genotype distributions from the HW 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 SPSS software ver.11 (SPSS Japan, Tokyo, Japan). Haplotype-based association analyses were performed with SNPAlyze software ver.6.5 (http://www.dynacom.co.jp/e/products/package/snpalyze/about.html). The measures of linkage disequilibrium (LD), denoted as D′ and r^2, were calculated from the haplotype frequency using the expectation-maximization (EM) algorithm. Haplotypes with frequencies of less than 1% were considered to be rare and were excluded from the analyses. All p values reported are two-tailed. We performed 100,000 permutations only for some significant haplotypes (e.g., rs2190208–rs1029564–rs17159614–rs121176601) and 10,000 permutations for the other haplotypes. OR and 95% CI were also calculated. To correct the critical p value for multiple testing, we used the spectral decomposition method of SNPSpD software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004; Li and Ji, 2005), which considers marker linkage disequilibrium information and generates an experiment-wide significance threshold required to keep the type I error rate at 5%.

3. Results

Genotype and allele distributions of the examined SNPs of *SEMA3D* in patients and controls are shown in Table 1. LD estimates of pairwise SNPs, expressed in D′ and r^2, are presented in Fig. 1. The genotype distributions did not significantly deviate from the HWE in patients and controls for any of the examined SNPs. For the non-synonymous polymorphism of *SEMA3D* (rs7800072), there were significant differences in both genotype ($\chi^2 = 8.7$, df = 2, $p = 0.013$) and allele ($\chi^2 = 7.3$, df = 1, $p = 0.0069$, OR = 0.76, 95% CI 0.63–0.93) distributions between patients and controls (Table 1). Furthermore, with respect to the other 8 SNPs (rs2190208, rs1029564,

Table 1
Genotype and Allelic Distribution of the *SEMA3D* 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)[c]	GF(df = 2)[d]	AF(df = 1)[e]
rs2190208	9986227				GG	GA	AA	G	A				
	5′ promoter	___	Schizophrenia	494	186 (0.38)	231 (0.47)	77 (0.16)	603 (0.61)	385 (0.39)	0.96	$\chi2 = 0.14, p = 0.71$	*p = 0.48*	*p = 0.59*
			Control	930	325 (0.35)	466 (0.50)	139 (0.15)	1116 (0.60)	744 (0.40)	(0.82–1.12)	$\chi2 = 1.79, p = 0.18$	$\chi2 = 1.48$	$\chi2 = 0.29$
rs1029564	9974131				AA	AC	CC	A	C				
	intron 1	12096	Schizophrenia	492	334(0.68)	140 (0.28)	18 (0.04)	808 (0.82)	176 (0.18)	0.78	$\chi2 = 0.48, p = 0.48$	***p = 0.028***	***p = 0.011***
			Control	931	565 (0.61)	324 (0.35)	42 (0.05)	1454 (0.78)	408 (0.22)	(0.64–0.94)	$\chi2 = 0.27, p = 0.61$	$\chi2 = 7.17$	$\chi2 = 6.40$
rs17159614	9959778				GG	GA	AA	G	A				
	intron 2	14353	Schizophrenia	495	289 (0.58)	181(0.37)	25 (0.05)	759 (0.77)	231 (0.23)	1.00	$\chi2 = 0.24, p = 0.62$	*p = 1.00*	*p = 0.96*
			Control	931	545 (0.59)	339 (0.36)	47(0.05)	1429(0.77)	433 (0.23)	(0.83–1.19)	$\chi2 = 0.38, p = 0.54$	$\chi2 = 0.0034$	$\chi2 = 0.0023$
rs12176601	9948019				TT	TA	AA	T	A				
	intron 2	11759	Schizophrenia	493	166(0.34)	244 (0.49)	83 (0.17)	576 (0.58)	410 (0.42)	1.21	$\chi2 = 0.17, p = 0.68$	***p = 0.029***	***p = 0.021***
			Control	917	375 (0.41)	403 (0.44)	139 (0.15)	1153 (0.63)	681 (0.37)	(1.03–1.41)	$\chi2 = 3.16, p = 0.08$	$\chi2 = 7.08$	$\chi2 = 5.35$
rs6966472	9933663				AA	AG	GG	A	G				
	intron 4	14356	Schizophrenia	493	381 (0.77)	103 (0.21)	9 (0.02)	865 (0.88)	121 (0.12)	0.73	$\chi2 = 0.43, p = 0.51$	***p = 0.023***	***p = 0.0075***
			Control	931	656 (0.70)	252 (0.27)	23 (0.02)	1564 (0.84)	298 (0.16)	(0.59–0.92)	$\chi2 = 0.04, p = 0.84$	$\chi2 = 7.59$	$\chi2 = 7.16$
rs17559978	9912136				CC	CT	TT	C	T				
	intron 7	21527	Schizophrenia	499	339 (0.68)	138 (0.28)	22 (0.04)	816 (0.82)	182 (0.18)	0.80	$\chi2 = 2.63, p = 0.10$	***p = 0.029***	***p = 0.025***
			Control	936	571 (0.61)	322 (0.34)	43 (0.05)	1464 (0.78)	408 (0.22)	(0.66–0.97)	$\chi2 = 0.08, p = 0.78$	$\chi2 = 7.11$	$\chi2 = 5.05$
rs17159577	9900238				CC	CT	TT	C	T				
	intron 10	11898	Schizophrenia	494	244 (0.49)	195 (0.39)	55 (0.11)	683 (0.69)	305 (0.31)	1.05	$\chi2 = 2.79, p = 0.09$	*p = 0.15*	*p = 0.60*
			Control	934	453 (0.49)	403 (0.43)	78 (0.08)	1309 (0.70)	559 (0.30)	(0.88–1.24)	$\chi2 = 0.77, p = 0.38$	$\chi2 = 3.78$	$\chi2 = 0.27$
rs17159556	9886562				GG	GT	TT	G	T				
	intron 10	13676	Schizophrenia	496	372 (0.75)	112 (0.23)	12 (0.02)	856(0.86)	136(0.14)	0.76	$\chi2 = 1.03, p = 0.31$	***p = 0.024***	***p = 0.012***
			Control	932	635 (0.68)	271 (0.29)	26 (0.03)	1541 (0.83)	323(0.17)	(0.61–0.94)	$\chi2 = 0.21, p = 0.65$	$\chi2 = 7.43$	$\chi2 = 6.29$
rs7800072	9863265				AA	AC	CC	A	C				
	exon 17	23297	Schizophrenia	502	342 (0.68)	140 (0.28)	20 (0.04)	824 (0.82)	180(0.18)	0.76	$\chi2 = 1.37, p = 0.24$	***p = 0.013***	***p = 0.0069***
	Lyn701Gln		Control	934	563 (0.60)	327 (0.35)	44 (0.05)	1453(0.78)	415 (0.22)	(0.63–0.93)	$\chi2 = 0.16, p = 0.69$	$\chi2 = 8.67$	$\chi2 = 7.31$

[a] Chromosome position was established from the dbSNP database.
[b] Without Bonferroni's correction.
[c] HWE; Hardy–Weinberg equilibrium.
[d] GF; Genotype distribution frequency.
[e] AF; Allele distribution frequency.

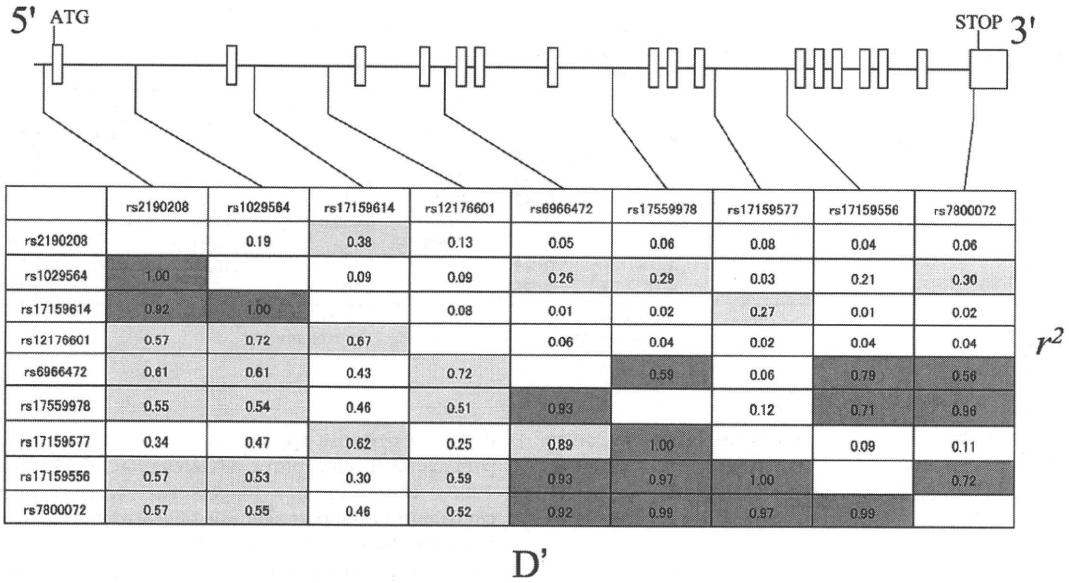

Fig. 1. The genetic structure of *SEMA3D* and location of the examined SNPs. The D′ and r^2 values between paired SNPs are shown in the diagram. The exonic regions are shown as white squares. The intensity of the box color corresponds to the strength of LD or r^2.

rs17159614, rs12176601, rs6966472, rs17559978, rs17159577, and rs17159556), several significant differences in genotype and allele distributions were observed (Table 1). To correct for multiple testing, we calculated the experiment-wide significance threshold required to keep the type I error rate at 5%. As a result, the corrected p value was calculated as 0.0085. The allelic associations with the SNPs rs7800072 (Lys701Gln) and rs6966472 remained significant after the correction (Table 1). Distinguishing between the carriers and the non-carriers with respect to the Gln701 allele for patients and controls, the protective effect became clearer ($p = 0.0033$).

The results of haplotype-based analyses are shown in Table 2. There were significant haplotypic associations of the SNPs in *SEMA3D* when comparing the schizophrenic patients and control subjects. In particular, the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601) showed a statistically significant association with schizophrenia (global permutation $p = 0.00001$). Concerning this haplotype analysis, global p values of 100,000 permutations, which corrected for multiple testing, were also significant. Furthermore, the haplotype frequency of GAGA was significantly higher in schizophrenia patients than in control subjects (0.376 and 0.291, permutation $p = 0.00005$), whereas those of GAGT, AAAA, and GCGA were significantly lower in schizophrenic patients than in controls (0.050 and 0.084, permutation $p = 0.0029$; 0.007 and 0.025, permutation $p = 0.0062$; 0.007 and 0.021, permutation $p = 0.020$, respectively) (Table 3).

When we performed stratified analysis of the data for rs7800072 by sex, a significant association was observed in women ($p = 0.0089$), but not in men ($p = 0.41$) (supplementary Tables 1 and 2). In the haplotype analysis, on the other hand, the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601) showed a statistically significant association in men (global permutation $p = 0.00001$), but was at a trend level in women (global permutation $p = 0.0699$). The haplotype frequency of GAGA

Table 2
Associations with schizophrenia of the 9 SNPs and haplotypes in *SEMA3D*.

SNP No.	dbSNP ID	Allele model	Haplotype p[a]							
		p value	2 Locus	3 Locus	4 Locus	5 Locus	6 Locus	7 Locus	8 Locus	9 Locus
SNP1	rs2190208	0.59								
			0.019							
SNP2	rs1029564	**0.011**		0.10						
			0.029		**0.00001**					
SNP3	rs17159614	0.96		**0.00002**		**0.00005**				
			0.0004		**0.0003**		**0.00007**			
SNP4	rs12176601	**0.021**		**0.0010**		**0.0001**		**0.0003**		
			0.035		**0.0006**		**0.0016**		**0.0007**	
SNP5	rs6966472	**0.0075**		0.053		**0.0004**		**0.0001**		**0.0007**
			0.023		0.098		**0.0001**		**0.0001**	
SNP6	rs17559978	**0.025**		**0.022**		0.061		**0.0004**		
			0.030		**0.025**		0.076			
SNP7	rs17159577	0.60		0.064		**0.024**				
			0.042		0.051					
SNP8	rs17159556	**0.012**		**0.028**						
			0.020							
SNP9	rs7800072	**0.0069**								

[a] global p value.

Table 3
Estimated haplotype frequencies and association significance for *SEMA3D*.

Haplotype	rs2190208	rs1029564	rs17159614	rs12176601	% of individuals					
					Overall	Control	Schizophrenia	$\chi 2$	*p value*	Permutation *p* value
1	G	A	G	A	0.321	0.291	0.376	20.40	**0.0000063**	**0.000050**
2	A	A	A	T	0.207	0.201	0.219	1.21	0.27	0.28
3	G	C	G	T	0.190	0.199	0.172	2.98	0.085	0.089
4	A	A	G	T	0.142	0.143	0.139	0.10	0.75	0.76
5	G	A	G	T	0.072	0.084	0.050	11.23	**0.00080**	**0.0029**
6	A	A	G	A	0.034	0.036	0.031	0.37	0.54	0.59
7	A	A	A	A	0.019	0.025	0.007	10.75	**0.0010**	**0.0062**
8	G	C	G	A	0.016	0.021	0.007	7.55	**0.0060**	**0.020**

	$\chi 2$	*p value*	Permutation *p* value	Replications
Global	46.07	**0.000000085**	**0.00001**	100000

was significantly higher in schizophrenia patients than in control subjects in both men (0.368 and 0.272, permutation $p = 0.00053$) and women (0.384 and 0.302, permutation $p = 0.003$).

4. Discussion

Our results provide the first evidence for the possible involvement of *SEMA3D* in the pathogenesis of schizophrenia. With respect to the non-synonymous (Lys701Gln) polymorphism, we found a significant preponderance of the Lys/Lys genotype and the Lys701 allele in schizophrenia patients compared with control subjects. In the haplotype-based analyses, we also obtained evidence for an association between *SEMA3D* and schizophrenia. Interestingly, the most significant haplotype, rs2190208–rs1029564–rs17159614–rs121176601, does not include rs7800072 (Lys701Gln) (see Fig. 1). Therefore, it is likely that at least one functional polymorphism other than rs7800072, which is in linkage disequilibrium to the haplotype, could be responsible for susceptibility to schizophrenia. In stratified analysis for rs7800072 by sex, the frequency of the Gln701 allele was significantly lower in schizophrenia patients than in control subjects in women (0.17 and 0.23, $p = 0.0088$) (supplementary Table 2). Likewise, this was also lower in men, but was not statistically significant (0.18 and 0.20, $p = 0.41$) (supplementary Table 1). Regarding analysis of the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601), there remained a statistical significance in men (global permutation $p = 0.00001$) and a tendency in women (global permutation $p = 0.0699$). In addition, the frequency of the most major haplotype (GAGA) was significantly higher in schizophrenia patients than in control subjects in both sexes. These inconsistent results between males and females are likely to have arisen from the lack of statistical power after dividing the sexes.

The neurodevelopmental hypothesis of schizophrenia proposes that abnormalities of brain development are involved in the pathogenesis of schizophrenia (Conrad and Scheibel, 1987; Weinberger, 1987; Murray, 1994; Waddington et al., 1998). In early brain developmental stages, a number of semaphorins play important roles in axonal repulsion, axonal attraction, neuronal cell migration, and axon pruning (reviewed in Kruger et al., 2005; Waimey and Cheng, 2006; Halloran and Wolman, 2006; Mann et al., 2007). Indeed, SEMA3D has been shown to act in axon guidance and cell migration during neuronal development (Wolman et al., 2004, 2007; Liu et al., 2004; Liu and Halloran, 2005; Sakai and Halloran, 2006; Takahashi et al., 2009). With respect to neuronal cell migration, neuronal disarray and abnormal migration in the neocortical white matter were reported in postmortem studies of patients with schizophrenia (Jakob and Beckmann, 1986; Akbarian et al., 1993). Regarding pruning, Feinberg proposed that schizophrenia may arise from excessive synaptic pruning during adolescence (Feinberg, 1982; Keshavan et al., 1994). Indeed, decreased density of dendritic spines was observed in the prefrontal cortex of patients with schizophrenia (Garey et al., 1998; Glantz and Lewis, 2000). These findings suggest that variants of *SEMA3D* may contribute to the pathogenesis of schizophrenia through affecting development of neural networks. The genotypic difference based on the Lys701Gln polymorphism of *SEMA3D* might lead to developmental differences in the brain; the Gln701 carriers would exhibit intrinsically greater protective effects against the development of schizophrenia than the Gln701 non-carriers. Although *SEMA3D* has not yet been well-studied, *SEMA3A* has been investigated in detail. In particular, an increase in the expression of SEMA3A has previously been associated with schizophrenia (Eastwood et al., 2003). Moreover, *PLXNA2*, which encodes one of the receptors for class 3 semaphorins, was identified as a candidate gene for schizophrenia in a genome-wide association study (Mah et al., 2006). Currently, this association is also supported by the meta-analysis of Allen et al. (2008). SEMA3A and SEMA3D belong to the same class and share the most similarity with each other of the class 3 semaphorin genes (Luo et al., 1995). These findings further strengthen the evidence for a possible role of *SEMA3D* in the development of schizophrenia.

It is possible that the amino acid change (Lys701Gln) may affect the function of SEMA3D protein and that this results in susceptibility to schizophrenia. Indeed, this is a substitution from a large and basic amino acid (Lys) to a medium-sized and polar one (Gln). This is likely to lead to functional differences between the two types of SEMA3D. One possibility is that this substitution might result in conformational change of SEMA3D and influence its affinity for its receptors. Another possibility is that the Lys701 and Gln701 variants of SEMA3D have different cellular localization. The basic domain of class 3 semaphorins electrostatically interacts with the proteoglycan components of the extracellular matrix (De Wit et al., 2005) and the granule matrix (de Wit et al., 2009). The substitution from the basic Lys701 to the non-basic Gln701 may affect such interactions between SEMA3D and these matrices. Alteration of the extracellular matrix may modify distribution of SEMA3D in neurons, and that of the granule matrix may affect secretion from secretory vesicles. The class 3 semaphorins not only act as axon guidance cues but also have key roles in synaptic formation and function. Therefore, these modified interactions could impact on the establishment of synaptic contacts and the formation of new synapses. Although the amino acid substitution (Lys701Gln) was predicted to be benign by Polyphen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/) programs, its actual effects should be elucidated by cell biological or biochemical approaches.

Accumulating evidence suggests that the semaphorins are regulatory factors of tumor progression and modulators of angiogenesis (reviewed in (Neufeld and Kessler, 2008) and (Capparuccia and Tamagnone, 2009)). Recently, SEMA3D was also reported to

possess anti-tumorigenic and anti-angiogenic properties (Kigel et al., 2008). The hypoactivity of *SEMA3D* could be linked to increased incidence of cancer. Previous studies and reviews have partially supported the idea that the incidence of cancer in patients with schizophrenia is reduced compared with the general population (Grinshpoon et al., 2005; Dalton et al., 2005; Catts et al., 2008). It is possible that semaphorins are related to the development of schizophrenia and also contribute to the associated lower incidence of cancer, and this topic warrants further investigation.

In conclusion, we found a significant association between the Lys701Gln polymorphism of *SEMA3D* and schizophrenia. In addition, the haplotype rs2190208–rs1029564–rs17159614–rs121176601, not including the Lys701Gln variant, was shown to be associated with schizophrenia, which suggests that some other polymorphisms of *SEMA3D* play a role in the pathogenesis of schizophrenia. Taking the previous molecular and developmental findings together with the present genetic findings, *SEMA3D* appears to be a promising candidate gene related to susceptibility to schizophrenia.

Conflict of interest

All authors declare no conflict of interest that could influence their work.

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Contributors

T.F. designed the study, performed genotyping of *SEMA3D*, made statistical analysis, managed literature search, interpreted the data, and wrote the manuscript. H.U. and N.Y. took part in genotyping. H.H., M.T., M.I., K.A., and T.H. collected samples and gave comments to the manuscript. H.K. organized recruitment and genotyping of schizophrenic patients and control subjects, and took part in analyzing the data and writing the manuscript.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.jpsychires.2010.05.004.

References

Akbarian S, Bunney Jr WE, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG. Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. Archives of General Psychiatry 1993;50:169–77.

Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nature Genetics 2008;40:827–34.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association (APA); 1994.

Budel S, Shim SO, Feng Z, Zhao H, Hisama F, Strittmatter SM. No association between schizophrenia and polymorphisms of the PlexinA2 gene in Chinese Han Trios. Schizophrenia Research 2008;99:365–6.

Capparuccia L, Tamagnone L. Semaphorin signaling in cancer cells and in cells of the tumor microenvironment–two sides of a coin. Journal of Cell Science 2009;122:1723–36.

Catts VS, Catts SV, O'Toole BI, Frost AD. Cancer incidence in patients with schizophrenia and their first-degree relatives–a meta-analysis. Acta Psychiatrica Scandinavica 2008;117:323–36.

Clark HF, Gurney AL, Abaya E, Baker K, Baldwin D, Brush J, et al. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. Genome Research 2003;13:2265–70.

Conrad AJ, Scheibel AB. Schizophrenia and the hippocampus: the embryological hypothesis extended. Schizophrenia Bulletin 1987;13:577–87.

Dalton SO, Mellemkjaer L, Thomassen L, Mortensen PB, Johansen C. Risk for cancer in a cohort of patients hospitalized for schizophrenia in Denmark, 1969–1993. Schizophrenia Research 2005;75:315–24.

De Wit J, De Winter F, Klooster J, Verhaagen J. Semaphorin 3A displays a punctate distribution on the surface of neuronal cells and interacts with proteoglycans in the extracellular matrix. Molecular and Cellular Neurosciences 2005;29:40–55.

de Wit J, Toonen RF, Verhage M. Matrix-dependent local retention of secretory vesicle cargo in cortical neurons. The Journal of Neuroscience 2009;29:23–37.

Eastwood SL, Law AJ, Everall IP, Harrison PJ. The axonal chemorepellant semaphorin 3A is increased in the cerebellum in schizophrenia and may contribute to its synaptic pathology. Molecular Psychiatry 2003;8:148–55.

Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, et al. Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. Human Molecular Genetics 2000;9:1049–57.

Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? Journal of Psychiatric Research 1982;17:319–34.

Fujii T, Iijima Y, Kondo H, Shizuno T, Hori H, Nakabayashi T, et al. Failure to confirm an association between the PLXNA2 gene and schizophrenia in a Japanese population. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2007;31:873–7.

Fujii T, Nakao F, Shibata Y, Shioi G, Kodama E, Fujisawa H, Takagi S. Caenorhabditis elegans PlexinA, PLX-1, interacts with transmembrane semaphorins and regulates epidermal morphogenesis. Development 2002;129:2053–63.

Fujisawa H. Discovery of semaphorin receptors, neuropilin and plexin, and their functions in neural development. Journal of Neurobiology 2004;59:24–33.

Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, et al. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. Journal of Neurology, Neurosurgery, and Psychiatry 1998;65:446–53.

Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Archives of General Psychiatry 2000;57:65–73.

Gregorio SP, Sallet PC, Do KA, Lin E, Gattaz WF, Dias-Neto E. Polymorphisms in genes involved in neurodevelopment may be associated with altered brain morphology in schizophrenia: preliminary evidence. Psychiatry Research 2009;165:1–9.

Grinshpoon A, Barchana M, Ponizovsky A, Lipshitz I, Nahon D, Tal O, et al. Cancer in schizophrenia: is the risk higher or lower? Schizophrenia Research 2005;73:333–41.

Halloran MC, Wolman MA. Repulsion or adhesion: receptors make the call. Current Opinion in Cell Biology 2006;18:533–40.

Idol JR, Addington AM, Long RT, Rapoport JL, Green ED. Sequencing and analyzing the t(1;7) reciprocal translocation breakpoints associated with a case of childhood-onset schizophrenia/autistic disorder. Journal of Autism and Developmental Disorders 2008;38:668–77.

Jakob H, Beckmann H. Prenatal developmental disturbances in the limbic allocortex in schizophrenics. Journal of Neural Transmission 1986;65:303–26.

Keshavan MS, Anderson S, Pettegrew JW. Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? the Feinberg hypothesis revisited. Journal of Psychiatric Research 1994;28:239–65.

Kigel B, Varshavsky A, Kessler O, Neufeld G. Successful inhibition of tumor development by specific class-3 semaphorins is associated with expression of appropriate semaphorin receptors by tumor cells. PLoS One 2008;3:e3287.

Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. Nature Reviews. Molecular Cell Biology 2005;6:789–800.

Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 2005;95:221–7.

Liu Y, Berndt J, Su F, Tawarayama H, Shoji W, Kuwada JY, Halloran MC. Semaphorin3D guides retinal axons along the dorsoventral axis of the tectum. The Journal of Neuroscience 2004;24:310–8.

Liu Y, Halloran MC. Central and peripheral axon branches from one neuron are guided differentially by Semaphorin3D and transient axonal glycoprotein-1. The Journal of Neuroscience 2005;25:10556–63.
Luo Y, Raible D, Raper JA. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. Cell 1993;75:217–27.
Luo Y, Shepherd I, Li J, Renzi MJ, Chang S, Raper JA. A family of molecules related to collapsin in the embryonic chick nervous system. Neuron 1995;14:1131–40.
Mah S, Nelson MR, Delisi LE, Reneland RH, Markward N, James MR, et al. Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. Molecular Psychiatry 2006;11:471–8.
Mann F, Chauvet S, Rougon G. Semaphorins in development and adult brain: Implication for neurological diseases. Progress in Neurobiology 2007;82:57–79.
Murray RM. Neurodevelopmental schizophrenia: the rediscovery of dementia praecox. The British Journal of Psychiatry. Supplement; 1994:6–12.
Neufeld G, Kessler O. The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nature Reviews. Cancer 2008;8:632–45.
Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American Journal of Human Genetics 2004;74:765–9.
Sakai JA, Halloran MC. Semaphorin 3d guides laterality of retinal ganglion cell projections in zebrafish. Development 2006;133:1035–44.
Takahashi K, Ishida M, Takahashi H. Expression of Sema3D in subsets of neurons in the developing dorsal root ganglia of the rat. Neuroscience Letters 2009;455:17–21.
Takahashi T, Strittmatter SM. Plexina1 autoinhibition by the plexin sema domain. Neuron 2001;29:429–39.
Takeshita M, Yamada K, Hattori E, Iwayama Y, Toyota T, Iwata Y, et al. Genetic examination of the PLXNA2 gene in Japanese and Chinese people with schizophrenia. Schizophrenia Research 2008;99:359–64.
Tastemir D, Demirhan O, Sertdemir Y. Chromosomal fragile site expression in Turkish psychiatric patients. Psychiatry Research 2006;144:197–203.
Waddington JL, Buckley PF, Scully PJ, Lane A, O'Callaghan E, Larkin C. Course of psychopathology, cognition and neurobiological abnormality in schizophrenia: developmental origins and amelioration by antipsychotics? Journal of Psychiatric Research 1998;32:179–89.
Waimey KE, Cheng HJ. Axon pruning and synaptic development: how are they perplexin? Neuroscientist 2006;12:398–409.
Wedenoja J, Loukola A, Tuulio-Henriksson A, Paunio T, Ekelund J, Silander K, et al. Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. Molecular Psychiatry 2008;13:673–84.
Wedenoja J, Tuulio-Henriksson A, Suvisaari J, Loukola A, Paunio T, Partonen T, et al. Replication of association between working memory and Reelin, a potential modifier gene in schizophrenia. Biological Psychiatry; 2009.
Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Archives of General Psychiatry 1987;44:660–9.
Wolman MA, Liu Y, Tawarayama H, Shoji W, Halloran MC. Repulsion and attraction of axons by semaphorin3D are mediated by different neuropilins in vivo. The Journal of Neuroscience 2004;24:8428–35.
Wolman MA, Regnery AM, Becker T, Becker CG, Halloran MC. Semaphorin3D regulates axon axon interactions by modulating levels of L1 cell adhesion molecule. The Journal of Neuroscience 2007;27:9653–63.
Yazdani U, Terman JR. The semaphorins. Genome Biology 2006;7:211.