Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 435 (2008) 34–39
Neuroscience Letters
www.elsevier.com/locate/neulet

Association between the brain-derived neurotrophic factor Val66Met polymorphism and brain morphology in a Japanese sample of schizophrenia and healthy comparisons

Tsutomu Takahashi [a,d], Michio Suzuki [a,d,*], Masahiko Tsunoda [a], Yukiko Kawamura [f,g], Nagahide Takahashi [f,h], Hiroshi Tsuneki [b], Yasuhiro Kawasaki [a,d], Shi-Yu Zhou [a,d,e], Soushi Kobayashi [b], Toshiyasu Sasaoka [b], Hikaru Seto [c], Masayoshi Kurachi [a,d], Norio Ozaki [f]

[a] Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[b] Department of Clinical Pharmacology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[c] Department of Radiology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[d] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan
[e] Department of Psychiatry and Medical Psychology, Dalian Medical University, Dalian, China
[f] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan
[g] Division of Clinical Science and Neuropsychopharmacology, Faculty of Pharmacy, Meijo University, Nagoya, Japan
[h] Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Mount Sinai School of Medicine, New York, USA

Received 18 November 2007; received in revised form 31 January 2008; accepted 4 February 2008

Abstract

Magnetic resonance imaging was used to investigate the relation between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and volumetric measurements for the medial temporal lobe structures (amygdala, hippocampus, and parahippocampal gyrus) and prefrontal subregions (the superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, ventral medial prefrontal cortex, orbitofrontal cortex, and straight gyrus) in a Japanese sample of 33 schizophrenia patients and 29 healthy subjects. For the controls, the Met carriers had significantly smaller parahippocampal and left superior frontal gyri than the Val homozygotes. The schizophrenia patients carrying the Met allele had a significantly smaller right parahippocampal gyrus than those with the Val/Val genotype, but the genotype did not affect the prefrontal regions in schizophrenia patients. These findings might reflect different genotypic effects of BDNF on brain morphology in schizophrenia patients and healthy controls, implicating the possible role of the brain morphology as an endophenotype for future genetic studies in schizophrenia.

Keywords: Brain-derived neurotrophic factor; Val66Met polymorphism; Schizophrenia; Magnetic resonance imaging; Parahippocampal gyrus; Prefrontal cortex

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, mediates differentiation and survival of neurons during development as well as synaptic plasticity in the mature nervous system [17,18,25]. A single nucleotide polymorphism (SNP) in the BDNF gene that produces an amino acid substitution (valine to methionine) at codon 66 (Val66Met) has been reported to affect the activity-dependent secretion of BDNF in cultured hippocampal neurons [4] and also human cognitive functioning [4,9,37]. Moreover, recent magnetic resonance imaging (MRI) studies in healthy subjects reported the effect of this functional SNP on brain morphology, with BDNF Met carriers having a reduced volume of the hippocampus [3,24,33], parahippocampal gyrus [20], or prefrontal cortex [24].

Several lines of evidence suggest a role for BDNF in the pathogenesis of schizophrenia. Recent postmortem studies in schizophrenia demonstrated decreased BDNF levels in the prefrontal cortex [10,40], and changes in the plasma BDNF levels were also reported in schizophrenia patients [8]. The association between the BDNF Val66Met polymorphism and treatment responses or clinical symptoms in schizophrenia [13,16,22]

* Corresponding author at: Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel.: +81 76 434 2281; fax: +81 76 434 5030.
E-mail address: suzukim@med.u-toyama.ac.jp (M. Suzuki).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.02.004

implies that the BDNF gene would be a plausible candidate for a schizophrenia-susceptibility gene.

Although previous genetic studies have not supported a significant role of the BDNF gene variants in the development of schizophrenia [15,19], several MRI studies have suggested different genotypic effects of BDNF on brain morphology in schizophrenia patients and healthy controls [1,11,12,33]. These studies have suggested that the effect of BDNF Val66Met polymorphism on medial temporal lobe [12,33] or frontal gray matter [11] volume is pronounced among schizophrenia patients as compared to healthy individuals, although the data are not entirely consistent [1]. These findings suggest a potential role for the investigation of the brain morphology as a neurobiological endophenotype in future genetic studies of schizophrenia [7], but first require further replication.

In this study, we used MRI to investigate the association between the BDNF Val66Met polymorphism and brain morphology in schizophrenia patients and healthy subjects. Based on previous MRI observations [3,20,24,33], regions of interest (ROIs) for the volumetric measurements were placed in the medial temporal and frontal lobe structures. We predicted from previous reports [11,12,33] that variation in the BDNF gene (Val66Met), a candidate locus for schizophrenia, would affect brain morphology differently in schizophrenia patients versus healthy subjects.

Thirty-three schizophrenia patients [20 males and 13 females, mean age = 25.6 ± 4.5 (S.D.) years (range, 19–36)] who met the ICD-10 diagnostic criteria for research [41] were recruited from the inpatient and outpatient clinics of the Department of Neuropsychiatry, Toyama University Hospital. All patients were receiving neuroleptic medication at the time of scanning [mean haloperidol equivalent dose = 12.2 ± 8.6 (S.D.) mg/day], with a mean duration of medication of 2.7 years (S.D. = 2.8). Fifteen patients were being treated with typical neuroleptics and 18 patients were receiving atypical neuroleptics. At the time of the MRI study, their mean scores on the scale for the assessment of negative symptoms (SANS) and the scale for the assessment of positive symptoms (SAPS) [2] were 49.7 (S.D. = 22.9) and 26.1 (S.D. = 24.7), respectively. The control subjects consisted of 29 healthy volunteers (17 males and 12 females) recruited from members of the community, hospital staff, and university students. Their mean age was 24.2 ± 6.1 (S.D.) years (range, 18–38). They were given a questionnaire consisting of 15 items concerning their family and past histories, and present illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. The minnesota multiphasic personality inventory (MMPI) was administered to all the control candidates, and they were excluded if any T-score for the validity scales or the clinical scales exceeded 70. This cohort is largely included in our previous MRI studies, which investigated the morphology in the temporal and frontal lobe structures in schizophrenia [21,32,35]. All subjects were right-handed Japanese and physically healthy at the time of the study, and none had a lifetime history of serious head trauma, neurological illness, serious medical or surgical illness, or substance abuse. The two diagnostic groups were matched for age, gender, height, and parental education. This study was approved by the ethical committees of Toyama University and Nagoya University. Written informed consent was obtained from all subjects.

Magnetic resonance images were obtained by utilizing a 1.5-T magnetom vision (Siemens Medical System, Inc., Erlangen, Germany) with a three-dimensional gradient-echo sequence fast low-angle shots (FLASH) yielding 160–180 contiguous T1-weighted slices of 1.0-mm thickness in the sagittal plane. The imaging parameters were: repetition time = 24 ms; echo time = 5 ms; flip angle = 40°; field of view = 256 mm; and matrix size = 256 × 256 pixels. The voxel size was 1.0 mm × 1.0 mm × 1.0 mm. Image processing for volumetric analysis has been described in detail elsewhere [34]. Briefly, on a Unix workstation (Silicon Graphics, Inc., Mountain View, CA), the image data were processed using the software package Dr. View 5.3 (Asahi Kasei Joho System Co., Ltd., Tokyo, Japan). Brain images were realigned in three dimensions to standardize for differences in head tilt during image acquisition and were then reconstructed into entire contiguous coronal images, with a 1-mm thickness, perpendicular to the anterior commissure-posterior commissure line. The whole cerebrum was manually separated from the brainstem and cerebellum. The signal-intensity histogram distributions from the T1-weighted images across the whole cerebrum were then used to semi-automatically segment the voxels into gray matter, white matter, and cerebrospinal fluid. The intracranial volume (ICV) was measured to correct for differences in head size as previously described [42]; the groups did not significantly differ in their ICV volumes.

The medial temporal structures (amygdala, hippocampus, and parahippocampal gyrus) and prefrontal sub-regions (the superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, ventral medial prefrontal cortex, orbitofrontal cortex, and straight gyrus) were manually traced on consecutive 1-mm coronal slices with the corresponding sagittal and axial planes simultaneously presented for reference. The procedures for delineation of these structures were described in detail previously [21,32]. The gray matter volumes of the prefrontal sub-regions were obtained by using the above-mentioned segmentation procedure. For the medial temporal lobe structures, volumes of gray and white matter were measured together. Three trained raters (S.Z., H.H., and L.N) measured the volumes of each ROI without knowledge of the subjects' identity, gender, and diagnosis. Intra- and inter-rater intraclass correlation coefficients in a subset of five randomly selected brains were over 0.92 for all ROIs.

Genomic DNA was extracted from EDTA-containing venous blood samples according to standard procedures. The genotyping of the Val66Met SNP (rs6265) of the BDNF gene was carried out using the polymerase chain reaction-restriction fragment length polymorphism assay. Sequences of primer pairs are Fw: 5′-ACTCTGGAGAGCGTGAATGG-3′ and Rv: 5′-CCGAACTTTCTGGTCCTCAT-3′. NlaIII was used for digestion.

Genotypic distribution and allelic frequencies were compared between schizophrenia patients and healthy subjects using the chi-square test. For the genotypic effects on brain morphology, each diagnostic group was examined separately based on the hypothesis that variation in the BDNF gene would affect brain

morphology differently in schizophrenia patients and healthy subjects. The relative volume [(absolute volume/ICV) × 100] for each region was analyzed using the repeated measures analysis of covariance (ANCOVA) with age and gender as covariates, genotype [Val homozygotes versus Met carriers (heterozygotes and Met homozygotes combined)] as a between-subject factor, and side (left versus right) as a within-subject variable. Heterozygotes and Met homozygotes were combined and categorized as Met carriers following the strategy used by Ho et al. [12]. The volumetric measurements for all ROIs in this study were normally distributed (Kolmogorov–Smirnov test). Post hoc Spjotvoll and Stoline tests, modified Tukey's tests for unequal sample size, were carried out to follow up the significant main effects or interactions yielded by ANCOVAs. Statistical significance was defined as $p < 0.05$.

The observed genotypic frequency of SNP was within the distribution expected according to Hardy–Weinberg equilibrium. As for the genotypic distribution, 12/33 schizophrenia patients (36.4%) and 13/29 controls (44.8%) were Val homozygotes, 6/33 patients (18.2%) and 5/29 controls (17.2%) were Met homozygotes, and 15/33 patients (45.5%) and 11/29 controls (37.9%) were heterozygotes, with no significant group difference (chi-square = 0.49, $p = 0.783$). No significant difference was observed in allelic frequencies between the patients and controls either (chi-square = 0.29, $p = 0.592$). For schizophrenia patients, no differences between the genotype groups were observed in age, education, parental education, numbers of hospitalization, onset age, illness duration, total score for SANS and SAPS, or medication status (dose, duration, and typical versus atypical). Although an association between smoking behavior and BDNF Val66Met polymorphism has been previously reported in schizophrenia [38], assessment of cigarette smoking behavior was not comprehensively undertaken in this sample.

Table 1 shows a comparison of the relative ROI volumes between the subjects with the Val/Val genotype and Met carriers. For the healthy controls, ANCOVA showed a significant main effect of genotype for the parahippocampal gyrus and a significant genotype-by-side interaction for the superior frontal gyrus, with the Met carriers having a smaller parahippocampal gyrus (post hoc test, $p = 0.014$) and smaller left superior frontal gyrus (post hoc test, $p = 0.010$) than the Val homozygotes (Fig. 1). For the schizophrenia patients, ANCOVA demonstrated a significant genotype-by-side interaction for the parahippocampal gyrus, showing that the Met carriers had a smaller right parahippocampal gyrus than the Val homozygotes (post hoc test, $p = 0.022$) (Fig. 1).

This volumetric MRI study investigated the effect of the BDNF Val66Met polymorphism on the prefrontal and medial temporal lobe structures in schizophrenia patients and healthy controls. We demonstrated that the Met carriers had a significantly smaller left superior frontal gyrus than the Val homozygotes among the controls but not the patients. For the parahippocampal gyrus, our results demonstrated that the Met allele is related to a reduction in volume bilaterally for controls, but only in the right hemisphere for the schizophrenia patients.

The present finding is consistent with the result of a previous MRI study using voxel-based morphometry that showed a similar association between the BDNF Val66Met polymorphism and the left superior frontal gyrus only for healthy controls [12]. The prefrontal cortex is an area that has received much attention in the search for the neural substrate of schizophrenia. In addition to a significant volume reduction [29,30,32], prefrontal abnormalities in schizophrenia have been implicated in negative symptoms and several cognitive impairments [6]. Although we did not find a specific genotypic effect of the BDNF on brain morphology in schizophrenia, a recent longitudinal MRI study demonstrated that BDNF Met allele contributed to frontal gray matter reduction over time in schizophrenia patients [11]. BDNF is known to support the survival and differentiation of dopaminergic neurons, and regulates the structural and functional maturation of the prefrontal cortex during young adulthood in the human brain [8,39]. Impairment of BDNF and related dopaminergic functions at this critical period in neurodevelopment, which could be influenced by variation in the BDNF gene, has important implications for the prefrontal pathology of schizophrenia. In addition, the findings of Ho et al. [11] support ongoing neuroplastic effects of BDNF beyond neurodevelopment. Our failure to find genotypic effects of BDNF on prefrontal gray matter volume in schizophrenia can possibly be explained by the fact

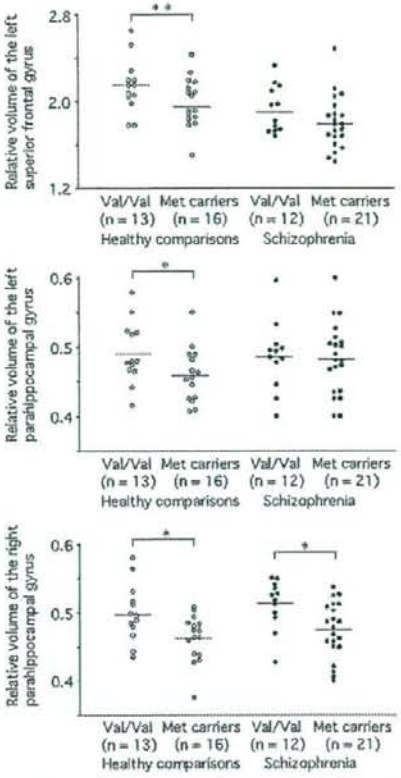

Fig. 1. Scatter plots of relative volumes (100 × absolute volume/intracranial volume) for the left superior frontal gyrus gray matter and the parahippocampal gyrus in healthy controls and schizophrenia patients. Horizontal lines indicate means. Post hoc comparisons: $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 1
BDNF genotypic differences in relative volume for regions of interest in schizophrenia patients and healthy controls

Brain region	Healthy comparisons		Analysis of covariance[a]				Schizophrenia patients		Analysis of covariance[b]			
	Val/Val ($N=13$)	Met carriers ($N=16$)	Genotype		Genotype × side		Val/Val ($N=12$)	Met carriers ($N=21$)	Genotype		Genotype × side	
			F	p	F	p			F	p	F	p
Amygdala			0.63	0.436	0.80	0.379			0.52	0.478	<0.01	0.965
Left	0.074 ± 0.011	0.078 ± 0.008					0.065 ± 0.006	0.066 ± 0.011				
Right	0.081 ± 0.007	0.075 ± 0.012					0.070 ± 0.006	0.071 ± 0.010				
Hippocampus			1.72	0.201	2.21	0.148			0.92	0.346	1.19	0.283
Left	0.208 ± 0.026	0.204 ± 0.020					0.201 ± 0.027	0.193 ± 0.025				
Right	0.224 ± 0.021	0.210 ± 0.018					0.218 ± 0.030	0.203 ± 0.024				
Parahippocampal gyrus			**5.22**	**0.031**	0.09	0.772			0.68	0.415	**4.93**	**0.034**
Left	0.492 ± 0.045	0.459 ± 0.038					0.487 ± 0.050	0.483 ± 0.052				
Right	0.499 ± 0.042	0.461 ± 0.034					0.512 ± 0.036	0.474 ± 0.041				
Superior frontal gyrus			0.70	0.410	**8.24**	**0.008**			1.30	0.264	0.06	0.811
Left	2.132 ± 0.249	1.980 ± 0.228					1.909 ± 0.214	1.775 ± 0.245				
Right	1.937 ± 0.273	1.956 ± 0.233					1.795 ± 0.226	1.679 ± 0.239				
Middle frontal gyrus			0.35	0.560	1.72	0.201			0.05	0.825	<0.01	0.968
Left	1.762 ± 0.340	1.867 ± 0.265					1.836 ± 0.246	1.764 ± 0.299				
Right	1.753 ± 0.361	1.766 ± 0.279					1.777 ± 0.184	1.708 ± 0.276				
Inferior frontal gyrus			0.14	0.716	0.08	0.781			0.43	0.518	0.47	0.498
Left	0.941 ± 0.174	0.915 ± 0.126					0.856 ± 0.111	0.840 ± 0.126				
Right	0.881 ± 0.140	0.865 ± 0.113					0.826 ± 0.134	0.782 ± 0.115				
Ventral medial PFC			0.30	0.590	2.73	0.110			0.35	0.558	2.44	0.129
Left	0.402 ± 0.083	0.399 ± 0.074					0.384 ± 0.064	0.364 ± 0.060				
Right	0.376 ± 0.051	0.417 ± 0.057					0.349 ± 0.061	0.364 ± 0.063				
Orbitofrontal cortex			0.01	0.905	0.23	0.636			1.37	0.252	1.28	0.267
Left	1.032 ± 0.112	1.016 ± 0.113					1.033 ± 0.095	0.986 ± 0.113				
Right	1.051 ± 0.086	1.024 ± 0.091					1.048 ± 0.063	0.969 ± 0.116				
Straight gyrus			1.15	0.294	2.13	0.156			0.32	0.576	0.55	0.466
Left	0.212 ± 0.032	0.210 ± 0.024					0.196 ± 0.022	0.192 ± 0.027				
Right	0.228 ± 0.033	0.205 ± 0.025					0.209 ± 0.032	0.196 ± 0.027				

PFC, prefrontal cortex; values represent means ± S.D.s. Relative volumes were calculated as follows: 100 × absolute volume/intracranial volume. Absolute volumes for the medial temporal structures and frontal regions in a larger sample were published elsewhere [32].

[a] Df = 1, 25 for the effect of genotype, and 1, 27 for genotype-by-side interaction.

[b] Df = 1, 29 for the effect of genotype, and 1, 31 for genotype-by-side interaction.

that the present cross-sectional study did not examine progressive brain morphologic changes. The possibility also exists that brain morphology in schizophrenia is more liable to be influenced by non-genetic factors such as antipsychotic medication [11,27]. This medication issue will be further discussed below.

For the parahippocampal gyrus, which is another structure that has been implicated in the pathophysiology of schizophrenia [5,26], we found a significant relationship between the BDNF genotype and the volume of this region only in the right hemisphere in schizophrenia patients. Our findings may be partly consistent with a previous MRI study showing that schizophrenia patients and nonpsychotic relatives from families with multiply affected ill members had significantly smaller right parahippocampal gyrus than controls [28]. However, our results did not accord with the previous MRI study [12], which showed a genotypic effect of BDNF only on the left parahippocampal gyrus in schizophrenia. The timing and course of brain morphologic changes in schizophrenia are not well understood [23] and brain changes related to genetic vulnerability of the illness are not necessarily static over time [11]. Thus, further longitudinal studies are needed to clarify the contribution of genetic and non-genetic factors to the neurobiology of schizophrenia.

As discussed above, the role of the BDNF Val66Met polymorphism in the prefrontal and medial temporal lobe structures in schizophrenia remains unclear. Additionally, we investigated only a single polymorphism in this study, though schizophrenia is thought to have a multifactorial etiology in which multiple susceptibility genes interact with environmental insults. Nevertheless, the present findings support the possibility that BDNF affects brain morphology differently in schizophrenia patients and healthy subjects, implicating brain morphology as a potential endophenotype for future genetic studies in schizophrenia.

In this study, we did not find a significant association between the BDNF Val66Met polymorphism and hippocampal volume in schizophrenia patients or healthy controls. In contrast, several MRI studies reported a smaller hippocampal volume in Met carriers [3,24,33], supporting the notion that the BDNF expressed abundantly in the hippocampus plays an important role in human memory formation [17,18,25]. This discrepancy might be partly explained by ethnic differences. In a recent MRI study, Inoue et al. [14] showed a lack of association between the BDNF Val66Met genotype and manually measured volumes of the amygdala and hippocampus in 116 healthy Japanese individuals. Another MRI study in healthy Japanese found no genotypic effect of the BDNF Val66Met polymorphism on hippocampal volume either, but demonstrated an association between this polymorphism and the volume of the parahippocampal gyrus as in the present study [20]. Furthermore, there seem to be ethnic differences in brain morphology [43] and also in the frequency of the BDNF Val66Met polymorphism, with healthy Japanese individuals carrying Met significantly more often than healthy Caucasians [31,36]. Thus, both present and previous findings suggest possible interethnic differences in the variation of the BDNF gene as well as in its genotypic effects on brain morphology especially for the medial temporal lobe structures.

A few possible confounding factors in the present study need to be addressed. First, all the schizophrenia patients were on antipsychotic medication, which might directly affect brain morphology [27]. Although duration of antipsychotic exposure in our sample was relatively short and there was no difference in medication status between the genotype groups, pharmacotherapy, particularly with typical antipsychotics, may cause gray matter reduction in cortical regions even over short periods [11,27]. Thus, medication effects may have influenced our findings, with particular regard to comparisons between healthy subjects and schizophrenia patients. Secondly, the relatively small number of subjects limited our ability to generalize the findings of the study. Failure to replicate previous findings of genotypic effect of the BDNF on hippocampal volume may be a result of the limited sample size of this study. Additionally, the weak association between the BDNF genotypes and brain morphology did not allow for statistical correction for multiple comparisons, representing a further limitation of the study. Thus, the results of the present study should be confirmed by an additional study with a large number of subjects without sustained antipsychotic treatment.

In conclusion, our preliminary findings suggest that a variation of the BDNF Val66Met polymorphism may affect brain morphology differently in schizophrenia patients and healthy controls in the left superior frontal and parahippocampal gyri. Although we did not observe a genotypic effect of this polymorphism on brain morphology specific to schizophrenia, our findings imply the possible role of brain morphology as an endophenotype for future genetic studies in schizophrenia.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research (No. 18591277, 19591346, 19390304, 18053011, and 1702501) from the Japanese Society for the Promotion of Science, and a Research Grant (17-2, 19230101) for Nervous and Mental Disorders from the Ministry of Health and Welfare, Japan. The authors are grateful to Prof. Hideki Origasa for suggestions on statistical analyses.

References

[1] I. Agartz, G.C. Sedvall, L. Terenius, B. Kulle, A. Frigessi, H. Hall, E.G. Jonsson, BDNF gene variants and brain morphology in schizophrenia, Am. J. Med. Genet. B: Neuropsychiatr. Genet. 141 (2006) 513–523.
[2] N.C. Andreasen, Scale for the Assessment of Negative Symptoms/Scale for the Assessment of Positive Symptoms [manual], University of Iowa Press, Iowa City, 1984.
[3] J.A. Bueller, M. Aftab, S. Sen, D. Gomez-Hassan, M. Burmeister, J.K. Zubieta, BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects, Biol. Psychiatr. 59 (2006) 812–815.
[4] M.F. Egan, M. Kojima, J.H. Callicott, T.E. Goldberg, B.S. Kolachana, A. Bertolino, E. Zaitsev, B. Gold, D. Goldman, M. Dean, B. Lu, D.R. Weinberger, The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function, Cell 112 (2003) 257–269.
[5] K.J. Friston, P.F. Liddle, C.D. Frith, S.R. Hirsch, R.S. Frackowiak, The left medial temporal region and schizophrenia: a PET study, Brain 115 (1992) 367–382.
[6] P.S. Goldman-Rakic, L.D. Selemon, Functional and anatomical aspects of prefrontal pathology in schizophrenia, Schizophr. Bull. 23 (1997) 437–458.

[7] I.I. Gottesman, T.D. Gould, The endophenotype concept in psychiatry: etymology and strategic intentions, Am. J. Psychiatr. 160 (2003) 636–645.
[8] O. Guillin, C. Demily, F. Thibaut, Brain-derived neurotrophic factor in schizophrenia and its relation with dopamine, Int. Rev. Neurobiol. 78 (2007) 377–395.
[9] A.R. Hariri, T.E. Goldberg, V.S. Mattay, B.S. Kolachana, J.H. Callicott, M.F. Egan, D.R. Weinberger, Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance, J. Neurosci. 23 (2003) 6690–6694.
[10] T. Hashimoto, S.E. Bergen, Q.L. Nguyen, B. Xu, L.M. Monteggia, J.N. Pierri, Z. Sun, A.R. Sampson, D.A. Lewis, Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia, J. Neurosci. 25 (2005) 372–383.
[11] B.C. Ho, N.C. Andreasen, J.D. Dawson, T.H. Wassink, Association between brain-derived neurotrophic factor Val66Met gene polymorphism and progressive brain volume changes in schizophrenia, Am. J. Psychiatry 164 (2007) 1890–1899.
[12] B.C. Ho, P. Milev, D.S. O'Leary, A. Librant, N.C. Andreasen, T.H. Wassink, Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers, Arch. Gen. Psychiatr. 63 (2006) 731–740.
[13] C.J. Hong, Y.W. Yu, C.H. Lin, S.J. Tsai, An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients, Neurosci. Lett. 349 (2003) 206–208.
[14] H. Inoue, H. Yamasue, M. Tochigi, M. Suga, T. Minato, O. Abe, H. Yamada, S. Aoki, N. Kato, K. Kasai, T. Sasaki, Lack of association between brain-derived neurotorophic factor val66met polymorphism and volume of amygdala and hippocampus in a Japanese population, Ann. Rep. Pharmacopsychiatry Res. 38 (2006) 99–104 [in Japanese].
[15] E.G. Jonsson, B. Edman-Ahlbom, A. Sillen, A. Gunnar, B. Kulle, A. Frigessi, M. Vares, B. Ekholm, B. Wode-Helgodt, J. Schumacher, S. Cichon, I. Agartz, G.C. Sedvall, H. Hall, L. Terenius, Brain-derived neurotrophic factor gene (BDNF) variants and schizophrenia: an association study, Prog. Neuropsychopharmacol. Biol. Psychiatry 30 (2006) 924–933.
[16] M.O. Krebs, O. Guillin, M.C. Bourdell, J.C. Schwartz, J.P. Olie, M.F. Poirier, P. Sokoloff, Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia, Mol. Psychiatry 5 (2000) 558–562.
[17] B. Lu, BDNF and activity-dependent synaptic modulation, Learn. Mem. 10 (2003) 86–98.
[18] B. Lu, A. Chow, Neurotrophins and hippocampal synaptic transmission and plasticity, J. Neurosci. Res. 58 (1999) 76–87.
[19] Y. Naoe, T. Shinkai, H. Hori, Y. Fukunaka, K. Utsunomiya, S. Sakata, C. Matsumoto, K. Shimizu, R. Hwang, O. Ohmori, J. Nakamura, No association between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and schizophrenia in Asian populations: Evidence from a case-control study and meta-analysis, Neurosci. Lett. 415 (2007) 108–112.
[20] K. Nemoto, T. Ohnishi, T. Mori, Y. Moriguchi, R. Hashimoto, T. Asada, H. Kunugi, The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology, Neurosci. Lett. 397 (2006) 25–29.
[21] L. Niu, M. Matsui, S.-Y. Zhou, H. Hagino, T. Takahashi, E. Yoneyama, Y. Kawasaki, M. Suzuki, H. Seto, T. Ono, M. Kurachi, Volume reduction of the amygdala in patients with schizophrenia: a magnetic resonance imaging study, Psychiatry Res. 132 (2004) 41–51.
[22] S. Numata, S. Ueno, I. Iga, K. Yamauchi, S. Hongwei, K. Ohta, S. Kinouchi, S. Shibuya-Tayoshi, S. Tayoshi, M. Aono, N. Kameoka, S. Sumitani, M. Tomotake, Y. Kaneda, T. Taniguchi, Y. Ishimoto, T. Ohmori, Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms, Neurosci. Lett. 401 (2006) 1–5.
[23] C. Pantelis, M. Yücel, S.J. Wood, D. Velakoulis, D. Sun, G. Berger, G.W. Stuart, A. Yung, L. Phillips, P.D. McGorry, Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia, Schizophr Bull. 31 (2005) 672–696.
[24] L. Pezawas, B.A. Verchinski, V.S. Mattay, J.H. Callicott, B.S. Kolachana, R.E. Straub, M.F. Egan, A. Meyer-Lindenberg, D.R. Weinberger, The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology, J Neurosci. 24 (2004) 10099–10102.
[25] M.M. Poo, Neurotrophins as synaptic modulators, Nat. Rev. Neurosci. 2 (2001) 24–32.
[26] G.W. Roberts, Schizophrenia: a neuropathological perspective, Br. J. Psychiatry 158 (1992) 8–17.
[27] H. Schrk, P. Falkai, Effects of antipsychotics on brain structure, Curr. Opin. Psychiatry 19 (2006) 145–150.
[28] L.J. Seidman, C. Pantelis, M.S. Keshavan, S.V. Faraone, J.M. Goldstein, N.J. Horton, N. Makris, P. Falkai, V.S. Caviness, M.T. Tsuang, A review and new report of medial temporal lobe dysfunction as a vulnerability indicator for schizophrenia: a magnetic resonance imaging morphometric family study of the parahippocampal gyrus, Schizophr. Bull. 29 (2003) 803–830.
[29] L.D. Selemon, J.E. Kleinman, M.M. Herman, P.S. Goldman-Rakic, Smaller frontal gray matter volume in postmortem schizophrenic brains, Am. J. Psychiatry 159 (2002) 1983–1991.
[30] M.E. Shenton, C.C. Dickey, M. Frumin, R.W. McCarley, A review of MRI findings in schizophrenia, Schizophr. Res. 49 (2001) 1–52.
[31] E. Shimizu, K. Hashimoto, M. Iyo, Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits, Am. J. Med. Genet. B: Neuropsychiatr. Genet. 126 (2004) 122–123.
[32] M. Suzuki, S.-Y. Zhou, T. Takahashi, H. Hagino, Y. Kawasaki, L. Niu, M. Matsui, H. Seto, M. Kurachi, Differential contributions of prefrontal and temporolimbic pathology to mechanisms of psychosis, Brain 128 (2005) 2109–2122.
[33] P.R. Szeszko, R. Lipsky, C. Mentschel, D. Robinson, H. Gunduz-Bruce, S. Sevy, M. Ashtari, B. Napolitano, R.M. Bilder, J.M. Kane, D. Goldman, A.K. Malhotra, Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation, Mol. Psychiatry 10 (2005) 631–636.
[34] T. Takahashi, Y. Kawasaki, K. Kurokawa, H. Hagino, S. Nohara, I. Yamashita, K. Nakamura, M. Murata, M. Matsui, M. Suzuki, H. Seto, M. Kurachi, Lack of normal structural asymmetry of the anterior cingulate gyrus in female patients with schizophrenia: a volumetric magnetic resonance imaging study, Schizophr. Res. 55 (2002) 69–81.
[35] T. Takahashi, M. Suzuki, S.-Y. Zhou, R. Tanino, H. Hagino, Y. Kawasaki, M. Matsui, H. Seto, M. Kurachi, Morphologic alterations of the parcellated superior temporal gyrus in schizophrenia spectrum, Schizophr. Res. 83 (2006) 131–143.
[36] M. Tochigi, T. Otowa, M. Suga, M. Rogers, T. Minato, H. Yamasue, K. Kasai, N. Kato, T. Sasaki, No evidence for an association between the BDNF Val66Met polymorphism and schizophrenia or personality traits, Schizophr. Res. 87 (2006) 45–47.
[37] S.J. Tsai, C.J. Hong, Y.W. Yu, T.J. Chen, Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females, Neuropsychobiology 49 (2004) 13–16.
[38] Z.R. Wang, D.F. Zhou, L.Y. Cao, Y.L. Tan, X.Y. Zhang, J. Li, L. Lu, G.Y. Wu, T.A. Kosten, T.R. Kosten, Brain-derived neurotrophic factor polymorphisms and smoking in schizophrenia, Schizophr. Res. 97 (2007) 299–301.
[39] M.J. Webster, C.S. Weickert, M.M. Herman, J.E. Kleinman, BDNF mRNA expression during postnatal development, maturation and aging of the human prefrontal cortex, Brain Res. Dev. Brain Res. 139 (2002) 139–150.
[40] C.S. Weickert, T.M. Hyde, B.K. Lipska, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia, Mol. Psychiatry 8 (2003) 592–610.
[41] World Health Organization, The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research, World Health Organization, Geneva, 1993.
[42] S.-Y. Zhou, M. Suzuki, H. Hagino, T. Takahashi, Y. Kawasaki, S. Nohara, I. Yamashita, H. Seto, M. Kurachi, Decreased volume and increased asymmetry of the anterior limb of the internal capsule in patients with schizophrenia, Biol. Psychiatry 54 (2003) 427–436.
[43] K. Zilles, R. Kawashima, A. Dabringhaus, H. Fukuda, T. Schormann, Hemispheric shape of European and Japanese brains: 3-D MRI analysis of intersubject variability, ethnical, and gender differences, Neuroimage 13 (2001) 262–271.

Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 1236–1242

Contents lists available at ScienceDirect

Progress in Neuro-sychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

The association of genotypic combination of the DRD3 and BDNF polymorphisms on the adhesio interthalamica and medial temporal lobe structures

Tsutomu Takahashi [a,d], Michio Suzuki [a,d,*], Masahiko Tsunoda [a], Yukiko Kawamura [f,g], Nagahide Takahashi [f,h], Nobuhisa Maeno [f,i], Yasuhiro Kawasaki [a,d], Shi-Yu Zhou [a,d,e], Hirofumi Hagino [a], Lisha Niu [a], Hiroshi Tsuneki [b], Soushi Kobayashi [b], Toshiyasu Sasaoka [b], Hikaru Seto [c], Masayoshi Kurachi [a,d], Norio Ozaki [f]

[a] Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[b] Department of Clinical Pharmacology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[c] Department of Radiology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[d] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan
[e] Department of Psychiatry and Medical Psychology, Dalian Medical University, Dalian, China
[f] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan
[g] Division of Clinical Science and Neuropsychopharmacology, Faculty of Pharmacy, Meijo University, Nagoya, Japan
[h] Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Mount Sinai School of Medicine, New York, USA
[i] Department of Brain Science and Molecular Imaging, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Obu, Japan

ARTICLE INFO

Article history:
Received 31 January 2008
Received in revised form 10 March 2008
Accepted 18 March 2008
Available online 25 March 2008

Keywords:
Adhesio interthalamica
Brain-derived neurotrophic factor
Dopamine D3 receptor
Gene–gene interaction
Magnetic resonance imaging
Schizophrenia

ABSTRACT

Abnormal neurodevelopment in midline structures such as the adhesio interthalamica (AI), as well as in the medial temporal lobe structures has been implicated in schizophrenia, while its genetic mechanism is unknown. This magnetic resonance imaging study investigated the effect of the genotypic combination of the dopamine D3 receptor (DRD3) Ser9Gly and brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms on the AI length and volumetric measures of the medial temporal lobe structures (amygdala, hippocampus, and parahippocampal gyrus) in 33 schizophrenia patients and 29 healthy controls. The subjects with a combination of the Ser/Ser genotype of DRD3 and Met-containing genotypes of BDNF (high-risk combination) had a shorter AI than those without it in the healthy controls, but not in the schizophrenia patients. The subjects carrying the high-risk combination had a smaller posterior hippocampus than those without it for both diagnostic groups. These genotypic combination effects on brain morphology were not explained by the independent effect of each polymorphism. These findings suggest the effect of gene–gene interaction between the DRD3 and BDNF variations on brain morphology in midline and medial temporal lobe structures, but do not support its specific role in the pathogenesis of schizophrenia.

1. Introduction

The adhesio interthalamica (AI), a narrow bridge of glial cells connecting the medial surfaces of the thalamus on each side, is variable in size among individuals and missing in about 20–30% of human brains (Kretschmann and Weinrich, 1992; Percheron, 2004). Although its role in humans is unclear, the AI develops during early gestation (O'Rahilly and Muller, 1990; Rosales et al., 1968) and is likely to be involved in dopaminergic regulation (Cheramy et al., 1984; Romo et al., 1984). While not consistently replicated (Ettinger et al., 2007; de Souza Crippa et al., 2006; Meisenzahl et al., 2000), the AI was found to be more often absent and shorter in schizophrenia compared with healthy subjects (Erbagci et al., 2002; Nopoulos et al., 2001; Snyder et al., 1998; Shimizu et al., in press; Takahashi et al., 2008). In addition, several magnetic resonance imaging (MRI) studies reported an association between the midline brain structures and the medial temporal morphology in schizophrenia (Kasai et al., 2004; Kwon et al., 1998; Takahashi et al., 2007, 2008). Thus, the AI findings in schizophrenia could be a marker of early developmental and dopaminergic abnormalities in the midline and associated medial temporal lobe structures. However, the genetic mechanism underlying this neurodevelopmental process is largely unknown.

The dopamine D3 receptor (DRD3) gene could be a strong candidate gene for schizophrenia-susceptibility considering the dopamine hypothesis of the illness (Meltzer and Stahl, 1976). Increased DRD3

Abbreviations: AI, adhesio interthalamica; ANOVA, Analysis of variance; ANCOVA, Analysis of covariance; BDNF, Brain-derived neurotrophic factor; CASH, Comprehensive Assessment of Symptoms and History; DRD3, Dopamine D3 receptor; ICC, Intraclass correlation coefficient; ICD-10, International Classification of Diseases, 10th edition; ICV, Intracranial volume; MRI, Magnetic resonance imaging; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SNP, single nucleotide polymorphism.

* Corresponding author. Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel.: +81 76 434 2281; fax: +81 76 434 5030.
E-mail address: suzukim@med.u-toyama.ac.jp (M. Suzuki).

0278-5846/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2008.03.014

expression in the brain of drug-free schizophrenia patients also implicates its role in the pathophysiology of the illness (Gurevich et al., 1997). The DRD3 Ser9Gly single nucleotide polymorphism (SNP), which causes higher dopamine binding affinity (Lundstrom and Turpin, 1996), has been shown to affect the response to antipsychotics (Scharfetter et al., 1999; Szekeres et al., 2004) or susceptibility to tardive dyskinesia (Bakker et al., 2006) in schizophrenia, although it is not consistently replicated (Liou et al., 2004; Malhotra et al., 1998). Several (Dubertret et al., 1998; Jonsson et al., 2003; Shaikh et al., 1996), but not all (Jonsson et al., 2004), meta-analyses have demonstrated the association between the Ser allele or Ser/Ser genotype and schizophrenia. Given the complex genetic background of schizophrenia (Sawa and Snyder, 2002), these inconsistencies might reflect interactions with other genes that regulate the expression of DRD3.

The genotypic interaction effect of DRD3 and brain-derived neurotrophic factor (BDNF) may be of interest in the search for the pathogenesis of schizophrenia because the BDNF supports the survival and differentiation of dopaminergic neurons by regulating DRD3 expression (Guillin et al., 2004, 2007). Although previous genetic studies testing associations between BDNF gene variants and schizophrenia have produced contradictory results (Jonsson et al., 2006), BDNF Val66Met SNP may weakly impact on clinical variables such as clinical symptoms or treatment response (Hong et al., 2003; Krebs et al., 2006; Numata et al., 2006) as well as on brain morphology (Ho et al., 2006; Szeszko et al., 2005) in schizophrenia. Interestingly, Gourion et al. (2005) demonstrated a significant interaction effect of the DRD3 Ser9Gly and BDNF Val66Met SNPs on the onset age of schizophrenia. To our knowledge, however, no studies in schizophrenia have examined the gene–gene interaction effect of these SNPs on quantitative neurobiological endophenotypes.

In summary, the current evidence suggests that 1) the genotypic combination of DRD3 and BDNF may play a role in the dopaminergic pathology of schizophrenia and that 2) the morphology of the AI and related medial temporal lobe structures may be a marker of early neurodevelopmental and dopaminergic deficits in schizophrenia. Based on these notions, we hypothesized that the interaction of DRD3 and BDNF would affect these brain structures specifically in schizophrenia. The current MRI study sought to test this hypothesis by examining the genotypic interaction effect of the DRD3 Ser9Gly and BDNF Val66Met SNPs on the length of the AI and volumetric measures of the medial temporal lobe structures (amygdala, hippocampus, and parahippocampal gyrus) in schizophrenia patients and healthy controls.

2. Methods

2.1. Subjects

Demographic and clinical data of the subjects in this study are shown in Table 1. This cohort was largely included in that in our previous MRI study, which investigated the morphology of the AI and medial temporal lobe structures in schizophrenia (Takahashi et al., 2008). All subjects were right-handed Japanese and physically healthy at the time of the study, and none had a lifetime history of serious head trauma, neurological illness, serious medical or surgical illness, or substance abuse. The two diagnostic groups were matched for age, gender, height, and parental education (Table 1).

Thirty-three schizophrenia patients who met the ICD-10 diagnostic criteria for research (World Health Organization, 1993) were recruited from the inpatient and outpatient clinics of the Department of Neuropsychiatry, Toyama University Hospital. Diagnoses were made following structured clinical interviews by psychiatrists with the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992). Clinical symptoms were rated at the time of scanning using the Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984). All patients were receiving neuroleptic medication at the time of scanning; 15 patients were treated with typical neuroleptics and 18 patients were receiving atypical neuroleptics. Patients were also receiving anticolinergic drugs ($N=28$), benzodiazepines ($N=25$), antidepressant ($N=1$), and/or carbamazepine ($N=2$).

Table 1
Clinical and genetic descriptions of healthy comparisons and patients with schizophrenia

Variable	Healthy comparisons	Schizophrenia patients	Group comparisons (one-way ANOVA or chi-square test)
	$N=29$	$N=33$	
Male/female	17/12	20/13	Chi-square=0.03, $p=0.874$
Age (years)	24.2±6.1	25.6±4.5	$F(1, 60)=1.01$, $p=0.319$
Height (cm)	166.0±7.0	165.5±7.8	$F(1, 60)=0.05$, $p=0.819$
Education (years)	15.4[a]±2.4	13.5±1.7	$F(1, 60)=13.45$, $p=0.001$
Parental education (years)	12.2±2.3	12.3±2.2	$F(1, 60)=0.10$, $p=0.759$
Age at onset (years)	–	22.2±4.4	–
Duration of illness (years)	–	3.5±3.6	–
Duration of medication (years)	–	2.7±2.8	–
Drug (mg/day, haloperidol equivalent)	–	12.2±8.6	–
Total SAPS score	–	26.1±24.7	–
Total SANS score	–	49.7±22.9	–
DRD3 genotypes (N; Ser homo/hetero/Gly homo)	12/16/1	15/15/2[b]	Chi-square=0.55, $p=0.759$
BDNF genotypes (N; Val homo/hetero/Met homo)	13/11/5	12/15/6	Chi-square=0.49, $p=0.783$

Values represent means±SDs. SANS, scale for the assessment of negative symptoms; SAPS, scale for the assessment of positive symptoms.
[a] $p<0.01$: compared to schizophrenia patients (Scheffé's test).
[b] DRD3 gene was not detected for one patient.

The control subjects consisted of 29 healthy volunteers recruited from members of the community, hospital staff, and university students. They were given a questionnaire consisting of 15 items concerning their personal (13 items; e.g., a history of obstetric complications, substantial head injury, seizures, neurological or psychiatric diseases, impaired thyroid function, hypertension, diabetes, and substance use) and family (2 items) histories of illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. The Minnesota Multiphasic Personality Inventory (New Japanese MMPI Committee, 1997) was administered to all the control candidates, and they were excluded if any T-score for the validity scales or the clinical scales exceeded 70. This study was approved by the ethical committees of Toyama University and Nagoya University. Written informed consent was obtained from all subjects.

2.2. Magnetic resonance imaging procedures

Magnetic resonance images were obtained by utilizing a 1.5-T Magnetom Vision (Siemens Medical System, Inc, Erlangen, Germany) with a three-dimensional gradient-echo sequence FLASH (fast low-angle shots) yielding 160–180 contiguous T1-weighted slices of 1.0-mm thickness in the sagittal plane. The number of slices acquired varied between subjects according to the head size of each case. The imaging parameters were: repetition time=24 ms; echo time=5 ms; flip angle=40°; field of view=256 mm; and matrix size=256×256 pixels. The voxel size was 1.0 mm^3. Image processing for volumetric analysis has been described in detail elsewhere (Takahashi et al., 2002). Briefly, on a Unix workstation (Silicon Graphics, Inc, Mountain View, CA, USA), the image data were processed using the software package Dr View 5.3

(Asahi Kasei Joho System Co, Ltd, Tokyo, Japan). Brain images were realigned in three dimensions to standardize for differences in head tilt during image acquisition and were then reconstructed into entire contiguous coronal images of 1-mm thickness, perpendicular to the anterior commissure–posterior commissure line. The whole cerebrum was manually separated from the brainstem and cerebellum. The signal-intensity histogram distributions from the T1-weighted images across the whole cerebrum were then used to semi-automatically segment the voxels into gray matter, white matter, and cerebrospinal fluid. The intracranial volume (ICV) was measured to correct for differences in head size as previously described (Zhou et al., 2003).

2.3. Assessment of regions of interest

To assess the length of the AI, the number of slices where an AI was clearly seen was counted on consecutive 1-mm coronal slices (Takahashi et al., 2008). Both intra- and inter (TT and KN)-rater intra-class correlation coefficients (ICCs) for the length of the AI in a subset of 30 randomly selected brains were over 0.97.

The medial temporal lobe structures (amygdala, hippocampus, and parahippocampal gyrus) were manually traced on consecutive 1-mm coronal slices with the corresponding sagittal and axial planes simultaneously presented for reference. Detailed delineation methods for these structures have been described elsewhere (Niu et al., 2004; Suzuki et al., 2005a,b). The volumes of gray and white matter in each of these structures including the parahippocampal gyrus were measured together. The inferior border of the amygdala in contact with the hippocampus head was determined by reference to the sagittal plane; the alveus was used to differentiate these structures. The hippocampus and the parahippocampal gyrus were subdivided into anterior and posterior parts at the level of the posterior edge of the mammillary body. Two trained raters (HH and LN) measured the volumes of the medial temporal lobe structures without knowledge of the subjects' identity, gender, or diagnosis. Inter- and intra-rater ICCs in five randomly selected brains were over 0.93.

2.4. DNA procedures

Genomic DNA was extracted from EDTA-containing venous blood samples according to standard procedures. The genotyping of the Val66Met SNP (rs6265) of the BDNF gene and the Ser9Gly SNP (rs6280) of the DRD3 gene were carried out using polymerase chain reaction-restriction fragment length polymorphism assays. Sequences of primer pairs and restriction enzymes were as follows; Fw: 5′-ACTCTGGAGAGCGTGAATGG-3′, Rv: 5′-CCGAACTTTCTGGTCCTCAT-3′, Nla III (rs6265); Fw: 5′-CTCTGCCCCACAGGTGTAGT-3′, Rv: 5′-CAAGCCCCAAAGAGTCTGAT-3′, Hae III (rs6280).

2.5. Statistical analysis

Statistical analysis was carried out using the software package STATISTICA for Macintosh (StatSoft, Tulsa, OK, USA). Genotype frequencies between schizophrenia patients and healthy comparisons were compared using chi-square test to test for Hardy–Weinberg equilibrium. Based on the previous observation (Gourion et al., 2005), the genotypic combination of the Ser/Ser genotype of the DRD3 gene and Met-containing genotypes of the BDNF gene was categorized as a "high-risk genotypic combination". The other combinations (i.e. Gly-containing genotypes of the DRD3 gene and/or Val/Val genotype of the BDNF gene) were categorized as "non-high-risk genotypic combinations".

In order to examine these genotype effects on AI, the absolute length of AI was analyzed by analysis of covariance (ANCOVA) with age, gender, and ICV as covariates, with diagnosis (schizophrenia patients versus controls) and genotypic combination (high-risk versus non-high-risk) as between-subject factors.

For the medial temporal lobe structures, the relative volumes [(absolute volume/ICV)× 100] for each ROI were analyzed using the repeated measures ANCOVA with age and gender as covariates, with diagnosis and genotypic combination as between-subject factors, and hemisphere (left versus right) as a within-subject variable. We used relative volumes for these ANCOVAs, but we considered main effects or interactions to be significant if the results did not change when the absolute volumes were used with age, gender, and ICV as covariates. The AI length and volumetric measures for all ROIs in this study were normally distributed (tested by the Kolmogorov–Smirnov test). The post hoc Scheffé's test was employed to follow up the significant main effects or interactions yielded by ANCOVAs. Statistical significance was defined as $p<0.05$.

3. Results

The observed genotype frequency of the SNPs was within the distribution as expected according to the Hardy–Weinberg equilibrium. No significant difference was observed in the genotypic distribution of the BDNF Val66Met SNP or the DRD3 Ser9Gly SNP between the healthy controls and schizophrenia patients (Table 1). The DRD3 gene was not detected for one schizophrenia patient, and this case was removed from subsequent analyses. As for the genotypic combination, 10/32 schizophrenia patients (31.3%) and 7/29 controls (24.1%) were categorized as having the high-risk combination, showing no significant group difference (chi-square=0.38, $p=0.536$).

For schizophrenia patients, no differences between the genotypic combination groups (high-risk versus non-high-risk) were observed for age, onset age, illness duration, total SANS or SAPS score, or medication status (dose, duration, and typical versus atypical).

Table 2 shows a comparison of the AI length and relative ROI volumes between the subjects with and without the high-risk genotypic combination. ANCOVA of the AI length showed a significant diagnosis-by-genotypic combination interaction, with subjects with the high-risk genotypic combination having a shorter AI than those without it for the healthy controls (post hoc test, $p=0.048$), but not for the schizophrenia patients (post hoc test, $p=0.981$) (Fig. 1).

For the medial temporal lobe structures, we found significant main effects of genotypic combination for the posterior hippocampus and posterior parahippocampal gyrus, showing that the subjects with high-risk genotypic combination had a smaller posterior hippocampus (post hoc test, $p=0.010$) and posterior parahippocampal gyrus (post hoc test, $p=0.013$) than those without it. When the absolute volumes were used for the statistical analyses, however, the genotypic combination effect was significant for the posterior hippocampus ($F=5.38$, $p=0.024$) but not for the posterior parahippocampal gyrus (Table 2). Thus, we considered only the effect on the posterior hippocampus to be significant.

We further tested whether these genotypic combination effects on the AI or posterior hippocampus could be explained by the independent effect of each SNP. No genotype effect was seen on these structures when the effect of DRD3 Ser9Gly SNP alone (Ser homozygotes versus Gly carriers) or BDNF Val66Met SNP alone (Met carriers versus Val homozygotes) was tested.

4. Discussion

To our knowledge, this is the first volumetric MRI study to report the gene–gene interaction effect of the DRD3 Ser9Gly and BDNF Val66Met SNPs on the brain morphology in schizophrenia patients and healthy controls. The subjects carrying the combination of the Ser/Ser genotype of DRD3 and Met-containing genotypes of BDNF had a shorter AI than those without it in the healthy controls, but not in the schizophrenia patients. On the other hand, the subjects with the same genotypic combination had a significantly smaller posterior hippocampus than those without it in both diagnostic groups. Of note, these

Table 2
Absolute AI length and relative volume for medial temporal lobe structures in schizophrenia patients and healthy comparisons with and without the high-risk genotypic combination[a]

Brain region	Healthy comparisons		Schizophrenia patients		Analysis of covariance[b]					
	Non-high-risk	High-risk	Non-high-risk	High-risk	Genotype		Diagnosis		Diagnosis × genotype	
	(N=22)	(N=7)	(N=22)	(N=10)	F	p	F	p	F	p
ICV (cm³)[c]	1469±123	1525±123	1498±159	1616±110	2.59	0.113	3.02	0.088	0.63	0.432
AI length (mm)[c]	10.9±2.9	8.4±4.0	6.5±3.3	6.0±3.6	0.45	0.504	2.08	0.155	5.33	0.025
Amygdala					0.04	0.846	14.09	<0.001	0.11	0.741
Left	0.076±0.010	0.076±0.008	0.067±0.006	0.064±0.014						
Right	0.077±0.011	0.082±0.008	0.070±0.007	0.073±0.012						
Anterior hippocampus					<0.01	0.963	<0.01	0.958	0.32	0.573
Left	0.058±0.017	0.054±0.018	0.051±0.021	0.049±0.014						
Right	0.061±0.022	0.060±0.016	0.062±0.022	0.070±0.028						
Posterior hippocampus					6.01	0.017	2.03	0.158	0.18	0.564
Left	0.151±0.022	0.143±0.015	0.147±0.021	0.137±0.020						
Right	0.159±0.024	0.143±0.015	0.150±0.026	0.127±0.023						
Anterior PHG					<0.01	0.957	8.72	0.012	0.75	0.390
Left	0.156±0.032	0.154±0.024	0.167±0.028	0.171±0.028						
Right	0.178±0.030	0.163±0.020	0.186±0.028	0.185±0.022						
Posterior PHG[d]					4.30	0.043	0.07	0.787	0.52	0.472
Left	0.326±0.031	0.294±0.025	0.316±0.035	0.308±0.046						
Right	0.309±0.030	0.287±0.044	0.307±0.031	0.293±0.036						

Values represent means±SDs.
AI, adhesio interthalamica; ICV, intracranial volume; PHG, parahippocampal gyrus.
[a] Relative volumes were calculated as follows: 100×absolute volume/intracranial volume. The genotypic combination of the Ser/Ser genotype of DRD3 gene and Met-containing genotypes of BDNF gene was categorized as "high-risk genotypic combination".
[b] $df=1, 54$ for the AI length and 1, 55 for the ICV and the volume of the medial temporal lobe structures.
[c] Age and gender were used as covariates.
[d] Main effect of genotype was not significant when the absolute volume was used with age, gender, and ICV as covariates ($F=2.65$, $p=0.109$).

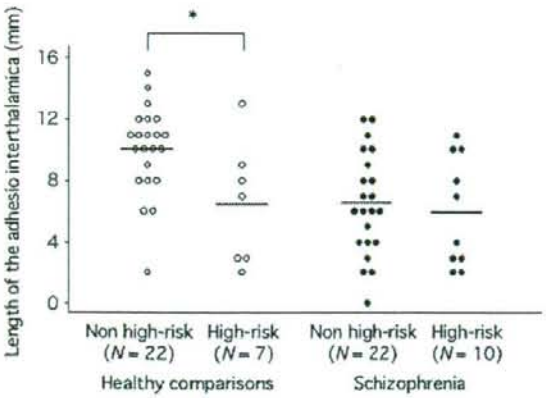

Fig. 1. Scatter plots of length of the adhesion interthalamica in healthy comparisons and schizophrenia patients. Horizontal lines indicate means. Post hoc comparisons: *$p<0.05$.

genotypic interaction effects were not explained by the independent effect of the DRD3 or BDNF. Thus, our findings suggest the effect of gene–gene interaction between the DRD3 and BDNF variations on brain morphology in midline and medial temporal lobe structures. Contrary to our prediction, however, we did not find a specific interaction effect of these genes on brain morphology in schizophrenia.

With regard to the association between these genotypic variations and schizophrenia or its clinical characteristics such as age at onset of psychosis, large-scale meta-analyses of the BDNF and DRD3 suggested each genotype effect was of small magnitude (e.g., Jonsson et al., 2003, 2006; Xu et al., 2007). Nevertheless, Gourion et al. (2005) reported a significant association between the combination of the Ser/Ser genotype of DRD3 and Met-containing genotypes of BDNF and earlier onset of schizophrenia. Although we did not find a significant genotypic interaction effect on the clinical variables in schizophrenia possibly due to the small sample size, these previous findings support the notion that schizophrenia is multifactorial in origin and cannot be easily explained by a single genetic component (Harrison and Weinberger, 2005; Sawa and Snyder, 2002).

The reported malformation of the AI in schizophrenia is likely to reflect early neurodevelopment and consequent dopaminergic abnormalities in the neural network including the thalamic region (Takahashi et al., 2008). The functional significance of the AI in human brain is obscure, but animal studies have shown that the AI is involved in regulating the release of dopamine in the basal ganglia (Cheramy et al., 1984; Romo et al., 1984). It is interesting to note that DRD3 mRNA is preferentially expressed in limbic-related and basal ganglia regions, as well as in the thalamus in the human brain (Suzuki et al., 1998). Low thalamic D2/D3 receptor binding in drug-naïve patients with schizophrenia demonstrated by positron emission tomography studies (Talvik et al., 2003; Buchsbaum et al., 2006) implied deficits in the thalamic dopaminergic system (Sanchez-Gonzalez et al., 2005; Garcia-Cabezas et al., 2007) in schizophrenia. Given the role of BDNF as a modulator of dopaminergic function during development by regulating DRD3 expression (Guillin et al., 2004, 2007), it can be hypothesized that the genotypic interaction effect of these genes is at least partly related to the thalamic dopaminergic pathology of schizophrenia, which could result in the differences in the prevalence or length of the AI in schizophrenia patients. In this study, however, we did not provide direct evidence for the genotypic combination effect of the DRD3 and BDNF SNPs on the AI in schizophrenia. A recent MRI study in monozygotic twins concordant or discordant for schizophrenia also failed to find genetic contribution to the AI abnormalities (Ettinger et al., 2007). Thus, the possible interaction effect of these genes on brain morphology, including the midline structures, should be further examined in schizophrenia.

Regarding the medial temporal lobe structures, our findings demonstrated that the combination of the Ser/Ser genotype of DRD3 and Met-containing genotypes of BDNF may contribute to the volume reduction of the hippocampus in both schizophrenia patients and healthy comparisons. This finding is largely in line with previous MRI studies that demonstrated the association between the Met allele of the BDNF gene and a reduced volume of the hippocampus in schizophrenia (Szeszko et al., 2005) or healthy subjects (Bueller et al., 2006; Pezawas et al., 2004; Szeszko et al., 2005), and further supports the role of BDNF as a regulating factor of DRD3. The medial temporal lobe has already decreased in volume by the onset of schizophrenia (Shenton et al., 2001; Vita et al., 2006) and several (Boos et al., 2007; Lawrie et al., 2001; Seidman et al., 2003) but not all (Goldman et al., 2008; Honea et al., 2008) MRI studies have demonstrated a similar reduction in subjects at genetic high-risk for developing schizophrenia. These findings suggest the heritability of the medial temporal lobe changes in schizophrenia, but these changes might represent only a weak intermediate phenotype for schizophrenia (Goldman et al., 2008; Honea et al., 2008). In fact, the previous MRI study (Szeszko et al., 2005) as well as the present study did not reveal statistically significant genotype-by-diagnosis interaction regarding the effect of the BDNF Val66Met SNP on the medial temporal morphology in schizophrenia and healthy controls. As for the DRD3 Ser9Gly SNP, to our knowledge, no volumetric MRI studies have investigated its genotype effect on the brain morphology in schizophrenia patients or in healthy subjects, but the Ser/Ser genotype was reported to contribute to the quality of eye tracking performance, another potential phenotypic marker of schizophrenia, in both schizophrenia patients and healthy subjects (Rybakowski et al., 2001). Taken together, the genetic effect of DRD3, BDNF, or a combination of these genes could contribute to the neurobiological phenotypic markers observed in schizophrenia such as the medial temporal abnormalities, but it seems likely that each effect on the pathogenesis of schizophrenia is weak and cannot explain all the differences in these phenotypic expressions between schizophrenia patients and healthy controls.

A few possible confounding factors in the present study need to be addressed. First, all schizophrenia patients in this study were on neuroleptic medication, which could have affected the brain morphology. Although there was no difference in medication status between the genotype groups (Ser/Ser versus Gly carrier for the DRD3 gene, Val/Val versus Met carrier for the BDNF gene, and high-risk versus non-high-risk genotypic combinations), the effects of medication may have biased our results, with particular regard to comparisons between healthy subjects and schizophrenia patients. In fact, our preliminary data showed a negative correlation between the AI length and daily medication dosage in a different sample of psychotic disorder patients (unpublished data). Secondly, this cross-sectional study did not examine progressive brain morphologic changes. The AI has been implicated in early neurodevelopment, but it also undergoes atrophy with age (Rosales et al., 1968), which we found to be accelerated in schizophrenia (unpublished data). Although the present study failed to find a specific interaction effect of the BDNF and DRD3 polymorphisms on brain morphology in schizophrenia, the possibility exists that these genetic variations contribute to brain changes over time in the illness (Ho et al., 2007). In addition to these potential confounding factors, this study was limited by the relatively small number of subjects carrying the combination of the Ser/Ser genotype of DRD3 and Met-containing genotypes of BDNF. Our unexpected finding of the interaction effect of these genes on the AI only in healthy comparisons may be partly related to the lower statistical power due to the small sample size. Thus, the present findings require replication in a larger sample without sustained neuroleptic medication in a longitudinal design. Furthermore, other genes for schizophrenia-susceptibility should be

included in future studies for gene–gene interaction effects on the pathogenesis of schizophrenia.

5. Conclusion

Our preliminary results demonstrated a significant genotypic interaction effect between the DRD3 Ser9Gly and BDNF Val66Met polymorphisms on the midline and associated medial temporal morphology. However, the present study did not find a specific genotypic effect on brain morphology in schizophrenia, implicating that the independent or interaction effects of these genetic variations are unlikely to play a major role in the pathogenesis of schizophrenia.

Acknowledgements

This study was supported in part by Grant-in-Aid for Scientific Research (No. 18591277, 19591346, 19390304, 18053011, 1702501) from the Japanese Society for the Promotion of Science, and a Research Grant (17-2, 19230101) for Nervous and Mental Disorders from the Ministry of Health and Welfare, Japan. The authors are grateful to Prof. Hideki Origasa, PhD, for helpful suggestions on statistical analyses.

References

Andreasen NC. Scale for the assessment of negative symptoms/scale for the assessment of positive symptoms [manual]. Iowa City: University of Iowa Press; 1984.
Andreasen NC, Flaum M, Arndt S. The Comprehensive Assessment of Symptoms and History (CASH): an instrument for assessing diagnosis and psychopathology. Arch Gen Psychiatry 1992;49:615–23.
Bakker PR, van Harten PN, van Os J. Antipsychotic-induced tardive dyskinesia and the Ser9Gly polymorphism in the DRD3 gene: a meta analysis. Schizophr Res 2006;83:185–92.
Boos HB, Aleman A, Cahn W, Pol HH, Kahn RS. Brain volumes in relatives of patients with schizophrenia: a meta-analysis. Arch Gen Psychiatry 2007;64:297–304.
Buchsbaum MS, Christian BT, Lehrer DS, Narayanan TK, Shi B, Mantil J, et al. D2/D3 dopamine receptor binding with [F-18]fallypride in thalamus and cortex of patients with schizophrenia. Schizophr Res 2006;85:232–44.
Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biol Psychiatry 2006;59:812–5.
Cheramy A, Romo R, Godeheu G, Glowinski J. Effects of electrical stimulation of various midline thalamic nuclei on the bilateral release of dopamine from dendrites and nerve terminals of neurons in the nigro-striatal dopaminergic pathways. Neurosci Lett 1984;44:193–8.
de Souza Crippa JA, Zuardi AW, Busarto GF, Sanches RF, Santos AC, Araújo D, et al. Cavum septum pellucidum and adhesio interthalamica in schizophrenia: an MRI study. Eur Psychiatry 2006;21:291–299.
Dubertret C, Gorwood P, Ades J, Feingold J, Schwartz JC, Sokoloff P. Meta-analysis of DRD3 gene and schizophrenia: ethnic heterogeneity and significant association in Caucasians. Am J Med Genet 1998;81:318–22.
Erbagci H, Yildirim H, Herken H, Gumusburun E. A magnetic resonance imaging study of the adhesio interthalamica in schizophrenia. Schizophr Res 2002;55:89–92.
Ettinger U, Picchioni M, Landau S, Matsumoto K, van Haren NE, Marshall N, et al. Magnetic resonance imaging of the thalamus and adhesio interthalamica in twins with schizophrenia. Arch Gen Psychiatry 2007;64:401–9.
Garcia-Cabezas MA, Rico B, Sanchez-Gonzalez MA, Cavada C. Distribution of the dopamine innervation in the macaque and human thalamus. NeuroImage 2007;34:965–84.
Goldman AL, Pezawas L, Mattay VS, Fischl B, Verchinski BA, Zoltick B, et al. Heritability of brain morphology related to schizophrenia: a large-scale automated magnetic resonance imaging segmentation study. Biol Psychiatry 2008;63:475–83.
Gourion D, Goldberger C, Leroy S, Bourdel MC, Olié JP, Krebs MO. Age at onset of schizophrenia: interaction between brain-derived neurotrophic factor and dopamine D3 receptor gene variants. NeuroReport 2005;16:1407–10.
Guillin O, Griffon N, Diaz J, Le Foll B, Bezard E, Gross C, et al. Brain-derived neurotrophic factor and the plasticity of the mesolimbic dopamine pathway. Int Rev Neurobiol 2004;59:425–44.
Guillin O, Demily C, Thibaut F. Brain-derived neurotrophic factor in schizophrenia and its relation with dopamine. Int Rev Neurobiol 2007;78:377–95.
Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN. Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. Arch Gen Psychiatry 1997;54:225–32.
Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 2005;10:40–68.
Ho BC, Milev P, O'Leary DS, Librant A, Andreasen NC, Wassink TH. Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. Arch Gen Psychiatry 2006;63:731–40.
Ho BC, Andreasen NC, Dawson JD, Wassink TH. Association between brain-derived neurotrophic factor Val66Met gene polymorphism and progressive brain volume changes in schizophrenia. Am J Psychiatry 2007;164:1890–9.
Honea RA, Meyer-Lindenberg A, Hobbs KB, Pezawas L, Mattay VS, Egan MF, et al. Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings. Biol Psychiatry 2008;63:465–74.
Hong CJ, Yu YW, Lin CH, Tsai SJ. An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. Neurosci Lett 2003;349:206–8.
Jonsson EG, Flyckt L, Burgert E, Crocq MA, Forslund K, Martila-Evenden M, et al. Dopamine D3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis. Psychiatr Genet 2003;13:1–12.
Jonsson EG, Kaiser R, Brockmoller J, Nimgaonkar VL, Crocq MA. Meta-analysis of the dopamine D3 receptor gene (DRD3) Ser9Gly variant and schizophrenia. Psychiatr Genet 2004;14:9–12.
Jonsson EG, Edman-Ahlbom B, Sillen A, Gunnar A, Kulle B, Frigessi A, et al. Brain-derived neurotrophic factor gene (BDNF) variants and schizophrenia: an association study. Prog Neuro-Psychopharmacol Biol Psychiatry 2006;30:924–33.
Kasai K, McCarley RW, Salisbury DF, Onitsuka T, Demeo S, Yurgelun-Todd D, et al. Cavum septi pellucidi in first-episode schizophrenia and first-episode affective psychosis: an MRI study. Schizophr Res 2004;71:65–76.
Krebs MO, Guillin O, Bourdell MC, Schwartz JC, Olie JP, Poirier MF, et al. Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. Mol Psychiatry 2000;5:558–62.
Kretschmann HJ, Weinrich W. Clinical neuroimaging and clinical neuroanatomy. 2nd ed. New York: Thieme Medical Publishers; 1992.
Kwon JS, Shenton ME, Hirayasu Y, Salisbury DF, Fischer IA, Dickey CC, et al. MRI study of cavum septi pellucidi in schizophrenia, affective disorder, and schizotypal personality disorder. Am J Psychiatry 1998;155:509–15.
Lawrie SM, Whalley HC, Abukmeil SS, Kestelman JN, Donnelly L, Miller P, et al. Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia. Biol Psychiatry 2001;49:811–23.
Liou YJ, Liao DL, Chen JY, Wang YC, Lin CC, Bai YM, et al. Association analysis of the dopamine D3 receptor gene ser9gly and brain-derived neurotrophic factor gene val66met polymorphisms with antipsychotic-induced persistent tardive dyskinesia and clinical expression in Chinese schizophrenic patients. Neuromolecular Med 2004;5:243–51.
Lundstrom K, Turpin MP. Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D3 receptor with the Semliki Forest virus system. Biochem Biophys Res Commun 1996;225:1068–72.
Malhotra AK, Goldman D, Buchanan RW, Rooney W, Clifton A, Kosmidis MH, et al. The dopamine D3 receptor (DRD3) Ser9Gly polymorphism and schizophrenia: a haplotype relative risk study and association with clozapine response. Mol Psychiatry 1998;3:72–5.
Meisenzahl EM, Frodl T, Zetzsche, T, Leinsinger G, Heiss D, Maag K, et al. Adhesio interthalamica in male patients with schizophrenia. Am J Psychiatry 2000;157:823–825.
Meltzer HY, Stahl SM. The dopamine hypothesis of schizophrenia: a review. Schizophr Bull 1976;2:19–76.
New Japanese MMPI Committee. Study of standardization for the new Japanese MMPI. Kyoto: San-Kyo-Bo; 1997 [in Japanese].
Niu L, Matsui M, Zhou SY, Hagino H, Takahashi T, Yoneyama E, et al. Volume reduction of the amygdala in patients with schizophrenia: a magnetic resonance imaging study. Psychiatry Res 2004;132:41–51.
Nopoulos PC, Rideout D, Crespo-Facorro B, Andreasen NC. Sex differences in the absence of massa intermedia in patients with schizophrenia versus healthy controls. Schizophr Res 2001;48:177–85.
Numata S, Ueno S, Iga J, Yamauchi K, Hongwei S, Ohta K, et al. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. Neurosci Lett 2006;401:1–5.
O'Rahilly R, Muller F. Ventricular system and choroid plexuses of the human brain during the embryonic period proper. Am J Anat 1990;189:285–302.
Percheron G. Thalamus. In: Paxinos G, Mai K, editors. The human nervous system. 2nd ed. California: Elsevier Academic Press; 2004. p. 592–675.
Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, et al. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 2004;24:10099–102.
Romo R, Cheramy A, Godeheu G, Glowinski J. Distinct commissural pathways are involved in the enhanced release of dopamine induced in the contralateral caudate nucleus and substantia nigra by unilateral application of GABA in the cat thalamic motor nuclei. Brain Res 1984;308:43–52.
Rosales RK, Lemay MJ, Yakovlev PI. The development and involution of massa intermedia with regard to age and sex. J Neuropathol Exp Neurol 1968;27:166.
Rybakowski JK, Borkowska A, Czerski PM, Hauser J. Dopamine D3 receptor (DRD3) gene polymorphism is associated with the intensity of eye movement disturbances in schizophrenic patients and healthy subjects. Mol Psychiatry 2001;6:718–24.
Sanchez-Gonzalez MA, Garcia-Cabezas MA, Rico B, Cavada C. The primate thalamus is a key target for brain dopamine. J Neurosci 2005;25:6076–83.
Sawa A, Snyder SH. Schizophrenia: diverse approaches to a complex disease. Science 2002;296:692–5.
Scharfetter J, Chaudhry HR, Hornik K, Fuchs K, Sieghart W, Kasper S, et al. Dopamine D3 receptor gene polymorphism and response to clozapine in schizophrenic Pakastani patients. Eur Neuropsychopharmacol 1999;10:17–20.
Seidman LJ, Pantelis C, Keshavan MS, Faraone SV, Goldstein JM, Horton NJ, et al. A review and new report of medial temporal lobe dysfunction as a vulnerability indicator for schizophrenia: a magnetic resonance imaging morphometric family study of the parahippocampal gyrus. Schizophr Bull 2003;29:803–30.
Shaikh S, Collier DA, Sham PC, Ball D, Aitchison K, Vallada H, et al. Allelic association between a Ser-9-Gly polymorphism in the dopamine D3 receptor gene and schizophrenia. Hum Genet 1996;97:714–9.

Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. Schizophr Res 2001;49:1–52.
Shimizu M, Fujiwara H, Hiraol K, Namiki C, Fukuyama H, Hayashi T, et al. Structural abnormalities of the adhesio interthalamica and mediodorsal nuclei of the thalamus in schizophrenia. Schizophr Res in press.
Snyder PJ, Bogerts B, Wu H, Bilder RM, Deoras KS, Lieberman JA. Absence of the adhesio interthalamica as a marker of early developmental neuropathology in schizophrenia: an MRI and postmortem histologic study. J Neuroimaging 1998;8:159–63.
Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedvall G. D3 dopamine receptor mRNA is widely expressed in the human brain. Brain Res 1998;779:58–74.
Suzuki M, Hagino H, Nohara S, Zhou SY, Kawasaki Y, Takahashi T, et al. Male-specific volume expansion of the human hippocampus during adolescence. Cereb Cortex 2005a;15:187–93.
Suzuki M, Zhou SY, Takahashi T, Hagino H, Kawasaki Y, Niu L, et al. Differential contributions of prefrontal and temporolimbic pathology to mechanisms of psychosis. Brain 2005b;128:2109–22.
Szekeres G, Kéri S, Juhász A, Rimanóczy A, Szendi I, Czimmer C, et al. Role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004;124:1–5.
Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S, et al. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry 2005;10:631–6.
Takahashi T, Kawasaki Y, Kurokawa K, Hagino H, Nohara S, Yamashita I, et al. Lack of normal structural asymmetry of the anterior cingulate gyrus in female patients with schizophrenia: a volumetric magnetic resonance imaging study. Schizophr Res 2002;55:69–81.
Takahashi T, Suzuki M, Hagino H, Niu L, Zhou SY, Nakamura K, et al. Prevalence of large cavum septi pellucidi and its relation to the medial temporal lobe structures in schizophrenia spectrum. Prog Neuro-Psychopharmacol Biol Psychiatry 2007;31:1235–41.
Takahashi T, Suzuki M, Nakamura K, Tanino R, Zhou S-Y, Hagino H, et al. Association between absence of the adhesio interthalamica and amygdala volume in schizophrenia. Psychiatry Res Neuroimaging 2008;162:101–11.
Talvik M, Nordström AL, Olsson H, Halldin C, Farde L. Decreased thalamic D2/D3 receptor binding in drug-naive patients with schizophrenia: a PET study with [11C] FLB 457. Int J Neuropsychopharmacol 2003;6:361–70.
Vita A, De Peri L, Silenzi C, Dieci M. Brain morphology in first-episode schizophrenia: a meta-analysis of quantitative magnetic resonance imaging studies. Schizophr Res 2006;82:75–88.
World Health Organization. The ICD-10 classification of mental and behavioural disorders: diagnostic criteria for research. Geneva: World Health Organization; 1993.
Xu MQ, St Clair D, Ott J, Feng GY, He L. Brain-derived neurotrophic factor gene C-270 T and Val66Met functional polymorphisms and risk of schizophrenia: a moderate-scale population-based study and meta-analysis. Schizophr Res 2007;91:6–13.
Zhou SY, Suzuki M, Hagino H, Takahashi T, Kawasaki Y, Nohara S, et al. Decreased volume and increased asymmetry of the anterior limb of the internal capsule in patients with schizophrenia. Biol Psychiatry 2003;54:427–36.

Available online at www.sciencedirect.com
ScienceDirect
Psychiatry Research: Neuroimaging 164 (2008) 90–94
PSYCHIATRY RESEARCH NEUROIMAGING
www.elsevier.com/locate/psychresns

Brief report

Prevalence and length of the adhesio interthalamica in schizophrenia spectrum disorders

Tsutomu Takahashi[a,c], Michio Suzuki[a,c,*], Shi-Yu Zhou[c,d], Kazue Nakamura[a], Ryoichiro Tanino[a], Yasuhiro Kawasaki[a,c], Marc L. Seal[e], Hikaru Seto[b], Masayoshi Kurachi[a,c]

[a] *Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[b] *Department of Radiology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[c] *Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan*
[d] *Department of Psychiatry and Medical Psychology, Dalian Medical University, Dalian, China*
[e] *Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne and Melbourne Health, Melbourne, Australia*

Received 13 September 2007; received in revised form 4 February 2008; accepted 5 March 2008

Abstract

We used magnetic resonance imaging to investigate the prevalence and length of the adhesio interthalamica (AI) in 72 schizophrenia patients, 47 schizotypal disorder patients, and 81 healthy controls. The AI was more often absent and shorter in both disorders than in controls, possibly reflecting common neurodevelopmental abnormalities in the schizophrenia spectrum.

Keywords: Schizotypal disorder; Magnetic resonance imaging; Thalamus

1. Introduction

Pathological deviations genetically and phenomenologically related to schizophrenia are grouped under the schizophrenia spectrum. This concept reflects the assumption that schizophrenia has a multifactorial etiology in which multiple susceptibility genes interact with environmental insults to yield a range of phenotypes (Siever and Davis, 2004). Subjects with schizophrenia spectrum disorders are thought to share neurodevelopmental abnormalities with schizophrenia as a common neurobiological basis for vulnerability factors (Siever and Davis, 2004).

The adhesio interthalamica (AI), a narrow bridge connecting the medial surfaces of the thalamus on each side, is variable in size among individuals and missing in about 20% of human brains (Carpenter and Sutin, 1983). While the finding has not been consistently replicated (e.g., Ettinger et al., 2007), the AI was reported to be more often absent in schizophrenia compared with healthy subjects (Snyder et al., 1998; Nopoulos et al., 2001; Takahashi et al., 2008), possibly reflecting its neurodevelopmental pathology. To our knowledge, however, it is unknown whether

* Corresponding author. Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel.: +81 76 434 2281; fax: +81 76 434 5030.
E-mail address: suzukim@med.u-toyama.ac.jp (M. Suzuki).

0925-4927/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pscychresns.2008.03.001

other subjects in the schizophrenia spectrum such as schizotypal (personality) disorder (SPD) share the AI abnormalities with schizophrenia.

This study used magnetic resonance imaging (MRI) to investigate the prevalence and length of the AI in schizophrenia patients, schizotypal disorder patients, and healthy controls.

2. Methods

2.1. Subjects

The cohort in this study is shown in Table 1. Among them, 62 schizophrenia patients and 63 controls were also included in our previous AI study (Takahashi et al., 2008). All subjects were right-handed and physically healthy, and none had a lifetime history of serious head trauma, neurological illness, serious medical or surgical illness, or substance abuse. They were screened by neuroradiologists for gross brain abnormalities, though subjects with a large cavum septi pellucidi were not excluded from this study.

Forty-seven schizotypal disorder patients were recruited among patients who visited our university hospital with schizotypal features accompanied by distress or associated problems in their lives; their sample characteristics have been described in detail previously (Takahashi et al., 2006). Based on data from the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992) and the Structured Clinical Interview for DSM-IV Axis II Disorders (First et al., 1997), subjects were diagnosed by the consensus of at least two psychiatrists; they all met the criteria for schizotypal disorder in ICD-10 as well as for SPD in DSM-IV. At the time of MRI scanning, 40 patients

Table 1
Demographic characteristics and adhesio interthalamica (AI) measures of healthy controls, patients with schizotypal disorder, and patients with schizophrenia

Variable	Healthy controls			Schizotypal disorder patients			Schizophrenia patients		
	All subjects	Male	Female	All subjects	Male	Female	All subjects	Male	Female
	(*N*=81)	(*N*=46)	(*N*=35)	(*N*=47)	(*N*=29)	(*N*=18)	(*N*=72)	(*N*=38)	(*N*=34)
Age (years)	24.5±5.7	25.0±5.8	23.8±5.7	25.0±5.4	25.2±5.8	24.6±4.9	26.2±5.6	25.6±4.7	27.0±6.5
Height (cm)	166.5±7.7	172.0±4.4[a]	159.3±4.5	165.9±8.7	171±5.6[a]	157.7±6.1	164.9±7.6	170.8±4.7[a]	158.3±4.0
Education (years)	16.0±2.5	16.9±2.8[b]	14.8±1.6[c]	13.1±2.0	13.3±1.9	12.8±2.2	13.5±1.9	13.5±1.8	13.4±1.9
Parental education (years)	12.9±2.3	13.1±2.4	12.5±2.3	12.3±1.7	12.4±1.6	12.2±1.9	12.0±2.1	12.2±1.9	11.9±2.4
Age at onset (years)	–	–	–	–	–	–	21.7±4.3	21.7±4.4	21.7±4.2
Duration of illness (years)	–	–	–	–	–	–	4.6±5.0	3.9±4.0	5.5±5.9
Duration of medication (years)	–	–	–	1.5±3.0	1.8±3.5	1.1±1.8	3.5±4.3[d]	2.9±3.2	4.3±5.1
Drug (mg/day, haloperidol equivalent)	–	–	–	4.8±5.7	5.0±5.5	4.5±6.3	11.5±9.4[d]	11.7±8.7	11.3±10.2
Total SAPS score	–	–	–	16.0±9.2	15.5±8.8	16.9±10.1	26.7±21.0[d]	24.9±22.2	28.7±19.6
Total SANS score	–	–	–	41.8±21.7	40.5±21.3	44.0±22.8	47.4±22.9	50.9±22.1	43.5±23.6
AI absent [e]; *N* (%)	7 (8.6)	6 (13.0)	1 (2.9)	11 (23.4)[f]	9 (31.0)	2 (11.1)	17 (23.6)[f]	14 (36.8)[g]	3 (8.8)
AI length (mm)	9.5±3.3[h]	8.4±3.5	10.8±2.6[i]	6.7±3.4	6.1±3.2	7.7±3.7[i]	6.6±3.3	5.3±3.2	8.1±2.8[i]
AI length [effect size (Cohen's *d*)]	–	–	–	0.84	0.69	0.97	0.88	0.92	1.00

Values represent means±S.D.s.
SANS, Scale for the Assessment of Negative Symptoms (Andreasen, 1984); SAPS, Scale for the Assessment of Positive Symptoms (Andreasen, 1984).
ANOVA followed by Scheffé's test was used except for the prevalence of the AI.
[a] $P<0.01$: compared with females.
[b] $P<0.01$: compared with male and female schizophrenia patients, male and female schizotypal patients, and female controls.
[c] $P<0.05$: compared with female schizotypal patients.
[d] $P<0.01$: compared with schizotypal patients.
[e] Chi-square test or Fisher's exact test was used.
[f] $P<0.01$: compared with controls.
[g] $P<0.01$: compared with female schizophrenia patients.
[h] $P<0.01$: compared with schizophrenia and schizotypal patients.
[i] $P<0.01$: compared with males.

were treated with low-dose antipsychotics, of which 14 were treated with typical neuroleptics and 26 received atypical neuroleptics. The remaining seven patients were neuroleptic-naïve.

The schizophrenia comparison group comprised 72 ICD-10 schizophrenia patients diagnosed by psychiatrists following structured clinical interviews with the CASH. Seventy-one patients were receiving neuroleptic medication; 38 were being treated with typical neuroleptics and 33 were receiving atypical neuroleptics.

The healthy comparisons consisted of 81 healthy volunteers recruited from members of the community ($N=29$), hospital staff ($N=20$), and university students ($N=32$). They were given a questionnaire consisting of 15 items concerning their family and past histories, as well as present illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. All control candidates were also screened using the Minnesota Multiphasic Personality Inventory. This study was approved by the regional ethics committee. Written informed consent was obtained from all subjects.

2.2. Magnetic resonance imaging procedures

MR scans were acquired with a 1.5-T Magnetom Vision (Siemens, Erlangen, Germany) with a 3D gradient-echo sequence FLASH yielding 160–180 contiguous 1-mm slices in the sagittal plane (TR=24 ms, TE=5 ms, Flip=40°, FOV=256 mm, Matrix=256×256, and Voxel size=1.0 mm^3). The image data were processed using the software package Dr. View (AJS, Tokyo, Japan) as described in detail elsewhere (Takahashi et al., 2006). Briefly, on a Unix workstation (Silicon Graphics, CA, USA), brain images were realigned in three dimensions and then reconstructed into entire contiguous coronal images, with a 1-mm thickness, perpendicular to the anterior commissure-posterior commissure line. The intracranial volume (ICV) was measured on 5-mm-thick sagittal slices as previously described (Zhou et al., 2003).

The number of slices where an AI was clearly seen was counted on reconstructed consecutive 1-mm coronal slices. Presence or absence of the AI was determined by viewing both coronal and axial 1-mm slices without a gap; when the AI could be identified on three or more slices in both views, we considered it as present (Takahashi et al., 2008). Intra- and inter-rater intraclass correlation coefficients for the AI length in a subset of 30 randomly selected brains (25 controls, 3 schizophrenia patients, and 2 schizotypal patients) were over 0.97. For the prevalence of the AI, intra- and inter-rater reliabilities were 100% (30/30 agreement, Kappa=1.00) and 97% (29/30 agreement, Kappa=0.79), respectively.

2.3. Statistical analysis

Chi-square tests, or Fisher's exact tests when expected cell sizes were less than five, were used for assessing the frequency of the AI. The AI length was analyzed using analysis of covariance (ANCOVA) with ICV and age as covariates, and with diagnosis and gender as between-subject factors. Post hoc Scheffé's test was employed. We used age as a covariate because there was a negative correlation between AI length and age for the controls (Spearman's $rho=-0.395$, $P<0.001$) and schizophrenia patients ($rho=-0.331$, $P=0.005$) but not for the schizotypal patients ($rho=-0.183$, $P=0.218$). The relationships between the length of the AI and clinical symptoms in schizophrenia and schizotypal disorder patients were examined by Pearson's partial correlation coefficients controlling for age, ICV, and duration and dosage of neuroleptic medication. Statistical significance was defined as $P<0.05$.

3. Results

The AI was absent in 8.6% (7/81) of the controls, 23.6% (17/72) of the schizophrenia patients, and 23.4% (11/47) of the schizotypal patients (Table 1), indicating a significant group difference between the controls and schizophrenia (chi-square=6.46, $P=0.011$) or schizotypal (chi-square=5.36, $P=0.021$) patients.

ANCOVA of the AI length revealed significant main effects for diagnosis ($F=17.82$, $df=2$, 192, $P<0.001$) and gender ($F=5.61$, $df=1$, 192, $P=0.019$); the schizophrenia ($P<0.001$) and schizotypal ($P<0.001$) patients had a shorter AI than controls, and males had a shorter AI than females ($P<0.001$). There was no gender-by-diagnosis interaction ($F=1.04$, $df=2$, 192, $P=0.357$).

The length of the AI was not correlated with total SANS and SAPS scores in the two patient groups.

4. Discussion

To our knowledge, this is the first MRI study to report the AI abnormalities in schizotypal subjects. We found that the AI was more often absent and shorter in both schizophrenia and schizotypal disorder patients as compared with control subjects, but there was no difference in its prevalence or length between these disorders. These findings suggest that the schizotypal subjects share AI abnormalities with patients with established schizophrenia.

Our finding of the AI malformation in the schizophrenia spectrum is thought to reflect neurodevelopmental

abnormalities because the AI develops during the early gestation period (Rosales et al., 1968). Although its functional significance in humans is unclear, animal studies have shown that the AI is involved in the regulation of the dopamine release of the basal ganglia (Romo et al., 1984). Low thalamic D2/D3 receptor binding (Buchsbaum et al., 2006), as well as thalamic volume reduction (Andreasen et al., 1994; Ettinger et al., 2001), in schizophrenia implicates deficits of the thalamic dopaminergic system (Sanchez-Gonzalez et al., 2005). Interestingly, first-degree relatives of schizophrenia patients (Seidman et al., 1999) or SPD subjects (Byne et al., 2001) share, at least partly, the thalamic volume reduction with schizophrenia patients. Together with these previous observations, our findings suggest that the AI abnormalities could be a marker of a disturbed neural network including the thalamic and related regions during neurodevelopment, which might be core components of the vulnerability to schizophrenia.

Regarding the gender effects, our results support a sexual dimorphism of the AI previously reported in healthy subjects (Allen and Gorski, 1991). Although there was no gender-by-diagnosis interaction in the AI length, our findings also support that absence of the AI in schizophrenia might be more evident in males (de Souza Crippa et al., 2006) because its absence was significantly more common in males than in females only for schizophrenia patients in this study (Table 1).

As discussed elsewhere (Takahashi et al., 2008), differences between this study and other studies in imaging techniques and criteria used to define the AI as absent/present (e.g., de Souza Crippa et al., 2006) limit our ability to generalize the findings of the present study. In addition, because neuroleptic medication might have affected our results and we could not measure the thalamic volume for technical reason, our findings require further replication.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research (No. 19591346) from the Japanese Society for the Promotion of Science, and a Research Grant (17-2, 18-6) for Nervous and Mental Disorders from the Ministry of Health and Welfare, Japan.

References

Allen, L.S., Gorski, R.A., 1991. Sexual dimorphism of the anterior commissure and massa intermedia of the human brain. Journal of Comparative Neurology 312, 97–104.

Andreasen, N.C., 1984. Scale for the Assessment of Negative Symptoms/ Scale for the Assessment of Positive Symptoms [Manual]. University of Iowa Press, Iowa City.

Andreasen, N.C., Flaum, M., Arndt, S., 1992. The Comprehensive Assessment of Symptoms and History (CASH): an instrument for assessing diagnosis and psychopathology. Archives of General Psychiatry 49, 615–623.

Andreasen, N.C., Arndt, S., Swayze II, V., Cizadlo, T., Flaum, M., O'Leary, D., Ehrhardt, J.C., Yuh, W.T., 1994. Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. Science 266, 294–298.

Buchsbaum, M.S., Christian, B.T., Lehrer, D.S., Narayanan, T.K., Shi, B., Mantil, J., Kemether, E., Oakes, T.R., Mukherjee, J., 2006. D2/D3 dopamine receptor binding with [F-18]fallypride in thalamus and cortex of patients with schizophrenia. Schizophrenia Research 85, 232–244.

Byne, W., Buchsbaum, M.S., Kemether, E., Hazlett, E.A., Shinwari, A., Mitropoulou, V., Siever, L.J., 2001. Magnetic resonance imaging of the thalamic mediodorsal nucleus and pulvinar in schizophrenia and schizotypal personality disorder. Archives of General Psychiatry 58, 133–140.

Carpenter, M.B., Sutin, J., 1983. Human Neuroanatomy, 8th ed. Williams & Wilkins, Baltimore, pp. 52–54.

de Souza Crippa, J.A., Zuardi, A.W., Busatto, G.F., Sanches, R.F., Santos, A.C., Araújo, D., Amaro, E., Hallak, J.E., Ng, V., McGuire, P.K., 2006. Cavum septum pellucidum and adhesio interthalamica in schizophrenia: an MRI study. European Psychiatry 21, 291–299.

Ettinger, U., Chitnis, X.A., Kumari, V., Fannon, D.G., Sumich, A.L., O'Ceallaigh, S., Doku, V.C., Sharma, T., 2001. Magnetic resonance imaging of the thalamus in first-episode psychosis. American Journal of Psychiatry 158, 116–118.

Ettinger, U., Picchioni, M., Landau, S., Matsumoto, K., van Haren, N.E., Marshall, N., Hall, M.H., Schulze, K., Toulopoulou, T., Davies, N., Ribchester, T., McGuire, P.K., Murray, R.M., 2007. Magnetic resonance imaging of the thalamus and adhesio interthalamica in twins with schizophrenia. Archives of General Psychiatry 64, 401–409.

First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B.W., Benjamin, L.S., 1997. Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II). American Psychiatric Publishing, Washington, DC.

Nopoulos, P.C., Rideout, D., Crespo-Facorro, B., Andreasen, N.C., 2001. Sex differences in the absence of massa intermedia in patients with schizophrenia versus healthy controls. Schizophrenia Research 48, 177–185.

Romo, R., Cheramy, A., Godeheu, G., Glowinski, J., 1984. Distinct commissural pathways are involved in the enhanced release of dopamine induced in the contralateral caudate nucleus and substantia nigra by unilateral application of GABA in the cat thalamic motor nuclei. Brain Research 308, 43–52.

Rosales, R.K., Lemay, M.J., Yakovlev, P.I., 1968. The development and involution of massa intermedia with regard to age and sex. Journal of Neuropathology and Experimental Neurology 27, 166.

Sanchez-Gonzalez, M.A., Garcia-Cabezas, M.A., Rico, B., Cavada, C., 2005. The primate thalamus is a key target for brain dopamine. Journal of Neuroscience 25, 6076–6083.

Seidman, L.J., Faraone, S.V., Goldstein, J.M., Goodman, J.M., Kremen, W.S., Toomey, R., Tourville, J., Kennedy, D., Makris, N., Caviness, V.S., Tsuang, M.T., 1999. Thalamic and amygdala-hippocampal volume reductions in first-degree relatives of patients with schizophrenia: an MRI-based morphometric analysis. Biological Psychiatry 46, 941–954.

Siever, L.J., Davis, K.L., 2004. The pathophysiology of schizophrenia disorders: perspective from the spectrum. American Journal of Psychiatry 161, 398–413.

Snyder, P.J., Bogerts, B., Wu, H., Bilder, R.M., Deoras, K.S., Liebermnan, J.A., 1998. Absence of the adhesio interthalamica as a marker of early developmental neuropathology in schizophrenia: an MRI and postmortem histologic study. Journal of Neuroimaging 8, 159–163.

Takahashi, T., Suzuki, M., Zhou, S.Y., Tanino, R., Hagino, H., Kawasaki, Y., Matsui, M., Seto, H., Kurachi, M., 2006. Morphologic alterations of the parcellated superior temporal gyrus in schizophrenia spectrum. Schizophrenia Research 83, 131–143.

Takahashi, T., Suzuki, M., Nakamura, K., Tanino, R., Zhou, S.Y., Hagino, H., Niu, L., Kawasaki, Y., Seto, H., Kurachi, M., 2008. Association between absence of the adhesio interthalamica and amygdala volume in schizophrenia. Psychiatry Research: Neuroimaging 162, 101–110.

Zhou, S.Y., Suzuki, M., Hagino, H., Takahashi, T., Kawasaki, Y., Nohara, S., Yamashita, I., Seto, H., Kurachi, M., 2003. Decreased volume and increased asymmetry of the anterior limb of the internal capsule in patients with schizophrenia. Biological Psychiatry 54, 427–436.

Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 1708–1714

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Adhesio interthalamica in individuals at high-risk for developing psychosis and patients with psychotic disorders

Tsutomu Takahashi [a,b,c,*], Murat Yücel [a,d], Alison R. Yung [d], Stephen J. Wood [a], Lisa J. Phillips [e], Gregor E. Berger [d,f], Anthony Ang [a], Bridget Soulsby [a], Patrick D. McGorry [d], Michio Suzuki [b,c], Dennis Velakoulis [a], Christos Pantelis [a]

[a] Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne and Melbourne Health, Victoria, Australia
[b] Department of Neuropsychiatry, University of Toyama, Toyama, Japan
[c] Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan
[d] ORYGEN Research Centre, Early Psychosis Prevention and Intervention Centre, Personal Assessment and Crisis Evaluation Clinic and the Department of Psychiatry, University of Melbourne, Victoria, Australia
[e] Department of Psychology, University of Melbourne, Victoria, Australia
[f] Department of Research and Education, The Schloessli Clinic, Oetwil am See, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 30 May 2008
Received in revised form 9 July 2008
Accepted 10 July 2008
Available online 15 July 2008

Keywords:
Adhesio interthalamica
High-risk
Lateral ventricles
Neurodevelopment
Schizophrenia

ABSTRACT

Abnormal neurodevelopment in midline structures such as the adhesio interthalamica (AI) has been reported in psychotic disorders, but it is unknown whether individuals at risk for the disorder share the AI findings observed in patients with florid psychosis. Magnetic resonance imaging of 162 patients with first-episode psychosis (FEP), 89 patients with chronic schizophrenia, 135 individuals at ultra high-risk (UHR) of psychosis (of whom 39 later developed psychosis), and 87 healthy controls were used to investigate the length and prevalence of the AI. The relation of the AI length to lateral ventricular enlargement was also explored. The patients with FEP and chronic schizophrenia as well as UHR individuals had a shorter AI than the controls, but there was no difference in the AI findings between the UHR individuals who did and did not subsequently develop psychosis. There was a negative correlation between the AI length and lateral ventricular volume in all the diagnostic groups. The absence of the AI was more common in the chronic schizophrenia patients when compared with all other groups. These results support the notion that the AI absence or shorter length could be a neurodevelopmental marker related to vulnerability to psychopathology, but also suggest that schizophrenia patients may manifest progressive brain changes related to ongoing atrophy of the AI after the onset.

1. Introduction

The adhesio interthalamica (AI), a narrow bridge of glial cells connecting the medial surfaces of the thalami, is variable in size among individuals and absent in about 20% of human brains (Kretschmann and Weinrich, 1992; Percheron, 2004). Although its role in humans is obscure, absence of the AI could represent early neurodevelopmental insult because the AI develops during early gestation (O'Rahilly and Müller, 1990; Rosales et al., 1968). The AI has been found to be smaller and more often absent in schizophrenia spectrum disorders compared with healthy subjects (Erbagci et al., 2002; Nopoulos et al., 2001; Shimizu et al., 2008; Snyder et al., 1998; Takahashi et al., 2008, in press), though the results were not consistently replicated (de Souza Crippa et al., 2006; Ettinger et al., 2007; Meisenzahl et al., 2000, 2002). In addition, several magnetic resonance imaging (MRI) studies indicated an association between the midline brain structures and ventricular enlargement in schizophrenia (Meisenzahl et al., 2002; Snyder et al., 1998; Takahashi et al., 2008). These observations suggest that the AI findings in psychotic disorders could be a marker of early developmental abnormalities in the midline and related brain regions.

These AI findings may not be fully explained by a neurodevelopmental anomaly, as the AI undergoes increasing atrophy especially after the third decade and even disappears in older individuals (Rosales et al., 1968). Our previous study found a negative correlation

Abbreviations: AI, adhesio interthalamica; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BPRS, Brief Psychiatric Rating Scale; CAARMS, Comprehensive Assessment of At Risk Mental States; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Revised Third Edition; EPPIC, Early Psychosis Prevention and Intervention Centre; FEP, first-episode psychosis; ICC, intraclass correlation coefficient; ICV, intracranial volume; MRI, magnetic resonance imaging; PACE, Personal Assessment and Crisis Evaluation; SANS, Scale for the Assessment of Negative Symptoms; SCID-P, Structured Clinical Interview for DSM-III-R Patient Edition; UHR, ultra high-risk; UHR-NP, ultra high-risk individuals who did not develop psychosis; UHR-P, ultra high-risk individuals who developed psychosis.

* Corresponding author. Melbourne Neuropsychiatry Centre, c/o National Neuroscience Facility, 161 Barry St, Carlton South, Victoria 3053, Australia. Tel.: +61 3 8344 1800; fax: +61 3 9348 0469.
E-mail address: tsutomu@med.u-toyama.ac.jp (T. Takahashi).

0278-5846/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2008.07.007

between the length of the AI and age for both schizophrenia patients and healthy controls (Takahashi et al., 2008). A direct comparison of the AI in large numbers of patients across different illness stages (i.e., prodromal subjects, first-episode and chronic schizophrenia) together with detailed examination of the association between the AI findings and age, illness duration, or neuroleptic medication is required to further elucidate neurodevelopmental and potential degenerative aspects of AI changes in psychotic disorders. Especially, brain changes in individuals at high-risk for developing psychosis, such as abnormalities in sulcus/gyral folding in the cingulate cortex (Yücel et al., 2003) or a higher prevalence of radiological findings (Borgwardt et al., 2006), could be related to pre-existing vulnerability to psychopathology as a consequence of early neurodevelopmental insult (Pantelis et al., 2005). To our knowledge, however, no MRI studies have examined the AI in clinical high-risk subjects of psychosis or examined whether patients with chronic schizophrenia exhibit more prominent AI changes compared with patients in the early stages of illness.

In this study, we used MRI to investigate the anterior–posterior length and prevalence of the AI and its relation to lateral ventricular volume in a large sample of patients with first-episode psychosis, chronic schizophrenia patients, and individuals at ultra high-risk for psychosis (UHR; Yung et al., 2003) compared with healthy comparison subjects. Based on previous studies, we predicted that patients with psychotic disorders, especially chronic schizophrenia, would have shorter AI or higher prevalence of its absence and that UHR subjects would exhibit similar AI changes to patients with established psychotic disorders.

2. Methods

2.1. Subjects

Inclusion criteria and demographic characteristics of 162 first-episode psychosis (FEP), 89 chronic schizophrenia, 135 ultra high-risk (UHR), and 87 control subjects, recruited from 1994 to 2001, have been described in detail elsewhere (Garner et al., 2005; Velakoulis et al., 1999, 2006). Briefly, the inpatients with FEP patients were recruited from the Early Psychosis Prevention and Intervention Centre (EPPIC; McGorry et al., 1996), were aged between 16 and 30 years, and were currently psychotic as reflected by the presence of at least one symptom (delusions, hallucinations, disorder of thinking or speech other than simple acceleration or retardation, or disorganized, bizarre, or markedly inappropriate behavior). Patients with chronic schizophrenia, who had more than 18 months of continuous illness, were recruited from the Adult Mental Health Rehabilitation services of the North Western Mental Health Program, Melbourne. The FEP and chronic schizophrenia patients received DSM-III-R diagnoses (American Psychiatric Association, 1994) based on medical record review and either the Royal Park Multidiagnostic Instrument for Psychosis (McGorry et al., 1989) or the Structured Clinical Interview for DSM-III-R, Patient Edition (SCID-P; Spitzer et al., 1990).

Based on these assessments administered during the initial treatment episode (median illness duration=27.0 days), the FEP patients were further divided into 4 subgroups: (1) schizophrenia (N=46), (2) schizophreniform psychosis (N=57), (3) affective psychosis (N=34), and (4) other psychoses (N=25) (Velakoulis et al., 2006). All FEP patients were neuroleptic-naïve prior to admission but 150 had received antipsychotic medication for a short period prior to scanning. Accurate values for duration of medication were not available, but mean duration of such a period in our centre is about 30 days (Velakoulis et al., 1999). The control subjects were recruited by approaching ancillary hospital staff and through advertisements. These controls were recruited from similar socio-demographic areas as the patients and did not have any personal or family history of psychiatric illness.

The UHR subjects were recruited from admissions to the Personal Assessment and Crisis Evaluation (PACE) Clinic. Criteria for identification of the UHR cohort and the rationale for these criteria have been fully described elsewhere (Yung et al., 2003, 2004a). The UHR subjects were assessed with the Brief Psychiatric Rating Scale (BPRS; Rhoades and Overall, 1988), the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1983), and the Comprehensive Assessment of At Risk Mental States (CAARMS; Yung et al., 2004b). All UHR subjects were aged 14 to 30 years and had not experienced a previous psychotic episode. After baseline scanning, they were monitored regularly for at least 12 months (maximum length of follow-up was 44 months) for the onset of full threshold psychosis based on operationalized criteria (Yung et al., 2003, 2004a) and were then divided into subgroups according to the outcome at 12 months; 39 UHR subjects (28.9%) developed psychosis (UHR-P) and 96 (71.1%) did not (UHR-NP). The predominant diagnosis in the psychosis group was schizophrenia spectrum (N=21), but there were also diagnoses of affective psychosis (N=13), and other psychoses (N=5). After the brain scan, 21 subjects started low-dose risperidone therapy and cognitive behavior therapy as part of a double-blind randomized study examining a 6-month therapeutic intervention to reduce the risk of progression to psychosis (McGorry et al., 2002). Most of the remaining UHR participants received case management and supportive therapy for at least six months.

All subjects were physically healthy, and none had a lifetime history of serious head trauma, neurological illness, serious medical or surgical illness, or DSM-III-R criteria of alcohol or substance abuse or dependence. The medial temporal lobe structures (Velakoulis et al., 2006) and lateral ventricular volume (Berger et al., 2007b; Pantelis et al., 2008) of the participants in this study have been examined previously. The regional ethics committee approved this study. After a complete description of the study, written informed consent was obtained from all subjects.

2.2. Magnetic resonance imaging procedures

MR scans were acquired with a 1.5-T GE Signa scanner (General Electric Medical Systems, Milwaukee, Wisconsin). A 3D volumetric spoiled gradient recalled echo in the steady state sequence generated 124 contiguous 1.5 mm coronal slices (TR=14.3 ms, TE=3.3 ms, Flip=30°, FOV=24×24 cm, Matrix=256×256, voxel dimension=0.9375×0.9375×1.5 mm). The intracranial volume (ICV) was measured to correct for differences in head size as previously described (Velakoulis et al., 2006); the five groups (healthy controls, UHR-NP, UHR-P, FEP, and chronic schizophrenia) did not significantly differ in their ICV volumes (Table 1).

2.3. Assessment of adhesio interthalamica (AI)

The image data were processed using the software package Dr. View (AJS, Tokyo, Japan). Brain images were realigned in three dimensions and reconstructed into contiguous coronal images, with a 0.9375-mm thickness, perpendicular to the AC–PC line. Then, the number of slices where an AI was clearly seen was counted on the reconstructed coronal slices. The length of the AI (mm) reported here was determined by the multiplication of the number of these slices by 0.9375. Presence or absence of the AI was determined by viewing both coronal and axial 0.9375-mm slices without gap; when the AI could be identified on three or more slices in both views, we considered it as present (Takahashi et al., 2008) (Fig. 1). Intra- and inter-rater intraclass correlation coefficients (ICCs) for the AI length in a subset of randomly selected 30 brains were over 0.97. For the presence of the AI, both intra- and inter-rater reliabilities were 100% (30/30 agreement).

2.4. Volumetric analysis of lateral ventricular volume

The volumes of lateral ventricles in both hemispheres were estimated as a whole using a semi-automated thresholding implemented by the MRIcro program (http://www.sph.sc.edu/comd/rorden/mricro.html). A seed was first placed at one spot within the