REFERENCES

1 Petrella JR, Coleman RE, Doraiswamy PM. Neuroimaging and early diagnosis of Alzheimer disease: a look to the future. *Radiology* 2003; **226**: 315–336.
2 Giannakopoulos P, Hof PR, Michel JP, Guimon J, Bouras C. Cerebral cortex pathology in aging and Alzheimer's disease: a quantitative survey of large hospital-based geriatric and psychiatric cohorts. *Brain Res* 1997; **25**: 217–245.
3 Morris R, Petrides M, Pandya DN. Architecture and connections of retrosplenial area 30 in the rhesus monkey (Macaca mulatta). *Eur J Neurosci* 1999; **11**: 2506–2518.
4 Shin J, Lee SY, Kim SJ, Kim SH, Cho SJ, Kim YB. Voxel-based analysis of Alzheimer's disease PET imaging using a triplet of radiotracers: PIB, FDDNP, and FDG. *Neuroimage* 2010; **52**: 488–496.
5 Chetelat G, Landeau B, Eustache F *et al*. Using voxel-based morphometry to map the structural changes associated with rapid conversion in MCI: a longitudinal MRI study. *NeuroImage* 2005; **27**: 934–946.
6 Whitwell JL, Shiung MM, Przybelski SA *et al*. MRI patterns of atrophy associated with progression to AD in amnestic mild cognitive impairment. *Neurology* 2008; **70**: 512–520.
7 Lajtha A. Controls of cerebral protein breakdown. *Adv Exp Med Biol* 1993; **341**: 107–117.
8 Shahbazian FM, Jacobs M, Lajtha A. Regional and cellular differences in rat brain protein synthesis in vivo and in slices during development. *Int J Dev Neurosci* 1986; **4**: 209–215.
9 Vorstrup S. Tomographic cerebral blood flow measurements in patients with ischemic cerebrovascular disease and evaluation of the vasodilatory capacity by the acetazolamide test. *Acta Neurol Scand Suppl* 1988; **114**: 1–48.
10 Kajimoto K, Oku N, Kimura Y *et al*. Crossed cerebellar diaschisis: a positron emission tomography study with L-[methyl-11C] methionine and 2-deoxy-2-18Ffluoro-D-Glucose. *Ann Nucl Med* 2007; **21**: 109–113.
11 Bustany P, Henry JF, Soussaline F, Comar D. Brain protein synthesis in normal and demented patients—a study by positron emission tomography with ^{11}C-L-Methionine. In: Magistretti PL, ed. *Functional Radionuclide Imaging of the Brain*. New York: Raven Press, 1983; 319–326.
12 Salmon E, Gregoire MC, Delfiore G *et al*. Combined study of cerebral glucose metabolism and [11C] methionine accumulation in probable Alzheimer's disease using positron emission tomography. *J Cereb Blood Flow Metab* 1996; **16**: 399–408.
13 Reiman EM, Chen K, Alexander GE *et al*. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. *Proc Natl Acad Sci USA* 2005; **102**: 8299–8302.
14 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
15 Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
16 Mohs RC, Rosen WG, Davis KL. The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. *Psychopharmacol Bull* 1983; **19**: 448–450.
17 Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry* 1982; **140**: 566–572.
18 Minoshima S, Koeppe RA, Frey KA, Kuhl DE. Anatomic standardization: linear scaling and nonlinear warping of functional brain images. *J Nucl Med* 1994; **35**: 1528–1537.
19 Minoshima S, Frey KA, Koeppe RA, Foster NL, Kuhl DE. A diagnostic approach in Alzheimer's disease using three-dimensional stereotactic surface projections of fluorine-18-FDG PET. *J Nucl Med* 1995; **36**: 1238–1248.
20 Ishii K, Willoch F, Minoshima S *et al*. Statistical brain mapping of 18F-FDG PET in Alzheimer's disease: validation of anatomic standardization for atrophied brains. *J Nucl Med* 2001; **42**: 548–557.
21 Messa C, Perani D, Lucignani G *et al*. High-resolution technetium-99m-HMPAO SPECT in patients with probable Alzheimer's disease: comparison with fluorine-18-FDG PET. *J Nucl Med* 1994; **35**: 210–216.
22 Matsuda H. Cerebral blood flow and metabolic abnormalities in Alzheimer's disease. *Ann Nucl Med* 2001; **15**: 85–92.
23 Coope DJ, Cizek J, Eggers C, Vollmar S, Heiss WD, Herholz K. Evaluation of primary brain tumors using 11C-methionine PET with reference to a normal methionine uptake map. *J Nucl Med* 2007; **48**: 1971–1980.
24 Gomez-Isla T, Price JL, McKeel DW, Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci* 1996; **16**: 4491–4500.
25 Gold G, Bouras C, Kovari E *et al*. Clinical validity of Braak neuropathological staging in the oldest-old. *Acta Neuropathologica* 2000; **99**: 579–582; discussion 583–584.
26 Chételat G, Villemagne VL, Bourgeat P *et al*. Relationship between atrophy and beta-amyloid deposition in Alzheimer disease. *Ann Neurol* 2010; **67**: 317–324.
27 Rose SE, Janke AL, Chalk JB. Gray and white matter changes in Alzheimer's disease: a diffusion tensor imaging study. *J Magn Reson Imaging* 2008; **27**: 20–26.
28 Vogt BA, Vogt LJ, Vrana KE *et al*. Multivariate analysis of laminar patterns of neurodegeneration in posterior cingulate cortex in Alzheimer's disease. *Exp Neurol* 1998; **153**: 8–22.

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Global cerebral hypoperfusion in preclinical stage of idiopathic normal pressure hydrocephalus ☆

Masahiko Takaya [a], Hiroaki Kazui [a,*], Hiromasa Tokunaga [a,b], Tetsuhiko Yoshida [a,c], Yumiko Kito [a,d], Tamiki Wada [a], Keiko Nomura [a], Eku Shimosegawa [e], Jun Hatazawa [e], Masatoshi Takeda [a]

[a] Department of Psychiatry, Osaka University Graduate School of Medicine, Japan
[b] Tokunaga clinic, Japan
[c] Department of Psychiatry, National Hospital Organization Osaka National Hospital, Japan
[d] Department of Psychiatry, Nissay Hospital, Japan
[e] Department of Nuclear Medicine and Tracer Kinetics, Osaka University Graduate School of Medicine, Japan

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ABSTRACT

In patients with idiopathic normal pressure hydrocephalus (iNPH), ventriculomegaly and narrowed subarachnoid spaces at the high convexity appear in magnetic resonance (MR) images before the occurrence of objective symptoms. In addition, quantitative regional cerebral blood flow (rCBF) has been reported to be reduced in iNPH patients with objective symptoms. To determine whether reduced rCBF is responsible for the appearance of symptoms, we compared rCBF in patients with suspected iNPH with no objective triad symptoms (NOS), iNPH patients with apparent objective triad symptoms (AOS) and normal control subjects (NC). Regional CBF was quantified in 35 Regions-of-interest (ROIs) by 123I-IMP single photon emission computed tomography (SPECT) using the autoradiography (ARG) method. Multiple comparisons showed that, in all brain regions examined except for in the frontal white matter, rCBF in the NOS group was significantly lower than that in the NC group, but in all brain regions, not significantly different from that of the AOS group. These results suggest that factors other than rCBF in the resting state are responsible for the occurrence of objective symptoms of iNPH.

1. Introduction

Normal pressure hydrocephalus (NPH) is a treatable syndrome accompanied by a progressive triad of gait disturbance, cognitive impairment and urinary incontinence resulting from ventricular enlargement, and is associated with normal cerebrospinal fluid (CSF) pressure [1]. Idiopathic NPH most commonly occurs in the sixth to eighth decades of life without an identifiable causative antecedent disease. Both ventriculomegaly and a narrowed subarachnoid at the high convexity are observed in brain magnetic resonance (MR) images in about one percent of normal elderly subjects as well as in patients with iNPH [2]. Quantitative regional cerebral blood flow (rCBF) seemed to be reduced in iNPH patients [3], which raises the question whether quantitative rCBF is also reduced in patients with pre-clinical stage iNPH.

Kitagaki et al. observed narrowed subarachnoid spaces at the high convexity and enlarged sylvian fissures, as well as ventriculomegaly, in patients with iNPH [4]. In fact, the narrowed subarachnoid spaces at the high convexity on computed tomography images or MR images was used as an inclusion criterion for iNPH patients in some previous studies [5–7]. A recent epidemiological study in Japan reported a combination of ventriculomegaly and narrowed subarachnoid spaces at the high convexity on MR images in 12 (1.5%) of 790 community-dwelling elderly subjects [2]. Eight (1.0%) of the subjects had none of the triad symptoms, leading the authors to diagnose the subjects as having "asymptomatic ventriculomegaly with features of idiopathic normal pressure hydrocephalus on MRI (AVIM)". Two (25%) of the eight subjects with AVIM developed cognitive impairment and/or gait disturbance during 4–8 years follow-up. Thus, AVIM may be a preclinical stage of iNPH and patients with AVIM might provide clues to the neuropathological mechanism of the triad symptoms of iNPH.

There is some evidence that the cerebral vasculature has a role in the occurrence of symptoms of NPH, especially in iNPH [8,9]. Several studies seemed to show an association between rCBF reduction and the appearance of clinical symptoms, although there were some inconsistencies in the data [3,7,10–19]. The inconsistencies can be attributed to a number of methodological problems: (1) relative rCBF

☆ The address of institution in which the work was carried out: Department of Psychiatry, and Department of Nuclear Medicine and Tracer Kinetics, Osaka University Graduate School of Medicine, D3, 2-2 Yamadaoka, Suita-city, Osaka 565-0871, Japan.

* Corresponding author. Department of Psychiatry, Osaka University Graduate School of Medicine, D3, 2-2 Yamadaoka, Suita-city, Osaka, 565-0871, Japan. Tel.: +81 6 6879 3051; fax: +81 6 6879 3059.
E-mail address: kazui@psy.med.osaka-u.ac.jp (H. Kazui).

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was analyzed in most of the studies [7,17,18], although quantitative rCBF could decrease over broad regions of the brain in patients with iNPH [3]. (2) In some of the studies, rCBF was analyzed by a voxel-wise comparison technique, such as statistical parametric mapping (SPM) [7,17] or three-dimensional stereotactic surface projections (3D-SSP) [18], although the brains of iNPH patients were too severely distorted to be mapped by the automated voxel-wise comparison technique [18]. (3) In studies using region of interest (ROI) analyses, the number of ROIs was limited [3]. (4) Some of the studies included not only patients with iNPH but also patients with secondary NPH [12,14,20]. (5) Some studies measured rCBF in iNPH patients that were experiencing small improvements of symptoms after shunt-operations [17].

Because hypoperfusion has been observed in several brain regions in iNPH patients with triad symptoms [11–13,21], we hypothesized that hypoperfusion would not be detected in the brains of suspected iNPH patients with no objective symptoms. To test this hypothesis, we recruited patients with iNPH-associated MR image features with and without objective triad symptoms. For controls, we used existing data for normal elderly subjects. We then compared the quantitative rCBF of the 16 brain regions among the three groups.

2. Methods

This study was approved by the Ethical Committee, Osaka University Graduate School of Medicine. Written informed consent was obtained from both subjects and their caregivers.

2.1. Subjects

For our study, we recruited patients with suspected iNPH from patients who visited the neuropsychological clinic in the Department of Neuropsychiatry of Osaka University Medical Hospital from 1 May 2007 to 31 December 2008. Inclusion criteria for the study were (1) age >60 years, (2) both ventricular dilatation (Evans index >0.3) and narrowed subarachnoid spaces at the high convexity without severe cortical atrophy, shown on MR images, (3) absence of diseases or conditions that could cause the clinical symptoms or radiological findings, (4) no history or evidence of conditions that might cause secondary NPH. During the above period, we recruited 14 patients, with a mean age of 73.1 ± 4.6 years (range, 64–80 years) and a mean educational attainment of 13.4 ± 2.9 years (range, 9–16 years).

The iNPH grading scale (iNPHGS) [22] is a clinician-rated scale to separately rate the severity of each of the triad symptoms of iNPH. The score of each domain ranges from 0 to 4. Zero indicates normal and one indicates having subjective symptoms without objective symptoms. Two to four indicate having apparent objective symptoms, in which higher scores indicate worse symptoms. In the gait domain, the condition of score 1 was complaint of dizziness of drift and dysbasia but no objective gait disturbance and that of score 2 was an unstable, but independent gait. In the cognitive domain, the condition of score 1 was a complaint of amnesia or inattention but no objective memory or attentional impairment and that of score 2 was amnesia or inattention, but no disorientation of time or place. In the urinary domain, the condition of score 1 was pollakiuria or urinary urgency and that of score 2 was occasional urinary incontinence (1–3 or more times per week but less than once per day).

We evaluated the triad symptoms of the 14 iNPH patients with iNPHGS and divided the patients into two groups according to the iNPHGS scores. One group consisted of seven patients whose iNPHGS scores for all triad domains were 0 or 1. We designated this group as suspected iNPH patients with no objective triad symptoms (NOS) (Fig. 1, Table 1). All seven patients had a score of 1 in at least one of the triad domains. They thus differ from AVIM individuals who were defined as showing no neurological symptoms or signs (although the scale by which symptoms were measured was not given) [2]. The NOS group consisted of six males and one female. Their mean age was 72.7 ± 5.7 years (range, 64–80 years), mean morbidity duration was 3.2 ± 2.4 years (range, 0.5–6 years) and mean educational attainment was 13.9 ± 2.5 years (range, 9–16 years).

The other group consisted of seven patients who had an iNPHGS score of 2 or more in at least in one of the triad domains and were called iNPH patients with apparent objective triad symptoms (AOS) (Table 1). The AOS group consisted of four males and three females. Their mean age was 73.6 ± 3.6 years (range, 68–79 years), mean morbidity duration was 2.9 ± 1.3 years (range, 1–5 years) and mean educational attainment of 12.9 ± 3.4 years (range, 9–16 years). The mean value of CSF opening pressure of the AOS group was 163.6 ± 25.4 mmH2O (range, 140–205), which met the criteria of normal pressure (70–245 mmH2O) [23]. The CSF of all of the AOS patients was clear and had no abnormalities. The symptoms of all of the AOS patients transiently improved after discharge of 30 ml of CSF [24]. Four of the patients subsequently underwent a lumbo-peritoneal shunt (LP-shunt) operation, and all of them improved as a result of it.

For single photon emission computed tomography (SPECT) data for normal subjects, we used existing SPECT data for 34 subjects. These 34 subjects were selected from the 147 normal controls for a previous study [25], according to the following criteria. They were aged 60 years or older and lived in their own homes or in homes for the independent elderly. The inclusion criteria of the normal elderly

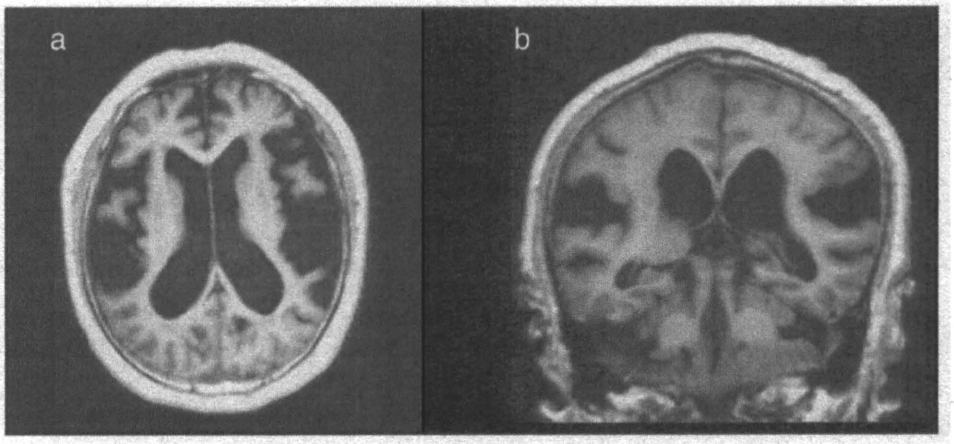

Fig. 1. Brain MR images of a NOS patient. (a) and (b) are MR images of an 80-year-old male patient with NOS (NOS 4). His MMSE score was 28/30. NOS: suspected iNPH patients with no objective symptoms. Lateral ventricle dilation (Evans index>0.3), narrowed subarachnoid spaces at the high convexity, and dilation of sylvian fissure and basal cistern are observed in the patient.

Table 1
Demographic data.

Subjects	Age	Sex	Chief complaint	Educational attainment	Mobidity duration
AOS1	75	F	Amnesia	10	2
AOS2	74	M	Gait disturbance	16	3
AOS3	68	F	Gait disturbance	9	1
AOS4	79	F	Gait disturbance	16	2
AOS5	71	M	Gait disturbance	16	4
AOS6	72	M	Gait disturbance	9	3
AOS7	76	M	Gait disturbance	14	5
NOS1	80	M	Amnestic complaint	13	5
NOS2	71	F	Amnestic complaint	14	6
NOS3	72	M	Amnestic complaint	9	3
NOS4	80	M	Feeling unstable walking	13	6
NOS5	72	M	Feeling unstable walking	16	1
NOS6	70	M	Feeling unstable walking	16	1
NOS7	64	M	Feeling unstable walking	16	0.5
NC1	73	M	NA	12	NA
NC2	79	M	NA	16	NA
NC3	70	M	NA	12	NA
NC4	79	M	NA	16	NA
NC5	68	F	NA	12	NA
NC6	71	F	NA	12	NA
NC7	78	F	NA	15	NA

M : male, F : female.
AOS : iNPH patients with apparent objective triad symptoms , NOS: suspected iNPH patients with no objective triad symptoms, NC : normal controls. The seven patients with AOS were numbered AOS1 to AOS7, the seven patients with NOS were numbered NOS1 to NOS7, and the seven normal controls were numbered NC1 to NC7.

control subjects in this study were having (1) the Mini-mental State Examination (MMSE) [26] scores of over 27, (2) ability to walk 1 km without assistance, (3) availability of reliable informants, (4) full scores on the Physical Self-Maintenance Scale (PSMS) and the Instrumental Activity of Daily Living Scale (IADL) [27] rated by their informants, (5) normal MR images, without ventriculomegaly or narrowed subarachnoid spaces at the high convexity, (6) normal Magnetic Resonance Angiography, and (7) willingness to undergo SPECT scan with the method as described in SPECT procedure. The exclusion criteria in this study were (1) existence of neurological or psychiatric disease in the past or in their current medical history, (2) head injury with unconsciousness for more than 1 h, and (3) undergoing active therapy for life-threatening cancers or poor condition due to a chronic disease. In the 34 normal elderly control subjects, 11 (seven males and four females) had undergone the same types of cognitive and gait tests that the NOS and AOS subjects had undergone. We selected four males and three females from these 11 subjects in the database that matched the NOS subjects in age (within 5 years). The normal controls (NC) had a mean age of 74.0 ± 4.6 years (range, 68–79 years) and a mean educational attainment of 13.6 ± 2.0 years (range, 12–16 years).

There was no significant difference among the three groups, with respect to age ($F(2,18) = 0.14$, $p = 0.87$, one-way ANOVA), sex ($p = 0.60$, Fisher's exact test) or educational attainment ($F(2,18) = 0.25$, $p = 0.78$, one-way ANOVA). There was also no significant difference between NOS and AOS in morbidity duration ($F(1,12) = 0.11$, $p = 0.74$, one-way ANOVA).

2.2. Evaluation of cognitive and gait functions

Cognitive examinations were administered to the iNPH patients, including MMSE [26], Frontal Assessment Battery (FAB) [28], selective attention test of Trail Making Test (TMT A) [29], Attention/Concentration (AC) subtest of the Wechsler Memory Scale-Revised (WMS-R) [30], and subtest of the picture recognition and subtest of the immediate and delayed recall of a short story of the Rivermead Behavioural Memory Test (RBMT) [31]. The MMSE is one of the most widely used screening instruments for dementia and provides a total score ranging from 0 to 30, with lower scores indicative of greater cognitive impairment [26]. The FAB is a simple tool for assessing frontal lobe symptoms [28]. TMT A is a neuropsychological test for evaluating psychomotor speed [29].

Gait disturbance was evaluated with the Timed Up & Go Test (TUG) [32], which has been used to evaluate walking ability [33,34] and gait disturbance in iNPH patients [22]. This test measures the total time it takes a subject to perform a series of movements, or, sitting in an armchair to stand up, walk forward 3 meters, and return to the seated position.

As for NC subjects, we gained the data of evaluations of cognitive and gait functions from the database for the previous study [25].

2.3. MR imaging procedure

MR imaging for patients with AOS and NOS, was performed on a 1.5-T system (Signa Excite HD 12.x, General Electric Medical Systems, Milwaukee). A three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin sagittal sections that cover the whole calvarium. The operating parameters were as follows: field of view = 240 mm, matrix = 256 × 256, 124 × 1.40 mm contiguous sections, TR = 12.55 ms, TE = 4.20 ms, and flip angle = 15°.

2.4. SPECT procedure

Regional CBF was quantitated by N-isopropyl-p-[123I] iodoamphetamine (123I-IMP) autoradiography (ARG) using a SPECT scanner (SPECT-2000H, Hitachi Medical Co., Tokyo, Japan). The scanner has a four-head rotating camera. The scanner, fitted with low-energy, medium-resolution collimators, has as intrinsic spatial resolution of 13-mm full width at half maximum (FWHM) in-plane and axially. Scanning was initiated 15 min after 1-min intravenous infusions of 167 MBq of [123I]IMP and physiological saline into a brachial artery, each delivered at a constant rate of 1.5 ml/min. Scan duration was 26.7 min (mid-scan time = 28.3 min). Two milliliters of arterial blood was taken from the opposite brachial artery at 10–14 min post-IMP administration. The radioactivity concentration of the blood was measured with a well counter cross-calibrated with SPECT. The partial pressures of O2 and CO2, and pH of the blood were measured with a blood gas tension analyzer. The projections of the SPECT scan were acquired by a 360° continuous rotation of the camera. The images were reconstructed using filtered back-projection with a Butterworth filter (Cut-off = 0.20, Nyquist; order = 10), in which attenuation was corrected numerically with an attenuation coefficient of 0.08 cm^{-1} [35].

Quantitative rCBF values were calculated based on the 2-compartment model analysis of IMP, with the assumption that the distribution volume (Vd) was 40.0 ml/ml. Regional CBF maps were calculated pixel by pixel (64 × 64 matrix size) from the SPECT data and the standard input function was calibrated by one-point arterial blood sampling.

2.5. Regions of interest (ROIs) analysis

We used the ROI method to evaluate rCBF in each subject and compared the rCBF among AOS, NOS, and NC subjects. Thirty-five ROIs were used in this study. Fig. 2 shows the location of the ROIs on brain template images. A monitor screen displayed each patient's MR image together with the corresponding brain slice templates. One investigator (M.T.), who specializes in neuroimaging, and who was blind to the subject's clinical information, manually placed the ROIs, each a circle 11 mm diameter on MR images of each subject, while referring to the brain template images. Then, he coregistered each MR image and the corresponding SPECT image using Neurological Statistical Image Analysis Software (NEUROSTAT) [36], and quantified the rCBF

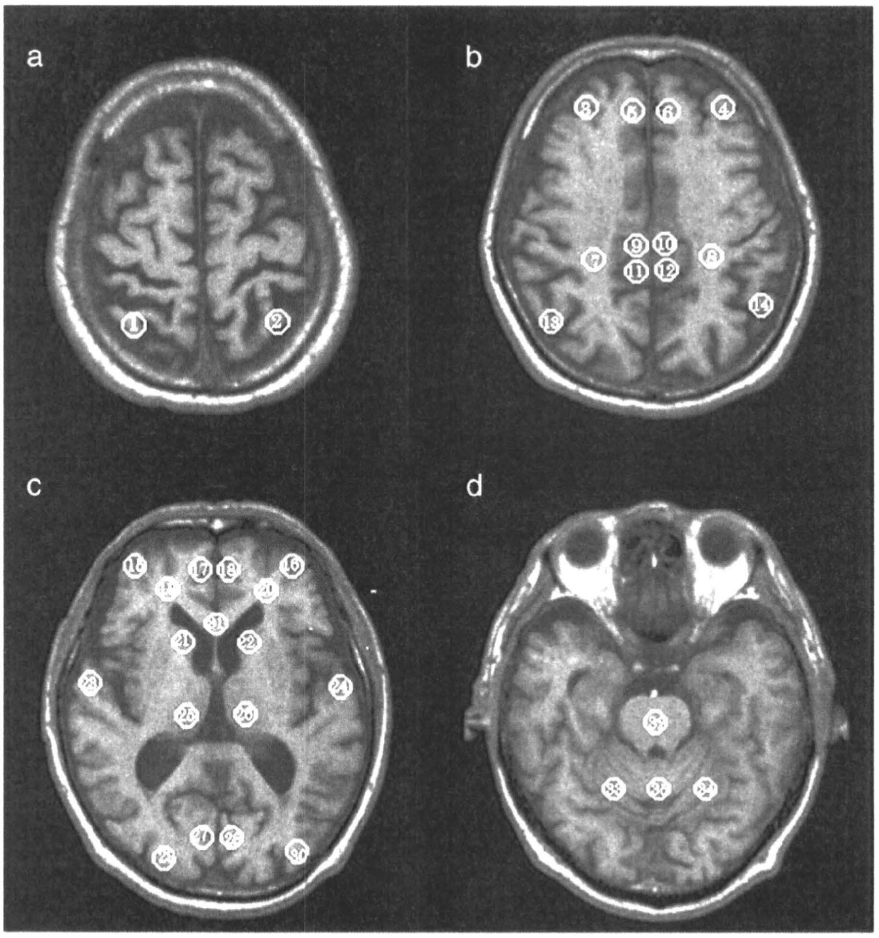

Fig. 2. Brain templates displaying ROIs on axial MR images of a normal brain Thirty-five regions of interest (ROIs), numbered from 1 to 35, are located in these four slices of MR images parallel to the AC-PC line. The 35 ROIs were lumped into 16 regions (Table 3) and quantitative rCBF was averaged for each region. (a) superior parietal lobe level ; (b) posterior cingulate gyrus and precuneus level ; (c) thalamus level ; (d) pons level.

of the 35 ROIs of each subject. ROI location and quantitative rCBF measurement were performed twice (test and retest) in order to determine the test–retest reliability, although only the data obtained in the first test were used in the comparisons of the three groups. We classified the 35 ROIs into 16 regions (listed in Table 3).

2.6. Statistical analysis

Demographic, clinical and rCBF data were analyzed with JMP8 (SAS Institute Inc, Cary, NC, USA). The NOS, AOS and NC groups were compared with one-way analysis of variance (ANOVA). A post-hoc Turkey-Kramer HSD test was used when appropriate. The level of statistical significance was set at $p<0.05$. Test–retest reliability was assessed by the intraclass correlation coefficient (ICC) [37].

3. Results

3.1. Cognitive and gait assessment

One-way ANOVA revealed significant differences among the three groups in all the cognitive tests (Table 2). Post-hoc tests showed that the scores of AOS were significantly worse than those of NC in all the cognitive tests. Although the mean scores of the NOS group were between those of the NC and AOS groups in all the cognitive tests, there were no significant differences between NOS and NC. The differences between NOC and AOS were significant in MMSE, FAB, the Attention/Concentration subtest of the WMS-R, and the immediate recall of a short story subtest of the RBMT, but not in TMT-A, or the subtests of picture recognition and delayed recall of a short story of the RBMT.

As for gait evaluation, the TUG scores of the NC and NOS groups were significantly lower (i.e. better) than those of the AOS group.

3.2. Comparison of quantitative rCBF

The ICCs, which assessed the test–retest reliability, was 0.96 for all 35 ROIs and ranged from 0.79 to 0.99 in 16 brain regions (Table 3), indicating high reliability of the ROI method used in this study.

One-way ANOVA showed significant differences in the quantitative rCBF in all regions among the three groups (Table 3). Post-hoc Tukey–Kramer HSD tests revealed that the quantitative rCBF of the NOS group was significantly less than that of the NC group in all regions except for in the frontal white matter, and that the quantitative rCBF of the AOS group was significantly less than that of the NC group in all regions (Table 3). Additionally no significant differences of quantitative rCBF were observed between the NOS and AOS groups in any of the regions (Table 3).

4. Discussion

In this study, we evaluated the quantitative rCBF of suspected iNPH patients who had specific MR image features of iNPH (enlarged sylvian fissures together with ventriculomegaly and narrowed subarachnoid spaces at the high convexity), but who did not have

Table 2
Results of cognitive and gait evaluations.

Cognitive and gait tests	Scores of tests			Statistical analysis		
	Average ± SD			One-way ANOVA	Tukey–Kramer HSD	
	AOS	NOS	NC	F value, p value		
MMSE (possible range 0–30)	21.1 ± 6.8	27.9 ± 1.3	29.1 ± 1.1	F(2,18) = 7.84, P = 0.0036	AOS<NOS[a]	AOS<NC[b]
FAB (possible range 0–18)	10.8 ± 2.6	14.4 ± 1.4	15.9 ± 1.3	F(2,17) = 13.06, P = 0.0004	AOS<NOS[b]	AOS<NC[c]
TMT A (seconds)	108.3 ± 67.4	53.1 ± 17.1	39.6 ± 17.0	F(2,17) = 5.44, P = 0.0149		AOS>NC[a]
WMS-R AC index	81.7 ± 17.7	101.9 ± 10.1	107.1 ± 11.7	F(2,17) = 6.47, P = 0.0081	AOS<NOS[a]	AOS<NC[b]
RBMT picture recognition (possible range 0–10)	8.2 ± 1.7	9.1 ± 0.9	10.0 ± 0	F(2,17) = 4.69, P = 0.0239		AOS<NC[a]
RBMT immediate story recall (possible range 0–25)	5.5 ± 3.1	11.1 ± 3.5	11.7 ± 2.3	F(2,17) = 8.20, P = 0.0032	AOS<NOS[b]	AOS<NC[b]
RMBTdelayed story recall (possible range 0–25)	4.1 ± 3.4	7.9 ± 4.6	9.8 ± 3.3	F(2,17) = 3.62, P = 0.0489		AOS<NC[a]
TUG (seconds)	14.3 ± 3.4	10.5 ± 1.5	10.5 ± 1.7	F(2,17) = 6.05, P = 0.0104	AOS>NOS[a]	AOS>NC[a]

AOS : iNPH patients with apparent objective triad symptoms, NOS: suspected iNPH patients with no objective triad symptoms, NC : normal controls.
MMSE: Mini-Mental State Examination (possible range 0–30), FAB: Frontal Assessment Battery (possible range 0–18), TMT A: Trail Making Test A (seconds), WMS-R AC: Wechsler Memory Scale Revised attention/concentration (index), RBMT picture: subtest of the picture recognition of the Rivermead Behavioural Memory Test (possible range 0–10), RBMT immediate story recall : subtest of the immediate story recall of the Rivermead Behavioural Memory Test (possible range 0–25), RBMT delayed story recall : subtest of the delayed story recall of the Rivermead Behavioural Memory Test (possible range 0–25), TUG : Timed Up & Go Test (seconds).
[a] p value<0.05.
[b] p value<0.01.
[c] p value<0.001.

Table 3
Intraclass correlation coefficients (ICCs) and quantitative rCBF of each brain region.

Brain region	ROI Number	ICC	Quantitative rCBF			One-way ANOVA		Tukey-Kramer HSD		
			Average (ml/100 g/min) ± SD					p value		
			AOS	NOS	NC	F (2,18)	p value	Between AOS and NC	Between NOS and NC	Between AOS and NOS
SPL	1, 2	0.85	18.0 ± 8.0	20.4 ± 3.9	37.0 ± 3.7	24.17	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.6924
IPL	13, 14	0.94	20.5 ± 8.1	22.5 ± 4.9	42.1 ± 4.0	28.43	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.7953
LFC	3, 4, 15, 16	0.99	22.8 ± 7.0	27.8 ± 5.2	47.7 ± 5.8	33.40	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.2915
LTC	23, 24	0.97	23.9 ± 2.9	21.4 ± 3.6	48.4 ± 5.5	90.13	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.5168
LOC	29, 30	0.99	28.4 ± 5.7	24.5 ± 4.8	43.1 ± 6.4	21.03	p<0.0001	AOS<NC p = 0.0003	NOS<NC p<0.0001	p = 0.4287
MFC	5, 6, 17, 18	0.99	27.3 ± 6.8	30.6 ± 7.5	49.2 ± 7.0	19.22	p<0.0001	AOS<NC p<0.0001	NOS<NC p = 0.0003	p = 0.6677
MOC	27, 28	0.97	35.2 ± 10.4	34.2 ± 5.1	54.6 ± 6.7	15.51	p = 0.0001	AOS<NC p = 0.0005	NOS<NC p = 0.0003	p = 0.9685
PCP	9, 10, 11, 12	0.89	30.4 ± 5.3	26.4 ± 7.5	47.4 ± 10.5	13.50	p = 0.0003	AOS<NC p = 0.0025	NOS<NC p = 0.0003	p = 0.6219
FWM	19, 20	0.98	19.5 ± 5.3	23.2 ± 5.8	30.7 ± 5.5	7.32	p = 0.0047	AOS<NC p = 0.0040	p = 0.0530	p = 0.4515
SC	7, 8	0.79	21.8 ± 5.2	18.9 ± 8.1	31.7 ± 5.1	8.02	p = 0.0032	AOS<NC p = 0.0216	NOS<NC p = 0.0034	p = 0.6753
GCC	31	0.92	12.4 ± 3.2	11.9 ± 5.7	22.5 ± 3.0	14.28	p = 0.0002	AOS<NC p = 0.0008	NOS<NC p = 0.0005	p = 0.9691
HCN	21, 22	0.97	21.9 ± 6.1	23.7 ± 4.4	43.8 ± 6.4	31.69	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.8240
TH	25, 26	0.99	25.1 ± 6.1	28.3 ± 5.2	54.6 ± 9.0	38.17	p<0.0001	AOS<NC p<0.0001	NOS<NC p<0.0001	p = 0.6611
PN	32	0.99	23.6 ± 9.1	28.1 ± 4.0	46.0 ± 5.8	22.34	p<0.0001	AOS<NC p<0.0001	NOS<NC p = 0.0002	p = 0.4203
HCB	33, 34	0.98	31.7 ± 11.6	33.9 ± 7.5	55.7 ± 9.3	13.25	p = 0.0003	AOS<NC p = 0.0006	NOS<NC p = 0.0014	p = 0.9083
VCB	35	0.96	33.5 ± 11.0	32.5 ± 5.3	56.1 ± 10.4	14.54	p = 0.0002	AOS<NC p = 0.0007	NOS<NC p = 0.0004	p = 0.9774

SPL, superior parietal lobe ; IPL, inferior parietal lobe ; LFC, lateral frontal cortex ; LTC, lateral temporal cortex ; LOC, lateral occipital cortex ; MFC, medial frontal cortex ; MOC, medial occipital cortex ; PCP, posterior cingulate gyrus and precuneus ; FWM ; frontal white matter ; SC, semioval center ; GCC, genu of corpus callosum ; HCN, head of caudate nucleus ; TH, thalamus ; PN, pons ; HCB, hemisphere of cerebellum ; VCB, vermis of cerebellum.
AOS : iNPH patients with apparent objective symptoms, NOS: suspected iNPH patients with no objective symptoms, NC : normal controls.
rCBF : regional cerebral blood flow.

any apparent objective triad symptoms. The NOS group did not significantly differ from the NC group in any of the cognitive or gait evaluations, although the lack of significant difference might be due to a type II error because of the small sample size of this study. In all brain regions examined except for in the frontal white matter, the quantitative rCBF of the NOS group was significantly less than that of the NC group. Although the difference of the quantitative rCBF in the frontal white matter between the NOS and NC groups did not reach the significant level, the trend (p value $= 0.0530$) that the quantitative rCBF of the NOS group was less than that of the NC group was shown in this region. The NOS group did not significantly differ from the AOS group in the quantitative rCBF in any of the regions, although the lack of significant difference might be due to a type II error because of the small sample size.

Two methods are generally used to compare rCBF values among different groups, the ROI method and voxel-based statistical image-analyzing methods. Advantages of the latter are that they can analyze rCBF automatically and absolutely in a user-independent fashion and can analyze rCBF of the whole brain easily, making it better than the ROI method for many subjects. A disadvantage of the voxel-based methods is that they work poorly with patients with severely distorted brains, which are difficult to normalize in SPM [7,17] or in 3D-SSP [18], leading to incorrect results. On the other hand, the ROI method can avoid the distortion problem by using MR image templates to locate the ROIs on anatomically precise regions. The main disadvantage of the ROI method is that it has an inherent arbitrariness based on how the regions are selected. For patients with iNPH-specific severely distorted brains, the ROI method is more suitable than the voxel-based statistical image-analyzing methods and was used to compare the rCBF values among the AOS, NOS, and NC subjects in this study. The ICCs for measuring the rCBF in different regions (0.79 to 0.99) were higher than those in other ROI analyses [38,39], and indicate high reliability of the ROI method used in this study. However, we could not completely erase the arbitrariness that necessarily accompanies the ROI method. Another problem was that an investigator, even though blinded to the subjects' clinical information, could distinguish MR images of the NC subjects from those of the AOS and NOS subjects. However, because the MR images of AOS and NOS were indistinguishable, the investigator was completely blind to whether iNPH-like MR images were those of AOS or NOS.

The NOS patients were probably in the very early stage of iNPH because their MR images showed enlarged sylvian fissures together with ventriculomegaly and narrowed subarachnoid spaces at the high convexity, which are not observed in patients with disease other than iNPH. "High (superior) convexity" was first used by Kitagaki, et al [4] to indicate the suprasylvian subarachnoid space. Although many researchers have used "high convexity" [2], the term has not been defined strictly. However, "high convexity" covers the superior parietal lobe. In addition, the mean scores of all the cognitive tests in the NOS group were located between those of the NC and AOS groups, although there were no significant differences between the NOS and NC groups in any of the cognitive tests. The NOS group showed no gait disturbance at all. The slight cognitive impairment with no gait disturbance in patients with NOS in this study was consistent with findings of the first report of AVIM [2]. In that study, two of the eight subjects who were diagnosed as AVIM at their first examination subsequently showed cognitive deterioration and were diagnosed as having iNPH.

Our finding that rCBF of the NOS group was reduced just as much as in the AOS group was not what we hypothesized. The fact that rCBF was equally reduced in the AOS and NOS groups suggests that factors other than reduced rCBF are involved in the manifestation of symptoms in iNPH patients. Possible factors include (1) low availability of striatal D2 receptor, which is associated with hypokinetic gait and anhednic mentation in iNPH patients [40], (2) the compressive effect of the ventricles on several brain areas [41–43], (3) apoptosis of neuronal cells [44], and (4) dysfunction of the neurons [45].

A limitation of our study is the small sample size. Another limitation is that our study was based only on cross sectional data, and not on longitudinal data. We intend to monitor the NOS group in this study to see if they develop objective iNPH symptoms. Third, we did not attempt to closely match the degrees of ventriculomegaly and narrowing of the sulci at the high convexity between the NOS and AOS patients. The degree of morphological change in the brain might be different between the two groups. These issues should be taken into consideration before the findings can be generalized.

In this study, we were unable to identify the neuroanatomical bases of the triad symptoms by quantitative rCBF analyses of NOS and AOS patients. Our results indicate that hypoperfusion developed in all brain regions before the appearance of the triad symptoms and was not correlated with the degree of symptoms in patients with iNPH. Further studies that compare factors other than rCBF between iNPH patients with NOS and AOS should provide some clues to the pathogenesis of iNPH.

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References

[1] Adams RD, F.C., Hakin S, Ojemann RG, Sweet WH. Symptomatic occult hydrocephalus with 'normal' cerebrospinal-fluid pressure. A treatable syndrome. N Eng J Med 1965;273:117–26.
[2] Iseki C, Kawanami T, Nagasawa H, Wada M, Koyama S, Kikuchi K, et al. Asymptomatic ventriculomegaly with features of idiopathic normal pressure hydrocephalus on MRI (AVIM) in the elderly: a prospective study in a Japanese population. J Neurol Sci 2009;277(1–2):54–7.
[3] Takeuchi T, Goto H, Izaki K, Tamura S, Tomii M, Sasanuma J, et al. Pathophysiology of cerebral circulatory disorders in idiopathic normal pressure hydrocephalus. Neurol Med Chir (Tokyo) 2007;47(7):299–306.
[4] Kitagaki H, Mori E, Ishii K, Yamaji S, Hirono N, Imamura T. CSF spaces in idiopathic normal pressure hydrocephalus: morphology and volumetry. AJNR Am J Neuroradiol 1998;19(7):1277–84.
[5] Boon AJ, Tans JT, Delwel EJ, Egeler-Peerdeman SM, Hanlo PW, Wurzer HA, et al. Dutch Normal-Pressure Hydrocephalus Study: randomized comparison of low- and medium-pressure shunts. J Neurosurg 1998;88(3):490–5.
[6] Savolainen S, Hurskainen H, Paljarvi L, Alafuzoff I, Vapalahti M. Five-year outcome of normal pressure hydrocephalus with or without a shunt: predictive value of the clinical signs, neuropsychological evaluation and infusion test. Acta Neurochir (Wien) 2002;144(6):515–23.
[7] Yoon B, Yang DW, Shim YS, Chung SW, Ahn KJ, O JH, et al. Voxel-based analysis of Tc-99m ECD brain perfusion SPECT in patients with normal pressure hydrocephalus. Appl Radiat Isot 2009;67(7–8):1377–81.
[8] Bradley Jr WG, Whittemore AR, Watanabe AS, Davis SJ, Teresi LM, Homyak M. Association of deep white matter infarction with chronic communicating hydrocephalus: implications regarding the possible origin of normal-pressure hydrocephalus. AJNR Am J Neuroradiol 1991;12(1):31–9.
[9] Greitz D, Hannerz J, Rahn T, Bolander H, Ericsson A. MR imaging of cerebrospinal fluid dynamics in health and disease. On the vascular pathogenesis of communicating hydrocephalus and benign intracranial hypertension. Acta Radiol 1994;35(3):204–11.
[10] Vorstrup S, Christensen J, Gjerris F, Sorensen PS, Thomsen AM, Paulson OB. Cerebral blood flow in patients with normal-pressure hydrocephalus before and after shunting. J Neurosurg 1987;66(3):379–87.
[11] Mamo HL, Meric PC, Ponsin JC, Rey AC, Luft AG, Seylaz JA. Cerebral blood flow in normal pressure hydrocephalus. Stroke 1987;18(6):1074–80.
[12] Larsson A, Bergh AC, Bilting M, Arlig A, Jacobsson L, Stephensen H, et al. Regional cerebral blood flow in normal pressure hydrocephalus: diagnostic and prognostic aspects. Eur J Nucl Med 1994;21(2):118–23.
[13] Kristensen B, Malm J, Fagerland M, Hietala SO, Johansson B, Ekstedt J, et al. Regional cerebral blood flow, white matter abnormalities, and cerebrospinal fluid hydrodynamics in patients with idiopathic adult hydrocephalus syndrome. J Neurol Neurosurg Psychiatry 1996;60(3):282–8.
[14] Tanaka A, Kimura M, Nakayama Y, Yoshinaga S, Tomonaga M. Cerebral blood flow and autoregulation in normal pressure hydrocephalus. Neurosurgery 1997;40(6):1161–5.

[15] Chang CC, Kuwana N, Ito S, Ikegami T. Prediction of effectiveness of shunting in patients with normal pressure hydrocephalus by cerebral blood flow measurement and computed tomography cisternography. Neurol Med Chir (Tokyo) 1999;39(12):841–5.
[16] Owler BK, Pickard JD. Normal pressure hydrocephalus and cerebral blood flow: a review. Acta Neurol Scand 2001;104(6):325–42.
[17] Mataro M, Poca MA, Salgado-Pineda P, Castell-Conesa J, Sahuquillo J, Diez-Castro MJ, et al. Postsurgical cerebral perfusion changes in idiopathic normal pressure hydrocephalus: a statistical parametric mapping study of SPECT images. J Nucl Med 2003;44(12):1884–9.
[18] Sasaki H, Ishii K, Kono AK, Miyamoto N, Fukuda T, Shimada K, et al. Cerebral perfusion pattern of idiopathic normal pressure hydrocephalus studied by SPECT and statistical brain mapping. Ann Nucl Med 2007;21(1):39–45.
[19] Miyamoto J, Imahori Y, Mineura K. Cerebral oxygen metabolism in idiopathic-normal pressure hydrocephalus. Neurol Res 2007;29(8):830–4.
[20] Meyer JS, T.H., Hardenberg JP, Dowell RE, M.S., Kitagawa Y, et al. Normal pressure hydrocephalus; influences on cerebral hemodynamic and cerebrospinal fluid pressure; Chemical autoregulation. Surg Neurol 1984;21:195–203.
[21] Meyer JS, Kitagawa Y, Tanahashi N, Tachibana H, Kandula P, Cech DA, et al. Evaluation of treatment of normal-pressure hydrocephalus. J Neurosurg 1985;62 (4):513–21.
[22] Kubo Y, Kazui H, Yoshida T, Kito Y, Kimura N, Tokunaga H, et al. Validation of grading scale for evaluating symptoms of idiopathic normal-pressure hydrocephalus. Dement Geriatr Cogn Disord 2008;25(1):37–45.
[23] Marmarou A, Bergsneider M, Relkin N, Klinge P, Black PM. Development of guidelines for idiopathic normal-pressure hydrocephalus: introduction. Neurosurgery 2005;57(3 Suppl):S1–3.
[24] Ishikawa M, Hashimoto M, Kuwana N, Mori E, Miyake H, Wachi A, et al. Guidelines for management of idiopathic normal pressure hydrocephalus. Neurol Med Chir (Tokyo) 2008;48:S1–S23 Suppl.
[25] Kimura N, Kazui H, Kubo Y, Yoshida T, Ishida Y, Miyoshi N, et al. Memory and physical mobility in physically and cognitively-independent elderly people. Geriatr Gerontol Int 2007;7:258–65.
[26] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3): 189–98.
[27] Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 1969;9(3):179–86.
[28] Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. Neurology 2000;55(11):1621–6.
[29] Reitan RM. Validity of the Trail Making Tast as an indicator of organic brain damage. Percept Mot Skills 1958;8:271–6.
[30] Russell EW. A multiple scoring method for the assessment of complex memory functions. J Consult Clin Psychol 1975;43:800–9.
[31] Wilson B, Cockburn J, Baddeley A, Hiorns R. The development and validation of a test battery for detecting and monitoring everyday memory problems. J Clin Exp Neuropsychol 1989;11(6):855–70.
[32] Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 1991;39(2):142–8.
[33] Gonzalez H, Sunnerhagen KS, Sjoberg I, Kaponides G, Olsson T, Borg K. Intravenous immunoglobulin for post-polio syndrome: a randomised controlled trial. Lancet Neurol 2006;5(6):493–500.
[34] Tice JA, Kanaya A, Hue T, Rubin S, Buist DS, Lacroix A, et al. Risk factors for mortality in middle-aged women. Arch Intern Med 2006;166(22):2469–77.
[35] Chang LT. Attenuation correction and imcomplete projection in single photon emission computed tomography. IEEE Trans Nucl Sci 1979;26:2780–9.
[36] Ogura T, Hida K, Masuzuka T, Saito H, Minoshima S, Nishikawa K. An automated ROI setting method using NEUROSTAT on cerebral blood flow SPECT images. Ann Nucl Med 2009;23(1):33–41.
[37] Bland JM, Altman DG. Measurement error and correlation coefficients. BMJ (Clinical Research Ed) 1996;313(7048):41–2.
[38] Salmi E, Aalto S, Hirvonen J, Langsjo JW, Maksimow AT, Oikonen V, et al. Measurement of GABAA receptor binding in vivo with [11C]flumazenil: a test–retest study in healthy subjects. Neuroimage 2008;41(2):260–9.
[39] Staley JK, van Dyck CH, Weinzimmer D, Brenner E, Baldwin RM, Tamagnan GD, et al. 123I-5-IA-85380 SPECT measurement of nicotinic acetylcholine receptors in human brain by the constant infusion paradigm: feasibility and reproducibility. J Nucl Med 2005;46(9):1466–72.
[40] Nakayama T, Ouchi Y, Yoshikawa E, Sugihara G, Torizuka T, Tanaka K. Striatal D2 receptor availability after shunting in idiopathic normal pressure hydrocephalus. J Nucl Med 2007;48(12):1981–6.
[41] Savolainen S, Laakso MP, Paljarvi L, Alafuzoff I, Hurskainen H, Partanen K, et al. MR imaging of the hippocampus in normal pressure hydrocephalus: correlations with cortical Alzheimer's disease confirmed by pathologic analysis. AJNR Am J Neuroradiol 2000;21(2):409–14.
[42] Mataro M, Matarin M, Poca MA, Pueyo R, Sahuquillo J, Barrios M, et al. Functional and magnetic resonance imaging correlates of corpus callosum in normal pressure hydrocephalus before and after shunting. J Neurol Neurosurg Psychiatry 2007;78 (4):395–8.
[43] Lee PH, Yong SW, Ahn YH, Huh K. Correlation of midbrain diameter and gait disturbance in patients with idiopathic normal pressure hydrocephalus. J Neurol 2005;252(8):958–63.
[44] Shibata M, Yamasaki N, Miyakawa T, Kalaria RN, Fujita Y, Ohtani R, et al. Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion. Stroke 2007;38(10):2826–32.
[45] Kavia RB, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. J Comp Neurol 2005;493(1):27–32.

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ORIGINAL ARTICLE

Frontal shift of posterior alpha activity is correlated with cognitive impairment in early Alzheimer's disease: A magnetoencephalography–beamformer study

Ryouhei ISHII, Leonides CANUET, Ryu KURIMOTO, Koji IKEZAWA, Yasunori AOKI, Michiyo AZECHI, Hidetoshi TAKAHASHI, Takayuki NAKAHACHI, Masao IWASE, Hiroaki KAZUI and Masatoshi TAKEDA

Department of Psychiatry, Osaka University Graduate School of Medicine, Suita City, Osaka, Japan

Correspondence: Dr Ryouhei Ishii MD PhD, Department of Psychiatry, Osaka University Graduate School of Medicine, 2-2 D3 Yamadaoka, Suita City, Osaka 565-0871 Japan. Email: ishii@psy.med.osaka-u.ac.jp

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Abstract

Background: Induced-oscillatory activity is considered a key factor for understanding functional processes in the brain. Magnetoencephalography (MEG) can measure oscillatory activity non-invasively with higher spatial resolution than electroencephalography (EEG). However, MEG has rarely been used to explore functional abnormalities that may represent state markers in patients with Alzheimer's disease (AD).

Methods: Thirteen patients with early AD and 14 age-matched normal controls participated in the present study. Magnetoencephalography activity was acquired during eyes-open and eyes-closed states. Alpha event-related synchronization (ERS) after eye closing was calculated and its cortical sources superimposed on each individual's magnetic resonance imaging (MRI) scan. The resulting functional image was converted into a Talairach-transformed anatomical brain image and group comparisons were made. We also assessed correlations between cortical ERS sources showing significant between-group differences in alpha activity and external clinical parameters, especially measures of cognitive function.

Results: The averaged alpha ERS after eye closing appeared dominantly in posterior brain regions in both patients with AD and healthy controls. However, there was a significant increase in alpha ERS in frontal regions, maximal over the prefrontal cortex, in patients with AD relative to controls, indicating a frontal shift of the posterior dominant MEG alpha rhythm in AD patients. This frontal ERS source in the alpha band was negatively correlated with Mini-Mental State Examination scores in the AD patient group.

Conclusions: The findings indicate that a frontal shift of alpha ERS elicited by an eyes-open/eyes-closed paradigm may be an early brain electromagnetic change in patients with AD, probably representing a physiological state marker of the disease. Furthermore, the results confirm that the beamformer with group comparison analysis is a useful tool with which to explore functional processes in the brain, as indicated by oscillatory activity changes.

Key words: *Alzheimer's disease, beamformer, correlation analysis, event-related synchronization, frontal cortex, magnetoencephalography, Mini-Mental State Examination.*

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder; it is characterized mainly by cognitive and intellectual deficits. Electroencephalography (EEG) has long been used as a diagnostic tool in AD.[1–3] Several EEG patterns of brain oscillatory activity have been reported in AD, including a slowing and diffusing of the posterior dominant alpha activity, an unclear alpha attenuation after eye opening, and an increase in delta and theta, as well as a decrease in

beta and gamma, activities in certain cortical regions.[1,2,4] However, in patients with early AD in particular, it is sometimes difficult to visualize these typical EEG findings.[1,2] Although magnetoencephalography (MEG) measures neural activity non-invasively with higher spatial resolution than EEG, little has been reported regarding MEG abnormalities in patients with AD. Most of these studies have used a single dipole fitting,[4,5] quantitative methods (e.g. frequency analysis approach),[6] or connectivity analysis[7] to explore brain abnormalities in AD.

Recently, with the advent of advanced time–frequency analysis, there interest has increased with regard to applying spatial filtering methods to analyze MEG data in several psychiatric and neurological disorders, including AD.[8–10] In particular, the beamformer, one of the spatial filtering methods, has given us an important insight into the dynamics of oscillatory activity in the brain.[11–14] We successfully used the beamformer for cortical mapping of source power changes after eye closing in patients with mild cognitive impairment (MCI) and AD, and found increased alpha event-related synchronization (ERS) in frontal regions specifically when comparing patients with early AD with healthy controls.[15] However, it is not clear whether this frontal shift of MEG alpha activity in the eyes closed state could be considered as an indicator of the degree of cognitive impairment in patients with AD, thus representing a state effect of the disease. In the present study, we aimed to determine possible correlations between changes in alpha oscillatory activity after eye closing and measures of cognitive function in patients with early AD and healthy controls.

METHODS

Thirteen patients with early AD and 14 age-matched normal controls were enrolled in the study. All AD patients were recruited from the outpatient clinic of the Department of Psychiatry at Osaka University Hospital. The study was performed in accordance with the Declaration of Helsinki and was approved by the hospital's Ethics Committee. Written informed consent was obtained from all participants. A diagnosis of probable AD was established according to the National Institute of Neurological and Communicative Disorders and Stroke/the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[16] The normal controls were healthy volunteers who had neither cognitive disturbances nor a history of neurological or psychiatric disorders. Both patients and controls were not taking any medication that may have affected the central nervous system at the time of recruitment and all subjects underwent brain magnetic resonance imaging (MRI) screening to exclude organic lesions. In order to assess cognitive function, the Mini-Mental State Examination (MMSE)[17] was performed on all patients and controls. In addition, the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-J cog)[18] and the Clinical Dementia Rating (CDR) scale[19] were performed on the patients.

The MEG data were recorded using a 64-channel whole-head magnetometer (NeuroSQUID Model 100; VSM Medtech CTF Systems, Port Coquitlam, Canada) in a magnetically shielded room. The MEG signals were digitized at 625 Hz and filtered using a combined 60-Hz notch filter and a 200-Hz low pass filter. During the recording, the participants were seated comfortably with the head positioned in the helmet-shaped Dewar. The localization of the subject's head relative to the sensor array was measured with three coils affixed to the nasion and preauricular points. Participants were asked to alternatively open and close their eyes for 10 s, which resulted in alpha synchronization mainly over occipital channels (Fig. 1). A total of eight trials was collected for each participant. Artifact rejection was performed off-line and all trials containing eye blinks were excluded. Brain Electrical Source Analysis (BESA) software[20] was used for source imaging of MEG data in the time-frequency domain. For each epoch, a 1-s MEG data interval in each condition was excluded from analysis to remove eye-blinking artifacts. Therefore, the event-related time frequency spectrum was calculated for a 9-s eyes-closed epoch (target interval) and compared with that of a 9-s eyes-open epoch (baseline interval; Fig. 2). The underlying cortical source was calculated using the Multiple Source Beamformer (MSBF) implemented in BESA (http://www.besa.de, accessed March 2008). This beamformer is a modified version of the linearly constrained minimum variance vector beamformer in the time-frequency domain, as described by Gross *et al.*[21] The beamformer images were superimposed on the individual's brain MRI. This results in functional three-dimensional (3D) images that reveal the locations of changes in oscillatory activity (as a percentage) during the target interval relative to the baseline interval.

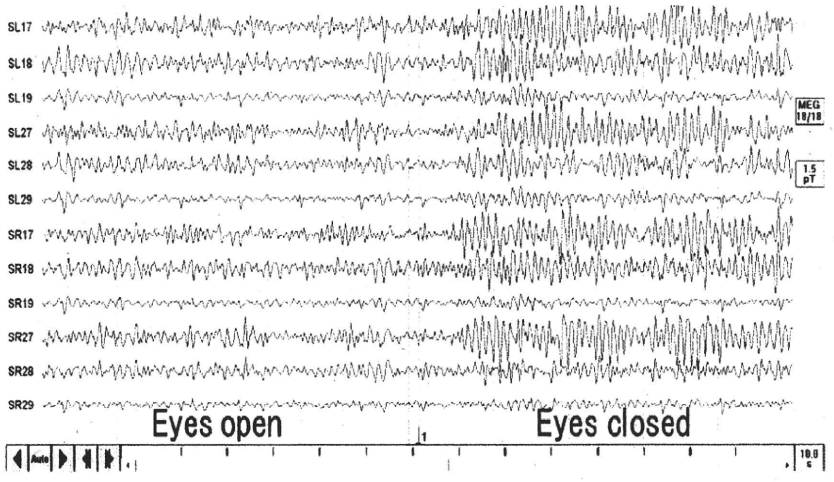

Figure 1 Magnetoencephalography (MEG) waveforms of an example trial during eyes open and eyes closed conditions in one normal control. Only channels over the posterior regions are shown. Alpha synchronization can be seen during the eyes closed condition.

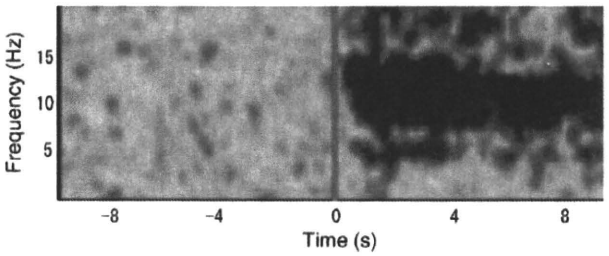

Figure 2 Time-frequency plot for a magnetoencephalography (MEG) channel overlying the occipital region in one normal control. The vertical line indicates the time of eye closing (change from the eyes open to the eyes closed condition). In the spectrogram, the *x*-axis denotes the time relative to the beginning of eyes closed condition (in s) and the *y*-axis denotes the frequency of oscillatory activity (Hz). Sustained alpha event-related synchronization can be seen after eye closing.

Table 1 Demographic and clinical characteristics of the patients with Alzheimer's disease and normal controls

	Early AD ($n = 13$)	Healthy controls ($n = 14$)
Age (years)	75.6 ± 5.0	71.2 ± 6.8
Sex (F/M)	9/4	8/6
MMSE score	22.1 ± 2.6	28.6 ± 1.5**
ADAS-cog	14.3 ± 3.3	
CDR score	0.8 ± 0.2	

Unless noted otherwise, data are presented as the mean ± SD. **$P < 0.001$ compared with patients with AD (Alzheimer's disease).
MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CDR, Clinical Dementia Rating scale.

Each acquired 3D image was imported into BrainVoyager QX (http://www.brainvoyager.com, accessed March 2008), transformed into a standardized Talairach brain (Talairach-transformed Montreal Neurological Institute (MNI) T_1-weighted brain template; http://www.bic.mni.mcgill.ca, accessed March 2008), and superimposed on a standard anatomical brain image. BrainVoyager QX,[22] originally developed for functional magnetic resonance imaging (fMRI) analysis, has been applied successfully to MEG data[9,15] because it allows us to import data from BESA software to perform statistical analysis of functional 3D images between groups. The averaged ERS of alpha activity was calculated for each group. Statistical group comparisons of 3D images and clinical variables were performed using unpaired, two-tailed *t*-test. The Chi-squared test was performed for independence of group and gender. For correlation of cortical sources of oscillatory activity changes with external clinical variables, especially with measures of cognitive function, the coordinates at the voxel with *t*-maxima (peak ERS value) at locations with significant alpha activity changes in the statistical maps were obtained. Then, the value at these coordinates in each individual's functional 3D image was determined for further analysis using Pearson's correlation coefficient. Data are given as the mean±SD and the level of significance was set at $P < 0.05$.

RESULTS

The demographic and clinical characteristics of patients with early AD and healthy controls are given in Table 1. There was no significant between-group difference in age and sex. Analysis of the results from neuropsychological tests revealed that patients with

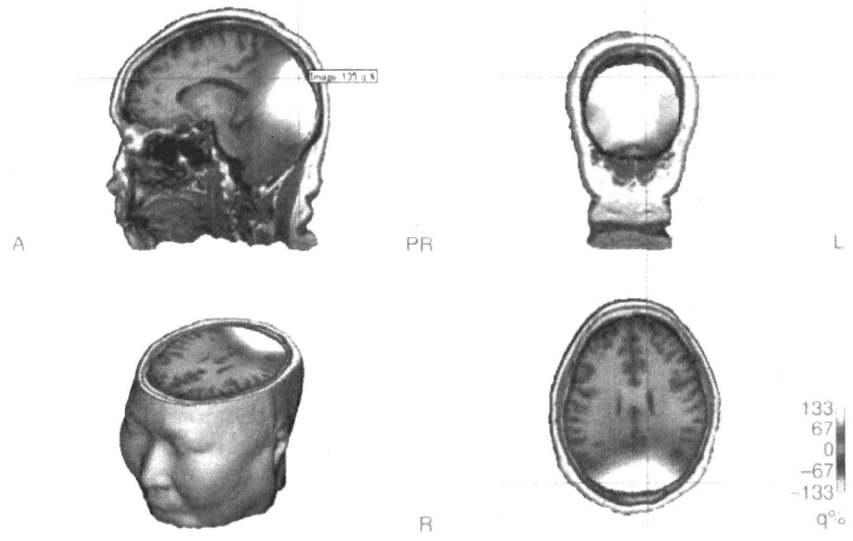

Figure 3 Multiple source beamformer analysis of alpha frequency band in one normal control. Color-coded maps show a posterior dominant alpha rhythm. P, posterior; A, anterior; L, left; R, right.

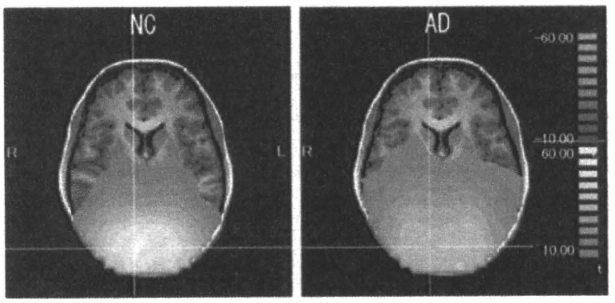

Figure 4 Averaged event-related synchronization of alpha activity in patients with Alzheimer's disease (AD) and normal controls (NC).

AD had a significantly lower MMSE scores compared with the control group. The peak frequency of alpha activity, as calculated by Fast Fourier Transform, was 9.3 ± 1.0 Hz in AD patients and 10.8 ± 1.3 Hz in controls. These findings indicate that neither patients nor controls showed slowing of the basic alpha rhythm.

The averaged alpha ERS appeared dominantly in posterior regions in the two groups (Figs 3,4). Compared with controls, patients with AD showed significantly increased alpha ERS in a broader area involving the bilateral frontal cortex, which was maximal over the right superior frontal gyrus ($t > 2.30$, $P < 0.05$; Fig. 5).

Correlation analysis of peak alpha ERS and clinical parameters and cognitive function measures indicated that, in the patient group, the MMSE score was negatively correlated with alpha ERS in the right prefrontal area, where significant between-group differences in oscillatory activity were found ($r = -0.697$, $P < 0.001$; Fig. 6). No significant correlation was observed between alpha ERS source in the frontal cortex and ADAS-J cog or CDR.

DISCUSSION

In the present study, changes in alpha oscillatory activity after eye closing were assessed in patients with early AD and age-matched healthy control subjects using MEG-beamformer and BrainVoyager for group comparisons. In addition, correlation analysis was performed between neural activity in cortical regions showing significant between-group differences and clinical parameters, including measures of cognitive function. Our findings revealed that although the averaged alpha ERS after eye closing appeared dominantly in posterior brain regions in both AD patients and healthy controls, there was increased alpha synchronization in frontal regions in AD patients, maximal over the right prefrontal cortex. Interestingly, this frontal ERS source in alpha band was negatively correlated with MMSE scores.

Eye opening/eye closing is deemed a simple task that induces modulation of EEG/MEG power. It is known that alpha reactivity or suppression during eye opening tends to decrease in several neurological disorders, including dementia.[23] Hence, the fact that the averaged alpha ERS in AD patients and healthy controls had a similar pattern was an unexpected finding.

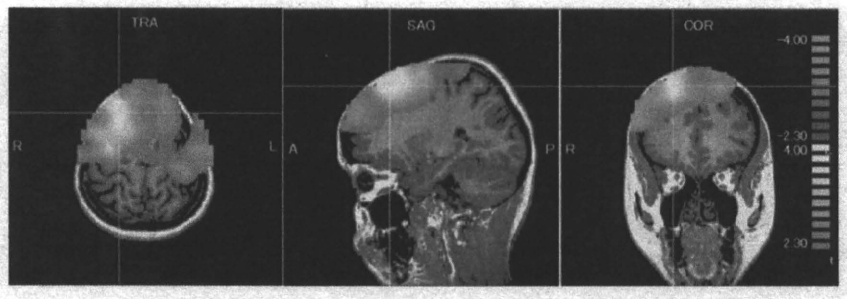

Figure 5 Group comparison of alpha event-related synchronization sources between patients with Alzheimer's disease (AD) and normal controls (NC) using BrainVoyager QX (http://www.brainvoyager.com, accessed March 2008). The color maps show cortical regions with significant between-group differences in alpha activity (threshold: $t > 2.30$; $P < 0.05$). TRA, transverse; SAG, sagittal; COR, coronal.

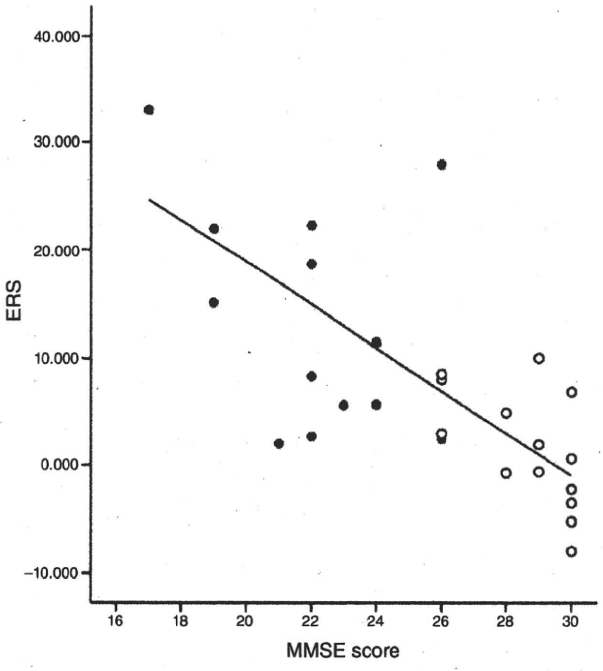

Figure 6 Correlation analysis between the peak alpha event-related synchronization (ERS) value (*t*-maxima) in the right prefrontal area and Mini-Mental State Examination (MMSE) scores in patients with Alzheimer's disease (AD; ●) and normal controls (○). A significant negative correlation was found for the AD patients ($\rho = -0.697$; $P < 0.001$).

This could be related to the existence of a slight difference in the degree of cognitive impairment between patients and controls, stemming from the enrollment of AD patients in the early stages of the disease, as indicated by the peak frequency of MEG alpha activity and the MMSE and CDR scores. The finding of a frontal shift of alpha ERS after eye closing characterizing patients with early AD in our study is consistent with previous EEG investigations reporting that anterior shift of alpha activity sources allowed for discrimination between patients with AD and those with MCI and healthy controls, despite the presence of decreased EEG alpha global field power in patients with AD.[24]

A striking finding with clinical implication is that the region with maximal alpha ERS in the frontal lobe, namely the right prefrontal cortex, showed a significant negative correlation with MMSE scores. This demonstrates an association of a frontal shift of the posterior alpha activity with cognitive impairment in AD. Thus, this abnormal oscillatory activity in frontal regions may represent a state effect of the disease. Recent evidence from a study of the functional significance of the frontal shift of event-related potential (ERP) amplitude with increasing age indicating that the elderly with most anterior distribution of neural activity showed the poorest recognition memory performance provides further support for our findings.[25] Others have reported frontal shift of cortical activity as a positive change in response to pharmacotherapy rather than an abnormal phenomenon following the administration of atypical antipsychotic drugs.[26] Surprisingly, unlike the MMSE, other measures of cognitive function, in particular the ADAS-J cognitive subscale and the CDR scale, were not correlated with the significant alpha ERS in the prefrontal cortex of patients with early AD. Although we do not have a clear explanation for this finding, it may indicate a higher sensitivity of the MMSE compared with the other two scales for the detection of early neurophysiological changes, such as abnormal alpha synchronization patterns in patients with AD.

In summary, the overall findings of the present study indicate that a frontal shift of alpha ERS may represent an early brain electromagnetic change in AD and suggest that a resting eyes open/eyes closed paradigm may elicit changes in alpha oscillatory power that could represent candidate physiological markers for this disorder. Moreover, our findings

demonstrate that MEG-beamformer and group comparison analysis are useful tools with which to explore functional processes in the brain, as indicated by source-power changes in oscillatory activity. Further neurophysiological investigations will be required to confirm these findings.

REFERENCES

1 Jeong J. EEG dynamics in patients with Alzheimer's disease. *Clin Neurophysiol* 2004; **115**: 1490–1505.
2 Rosen I. Electroencephalography as a diagnostic tool in dementia. *Dement Geriatr Cogn Disord* 1997; **8**: 110–116.
3 Oshima N, Chinzei M, Kikuchi E, Hayashida M. Preliminary application of processed electroencephalogram monitoring to differentiate senile dementia from depression. *Psychogeriatrics* 2009; **9**: 116–120.
4 Fernandez A, Maestu F, Amo C *et al*. Focal temporoparietal slow activity in Alzheimer's disease revealed by magnetoencephalography. *Biol Psychiatry* 2002; **52**: 764–770.
5 Fernandez A, Turrero A, Zuluaga P *et al*. Magnetoencephalographic parietal delta dipole density in mild cognitive impairment: Preliminary results of a method to estimate the risk of developing Alzheimer disease. *Arch Neurol* 2006; **63**: 427–430.
6 Berendse HW, Verbunt JP, Scheltens P, van Dijk BW, Jonkman EJ. Magnetoencephalographic analysis of cortical activity in Alzheimer's disease: A pilot study. *Clin Neurophysiol* 2000; **111**: 604–612.
7 Stam CJ, Jones BF, Manshanden I *et al*. Magnetoencephalographic evaluation of resting-state functional connectivity in Alzheimer's disease. *Neuroimage* 2006; **32**: 1335–1344.
8 Osipova D, Ahveninen J, Jensen O, Ylikoski A, Pekkonen E. Altered generation of spontaneous oscillations in Alzheimer's disease. *Neuroimage* 2005; **27**: 835–841.
9 Canuet L, Ishii R, Iwase M *et al*. Working memory abnormalities in chronic interictal epileptic psychosis and schizophrenia revealed by magnetoencephalography. *Epilepsy Behav* 2010; **17**: 109–119.
10 Canuet L, Ishii R, Iwase M *et al*. Cephalic auras of supplementary motor area origin: An ictal MEG and SAM(g2) study. *Epilepsy Behav* 2008; **13**: 570–574.
11 Ishii R, Shinosaki K, Ukai S *et al*. Medial prefrontal cortex generates frontal midline theta rhythm. *Neuroreport* 1999; **10**: 675–679.
12 Ishii R, Canuet L, Ochi A *et al*. Spatially filtered magnetoencephalography compared with electrocorticography to identify intrinsically epileptogenic focal cortical dysplasia. *Epilepsy Res* 2008; **81**: 228–232.
13 Ishii R, Canuet L, Herdman A *et al*. Cortical oscillatory power changes during auditory oddball task revealed by spatially filtered magnetoencephalography. *Clin Neurophysiol* 2009; **120**: 497–504.
14 Shinosaki K, Yamamoto M, Ukai S *et al*. Desynchronization in the right auditory cortex during musical hallucinations: A MEG study. *Psychogeriatrics* 2003; **3**: 88–92.
15 Kurimoto R, Ishii R, Canuet L *et al*. Event-related synchronization of alpha activity in early Alzheimer's disease and mild cognitive impairment: An MEG study combining beamformer and group comparison. *Neurosci Lett* 2008; **443**: 86–89.
16 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS–ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
17 Folstein MF, Folstein SE, Mchugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
18 Homma A, Fukuzawa K, Tsukada Y, Ishii T, Hasegawa K, Mohs RC. Development of a Japanese version of Alzheimer's disease Assessment Scale (ADAS). *Jpn J Geriatr Psychiatry* 1992; **3**: 647–655 (in Japanese).
19 Morris JC. The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology* 1993; **43**: 2412–2214.
20 Scherg M, Berg P. New concepts of brain source imaging and localization. *Electroencephalogr Clin Neurophysiol* 1996; **46** (Suppl.): 127–137.
21 Gross J, Kujala J, Hamalaine M, Timmermann L, Schnitzler A, Salmelin R. Dynamic imaging of coherent sources: Studying neural interactions in the human brain. *Proc Natl Acad Sci USA* 2001; **98**: 694–699.
22 Goebel R, Esposito F, Formisano E. Analysis of functional image analysis contest (FIAC) data with brainvoyager QX: From single-subject to cortically aligned group general linear model analysis and self-organizing group independent component analysis. *Hum Brain Mapp* 2006; **27**: 392–401.
23 van der Hiele K, Vein AA, van der Welle A *et al*. EEG and MRI correlates of mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* 2007; **28**: 1322–1329.
24 Huang C, Walhund LO, Dierks T, Julin P, Winblad B, Jelic V. Discrimination of Alzheimer's disease and mild cognitive impairment by equivalent EEG sources: A cross-sectional and longitudinal study. *Clin Neurophysiol* 2000; **111**: 1961–1967.
25 Walhovd KB, Fjell AM, Reinvang I *et al*. The functional and structural significance of the frontal shift in the old/new ERP effect. *Brain Res* 2006; **1081**: 156–170.
26 Yamada K, Isotani T, Irisawa S *et al*. EEG global field power spectrum changes after a single dose of atypical antipsychotics in healthy volunteers. *Brain Topogr* 2004; **16**: 281–285.

RESEARCH ARTICLE

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The Impact of a Genome-Wide Supported Psychosis Variant in the *ZNF804A* Gene on Memory Function in Schizophrenia

Ryota Hashimoto,[1,2,3]* Kazutaka Ohi,[2,3] Yuka Yasuda,[2,3] Motoyuki Fukumoto,[2,3] Masao Iwase,[2] Naomi Iike,[2,3] Michiyo Azechi,[2] Koji Ikezawa,[2] Masahiko Takaya,[2] Hidetoshi Takahashi,[2] Hidenaga Yamamori,[2,4] Tomo Okochi,[5] Hitoshi Tanimukai,[2] Shinji Tagami,[2] Takashi Morihara,[2] Masayasu Okochi,[2] Toshihisa Tanaka,[2] Takashi Kudo,[2] Hiroaki Kazui,[2] Nakao Iwata,[3,5] and Masatoshi Takeda[2]

[1]Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Suita, Osaka, Japan

[2]Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

[3]CREST (Core Research for Evolutionary Science and Technology) of JST (Japan Science and Technology Agency), Tokyo, Japan

[4]Department of Molecular Neuropsychiatry, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

[5]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

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A recent genome-wide association study showed that a variant (rs1344706) in the *ZNF804A* gene was associated with schizophrenia and bipolar disorder. Replication studies supported the evidence for association between this variant in the *ZNF804A* gene and schizophrenia and that this variant is the most likely susceptibility variant. Subsequent functional magnetic resonance imaging studies in healthy subjects demonstrated the association of the high-risk *ZNF804A* variant with neural activation during a memory task and a theory of mind task. As these cognitive performances are disturbed in patients with schizophrenia, this gene may play a role in cognitive dysfunction in schizophrenia. The aim of the current study was to investigate the potential relationship between this *ZNF804A* polymorphism and memory function. The effects of the high-risk *ZNF804A* genotype, diagnosis, and genotype–diagnosis interaction on verbal memory, visual memory (VisM), attention/concentration, and delayed recall (measured by the Wechsler Memory Scale-Revised) were analyzed by two-way analysis of covariance in 113 patients with schizophrenia and 184 healthy subjects. Consistent with previous studies, patients with schizophrenia exhibited poorer performance on all indices as compared to healthy control subjects ($P < 0.001$). A significant *ZNF804A* genotype–diagnosis interaction was found for VisM performance ($P = 0.0012$). Patients with the high-risk T/T genotype scored significantly lower on VisM than G carriers did ($P = 0.018$). In contrast, there was no genotype effect for any index in the healthy control subjects ($P > 0.05$). Our data suggest that rs1344706 may be related to memory dysfunction in schizophrenia. © 2010 Wiley-Liss, Inc.

Key words: ZNF804A; memory; schizophrenia; polymorphism; rs1344706

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*Correspondence to:
Dr. Ryota Hashimoto, M.D., Ph.D., Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, D3, 2-2, Yamadaoka, Suita, Osaka 5650871, Japan. E-mail: hashimor@psy.med.osaka-u.ac.jp

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INTRODUCTION

Schizophrenia (OMIM: 181500) is a common complex psychiatric disease with a lifetime risk of approximately 1%. There are strong genetic components of this disease, with an estimated heritability of approximately 80% [Cardno and Gottesman, 2000; Tsuang, 2000]. In a genome-wide association study and follow-up studies, a single nucleotide polymorphism (SNP) in the *ZNF804A* gene (rs1344706) was found to be associated with schizophrenia and bipolar disorder [O'Donovan et al., 2008]. Subsequent replication studies demonstrated the association between schizophrenia and the *ZNF804A* gene and that rs1344706 remained the most strongly associated marker in the gene after fine mapping of *ZNF804* locus [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010].

The *ZNF804A* gene (OMIM: 612282) is located on chromosome 2q32.1 and consists of four exons and three introns spanning 341 kb. Although little is known about the encoded protein and its function, the sequence contains predicted zinc ion and DNA-binding domains, suggesting a role in the regulation of gene expression. Two imaging genetics studies using functional magnetic resonance imaging (fMRI) have demonstrated associations between the high-risk *ZNF804A* variant and neural activation during a memory task and a theory of mind task in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. The high-risk *ZNF804A* variant had impact on brain functional dysconnectivity between dorsolateral prefrontal cortex (DLPFC) and hippocampal formation during an N-back memory task in healthy subjects [Esslinger et al., 2009]. This altered connectivity between DLPFC and hippocampal formation might be a basis of human memory function.

Patients with schizophrenia have pronounced deficits in aspects of neurocognitive function such as speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition [Nuechterlein et al., 2004]. Cognitive impairments are strongly related to functioning in areas such as work, social relationships, and independent living in schizophrenia. The lack of marked cognitive benefit of present antipsychotics has led to the investigation of alternative drugs and mechanisms for the treatment of these impairments [Buchanan et al., 2007]. Intermediate phenotypes/endophenotypes represent simpler clues to genetic underpinnings than the disease syndrome itself, promoting the view that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward and successful genetic analysis [Gottesman and Gould, 2003; Preston and Weinberger, 2005]. Memory deficits are prominent trait markers of schizophrenia, with impairments also observed in first-degree relatives [Snitz et al., 2006]. Genetic risk for schizophrenia could affect functional activity in the brain; such changes have been shown to mediate disturbed memory function [Meyer-Lindenberg and Weinberger, 2006]. In the present study, we examined the effect of the genome-wide supported variant in the *ZNF804A* gene on memory functions in patients with schizophrenia.

MATERIALS AND METHODS

Sample Description

The subjects of this study consisted of 113 patients with schizophrenia [53.1% males, mean age ± standard deviation: 38.3 ± 12.1 years] and 184 healthy control subjects [47.8% males, 36.2 ± 11.5 years]. The sex ratio and mean age did not differ significantly between patients and control subjects ($P > 0.05$), whereas the years of education were significantly lower among patients with schizophrenia (14.2 ± 2.4) than among control subjects (15.4 ± 2.4) [$z = -4.20$, $P < 0.001$]. All subjects were biologically unrelated Japanese individuals. Subjects were excluded from this study if they had neurological or medical conditions that could affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders, or mental retardation. Cases were both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia. Healthy control subjects were recruited through local advertisements at Osaka University. Psychiatrically, medically, and neurologically healthy control subjects were evaluated using the DSM-IV-Non-Patient version of the Structured Clinical Interview to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication [Ohi et al., 2009]. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Genotyping

We selected rs1344706 in the *ZNF804A* gene because this SNP has been found to be associated with schizophrenia and bipolar disorder in genome-wide association and follow-up studies [O'Donovan et al., 2008] and the four replication studies confirmed the association [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010]. Furthermore, this SNP was related to functional brain activity in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA) as described previously [Hashimoto et al., 2006, 2007]. Detailed information on the PCR conditions is available upon request.

Phenotype Measures

A full version of the Wechsler Memory Scale-Revised (WMS-R) [Sugishita, 2001], which is generally used to measure memory functions, was administered to the subjects. The four indices of the WMS-R, that is, verbal memory (VerM), visual memory (VisM), attention/concentration (AC), and delayed recall (DR), were used for the analysis. Psychiatric symptoms in patients with schizophrenia were evaluated using the positive and negative syndrome scale (PANSS) [Kay et al., 1987].

TABLE I. Demographic and Clinical Characteristics of Patients with Schizophrenia and Controls

	Schizophrenia (n = 113)						Control (n = 184)					
	T/T (n = 21)		G carrier (n = 92)				T/T (n = 44)		G carrier (n = 140)			
Variables	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**
Age (years)	38.1	11.2	38.4	12.4	0.99	0.01	36.5	10.8	36.1	11.8	0.68	0.42
Sex (male/female)[a]	10/11		49/43		0.94	0.01	24/20		64/76		0.31	1.05
Education (years)	14.2	2.2	14.2	2.4	0.80	0.25	14.7	1.9	15.6	2.5	**0.05**	1.99
CPZeq (mg/day)	586.2	518.6	535.9	443.1	0.95	0.06	—	—	—	—	—	—
Age at onset (years)	23.7	10.2	24.2	8.6	0.76	0.31	—	—	—	—	—	—
Duration of illness (years)	14.4	9.7	14.2	11.1	0.74	0.33	—	—	—	—	—	—
Positive symptoms[b,c]	16.0	7.9	18.2	5.5	0.10	1.62	—	—	—	—	—	—
Negative symptoms[b,c]	18.6	7.5	18.6	7.0	0.89	0.14	—	—	—	—	—	—

CPZeq, chlorpromazine equivalents of total antipsychotics; [b]PANSS, positive and negative syndrome scale; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G and G/T genotypes of rs1344706. Differences in clinical characteristics between genotype groups were analyzed using the Mann–Whitney *U*-test, except for [a]χ^2 test. [c]T/T: n = 18; G carrier: n = 84. A significant *P*-value is shown as bold face and underlined.

Statistical Analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and control subjects as well as between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2 test for goodness of fit. No deviation from Hardy–Weinberg equilibrium was detected in cases or in controls ($P > 0.05$). To examine the effect of ZNF804A rs1344706 genotype on memory function, the effects of *ZNF804A* genotype, diagnosis, and genotype–diagnosis interactions on four memory domains were analyzed by a two-way analysis of variance (ANOVA). In further analysis to control for confounding factors, the genotype effects, diagnosis effects, and genotype–diagnosis interactions on the memory functions were adjusted by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates (the scores of indices were previously corrected by age). When genotype–diagnosis interaction was found, cases and controls were separately analyzed by ANOVA and ANCOVA. The Bonferroni correction was applied for multiple testing on four indices of the WMS-R to avoid type I error. Standardized effect sizes were calculated using Cohen's *d* method (http:www.uccs.edu/faculty/lbecker). The significance level for statistical tests was set at two-tailed $P < 0.05$.

RESULTS

The Effect of the High-Risk *ZNF804A* Polymorphism on Memory Functions

We examined potential associations between the *ZNF804A* genotype and memory functions in patients with schizophrenia and healthy controls. There was no difference in age, sex, chlorpromazine equivalents of total antipsychotics, age at onset, duration of illness, or PANSS scores between genotype groups. The only difference in demographic variables was a significantly greater number of years of education in the control groups ($z = 1.99$, $P = 0.05$; Table I). The *ZNF804A* genotype effects, diagnosis effects, and genotype–diagnosis interactions on memory functions are shown in Table II. We found significant effects of diagnosis (VerM: $F_{1,293} = 146.91$, $P < 0.001$; adjusted $F_{1,291} = 133.70$, $P < 0.001$, VisM: $F_{1,293} = 114.30$, $P < 0.001$; adjusted $F_{1,291} = 103.87$, $P < 0.001$, AC: $F_{1,293} = 53.46$, $P < 0.001$; adjusted $F_{1,291} = 48.59$; $P < 0.001$, DR: $F_{1,293} = 200.36$, $P < 0.001$; adjusted $F_{1,291} = 186.09$, $P < 0.001$) and genotype–diagnosis interaction (VisM: $F_{1,293} = 8.21$, $P = 0.0045$, adjusted $F_{1,291} = 10.76$, $P = 0.0012$). Significant genotype effects were only found for VisM ($F_{1,293} = 4.46$, $P = 0.036$, adjusted $F_{1,291} = 3.40$, $P = 0.066$). The effect of diagnosis and the diagnosis–genotype interaction remained positive after correction for multiple tests (corrected *P*-values, VerM: $P < 0.001$, VisM: $P < 0.001$, AC: $P < 0.001$, DR: $P < 0.001$, interaction in VisM: $P = 0.0048$), whereas the genotype effect on VisM did not remain after correction for multiple tests ($P > 0.14$). Patients with schizophrenia displayed lower scores on all memory indices than did controls, and the effect sizes of VerM, VisM, AC, and DR were −1.72, −1.21, −1.17, and −1.89, respectively. As a genotype–diagnosis interaction was found for VisM, we separately analyzed the effects of genotype on VisM in patients and controls (Fig. 1). There was a significant genotype effect in patients with schizophrenia ($F_{1,111} = 5.05$, $P = 0.027$; adjusted $F_{1,109} = 5.82$, $P = 0.018$), whereas there was no genotype effect in controls ($F_{1,182} = 0.88$, $P = 0.35$; adjusted $F_{1,180} = 1.43$, $P = 0.23$). The patients with the high-risk T/T genotype scored significantly lower on VisM than did those who carry a G genotype (effect size: −0.56).

When the two genotypes were divided into three genotype groups (patients with T/T genotype, T/G genotype, and G/G genotype), the patients with the high-risk T/T genotype scored significantly lower on VisM than patients with the T/G genotype (adjusted $F_{1,68} = 8.59$, $P = 0.0046$) and marginally lower than patients with the G/G genotype (adjusted $F_{1,58} = 2.89$, $P = 0.09$;

TABLE II. Effects of the *ZNF804A* Genotype on Memory Function Determined Using WMS-R

	Schizophrenia (n = 113)				Control (n = 184)				ANOVA				ANCOVA (adjusted)							
	T/T (n = 21)		G carrier (n = 92)		T/T (n = 44)		G carrier (n = 140)		Diagnosis effect		Genotype effect		Interaction		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$
VerM	82.6	14.6	84.6	18.3	110.2	14.1	111.3	13.0	**$<10^{-3}$**	**146.9**	0.50	0.45	0.83	0.04	**$<10^{-3}$**	**133.7**	0.78	0.08	0.47	0.52
VisM	81.7	18.2	92.3	19.7	110.5	8.3	108.9	10.3	**$<10^{-3}$**	**114.3**	**0.036**	**4.46**	**0.0045**	**8.21**	**$<10^{-3}$**	**103.9**	0.066	3.40	**0.0012**	**10.8**
AC	92.0	16.5	90.9	15.2	105.1	13.6	109.2	13.9	**$<10^{-3}$**	**53.5**	0.48	0.50	0.23	1.44	**$<10^{-3}$**	**48.6**	0.54	0.37	0.28	1.17
DR	77.1	18.4	82.6	19.5	111.7	12.7	112.0	11.8	**$<10^{-3}$**	**200.4**	0.20	1.65	0.25	1.31	**$<10^{-3}$**	**186.1**	0.35	0.88	0.10	2.71

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration; DR, delayed recall; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G or G/T genotype of rs1344706. The effects of the ZNF804A genotype and the effects of diagnosis on the memory function were analyzed by a two-way analysis of variance (ANOVA). Adjusted effects of genotype were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

FIG. 1. The association between the high-risk *ZNF804A* genotype and visual memory in patients with schizophrenia. X-axis: gray bars, individuals with T/T genotype of rs1344706; white bars, individuals with a G allele (G/T and G/G genotypes) of rs1344706. Y-axis: scores of visual memory from the WMS-R. Error bars represent standard errors of the mean. *$P < 0.05$, compared with patients with a G allele.

Table III). However, there was no significant difference in scores between patients with the T/G genotype and G/G genotype ($F_{1,88} = 1.39$, $P = 0.24$).

DISCUSSION

In the present study, we first demonstrated an association between the high-risk *ZNF804A* SNP and memory performance in patients with schizophrenia. We provided evidence that patients with the high-risk T/T genotype had lower performance on VisM than patients who carry a G allele. The effect size of the difference in VisM scores between patients with the T/T genotype and G carriers was −0.56; this effect is typically considered a medium-sized effect. We do not know why we found the genotype effect on only VisM. A possible explanation is that a previous study reported suggestive linkage evidence for the VisM on 2q36 near the locus of the *ZNF804A* gene [Paunio et al., 2004]. Another possibility is that this SNP is associated with connectivity during N-back memory task, which is an fMRI task using visual cue [Esslinger et al., 2009]. This study showed no effect of genotype on a memory task in healthy subjects, which is consistent with our data [Esslinger et al., 2009].

A linear genotype effect on connectivity in DLPFC and hippocampal formation during a memory task was found in healthy control subjects in an fMRI study [Esslinger et al., 2009]. These data

TABLE III. Effects of the *ZNF804A* Genotype on Memory Performance

	Schizophrenia (n = 113)						Control (n = 184)						ANCOVA (adjusted)					
	T/T (n = 21)		T/G (n = 51)		G/G (n = 41)		T/T (n = 44)		T/G (n = 85)		G/G (n = 55)		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$
VerM	82.6	14.6	83.9	19.1	85.5	17.3	110.2	14.1	112.5	12.8	109.4	13.2	$<\mathbf{10^{-3}}$	**168.5**	0.61	0.49	0.52	0.66
VisM	81.7	18.2	93.2	20.2	91.1	19.3	110.5	8.3	108.4	10.0	109.7	10.9	$<\mathbf{10^{-3}}$	**111.6**	0.15	1.94	**0.0028**	**5.99**
AC	92.0	16.5	89.9	14.6	92.1	15.9	105.1	13.6	109.8	14.8	108.2	12.4	$<\mathbf{10^{-3}}$	**70.7**	0.84	0.18	0.45	0.81
DR	77.1	18.4	83.1	20.8	82.0	18.1	111.7	12.7	113.2	11.6	110.2	12.0	$<\mathbf{10^{-3}}$	**227.5**	0.18	1.71	0.23	1.47

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration: DR, delayed recall; SD, standard deviation. T/T, T/G, G/G: individuals with three genotypes of rs1344706. Adjusted effects of three genotypes were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

might indicate that quantitative traits (i.e., brain physiological activity measured by fMRI) are closer to the genetic substrate than behavioral traits, such as neuropsychological functions and psychiatric disorders, and should be observable in genetically at-risk but behaviorally unaffected individuals [Meyer-Lindenberg and Weinberger, 2006]. Such physiological quantitative traits are likely to influence a neuropsychological trait, memory performance, in patients with schizophrenia, however, they might not affect memory performance in healthy subjects. This phenomena suggests that the high-risk SNP in the *ZNF804A* gene might be related to the neuropsychological disturbance in schizophrenia.

There were several limitations to this study. Although the sample was moderate in size, it might not be representative of the schizophrenic population. A false-positive association cannot be excluded as a possibility in our study, despite the precautions of ethnic matching and correction for multiple testing. The effects of the ZNF804A gene on VisM could be an epiphenomenon of the severity of the disease and/or medication. In conclusion, we found an effect of the high-risk *ZNF804A* SNP on VisM in schizophrenia. Further research will be required to clarify the role of the high-risk *ZNF804A* SNP in the pathophysiology of schizophrenia.

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REFERENCES

Buchanan RW, Freedman R, Javitt DC, Abi-Dargham A, Lieberman JA. 2007. Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. Schizophr Bull 33(5):1120–1130.

Cardno AG, Gottesman II. 2000. Twin studies of schizophrenia: From bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17.

Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C, Haddad L, Mier D, Opitz von Boberfeld C, Raab K, Witt SH, Rietschel M, Cichon S, Meyer -Lindenberg A. 2009. Neural mechanisms of a genome-wide supported psychosis variant. Science 324(5927):605.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. 2006. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15(20): 3024–3033.

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, Nakajima M, Tanaka K, Kawagishi N, Nemoto K, Mori T, Ohnishi T, Noguchi H, Hori H, Suzuki T, Iwata N, Ozaki N, Nakabayashi T, Saitoh O, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Kunugi H, Baba A. 2007. Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Mol Psychiatry 12(11):1026–1032.

Kay SR, Fiszbein A, Opler LA. 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13(2):261–276.

Meyer-Lindenberg A, Weinberger DR. 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci 7(10):818–827.

Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. 2004. Identification of separable cognitive factors in schizophrenia. Schizophr Res 72(1):29–39.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L, Dwyer S, Holmans P, Marchini JL, Spencer CC, Howie B, Leung HT, Hartmann AM, Möller HJ, Morris DW, Shi Y, Feng G, Hoffmann P, Propping P, Vasilescu C, Maier W, Rietschel M, Zammit S, Schumacher J, Quinn EM, Schulze TG, Williams NM, Giegling I, Iwata N, Ikeda M, Darvasi A, Shifman S, He L, Duan J, Sanders AR, Levinson DF, Gejman PV, Cichon S, Nöthen MM, Gill M, Corvin A, Rujescu D, Kirov G, Owen MJ, Buccola NG, Mowry BJ, Freedman R, Amin F, Black DW, Silverman JM, Byerley WF, Cloninger CR, Molecular Genetics of Schizophrenia Collaboration. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9):1053–1055.

Ohi K, Hashimoto R, Yasuda Y, Kiribayashi M, Iike N, Yoshida T, Azechi M, Ikezawa K, Takahashi H, Morihara T, Ishii R, Tagami S, Iwase M, Okochi M, Kamino K, Kazui H, Tanaka T, Kudo T, Takeda M. 2009. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. Genes Brain Behav 8(4):473–480.

Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T, Cannon TD, Lonnqvist J, Peltonen L. 2004. Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. Hum Mol Genet 13(16):1693–1702.

Preston GA, Weinberger DR. 2005. Intermediate phenotypes in schizophrenia: A selective review. Dialogues Clin Neurosci 7(2):165–179.

Riley B, Thiselton D, Maher BS, Bigdeli T, Wormley B, McMichael GO, Fanous AH, Vladimirov V, O'Neill FA, Walsh D, Kendler KS. 2010. Replication of association between schizophrenia and ZNF804A in the Irish case–control study of schizophrenia sample. Mol Psychiatry 15(1):29–37.

Snitz BE, Macdonald AW III, Carter CS. 2006. Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: A meta-analytic review of putative endophenotypes. Schizophr Bull 32(1): 179–194.

Steinberg S, Mors O, Børglum AD, Gustafsson O, Werge T, Mortensen PB, Andreassen OA, Sigurdsson E, Thorgeirsson TE, Böttcher Y, Olason P, Ophoff RA, Cichon S, Gudjonsdottir IH, Pietiläinen OP, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Athanasiu L, Suvisaari J, Lonnqvist J, Paunio T, Hartmann A, Jürgens G, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Breuer R, Möller HJ, Giegling I, Glenthøj B, Rasmussen HB, Mattheisen M, Bitter I, Réthelyi JM, Sigmundsson T, Fossdal R, Thorsteinsdottir U, Ruggeri M, Tosato S, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Walshe M, Bramon E, Vassos E, Li T, Fraser G, Walker N, Toulopoulou T, Yoon J, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Peltonen L, Rujescu D, Collier DA, Stefansson H, St Clair D, Stefansson K. 2010. Expanding the range of ZNF804A variants conferring risk of psychosis. Mol Psychiatry (online publication).

Sugishita M. 2001. Japanese Wechsler Memory Scale-Revised. Tokyo: Nihonbunkakagakusha.

Tsuang M. 2000. Schizophrenia: Genes and environment. Biol Psychiatry 47(3):210–220.

Walter H, Schnell K, Erk S, Arnold C, Kirsch P, Esslinger C, Mier D, Schmitgen MM, Rietschel M, Witt SH, Nothen MM, Cichon S, Meyer-Lindenberg A. 2010. Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. Mol Psychiatry (online publication).

Williams HJ, Norton N, Dwyer S, Moskvina V, Nikolov I, Carroll L, Georgieva L, Williams NM, Morris DW, Quinn EM, Giegling I, Ikeda M, Wood J, Lencz T, Hultman C, Lichtenstein P, Thiselton D, Maher BS, Malhotra AK, Riley B, Kendler KS, Gill M, Sullivan P, Sklar P, Purcell S, Nimgaonkar VL, Kirov G, Holmans P, Corvin A, Rujescu D, Craddock N, Owen MJ, O'Donovan MC, Molecular Genetics of Schizophrenia Collaboration (MGS) International Schizophrenia Consortium (ISC), SGENE-plus, GROUP. 2010. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry (online publication).

Zhang R, Lu SM, Qiu C, Liu XG, Gao CG, Guo TW, Valenzuela RK, Deng HW, Ma J. 2010. Population-based and family-based association studies of ZNF804A locus and schizophrenia. Mol Psychiatry (online publication).