sample is required to replicate and expand the current preliminary results.

In summary, the present study demonstrated significant gray matter reduction of the anterior cingulate gyrus in first-episode schizophrenia. We also suggested the possibility that such morphological change may exist prior to the onset of psychosis in some individuals, implying the potential role of neuroimaging methods in the prediction of future transition and effective intervention for high-risk subjects.

ACKNOWLEDGMENTS

This research was supported in part by Grants-in-Aid for Scientific Research (C) (No. 22591275, 24591699) and Grants-in-Aid for Scientific Research (B) (No. 24390281) from the Japanese Society for the Promotion of Science, Health and Labour Sciences Research Grants (Comprehensive Research on Disability, Health and Welfare, H23-Seishin-Ippan-002 and H23-Seishin-Ippan-009), and a Research Grant from the JSPS Asian Core Program.

REFERENCES

Andreasen, N. C. (1983). *The Scale for the Assessment of Negative Symptoms (SANS).* Iowa City: University of Iowa.

Andreasen, N. C. (1984). *The Scale for the Assessment of Positive Symptoms (SAPS).* Iowa City: University of Iowa.

Andreasen, N. C., Flaum, M., and Arndt, S. (1992). The comprehensive assessment of symptoms and history (CASH): an instrument for assessing diagnosis and psychopathology. *Arch. Gen. Psychiatry* 49, 615–623.

Andreasen, N. C., Nopoulos, P., Magnotta, V., Pierson, R., Ziebell, S., and Ho, B. C. (2011). Progressive brain change in schizophrenia: a prospective longitudinal study of first-episode schizophrenia. *Biol. Psychiatry* 70, 672–679.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage* 38, 95–113.

Ashburner, J., and Friston, K. J. (2005). Unified segmentation. *Neuroimage* 26, 839–851.

Ashburner, J., and Friston, K. J. (2009). Computing average shaped tissue probability templates. *Neuroimage* 45, 333–341.

Birchwood, M., Todd, P., and Jackson, C. (1998). Early intervention in psychosis: the critical-period hypothesis. *Br. J. Psychiatry Suppl.* 172, 53–59.

Borgwardt, S. J., Riecher-Rössler, A., Dazzan, P., Chitnis, X., Aston, J., Drewe, M., et al. (2007). Regional gray matter volume abnormalities in the at risk mental state. *Biol. Psychiatry* 61, 1148–1156.

Ellison-Wright, I., Glahn, D. C., Laird, A. R., Thelen, S. M., and Bullmore, E. (2008). The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. *Am. J. Psychiatry* 165, 1015–1023.

Fornito, A., Yung, A. R., Wood, S. J., Phillips, L. J., Nelson, B., Cotton, S., et al. (2008). Anatomic abnormalities of the anterior cingulate cortex before psychosis onset: an MRI study of ultra-high-risk individuals. *Biol. Psychiatry* 64, 758–765.

Fusar-Poli, P., Bonoldi, I., Yung, A. R., Borgwardt, S., Kempton, M. J., Valmaggia, L., et al. (2012). Predicting psychosis, meta-analysis of transition outcomes in individuals at high clinical risk. *Arch. Gen. Psychiatry* 69, 220–229.

Fusar-Poli, P., Borgwardt, S., Crescini, A., Deste, G., Kempton, M. J., Lawrie, S., et al. (2011). Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. *Neurosci. Biobehav. Rev.* 35, 1175–1185.

Gaser, C. (2009). *Voxel-Based Morphometry Toolbox, Version 8 (vbm8).* Available at: http://dbm.neuro.uni-jena.de

Girgis, R. R., Diwadkar, V. A., Nutche, J. J., Sweeney, J. A., Keshavan, M. S., and Hardan, A. Y. (2006). Risperidone in first-episode psychosis: a longitudinal, exploratory voxel-based morphometric study. *Schizophr. Res.* 82, 89–94.

Hayasaka, S., Phan, K. L., Liberzon, I., Worsley, K. J., and Nichols, T. E. (2004). Nonstationary cluster-size inference with random field and permutation methods. *Neuroimage* 22, 676–687.

Jones, D. K., Symms, M. R., Cercignani, M., and Howard, R. J. (2005). The effect of filter size on VBM analyses of DT-MRI data. *Neuroimage* 26, 546–554.

Klein, A., Anderson, J., Ardekani, B. A., Ashburner, J., Avants, B., Chiang, M. C., et al. (2009). Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. *Neuroimage* 46, 786–802.

Koo, M. S., Levitt, J. L., Salisbury, D. F., Nakamura, M., Shenton, M. E., and McCarley, R. W. (2008). A cross-sectional and longitudinal magnetic resonance imaging study of cingulate gyrus gray matter volume abnormalities in first-episode schizophrenia and first-episode affective psychosis. *Arch. Gen. Psychiatry* 65, 746–760.

Lappin, J. M., Morgen, K., Morgen, C., Hutchison, G., Chitnis, X., Sucking, J., et al. (2006). Gray matter abnormalities associated with duration of untreated psychosis. *Schizophr. Res.* 83, 145–153.

Leung, M., Cheung, C., Yu, K., Yip, B., Sham, P., Li, Q., et al. (2011). Gray matter in first-episode schizophrenia before and after antipsychotic drug treatment. Anatomical likelihood estimation meta-analyses with sample size weighting. *Schizophr. Bull.* 37, 199–211.

Lieberman, J. A., Tollefson, G. D., Charles, C., Zipursky, R., Sharma, T., Kahn, R. S., et al. (2005). Antipsychotic drug effects on brain morphology in first-episode schizophrenia. *Arch. Gen. Psychiatry* 62, 361–370.

Marshall, M., Lewis, S., Lockwood, A., Drake, R., Jones, P., and Croudace, T. (2005). Association between duration of untreated psychosis and outcome in cohorts of first-episode patients: a systematic review. *Arch. Gen. Psychiatry* 62, 975–983.

McGlashan, T. H., Zipursky, R. B., Perkins, D., Addington, J., Miller, T., Woods, S. W., et al. (2006). Randomized, double-blind trial of olanzapine versus placebo in patients prodromally symptomatic for psychosis. *Am. J. Psychiatry* 163, 790–799.

McGorry, P. D., Hickie, I. B., Yung, A. R., Pantelis, C., and Jackson, H. J. (2006). Clinical staging of psychiatric disorders: a heuristic framework for choosing earlier, safer and more effective interventions. *Aust. N. Z. J. Psychiatry* 40, 616–622.

McGorry, P. D., Yung, A. R., Phillips, L. J., Yuen, H. P., Francey, S., Cosgrave, E. M., et al. (2002). Randomized controlled trial of interventions designed to reduce the risk of progression to first-episode psychosis in a clinical sample with subthreshold symptoms. *Arch. Gen. Psychiatry* 59, 921–928.

Mechelli, A., Riecher-Rössler, A., Meisenzahl, E. M., Tognin, S., Wood, S. J., Borgwardt, S. J., et al. (2011). Neuroanatomical abnormalities that predate the onset of psychosis: a multicenter study. *Arch. Gen. Psychiatry* 68, 489–495.

Meisenzahl, E. M., Koutsouleris, N., Gaser, C., Bottelender, R., Schmitt, G. J. E., McGuire, P., et al. (2008). Structural brain alterations in subjects at high-risk of psychosis: a voxel-based morphometric study. *Schizophr. Res.* 102, 150–162.

Mizuno, M., Suzuki, M., Matsumoto, K., Murakami, M., Takeshi, K., Miyakoshi, T., et al. (2009). Clinical practice and research activities for early psychiatric intervention at Japanese leading centres. *Early Interv. Psychiatry* 3, 5–9.

Pantelis, C., Velakoulis, D., McGorry, P. D., Wood, S. J., Suckling, J., Phillips, L. J., et al. (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet* 361, 281–288.

Perkins, D. O., Gu, H., Boteva, K., and Lieberman, J. A. (2005). Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. *Am. J. Psychiatry* 162, 1785–1804.

Salgado-Pineda, P., Baeza, I., Pérez-Gómez, M., Vendrell, P., Junqué, C., Bargalló, N., et al. (2003). Sustained attention impairment correlates to gray matter decreases in first episode neuroleptic-naive schizophrenic patients. *Neuroimage* 19, 365–375.

Salmond, C. H., Asburner, J., Vargha-Khadem, F., Connelly, A., Gadian, D. G., and Fristion, K. J. (2002). Distributional assumptions in voxel-based morphometry. *Neuroimage* 17, 1027–1030.

Schultz, C. C., Koch, K., Wagner, G., Roebel, M., Schachtzabel, C., Gaser, C., et al. (2010). Reduced cortical thickness in first episode schizophrenia. *Schizophr. Res.* 116, 204–209.

Shepherd, A. M., Laurens, K. R., Matheson, S. L., Carr, S. M., and Green, M. J. (2012). Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. *Neurosci. Biobehav. Rev.* 36, 1342–1356.

Smieskova, R., Fusar-Poli, P., Allen, P., Bendfeldt, K., Stieglitz, R. D., Drewe, J., et al. (2010). Neuroimaging predictors of transition to psychosis–a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* 34, 1207–1222.

Steen, R. G., Mull, C., McClure, R., Hamer, R. M., and Lieberman, J. A. (2006). Brain volume in first episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br. J. Psychiatry* 188, 510–518.

Takahashi, T., Suzuki, M., Tanino, R., Zhou, S.-Y., Hagino, H., Niu, L., et al. (2007). Volume reduction of the left planum temporal gray matter associated with long duration of untreated psychosis in schizophrenia: preliminary report. *Psychiatry Res.* 154, 209–219.

Takahashi, T., Suzuki, M., Zhou, S.-Y., Tanino, R., Nakamura, K., Kawasaki, Y., et al. (2010). A follow-up MRI study of the superior temporal subregions in schizotypal disorder and first-episode schizophrenia. *Schizophr. Res.* 119, 65–74.

Takahashi, T., Zhou, S.-Y., Nakamura, K., Tanino, R., Furuichi, A., Kido, M., et al. (2011). A follow-up MRI study of the fusiform gyrus and middle and inferior temporal gyri in schizophrenia spectrum. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 1957–1964.

Talairach, J., and Tournoux, P. (1988). *Co-Planar Stereotaxic Atlas of the Human Brain.* New York: Thieme Medical Publishers.

Toru, M. (2001). *Psychotropic Manual,* 2nd Edn. Tokyo: Igaku-Shoin. (in Japanese).

Vita, A., De Peri, L., Silenzi, C., and Dicci, M. (2006). Brain morphology in first-episode schizophrenia: a meta-analysis of quantitative magnetic resonance imaging studies. *Schizophr. Res.* 82, 75–88.

Weiser, M. (2011). Early intervention for schizophrenia: the risk-benefit ratio of antipsychotic treatment in the prodromal phase. *Am. J. Psychiatry* 168, 761–763.

Woods, S. W., Tully, E. M., Walsh, B. C., Hawkins, K. A., Callahan, J. L., Cohen, S. J., et al. (2007). Aripiprazole in the treatment of the psychosis prodrome: an open-label pilot study. *Br. J. Psychiatry* 191, 96–101.

World Health Organization. (1993). *The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research.* Geneva: World Health Organization.

Worsley, K. J., Andermann, M., Koulis, T., MacDonald, D., and Evans, A. C. (1999). Detecting changes in nonisotropic images. *Hum. Brain Mapp.* 8, 98–101.

Yung, A. R., Phillips, L. J., and McGorry, P. D. (2004). *Treating Schizophrenia in the Prodromal Phase.* London: Taylor & Francis.

Yung, A. R., Phillips, L. J., Yuen, H. P., Francey, S. M., McFarlane, C. A., Hallgren, M., et al. (2003). Psychosis prediction: 12-month follow up of a high-risk ("prodromal") group. *Schizophr. Res.* 60, 21–32.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 December 2012; accepted: 01 March 2013; published online: 18 March 2013.

Citation: Nakamura K, Takahashi T, Nemoto K, Furuichi A, Nishiyama S, Nakamura Y, Ikeda E, Kido M, Noguchi K, Seto H and Suzuki M (2013) Gray matter changes in subjects at high risk for developing psychosis and first-episode schizophrenia: a voxel-based structural MRI study. Front. Psychiatry 4:16. doi: 10.3389/fpsyt.2013.00016

This article was submitted to Frontiers in Schizophrenia, a specialty of Frontiers in Psychiatry.

PLOS ONE

Mismatch Negativity and Cognitive Performance for the Prediction of Psychosis in Subjects with At-Risk Mental State

Yuko Higuchi[1], Tomiki Sumiyoshi[1]*, Tomonori Seo[1], Tomohiro Miyanishi[1], Yasuhiro Kawasaki[2], Michio Suzuki[1]

1 Department of Neuropsychiatry, University of Toyama Graduate School of Medicine and Pharmaceutical Science, Toyama, Japan, **2** Department of Neuropsychiatry, Kanazawa Medical University, Uchinada-machi, Japan

Abstract

Background: A shorter duration of untreated psychosis has been associated with better prognosis in schizophrenia. In this study, we measured the duration mismatch negativity (dMMN), an event-related potential, and cognitive performance in subjects with at-risk mental state (ARMS), patients with first-episode or chronic schizophrenia, and healthy volunteers. The main interest was to determine if these neurocognitive measures predict progression to overt schizophrenia in ARMS subjects.

Methodology/Principal Findings: Seventeen ARMS subjects, meeting the criteria of the Comprehensive Assessment of At-Risk Mental State, 31 schizophrenia patients (20 first-episode and 11 chronic) and healthy controls (N = 20) participated in the study. dMMN was measured by an auditory odd-ball paradigm at baseline. Neuropsychological performance was evaluated by the Japanese version of the Brief assessment of cognitive function of schizophrenia (BACS-J). The first-episode schizophrenia group showed significantly smaller amplitudes at frontal electrodes than did control subjects whereas chronic patients elicited smaller amplitudes at frontal and central electrodes, consistent with previous reports. During the follow-up period, 4 out of the 17 ARMS subjects transitioned to schizophrenia (converters) while 13 did not (non-converters). Specifically, dMMN amplitudes of non-converters did not differ from those of healthy controls, while converters showed significantly smaller dMMN amplitudes at some electrodes compared to control subjects. Converters performed significantly worse on tests of working memory, verbal fluency, and attention/information processing than did non-converters. There was a significant positive correlation between dMMN amplitudes at the frontal electrodes and verbal fluency, as measured by the BACS, in the AMRS subjects as a whole.

Conclusions/Significance: ARMS subjects who later developed schizophrenia elicited smaller dMMN amplitudes to begin with, compared to non-converters. Notably, we have provided the first evidence for the ability of verbal fluency to predict dMMN amplitudes in ARMS subjects. These findings are expected to add to the efforts for early diagnosis and intervention of schizophrenia.

Citation: Higuchi Y, Sumiyoshi T, Seo T, Miyanishi T, Kawasaki Y, et al. (2013) Mismatch Negativity and Cognitive Performance for the Prediction of Psychosis in Subjects with At-Risk Mental State. PLoS ONE 8(1): e54080. doi:10.1371/journal.pone.0054080

Editor: Tadafumi Kato, RIKEN Brain Science Institution, Japan

Received August 16, 2012; **Accepted** December 5, 2012; **Published** January 17, 2013

Funding: This work was supported by grants-in-aid for Scientific Research from Japan Society for the Promotion of Science, grants-in-aid from the Ministry of Health, Labour and Welfare, Japan, and SENSHIN Medical Research Foundation.The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: tomikisumiyoshi840@hotmail.com

Introduction

Schizophrenia usually develops around the adolescence period, with the whole life risk of about 0.85% [1]. Patients with schizophrenia suffer from positive symptoms (hallucination, delusion, thought disturbance, and etc.), negative symptoms (blunted affect, lack of volition, social withdrawal and etc.), and impairment in a range of cognitive domains, e.g. several types of memory, executive function, attention, verbal fluency [2,3,4,5]. Especially, cognitive function is considered to be a major determinant of outcome, including quality of life and social function [6]. It is interesting that the classification of cognitive domains differs across neuropsychological test batteries. For example, verbal fluency is categorized as an independent domain in the Brief Assessment of Cognition in Schizophrenia (BACS) [7,8], while it is regarded as one of the components of processing speed (of information) in the Measurement and Treatment Research to Improve Cognition in Schizophrenia – Consensus Cognitive Battery [9].

In order to achieve satisfactory long-term outcome, early detection, intervention and treatment of schizophrenia are needed. Specifically, a shorter duration of untreated psychosis (DUP) has been associated with a greater response to antipsychotic drugs in terms of symptoms and quality of life [10]. Prolonged DUP is also associated with decreased levels of social functions, for example, work function and communication skills, as well as longer

Table 1. Demographic and clinical data and dMMN amplitude.

	Healthy controls(n = 20)	ARMS(n = 17)	First episode schizophrenia(n = 20)	Chronic schizophrenia (n = 11)
Male/female	14/6	4/13	9/11	6/5
Age (years)	25.4 (6.9), range 16–45	19.4 (4.4)*, range, 15–29	27.2 (7.3), range 16–38	28.1 (8.0), range 18–44
Age of onset (years)	–	–	26.5 (7.1)	20.2 (4.7)
Duration of illness (years)	–	–	0.65 (0.51)	7.9 (6.9)
Drug dose a)	–	0.1(0.4)	2.1(2.3)	3.2 (2.4)
SAPS	–	13.2 (9.3)	15.7 (13.1)	17.6 (19.1)
SANS	–	50.3 (20.1)	53.8 (25.9)	51.5 (26.1)
dMMN amplitude[μV]				
F3	7.5 (1.3)	7.6 (2.2)	5.3 (1.5) **	4.5 (1.0) **
F4	7.3 (1.2)	7.5 (2.1)	5.6 (1.8) *	5.0 (1.3) **
Fz	7.9 (1.1)	7.9 (2.1)	5.6 (1.7) **	5.1 (1.7) **
Cz	6.6 (1.5)	6.6 (2.2)	5.1 (1.5)	4.2 (1.7) **
Pz	4.5 (1.7)	4.2 (2.0)	3.5 (1.2)	2.5 (1.0) **

Values represent mean (SD).
a) Risperidone equivalent [mg/day].
ARMS, at-risk mental state.
SAPS, Scale for the Assessment of Positive Symptoms;
SANS, Scale for the Assessment of Negative Symptoms.
*p<0.05 and ** p<0.01, compared to healthy control.
doi:10.1371/journal.pone.0054080.t001

hospitalization [11,12,13,14,15,16,17]. In this context, it was reasonable that recent efforts have been directed to subjects with "at-risk mental state (ARMS)" or "ultra-high risk patients." [18].

For the purpose of early diagnosis, objective biomarkers, particularly, those based on brain morphology, neurophysiology, and neuropsychology have been reported to provide useful information [19,20,21,22,23,24]. Neurophysiological measurements, such as event-related potentials (ERPs), have been suggested to provide a biological substrate for some aspects of cognitive disturbances of schizophrenia. Especially, P300, mismatch negativity (MMN), or N400 etc. are widely used ERPs for this purpose. For example, schizophrenia patients show smaller amplitudes of P300 than normal control subjects [25,26,27]. Reduction of P300 amplitudes has been also noted in subjects with ARMS, part of which develops schizophrenia [28]. P300 has been shown to be affected by various factors, including medication [20,29,30] suggesting the utility as a state marker of psychotic disorders.

MMN is another component of ERPs generated in response to occasional variations (e.g., duration, frequency, intensity) of acoustic stimuli, and is suggested to reflect pre-attentive cognitive operations [31,32]. MMN amplitudes have been shown to be decreased in patients with schizophrenia, as indicated by a recent meta-analysis [33] reporting a large effect size. Unlike the case with P300, MMN amplitudes are generally not affected by psychotropic drug, for example benzodiazepines [34], dopamine antagonists [35]. For these reasons, MMN is considered to provide a trait marker for schizophrenia.

There are several types of MMNs, such as duration MMN (dMMN) and frequency MMN (fMMN), based on the mode of presentation of stimuli. Attenuation of the fMMN amplitude, resulting from changes in the frequency of stimuli, reflects the progress of the disease, i.e. a function of duration of the illness. On the other hand, deficits of dMMN deficiency, resulting from changes in the duration of stimuli, may be more closely linked to the genetic aspect of schizophrenia [36]. Thus, impairment of dMMN is greater than that of fMMN [37], with the latter emerging only in the chronic, but not early stage of schizophrenia [38,39].

Recently, dMMN amplitudes have been shown to be reduced already in the prodromal stage of schizophrenia. Thus, Jahshan et al (2011) found dMMN amplitudes in subjects with at-risk for psychosis patients were reduced compared to normal controls, but the deficits were milder than those in patients with first episode schizophrenia [40]. Atkinson et al (2011) report that dMMN amplitudes were reduced as early as in the ultra-high risk stage [41]. This finding was extended by Bodatsch et al (2011) [42] and Shaikh et al (2012) [43], who observed smaller dMMN amplitudes in drug-naïve subjects with ARMS who later converted to overt psychosis, compared to those in non-converters. Thus, reduced dMMN amplitudes have been regarded to provide a biomarker to predict the development of schizophrenia.

Cognitive impairment, a core symptom of schizophrenia, is present at onset of illness [44], and is closely related to functional outcome [45]. Carrion et al. (2011) observed that cognitive and functional impairments are already evident in ultra-high risk patients before the onset of psychosis. Specifically, attention/processing speed was found to predict progression to psychosis [46]. On the other hand, Frommann et al. (2011) report prodromal patients were impaired in all neurocognitive domains, such as learning memory, executive control, processing speed, and working memory. These findings indicate neuropsychological measures, particularly attention/processing speed, provide another cognitive modality to identify high-risk people vulnerable to developing overt schizophrenia [47].

To date, little information is available about the relationship between neurophysiological indices, e.g. dMMN, and neuropsychological performance. So far, Lin et al (2012) investigated the correlation between neuropsychological performance and MMN amplitudes only in patients with schizophrenia [21]. For example, demonstration of the ability of some measures of neuropsychological performance, e.g. attention/information processing and

Table 2. Comparison between converters and non-converters of ARMS subjects.

	ARMS (n = 17)		Analyze of variance (df = 1,16), Group Effect	
	Non-C. (n = 13)	Conv. (n = 4)	F	p
Male/female	2/11	2/2		
Age [years]	18.5 (3.8), range 15–29	22.3 (5.6), range 17–30		
Drug dose [a)]	–	0.5 (0.7)		
SAPS	11.4 (9.3)	18.0 (8.6)		
SANS	42.9 (15.9)	69.0 (18.4) *		
dMMN amplitude[μV]				
F3	8.2 (2.0)	5.6 (1.7)	3.78	n.s.
F4	8.2 (1.6)	5.2 (1.8)	10.61	0.05
Fz	8.6 (1.6)	5.7 (2.0)	8.25	0.01
Cz	7.3 (1.8)	4.3 (1.7)	8.31	0.01
Pz	4.8 (1.8)	2.4 (1.2)	4.74	0.04
BACS-J				
Verbal memory	51.0 (7.8)	47.2 (11.3)	0.57	n.s.
Working memory	19.1 (3.2)	14.7 (2.2)	6.33	0.02
Motor function	69.3 (12.5)	60.5 (9.0)	1.66	n.s.
Verbal fluency	46.7 (12.1)	29.0 (9.5)	7.03	0.01
Attention	74.0 (12.7)	56.2 (5.8)	7.05	0.01
Executive function	17.8 (2.1)	18.5 (2.6)	0.24	n.s.

Values represent mean (SD).
a) Risperidone equivalent [mg/day].
ARMS, at-risk mental state.
Non-C., ARMS non-converters; Conv., ARMS converters.
SAPS, Scale for the Assessment of Positive Symptoms;
SANS, Scale for the Assessment of Negative Symptoms;
BACS-J, Brief Assessment of Cognition in Schizophrenia, Japanese version.
*p<0.05 compared to Non-C. (student's t-test).
doi:10.1371/journal.pone.0054080.t002

verbal fluency, to predict dMMN activity would greatly facilitate the early intervention practice, as the former indices require only a limited time constraint. Moreover, such evidence, if obtained, would help more precisely identify biological features of the prodromal phase of schizophrenia.

In this study, we measured dMMN amplitudes and cognitive performance in subjects with ARMS, first episode schizophrenia, or chronic phase of the illness, and compared them with those of normal control subjects. Specifically, we compared the results from ARMS subjects who later developed schizophrenia (converters) and those who did not (non-converters). The hypotheses tested were; 1) if correlations exist between the decrease in dMMN amplitudes and the impairment of neuropsychological performance in subjects with ARMS, and 2) if the impairments of neurophysiological and neuropsychological functions would similarly predict progression to overt psychosis in these subjects.

Methods

Ethics Statement

This protocol was approved by the Committee on Medical Ethics of the University of Toyama. After complete and detail description of the study to the subjects, written informed consent was obtained.

Clinical staff explained the nature of the study to the subjects, the risks and benefits, and the option not to participate in research. If the mental status of a subject was impaired to the point where s/he could not understand these issues, the subject was not approached to be in the research. In case there was a possibility that the capacity of a participant to consent was compromised, an additional consent was obtained from next of kin, care takers, or guardians of such subject.

Participants

Diagnosis was made based on the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia and the Comprehensive Assessment of At-Risk Mental State (CAARMS) for ARMS [48], by experienced psychiatrists. Most of these subjects were referred from "Psychiatric Health and Welfare Center of Toyama (PHWCT). Seventeen ARMS subjects followed at the University of Toyama Hospital participated in this study. [male/female = 4/13; mean (S.D.) age = 19.4(4.4)]. Thirty-one schizophrenia patients also participated in this study. Patients with duration of illness less than two years were defined as first episode schizophrenia (FES) [n = 20; male/female = 9/11; mean (S.D.) age = 27.2(7.3)], while those with duration of illness 2 years or longer were defined as chronic schizophrenia (CS) [n = 11; male/female = 6/5; mean (S.D.) age = 28.1(8.0)]. We recruited normal control subjects from the community by advertisements. They are healthy volunteers [n = 20; male/female = 14/6; mean (S.D.) age = 25.4(6.9)] without any personal history of psychiatric illnesses, including schizophrenia or other psychotic disorders.

All participants were right-handed. A psychiatric and treatment history was obtained from the subjects, families, and medical

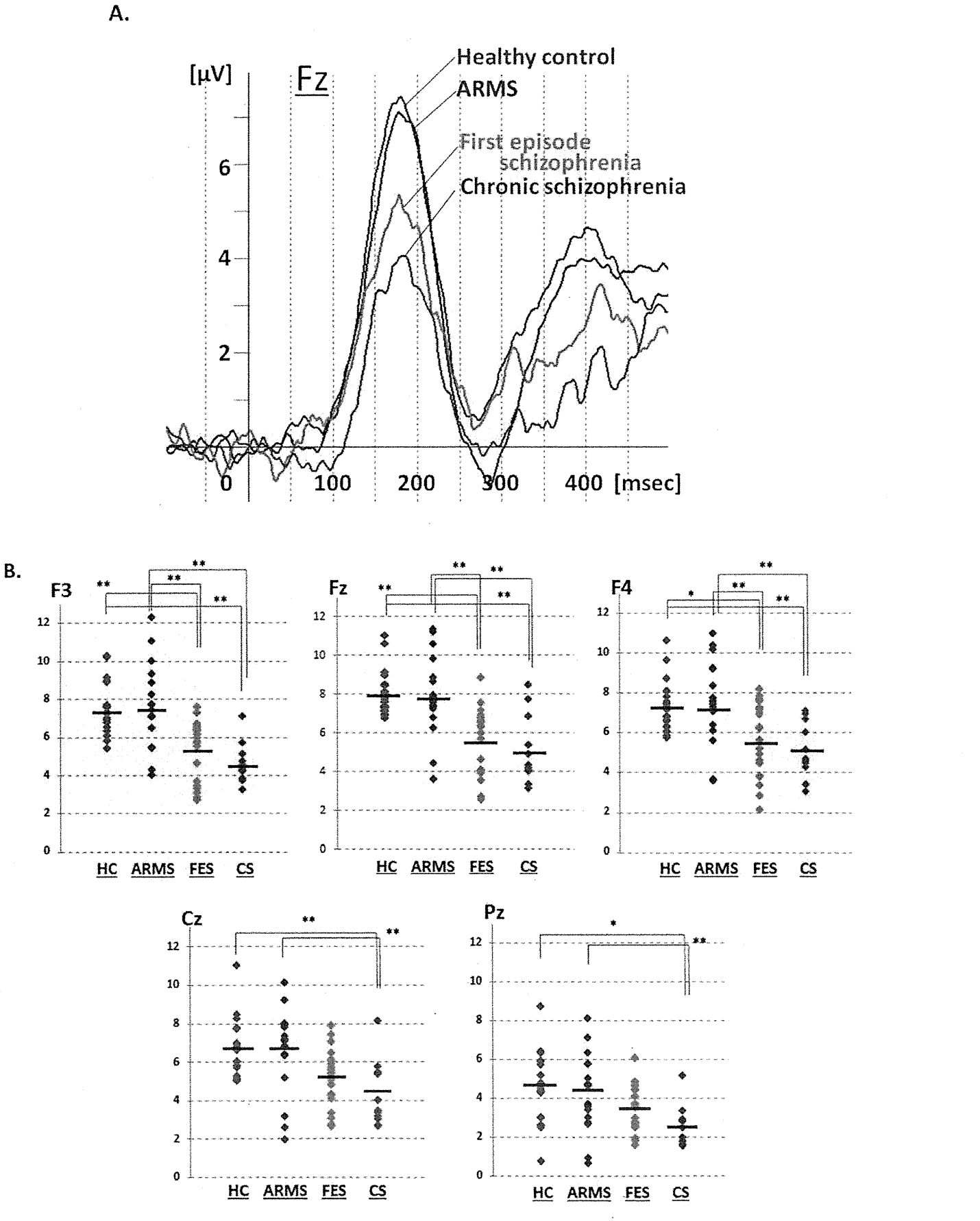

A.
[μV]
Fz
Healthy control
ARMS
First episode schizophrenia
Chronic schizophrenia
6
4
2
0
100
200
300
400 [msec]
B.
F3
Fz
F4
Cz
Pz
HC ARMS FES CS

Figure 1. Duration mismatch negativity (dMMN) waveform at Fz and scatterplots of dMMN amplitudes for all subjects. A. Waveforms are presented for healthy controls (HC, blue line), at-risk mental state (ARMS, red line), first episode schizophrenia (FES, light green line) and chronic schizophrenia (CS, dark green line). B. Distribution of amplitudes are presented for healthy controls (HC, blue dots), ARMS (red dots), first episode schizophrenia (light green dots) and chronic schizophrenia (dark green dots). * $p<0.05$ and ** $p<0.01$, compared to each groups.
doi:10.1371/journal.pone.0054080.g001

records. Subjects with a current history of substance abuse or dependence, seizure or head injury were excluded from the study. Eligible patients had a complete physical examination and standard laboratory testing was normal. Demographic data at baseline evaluation are shown in Table 1.

ARMS subjects were followed-up continuously at the hospital. Four out of the 17 ARMS subjects transitioned to schizophrenia during the observation period. When DSM-IV criteria were met, e.g. auditory hallucinations persisted or any delusion (for example, disturbance of the self) clearly observed, the subject was regarded to have converted to schizophrenia (converters; Conv.). Subjects who did not develop psychosis were defined as non-converters (Non-C.). The average observation period for ARMS subjects was 2.1±1.1 (Non-C.; 1.6±0.8) years.

Clinical Assessment

The Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) [49] were administered by an experienced psychiatrist. These data are shown in Table 1.

Neuropsychological Assessments

Neuropsychological performance, measured by the Japanese version of the BACS (BACS-J) [8], was evaluated by experienced psychiatrists or psychologists. The BACS-J cognitive battery uses the following assessments in the respective targeted domains: list learning (verbal memory), digit sequencing task (working memory), token motor task (motor function), category fluency and letter fluency (verbal fluency), symbol coding (attention and processing speed), and the Tower of London test (executive function), as shown in Table 1.

Electroencephalogram Recording

Electroencephalograms (EEGs) were recorded based on the previous report of our laboratory [20,30,50,51,52,53]. A 32-channel DC-amplifier (EEG-2100 version 2.22J,Nihon Kohden Corp., Tokyo, Japan), according to the international 10–20 system was used, and recordings were performed using an electro cap (Electrocap Inc., Eaton, OH) in a sound-attenuated room. Data were collected with a sampling rate of 500 Hz. All electrodes were referred to the average amplitude of the ear electrodes (bandwidth = 0.53–120 Hz, 60 Hz notch filter). Electrode impedance was less than 5 kΩ.

Measurements of dMMN were based on our previous report [53]. One thousand auditory stimuli were delivered binaurally through headphones with inter-stimulus intervals 500 msec. Standard/target tones of 50/100 msec duration were randomly presented with the presentation probability of 0.9/0.1. All tones were 60 dB, 1000 Hz and with a rise-fall time of 10 msec. The subjects were requested to watch silent animation movie (Tom and Jerry) and pay attention to the monitor and ignore the tones.

Averaging of ERP waves and related procedures were performed using Vital Tracer and EPLYZER II software (Kissei Comtec, Co. Ltd. Nagano, Japan). Epochs were 600 msec, including a 100-msec pre-stimulus baseline. Eye movement artifacts (blinks and eye movements) were manually rejected. MMN waveforms were obtained by subtract standard waveforms from target ones. ERP component peaks were identified within the 150–250 msec search windows. We selected F3, F4, Fz, Cz and Pz electrodes for analysis, based on our previous report [49].

Statistical Methods

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 19.0 (SPSS Japan Inc., Tokyo, Japan). In order to investigate group differences in MMN, repeated measures analysis of variance (ANOVA) with electrode site as within-subject variable and diagnostic group as between-subject variable was performed. BACS-J domain scores were analyzed with a two-way ANOVA with BACS-J domains as the within factor and group as the between factor. Group×electrode interactions and group×BACS-J domain score interactions were decomposed using one-way ANOVA, with Bonferroni correction. Relationships between MMN amplitudes at the Fz electrode and BACS -J domain scores were analyzed using Spearman rank correlations.

Raters (psychiatrist, psychologist) were not informed of subjects' profiles and diagnosis.

Results

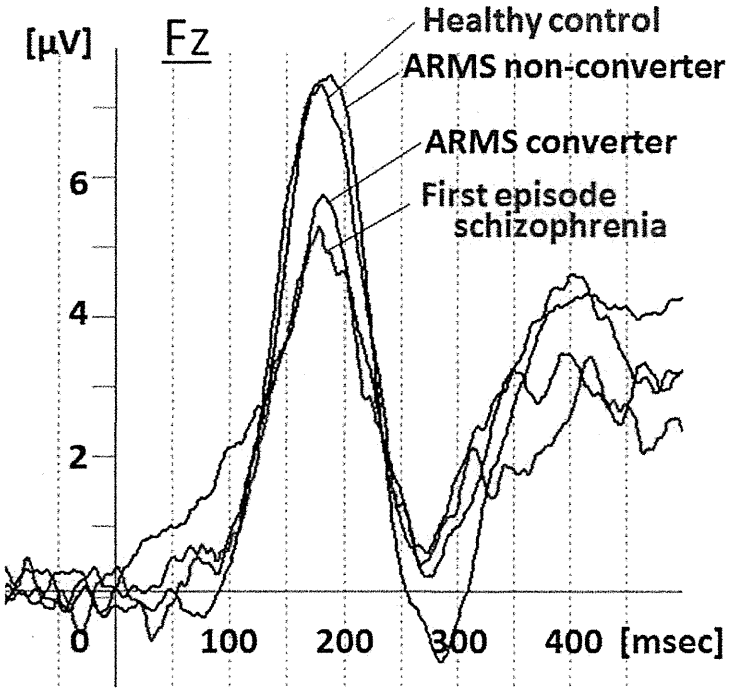
Subjects' Profile

Demographic data of participants are shown in Tables 1 and 2. There was significant group difference in age [$F(3,64)=5.51$, $p=0.02$]. The ARMS group was significantly younger than other groups. The female to male ratio in the ARMS group was significantly greater than that in the normal control group [$\chi^2=7.94$, $p=0.004$]. There was no difference between Conv. and Non-C. in age ($p=0.14$). The male/female ratio of Conv. was greater than Non-C.[$\chi^2=4.41$, $p=0.01$]. Fourteen out of 17 ARMS subjects were not taking any medication, and 3 were prescribed a small dose of risperidone (1.5 mg/day), aripiprazole (6 mg/day), and sulpiride (150 mg/day), respectively, for (or to prevent) acute psychosis episodes (sometimes with strong agitation), based on the criteria of International Early Psychosis Association Writing Group [54]. MMN recordings for these subjects were conducted shortly after medications were started (9, 15 and 27 days). All of the three subjects subsequently developed schizophrenia. Schizophrenia patients were taking the following treatment; FES (no medication 7, risperidone 3, perospirone 3, aripiprazole 2, olanzapine 1, sulpiride 1, blonanserin+quetiapine 1, risperidone+quetiapine 1, risperidone+zotepine 1.), CS (no medication 1, perospirone 3, risperidone 2, olanzapine 2, zotepine 1, perospirone+olanzapine 1, perospirone+aripiprazole 1). There were no differences between ARMS, FES and CS groups in SAPS [$F(2,47)=0.457$, $p=0.636$] and SANS [$F(2,47)=0.118$, $p=0.889$] scores. Conv. and Non-C. groups did not differ in the SAPS score. However, Conv. group showed a significantly higher score of SANS than Non-C. group (69.0±18.4 vs. 42.9±15.9, $p=0.02$).

Comparisons of dMMN Amplitudes between Healthy Controls vs. ARMS vs. Schizophrenia

dMMN data are shown in Table 1 and Figure 1. Grand average waveforms in the Fz lead and scatterplots for the electrodes sites are shown in Figure 1A and 1B. ARMS subjects showed dMMN amplitudes similar to those of healthy control subjects. On the

A.


[μV]
Fz
Healthy control
ARMS non-converter
ARMS converter
First episode schizophrenia
6
4
2
0
100
200
300
400 [msec]

B.

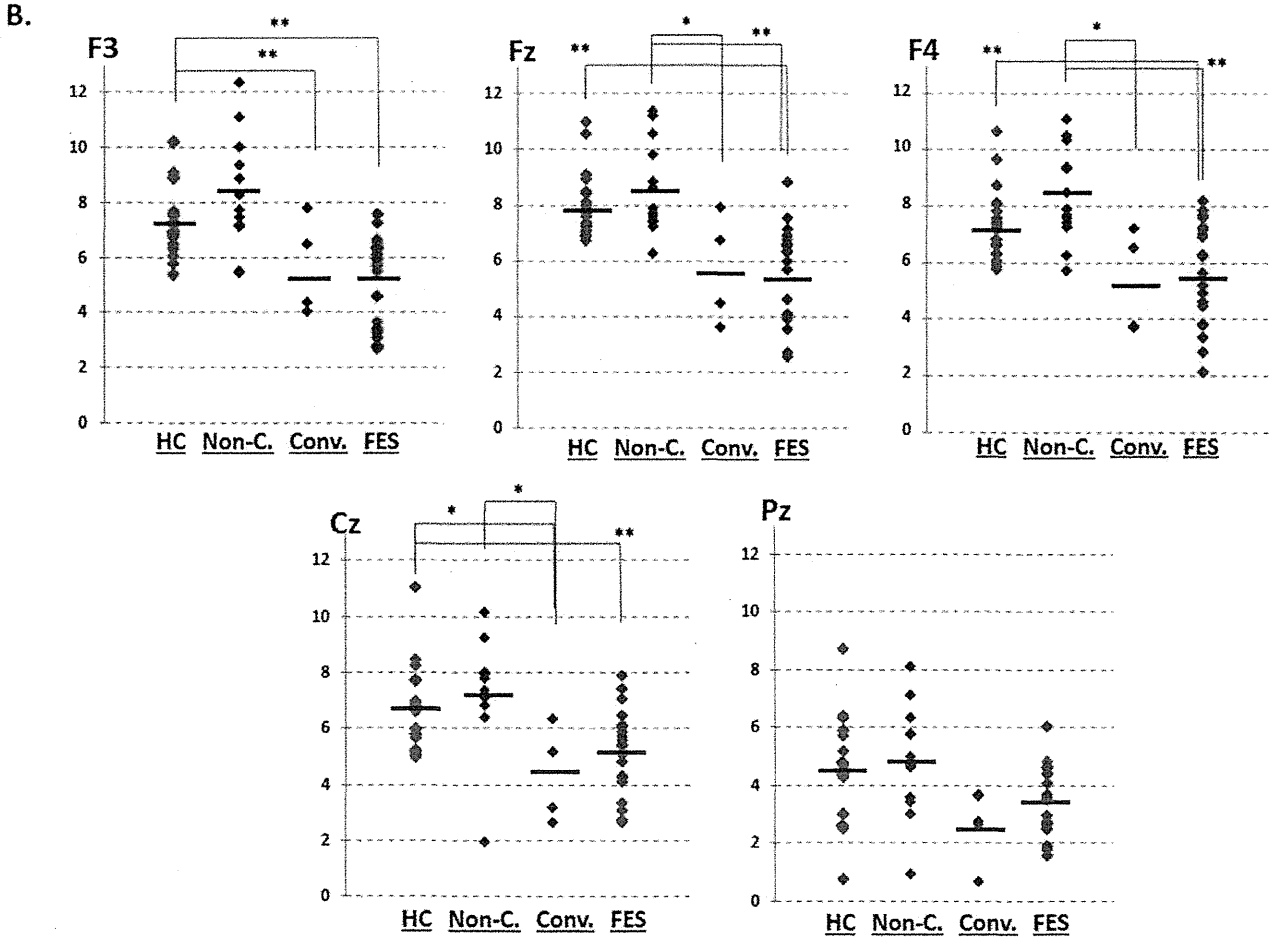

F3
Fz
F4
Cz
Pz
HC Non-C. Conv. FES

Figure 2. dMMN waveform at Fz and scatterplots of dMMN amplitude for at-risk mental state (ARMS,) healthy control (HC) and first episode schizophrenia (FES) subjects. A. Waveforms are presented for healthy controls (blue line), ARMS, converters (Conv.) and non-converter (Non-C.) (black lines), FES (light green line). B. Distribution of amplitudes are presented for healthy controls (blue dots), ARMS, converters (Conv.) and non-converter (Non-C.) (black dots), FES (light green dots). * $p<0.05$ and ** $p<0.01$, compared to each groups.
doi:10.1371/journal.pone.0054080.g002

other hand, FES group showed significantly smaller dMMN amplitudes at frontal electrodes (F3, F4 and Fz). Patients with CS showed greater amplitude reductions at all electrodes compared to healthy controls.

Comparisons of dMMN Amplitudes: Conv. vs. Non-C

Conv. subjects showed significant reduction in dMMN amplitudes at F4, Fz, Cz, and Pz electrode sites compared with Non-C. subjects (Table 2, Figure 2A). Waveforms of Conv. were similar to those of first-episode schizophrenia. By contrast, waveforms of Non-C. resembled to those of healthy controls (Figure 2A). Scatterplots of dMMN amplitudes are shown in Figure 2B. Non-C. subjects elicited larger dMMN amplitudes compared to those of Conv. Amplitudes of Non-C. did not differ from those of healthy controls. On the other hand, Conv. showed significantly smaller dMMN amplitudes at F3 and Cz compared to control subjects. There were no differences in dMMN amplitudes at any electrode between Conv. and FES subjects.

Neuropsychological Measurements: Conv. vs. Non-C

Conv. subjects demonstrated significantly smaller BACS-J scores compared to Non-Conv. subjects for working memory, verbal fluency, and attention (Table 2, Figure 3).

Relationship between Cognitive Performance and dMMN Amplitudes in ARMS subjects

Figure 4 demonstrates correlations between dMMN amplitudes and BACS scores in subjects with ARMS. Significant positive correlations were noted for verbal fluency ($r=0.546$, $p=0.02$; Figure 4A), but not other cognitive domains (data not shown). Also, scores of letter fluency task and category fluency task from the BACS-J [55] were significantly correlated with dMMN amplitudes in subjects with ARMS (Figure 4B,C).

Discussion

To our knowledge, this study is the first to report a relationship between dMMN amplitudes and neuropsychological performance in individuals with ARMS. ARMS subjects who later converted to overt schizophrenia elicited reduced dMMN amplitudes at frontal

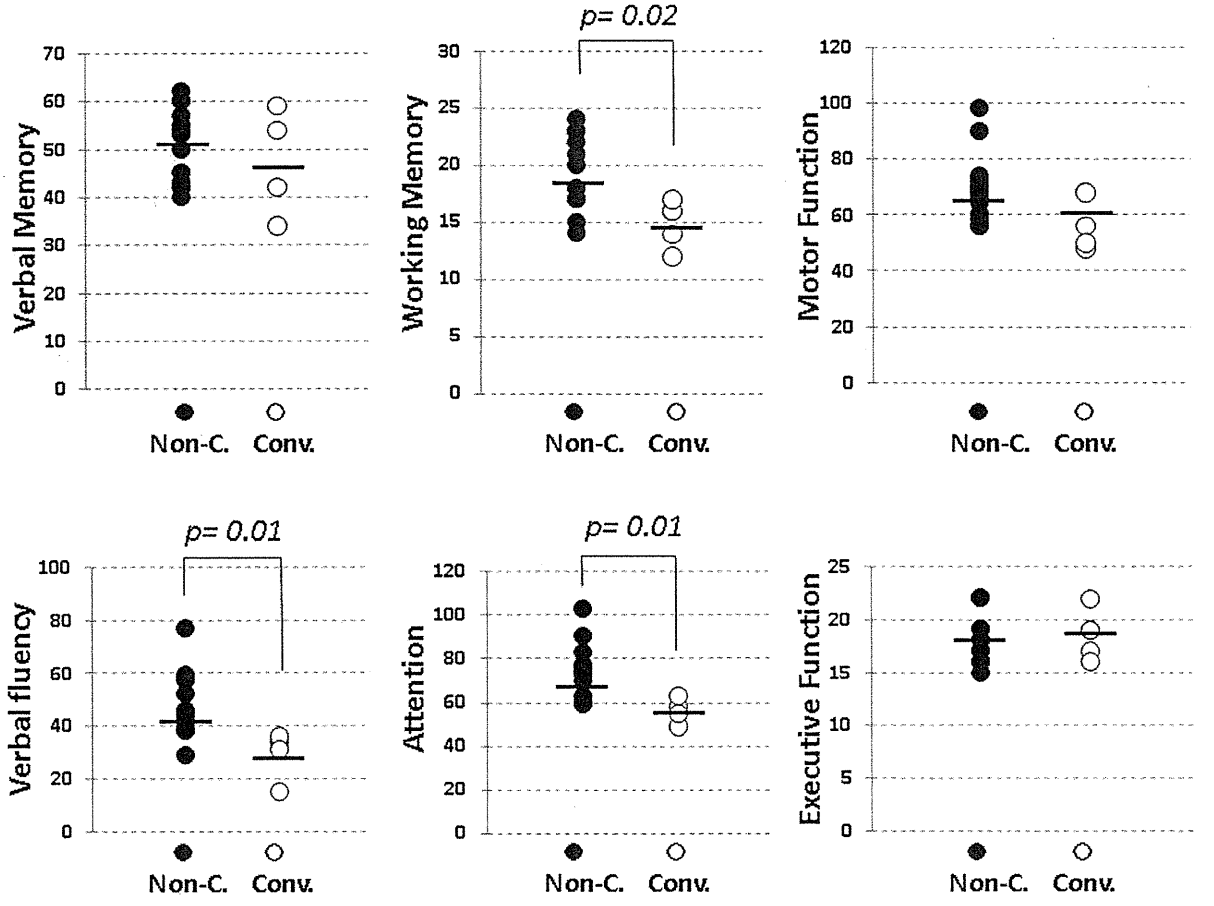


Figure 3. Scatterplot of the score of BACS-J for ARMS subjects. Black symbols(●) and white ones(○) represent scores of non-converters and converters, respectively.
doi:10.1371/journal.pone.0054080.g003

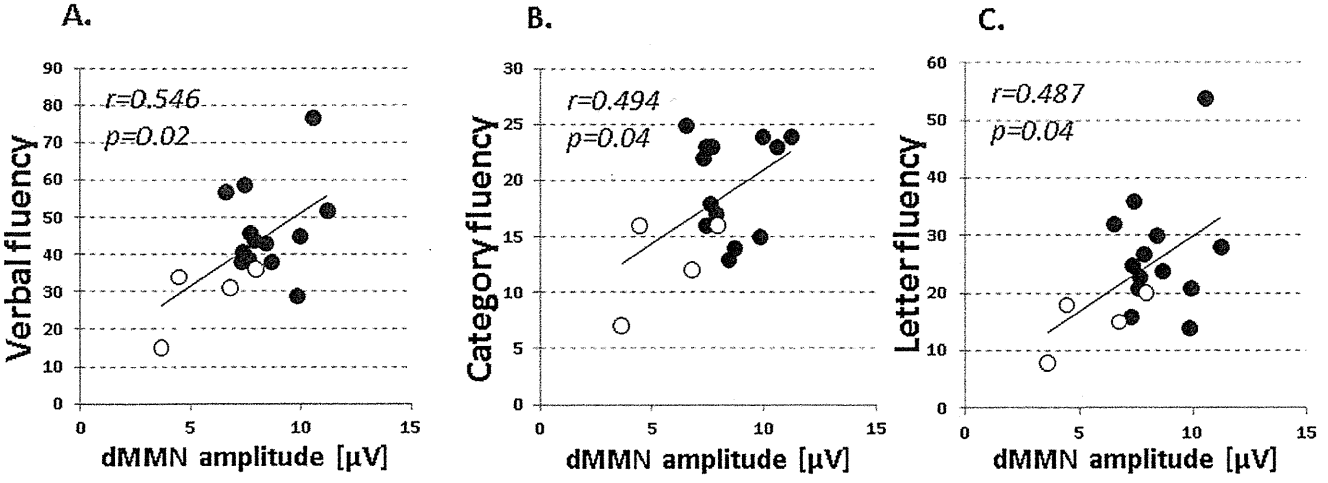


Figure 4. Correlations between dMMN amplitudes at Fz lead and performance on the verbal fluency tasks from the BACS-J in ARMS subjects. Black and white symbols represent scores of non-converters and converters, respectively. Relationships were analyzed using Pearson's product-moment correlation coefficient.
doi:10.1371/journal.pone.0054080.g004

and central leads compared with non-converters and normal subjects, consistent with previous reports [42,43]. In addition, verbal fluency, working memory and attention/information processing were more greatly impaired in converters compared to non-converters at baseline. Further a significant correlation was noted between performance on verbal fluency tasks and dMMN amplitudes in ARMS subjects. First episode schizophrenia patients showed significantly smaller dMMN amplitudes than ARMS subjects and healthy controls, consistent with previous observations [40,56]. Yung et al. (2003) [57] report that 10–40% of ARMS patients develop schizophrenia, consistent with our observations that 4 out of 17 (23.5%) subjects progressed to overt psychosis. Some previous studies report that ARMS subjects elicit reduced dMMN amplitudes, but with a lesser degree compared to patients with established schizophrenia [40,41,42]. By contrast, dMMN amplitudes of the entire ARMS subjects in the present study were not significantly different from those of healthy controls (Figure 1). One of the reasons for this discrepancy is the difference in age and the percentage of gender, as implicated by some previous studies [32,58,59].

The score of SANS/SAPS of ARMS were similar to schizophrenia (Table 1). We consider it was due, mainly, to the nature of the ARMS subjects studied here. Most of these subjects were referred from PHWCT. The PHWCT, a component of the Consultation and Support Service in Toyama(CAST), includes the Local Support Center for Social Withdrawal Young People that advertises its activity using internet home page and pamphlets. These systems mainly receive consultations from the family members of subjects with social withdrawal and/or disability. This may be why the ARMS subjects studied here elicited relatively severe negative symptoms comparable to those in subjects with overt schizophrenia. With regard to SAPS scores, part of the schizophrenia patients in this study had already been medicated, which may have decreased positive symptoms in these subjects. This may make the SAPS scores for ARMS group and schizophrenia groups look somewhat similar.

Compared to non-converters, dMMN amplitudes in converters were significantly reduced at F4, Fz, Cz and Pz leads (Table 2). This finding suggests dMMN amplitudes may be able to differentiate high-risk individuals who convert to schizophrenia from those who do not. Therefore, these electrophysiological findings are expected to facilitate early intervention of schizophrenia.

MMN is a pre-attentional response to a change of stimuli, and plays a critical role in establishing learning and memory. This electrophysiological event has been suggested to be generated by the glutamate (Glu)/N-methyl-D-aspartate (NMDA) system [60]. This theory is supported by the observation that administration of an NMDA-receptor antagonist (phencyclidine, MK-801 etc.) abolishes MMN in monkeys [61] and rats [62,63]. The pathophysiology of schizophrenia has been shown to be associated with the dysfunction of signal transduction through NMDA receptors [64]. Accordingly, Stone et al. (2009) report that ARMS subjects elicited reduced Glu levels in the thalamus, which was correlated with the gray matter volume of frontal and temporal lobes [65], the brain structures suggested to be involved in MMN generation [66,67]. In fact, the results of the present study (Table 2, Figure 2) indicate the ability of diminished dMMN to predict the development of schizophrenia, as in some previous reports [36–39], suggesting impaired NMDA-mediated transmissions provide an endophenotype for subjects vulnerable to the illness.

Neuropsychological deficits have been shown to exist in the early stage of schizophrenia [46,47]. In this study, neuropsychological performance, as measured by the BACS, differentiated between converters and non-converters in ARMS subjects. Compared with non-converters, scores of working memory, verbal fluency and attention in converters were significantly less for converters (Table 2, Figure 3). These results indicate cognitive abilities, particularly those requiring attention/information processing speed, provides a sensitive marker predicting the development of schizophrenia in vulnerable individuals.

The major finding of the present study was the ability of performance on the verbal fluency tasks to predict dMMN amplitudes in subjects with ARMS (Figure 4). The implications of these observations include the possibility of enhancing accuracy to identify subjects diagnosed with "ultra-high risk" who later develop psychosis. Another advantage is that some neuropsychological tests, which only require a shorter time constraint, could substitute for electrophysiological measurements, e.g. ERPs. In fact, verbal fluency test only requires less than 5 minutes. The easiness of assessment would facilitate the screening for subjects whose psychiatric conditions would not allow them to undergo

ERPs measurement, which generally takes more than 30 minutes. On the other hand, neuropsychological evaluations may sometimes be influenced by motivation of examinees. Therefore, combined administration of neurophysiological and neuropsychological assessments would facilitate screening procedures, depending on the condition of patients. In sum, these efforts are likely to lead to improvement of functional outcome for vulnerable subjects through early intervention by objective probes with greater sensitivity and specificity.

In conclusion, this study confirmed that ARMS subjects who later develop schizophrenia elicit smaller dMMN amplitudes to begin with, compared to non-converters. Notably, we have provided the first evidence for the ability of verbal fluency or attention/information processing to predict dMMN amplitudes in ARMS subjects. These findings are expected to add to the efforts for early diagnosis and intervention of schizophrenia.

Limitations

The main limitations of this study include that ARMS subjects were younger and had a larger female/male ratio compared to other groups. Clearly, further study with a larger number of matched subjects is warranted. Part of ARMS subjects was taking antipsychotic drugs which is another limitation of the study.

The observation periods of Non-C. were relatively short (1.6±0.8 year), compared to similar studies [42,43], which might be another limitation.

Author Contributions

Conceived and designed the experiments: YH T. Sumiyoshi. Performed the experiments: YH T. Seo TM. Analyzed the data: YH YK. Contributed reagents/materials/analysis tools: T. Sumiyoshi MS. Wrote the paper: YH T. Sumiyoshi.

References

1. Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, et al. (1992) Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychol Med Monogr Suppl 20: 1–97.
2. Meltzer HY, Sumiyoshi T (2003) Atypical antipsychotic drugs improve cognition in schizophrenia. Biol Psychiatry 53: 265–267; author reply 267–268.
3. Sumiyoshi T, Jayathilake K, Meltzer HY (2003) The effect of melperone, an atypical antipsychotic drug, on cognitive function in schizophrenia. Schizophr Res 59: 7–16.
4. Harvey PD, Green MF, Keefe RS, Velligan DI (2004) Cognitive functioning in schizophrenia: a consensus statement on its role in the definition and evaluation of effective treatments for the illness. J Clin Psychiatry 65: 361–372.
5. Heinrichs RW, Zakzanis KK (1998) Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12: 426–445.
6. Green MF (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 153: 321–330.
7. Keefe RS, Goldberg TE, Harvey PD, Gold JM, Poe MP, et al. (2004) The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr Res 68: 283–297.
8. Kaneda Y, Sumiyoshi T, Keefe R, Ishimoto Y, Numata S, et al. (2007) Brief assessment of cognition in schizophrenia: validation of the Japanese version. Psychiatry Clin Neurosci 61: 602–609.
9. Green MF, Nuechterlein KH, Kern RS, Baade LE, Fenton WS, et al. (2008) Functional co-primary measures for clinical trials in schizophrenia: results from the MATRICS Psychometric and Standardization Study. Am J Psychiatry 165: 221–228.
10. Perkins DO, Gu H, Boteva K, Lieberman JA (2005) Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. Am J Psychiatry 162: 1785–1804.
11. Loebel AD, Lieberman JA, Alvir JM, Mayerhoff DI, Geisler SH, et al. (1992) Duration of psychosis and outcome in first-episode schizophrenia. Am J Psychiatry 149: 1183–1188.
12. Melle I, Larsen TK, Haahr U, Friis S, Johannesen JO, et al. (2008) Prevention of negative symptom psychopathologies in first-episode schizophrenia: two-year effects of reducing the duration of untreated psychosis. Arch Gen Psychiatry 65: 634–640.
13. Yamazawa R, Nemoto T, Kobayashi H, Chino B, Kashima H, et al. (2008) Association between duration of untreated psychosis, premorbid functioning, and cognitive performance and the outcome of first-episode schizophrenia in Japanese patients: prospective study. Aust N Z J Psychiatry 42: 159–165.
14. Galderisi S, Mucci A, Bitter I, Libiger J, Bucci P, et al. (2012) Persistent negative symptoms in first episode patients with schizophrenia: Results from the European First Episode Schizophrenia Trial. Eur Neuropsychopharmacol.
15. Chang WC, Hui CL, Tang JY, Wong GH, Lam MM, et al. (2011) Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. Schizophr Res 133: 22–28.
16. Edwards J, McGorry PD, Waddell FM, Harrigan SM (1999) Enduring negative symptoms in first-episode psychosis: comparison of six methods using follow-up data. Schizophr Res 40: 147–158.
17. Malla AK, Norman RM, Takhar J, Manchanda R, Townsend L, et al. (2004) Can patients at risk for persistent negative symptoms be identified during their first episode of psychosis? J Nerv Ment Dis 192: 455–463.
18. McGorry PD, Nelson B, Amminger GP, Bechdolf A, Francey SM, et al. (2009) Intervention in individuals at ultra-high risk for psychosis: a review and future directions. J Clin Psychiatry 70: 1206–1212.
19. Kawasaki Y, Suzuki M, Kherif F, Takahashi T, Zhou SY, et al. (2007) Multivariate voxel-based morphometry successfully differentiates schizophrenia patients from healthy controls. Neuroimage 34: 235–242.
20. Higuchi Y, Sumiyoshi T, Kawasaki Y, Matsui M, Arai H, et al. (2008) Electrophysiological basis for the ability of olanzapine to improve verbal memory and functional outcome in patients with schizophrenia: a LORETA analysis of P300. Schizophr Res 101: 320–330.
21. Lin YT, Liu CM, Chiu MJ, Liu CC, Chien YL, et al. (2012) Differentiation of schizophrenia patients from healthy subjects by mismatch negativity and neuropsychological tests. PLoS One 7: e34454.
22. Nakamura K, Kawasaki Y, Suzuki M, Hagino H, Kurokawa K, et al. (2004) Multiple structural brain measures obtained by three-dimensional magnetic resonance imaging to distinguish between schizophrenia patients and normal subjects. Schizophr Bull 30: 393–404.
23. Takayanagi Y, Takahashi T, Orikabe L, Mozue Y, Kawasaki Y, et al. (2011) Classification of first-episode schizophrenia patients and healthy subjects by automated MRI measures of regional brain volume and cortical thickness. PLoS One 6: e21047.
24. Takahashi T, Zhou SY, Nakamura K, Tanino R, Furuichi A, et al. (2011) A follow-up MRI study of the fusiform gyrus and middle and inferior temporal gyri in schizophrenia spectrum. Prog Neuropsychopharmacol Biol Psychiatry 35: 1957–1964.
25. Kawasaki Y, Maeda Y, Higashima M, Nagasawa T, Koshino Y, et al. (1997) Reduced auditory P300 amplitude, medial temporal volume reduction and psychopathology in schizophrenia. Schizophr Res 26: 107–115.
26. Roth WT, Pfefferbaum A, Horvath TB, Berger PA, Kopell BS (1980) P3 reduction in auditory evoked potentials of schizophrenics. Electroencephalogr Clin Neurophysiol 49: 497–505.
27. Bruder GE, Tenke CE, Towey JP, Leite P, Fong R, et al. (1998) Brain ERPs of depressed patients to complex tones in an oddball task: relation of reduced P3 asymmetry to physical anhedonia. Psychophysiology 35: 54–63.
28. Ozgurdal S, Gudlowski Y, Witthaus H, Kawohl W, Uhl I, et al. (2008) Reduction of auditory event-related P300 amplitude in subjects with at-risk mental state for schizophrenia. Schizophr Res 105: 272–278.
29. Umbricht D, Javitt D, Novak G, Bates J, Pollack S, et al. (1998) Effects of clozapine on auditory event-related potentials in schizophrenia. Biol Psychiatry 44: 716–725.
30. Sumiyoshi T, Higuchi Y, Itoh T, Matsui M, Arai H, et al. (2009) Effect of perospirone on P300 electrophysiological activity and social cognition in schizophrenia: a three-dimensional analysis with sloreta. Psychiatry Res 172: 180–183.
31. Naatanen R, Paavilainen P, Rinne T, Alho K (2007) The mismatch negativity (MMN) in basic research of central auditory processing: a review. Clin Neurophysiol 118: 2544–2590.
32. Naatanen R, Kujala T, Escera C, Baldeweg T, Kreegipuu K, et al. (2012) The mismatch negativity (MMN)–a unique window to disturbed central auditory processing in ageing and different clinical conditions. Clin Neurophysiol 123: 424–458.
33. Umbricht D, Krljes S (2005) Mismatch negativity in schizophrenia: a meta-analysis. Schizophr Res 76: 1–23.
34. Kasai K, Yamada H, Kamio S, Nakagome K, Iwanami A, et al. (2002) Do high or low doses of anxiolytics and hypnotics affect mismatch negativity in schizophrenic subjects? An EEG and MEG study. Clin Neurophysiol 113: 141–150.
35. Leung S, Croft RJ, Baldeweg T, Nathan PJ (2007) Acute dopamine D(1) and D(2) receptor stimulation does not modulate mismatch negativity (MMN) in healthy human subjects. Psychopharmacology (Berl) 194: 443–451.
36. Naatanen R, Kahkonen S (2009) Central auditory dysfunction in schizophrenia as revealed by the mismatch negativity (MMN) and its magnetic equivalent MMNm: a review. Int J Neuropsychopharmacol 12: 125–135.
37. Michie PT, Budd TW, Todd J, Rock D, Wichmann H, et al. (2000) Duration and frequency mismatch negativity in schizophrenia. Clin Neurophysiol 111: 1054–1065.
38. Todd J, Michie PT, Schall U, Karayanidis F, Yabe H, et al. (2008) Deviant matters: duration, frequency, and intensity deviants reveal different patterns of

mismatch negativity reduction in early and late schizophrenia. Biol Psychiatry 63: 58–64.
39. Salisbury DF, Shenton ME, Griggs CB, Bonner-Jackson A, McCarley RW (2002) Mismatch negativity in chronic schizophrenia and first-episode schizophrenia. Arch Gen Psychiatry 59: 686–694.
40. Jahshan C, Cadenhead KS, Rissling AJ, Kirihara K, Braff DL, et al. (2012) Automatic sensory information processing abnormalities across the illness course of schizophrenia. Psychol Med 42: 85–97.
41. Atkinson RJ, Michie PT, Schall U (2012) Duration mismatch negativity and P3a in first-episode psychosis and individuals at ultra-high risk of psychosis. Biol Psychiatry 71: 98–104.
42. Bodatsch M, Ruhrmann S, Wagner M, Muller R, Schultze-Lutter F, et al. (2011) Prediction of psychosis by mismatch negativity. Biol Psychiatry 69: 959–966.
43. Shaikh M, Valmaggia L, Broome MR, Dutt A, Lappin J, et al. (2012) Reduced mismatch negativity predates the onset of psychosis. Schizophr Res 134: 42–48.
44. Saykin AJ, Shtasel DL, Gur RE, Kester DB, Mozley LH, et al. (1994) Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. Arch Gen Psychiatry 51: 124–131.
45. Green MF, Kern RS, Heaton RK (2004) Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr Res 72: 41–51.
46. Carrion RE, Goldberg TE, McLaughlin D, Auther AM, Correll CU, et al. (2011) Impact of neurocognition on social and role functioning in individuals at clinical high risk for psychosis. Am J Psychiatry 168: 806–813.
47. Frommann I, Pukrop R, Brinkmeyer J, Bechdolf A, Ruhrmann S, et al. (2011) Neuropsychological profiles in different at-risk states of psychosis: executive control impairment in the early–and additional memory dysfunction in the late–prodromal state. Schizophr Bull 37: 861–873.
48. Yung AR, Yuen HP, McGorry PD, Phillips LJ, Kelly D, et al. (2005) Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. Aust N Z J Psychiatry 39: 964–971.
49. Andreasen NC (1990) Methods for assessing positive and negative symptoms. Mod Probl Pharmacopsychiatry 24: 73–88.
50. Itoh T, Sumiyoshi T, Higuchi Y, Suzuki M, Kawasaki Y (2011) LORETA analysis of three-dimensional distribution of delta band activity in schizophrenia: relation to negative symptoms. Neurosci Res 70: 442–448.
51. Sumiyoshi T, Higuchi Y, Kawasaki Y, Matsui M, Kato K, et al. (2006) Electrical brain activity and response to olanzapine in schizophrenia: a study with LORETA images of P300. Prog Neuropsychopharmacol Biol Psychiatry 30: 1299–1303.
52. Kawasaki Y, Sumiyoshi T, Higuchi Y, Ito T, Takeuchi M, et al. (2007) Voxel-based analysis of P300 electrophysiological topography associated with positive and negative symptoms of schizophrenia. Schizophr Res 94: 164–171.
53. Higuchi Y, Sumiyoshi T, Kawasaki Y, Ito T, Seo T, et al. (2010) Effect of tandospirone on mismatch negativity and cognitive performance in schizophrenia: a case report. J Clin Psychopharmacol 30: 732–734.
54. International Early Psychosis Association Writing Group (2005) International clinical practice guidelines for early psychosis. Br J Psychiatry Suppl 48: s120–124.
55. Kaneda Y, Sumiyoshi T, Nakagome K, et al. (2008) The brief assessment of cognition in schizophrenia Japanese version (BACS-J). Seishin Igaku 50: 913–917.
56. Kaur M, Battisti RA, Ward PB, Ahmed A, Hickie IB, et al. (2011) MMN/P3a deficits in first episode psychosis: comparing schizophrenia-spectrum and affective-spectrum subgroups. Schizophr Res 130: 203–209.
57. Yung AR, Phillips LJ, Yuen HP, Francey SM, McFarlane CA, et al. (2003) Psychosis prediction: 12-month follow up of a high-risk ("prodromal") group. Schizophr Res 60: 21–32.
58. Ikezawa S, Nakagome K, Mimura M, Shinoda J, Itoh K, et al. (2008) Gender differences in lateralization of mismatch negativity in dichotic listening tasks. Int J Psychophysiol 68: 41–50.
59. Matsubayashi J, Kawakubo Y, Suga M, Takei Y, Kumano S, et al. (2008) The influence of gender and personality traits on individual difference in auditory mismatch: a magnetoencephalographic (MMNm) study. Brain Res 1236: 159–165.
60. Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, et al. (2000) Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. Arch Gen Psychiatry 57: 1139–1147.
61. Javitt DC, Steinschneider M, Schroeder CE, Arezzo JC (1996) Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: implications for schizophrenia. Proc Natl Acad Sci U S A 93: 11962–11967.
62. Tikhonravov D, Neuvonen T, Pertovaara A, Savioja K, Ruusuvirta T, et al. (2008) Effects of an NMDA-receptor antagonist MK-801 on an MMN-like response recorded in anesthetized rats. Brain Res 1203: 97–102.
63. Eriksson J, Villa AE (2005) Event-related potentials in an auditory oddball situation in the rat. Biosystems 79: 207–212.
64. Brzustowicz LM, Simone J, Mohseni P, Hayter JE, Hodgkinson KA, et al. (2004) Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. Am J Hum Genet 74: 1057–1063.
65. Stone JM, Day F, Tsagaraki H, Valli I, McLean MA, et al. (2009) Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume. Biol Psychiatry 66: 533–539.
66. Turetsky BI, Calkins ME, Light GA, Olincy A, Radant AD, et al. (2007) Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophr Bull 33: 69–94.
67. Opitz B, Rinne T, Mecklinger A, von Cramon DY, Schroger E (2002) Differential contribution of frontal and temporal cortices to auditory change detection: fMRI and ERP results. Neuroimage 15: 167–174.

Psychiatry and Clinical Neurosciences 2013; 67: 540–548 doi:10.1111/pcn.12093

Regular Article

Increased pituitary volume in subjects at risk for psychosis and patients with first-episode schizophrenia

Tsutomu Takahashi, MD, PhD,[1,3]* Kazue Nakamura, MD, PhD,[1] Shimako Nishiyama, PhD,[1] Atsushi Furuichi, MD, PhD,[1] Eiji Ikeda, MD, PhD,[1] Mikio Kido, MD,[1] Yumiko Nakamura, MD,[1] Yasuhiro Kawasaki, MD, PhD,[4] Kyo Noguchi, MD, PhD,[2] Hikaru Seto, MD, PhD[2] and Michio Suzuki, MD, PhD[1,3]

Departments of [1]Neuropsychiatry and [2]Radiology, University of Toyama, Toyama, [3]Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo and [4]Department of Neuropsychiatry, Kanazawa Medical University, Uchinada, Japan

Aim: Enlarged pituitary gland has been reported in schizophrenia, possibly reflecting hypothalamic–pituitary–adrenal hyperactivity. The aim of the present study was to examine whether individuals at risk of psychosis also have similar changes.

Methods: Magnetic resonance imaging was used to examine the pituitary volume in 22 individuals with at-risk mental state (ARMS; 11 male, 11 female), 64 first-episode patients with schizophrenia (FESz; 37 male, 27 female), and 86 healthy controls. The control subjects were divided into age- and gender-matched controls for ARMS (11 male, 11 female) and FESz (37 male, 27 female).

Results: Both the ARMS and FESz groups had a larger pituitary volume compared with matched controls, but no difference was found between the ARMS and FESz subjects. There was no association between the pituitary volume and clinical variables (symptom measures at scanning, daily dosage or duration of antipsychotic medication) in either clinical group. The pituitary volume did not differ significantly between the ARMS individuals who later developed schizophrenia ($n = 5$) and those who did not ($n = 17$). The pituitary volume was larger in women than in men for all diagnostic groups.

Conclusion: The finding of increased pituitary volume in both ARMS and FESz subjects may reflect a common vulnerability to stress in early psychosis. Further work in a larger ARMS sample is required to examine the possible relationship between pituitary volume and emergence of psychosis.

Key words: at-risk mental state, hypothalamic–pituitary–adrenal axis, magnetic resonance imaging, pituitary gland, schizophrenia.

HYPOTHALAMIC–PITUITARY–ADRENAL (HPA) axis hyperactivity is thought to reflect stress-related hormonal dysregulation and has been described in schizophrenia.[1,2] Although not consistently replicated,[3] previous magnetic resonance imaging (MRI) studies have generally demonstrated enlarged pituitary volume[4–6] with ongoing expansion[7,8] early in the course of schizophrenia, presumably reflecting activation of the hormonal stress response. The patients may also exhibit pituitary atrophy during later courses,[9–11] possibly as a result of prolonged HPA activation.[12] Interestingly, recent neuroendocrine findings in clinical subjects at high risk for developing psychosis (i.e. at-risk mental state; ARMS[13]), such as the association of cortisol level with prodromal or psychotic symptoms[14–17] as well as with

Correspondence: Tsutomu Takahashi, MD, PhD, Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Email: tsutomu@med.u-toyama.ac.jp
Received 5 September 2012; revised 21 March 2013; accepted 28 March 2013.

progression to psychosis,[18] suggest that HPA axis dysfunction may pre-date the onset of psychosis in at least some individuals.[19]

In contrast to these hormonal investigations, there have been only a few MRI studies addressing pituitary volume changes prior to psychosis onset and the results have been inconsistent. In the first MRI study of the pituitary gland in clinical high-risk subjects, Garner *et al.* found no significant volume difference between the ARMS subjects (as a whole or those who later developed psychosis) and controls, but pituitary enlargement was associated with later transition to psychosis (predominantly affective psychosis).[4] They also examined the possible relationship of the pituitary volume to anxiety/depressive or psychotic symptoms, but found no significant results. Thompson *et al.* showed that pituitary volume in ARMS did not correlate with the experience of stressful events, plasma cortisol level, or clinical symptoms, but that study lacked a healthy comparison group.[17] A recent study by Büschlen *et al.* did not replicate a significant difference in the pituitary volume between ARMS with and without transition,[4] but their data (controls < ARMS without later transition < ARMS with transition and first-episode psychosis)[20] were in line with hypothesized pituitary enlargement with the emergence of psychosis. Thus, it remains unclear from the current evidence whether these high-risk subjects have significant pituitary volume changes as compared with controls and whether their pituitary volume is related to clinical characteristics.

The present MRI study investigated the pituitary volume in subjects with ARMS and first-episode schizophrenia (FESz) compared with age- and gender-matched healthy controls. On the basis of previous MRI and neuroendocrine findings suggesting HPA hyperactivity prior to the onset of overt psychosis,[19] we predicted that both ARMS and FESz subjects would have increased pituitary volume compared with matched controls. We also explored the relationship between the pituitary volume and clinical characteristics (e.g. symptom severity, later transition into psychosis, and antipsychotic medication) in these participants.

METHODS

Participants

Twenty-two ARMS subjects were recruited from the Consultation Support Service in Toyama (CAST), which was launched in 2006 as a specialized clinical setting to study and treat young people (aged 15–30 years) at risk for developing psychosis.[21] The ARMS subjects, who had no previous episode of overt psychosis and no clear diagnosis of major depression or borderline personality disorder, were diagnosed according to the Comprehensive Assessment of At Risk Mental States (CAARMS);[13] inclusion into the study required one or more of (i) attenuated psychotic symptoms defined by subthreshold intensity or frequency ($n = 21$); (ii) brief limited intermittent psychotic symptoms with spontaneous resolution ($n = 2$); and/or (iii) family history of psychosis or a personal history of schizotypal personality disorder accompanied by a decline in general functioning ($n = 1$). At intake, they were also assessed using the Beck Depression Inventory (BDI) and State Trait Anxiety Inventory (STAI) (Table 1).[23,24] Eighteen ARMS subjects were antipsychotic naïve at the time of scanning, but three subjects were receiving low doses of atypical antipsychotics (risperidone, blonanserin, or aripiprazole) and one was treated with sulpiride. They were also receiving benzodiazepines ($n = 3$), antidepressants ($n = 1$), and/or tandospirone ($n = 3$). The mental condition of each subject was regularly assessed by experienced psychiatrists to check for the emergence of full-blown psychosis at outpatient clinics of the Department of Neuropsychiatry of Toyama University Hospital; five (22.7%) of the ARMS subjects in this group developed schizophrenia fulfilling ICD-10 research criteria[25] and 17 (77.3%) did not develop psychosis during follow up (mean clinical follow-up period after scanning, 15.6 ± 17.4 months).

Sixty-four FESz patients who fulfilled the ICD-10 research criteria,[25] with illness duration ≤1 year ($n = 48$) or under first psychiatric hospitalization ($n = 16$) at the time of scanning,[26–29] were recruited from inpatient and outpatient clinics of the Department of Neuropsychiatry of Toyama University Hospital (Table 1). The diagnosis of schizophrenia was confirmed for all patients at least 6 months after illness onset based on information obtained from a detailed chart review as well as their clinical symptoms rated at the time of scanning. They were also screened for other neuropsychiatric conditions (e.g. depressive/manic symptoms) by experienced psychiatrists. All but two of the patients were on antipsychotic medication; 18 were treated with typical antipsychotics, 43 were receiving atypical antipsychotics and one received both typical and atypical antipsychotics.

Table 1. ARMS and FESz subject data vs matched controls (mean ± SD)

Parameters	ARMS (11 M, 11 F)	Controls (11 M, 11 F)	Group comparisons
Age (years)	19.1 ± 4.1	19.4 ± 4.2	$F(1,42) = 0.05$, $P = 0.830$
Height (cm)	162.2 ± 9.5	164.3 ± 8.5	$F(1,42) = 0.62$, $P = 0.436$
Education (years)	11.1 ± 1.6	13.1 ± 2.6	$F(1,42) = 8.99$, $P = 0.005$
Parental education (years)	13.8 ± 1.7	12.4 ± 1.6	$F(1,42) = 7.68$, $P = 0.008$
Medication dose (HPD equiv., mg/day)†	2.2 ± 3.1 ($n = 4$)	–	–
Duration of medication (months)	2.3 ± 4.1 ($n = 4$)	–	–
Time between intake and scan (days)	50.8 ± 74.4	–	–
Time between scan and onset (months)	8.2 ± 9.9 ($n = 5$)	–	–
STAI trait at intake‡	65.3 ± 10.9	–	–
STAI state at intake‡	58.4 ± 11.3	–	–
BDI at intake‡	24.1 ± 10.0	–	–
SAPS total at scanning	20.4 ± 10.9	–	–
SANS total at scanning	48.5 ± 19.4	–	–
	FESz (37 M, 27 F)	Controls (37 M, 27 F)	Group comparisons
Age (years)	24.0 ± 4.7	25.1 ± 5.0	$F(1,126) = 1.64$, $P = 0.203$
Height (cm)	164.9 ± 7.6	167.0 ± 7.5	$F(1,126) = 2.60$, $P = 0.109$
Education (years)	13.5 ± 1.9	16.5 ± 2.6	$F(1,126) = 57.55$, $P < 0.001$
Parental education (years)	13.0 ± 2.0	13.2 ± 2.5	$F(1,124) = 0.50$, $P = 0.482$
Onset age (years)	23.1 ± 4.7	–	–
Illness duration (months)	11.2 ± 12.2	–	–
Medication dose (HPD equiv., mg/day)	10.3 ± 8.8	–	–
Duration of medication (months)	8.3 ± 12.6	–	–
SAPS total at scanning	27.3 ± 21.9	–	–
SANS total at scanning	53.1 ± 25.2	–	–

†Different typical and atypical antipsychotic dosages are converted into HPD equivalents using the guideline by Toru.[22]
‡Data missing for one participant. ARMS, at-risk mental state; BDI, Beck Depression Inventory; FESz, first-episode schizophrenia; HPD, haloperidol; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; STAI, State Trait Anxiety Inventory.

The control subjects consisted of 86 healthy volunteers recruited from the community, hospital staff, and university students. Given the sexual dimorphism (male < female) and age-related atrophy of the pituitary gland,[30–32] the control subjects comprised two groups that were age- and gender-matched for ARMS ($n = 22$) and for FESz ($n = 64$), respectively (Table 1). Although the controls did not receive a full diagnostic interview, they were given a questionnaire consisting of 15 items concerning their personal (13 items; e.g. a history of obstetric complications, substantial head injury, seizures, neurological or psychiatric diseases, impaired thyroid function, hypertension, diabetes, and substance use) and family (two items) histories of illness.[33] They did not have any personal or family history of psychiatric illness among their first-degree relatives.

All subjects in this study (ARMS, FESz, and controls) were screened using the same exclusion criteria (except family history of psychiatric illness, which was applied only to controls). They were right-handed and physically healthy at the time of the study, and none had a history of serious head trauma, severe obstetric complications, neurological illness, substance abuse disorder, or serious medical disease (e.g. impaired thyroid function, hypertension, and diabetes). The FESz and ARMS participants were screened for these conditions using a detailed chart review at scanning (FESz) or direct interview at study intake (ARMS). None of the participants was pregnant or taking exogenous estrogens at the time of the study, but hormone levels as well as menstrual cycle in female subjects were not assessed in this study. All participants were

Table 2. Intracranial and pituitary volume (mean ± SD)

Variables	ARMS (11 M, 11 F)	Controls for ARMS (11 M, 11 F)	FESz (37 M, 27 F)	Controls for FESz (37 M, 27 F)
Intracranial volume (cm^3)	1460 ± 132	1500 ± 146	1500 ± 147	1502 ± 150
Pituitary volume (mm^3)	763 ± 124†	697 ± 143	802 ± 153†	708 ± 140

†Significantly larger than age- and gender-matched controls. Statistical analysis for the pituitary gland was based on relative volume. Analysis of covariance with age as a covariate and with diagnosis as a between-subject factor was used for the intracranial volume. ARMS, at risk mental state; FESz, first-episode schizophrenia.

also screened for gross brain abnormalities by neuroradiologists.

The clinical symptoms of the ARMS and FESz subjects were rated at the time of scanning using the Scale for the Assessment of Negative Symptoms and the Scale for the Assessment of Positive Symptoms (SANS/SAPS).[34] Of the 172 participants in this study, 60 controls (35 male) and 37 schizophrenia patients (21 male) were also included in our previous pituitary study.[35] This study was approved by the Committee on Medical Ethics of Toyama University. After a complete description of the study was provided, written informed consent was obtained from all subjects.

Magnetic resonance imaging procedures

The subjects were scanned on a 1.5-T Magnetom Vision (Siemens Medical System, Erlangen, Germany) with a 3-D gradient-echo sequence fast low-angle shots (FLASH) yielding 160–180 contiguous T1-weighted slices of 1.0-mm thickness in the sagittal plane. The imaging parameters were as follows: repetition time, 24 ms; echo time, 5 ms; flip angle, 40°; field of view, 256 mm; and matrix size, 256 × 256 pixels. The voxel size was 1.0 × 1.0 × 1.0 mm. The scanner was calibrated weekly with the same phantom to ensure measurement stability.

To assess the pituitary volume, the images were processed on a Linux PC (Fujitsu, Tokyo, Japan) using Dr. View software (AJS, Tokyo, Japan). Brain images were realigned in three dimensions to standardize for differences in head tilt during image acquisition and were then reconstructed into entire contiguous coronal images of 1-mm thickness perpendicular to the anterior commissure–posterior commissure line. The signal intensity histogram distributions from the T1-weighted images across the whole cerebrum were then used to semi-automatically segment the voxels into brain tissue components and cerebrospinal fluid. The intracranial volume (ICV) was measured to correct for differences in head size as described previously;[36] there were no significant group differences for ICV (ARMS vs their controls, $F(1,41) = 0.88$, $P = 0.353$; FES vs their controls, $F(1,125) < 0.01$, $P = 0.984$; and FES vs ARMS vs all controls, $F(2,168) = 0.43$, $P = 0.654$; Table 2).

Pituitary measurements

The pituitary gland volume was manually traced on consecutive 1-mm coronal slices based on a method used by Garner *et al.*[4] Briefly, we traced around the usually well-defined borders of the anterior and posterior pituitary: the diaphragma sellae, superiorly; the sphenoid sinus, inferiorly; and the cavernous sinuses, bilaterally. As presented in Figure 1, the pituitary stalk was excluded from the tracings, but we included a posterior bright spot, corresponding to the posterior pituitary (the intensity of which is thought to reflect the vasopressin concentration). All measurements were carried out by a trained rater (TT) without knowledge of the subjects' identities or the times of their scans. To determine the reliability of the measurement, a second rater (VL) measured the pituitary volume in a subset of 10 randomly selected brains. Each pituitary volume in these 10 brains was then remeasured after at least 4 weeks by the first rater. Inter- (TT and VL) and intra-rater intraclass correlation coefficients were >0.93.

Statistical analysis

The relative volume of the pituitary gland ([absolute volume/ICV) × 100]) was analyzed using analysis of

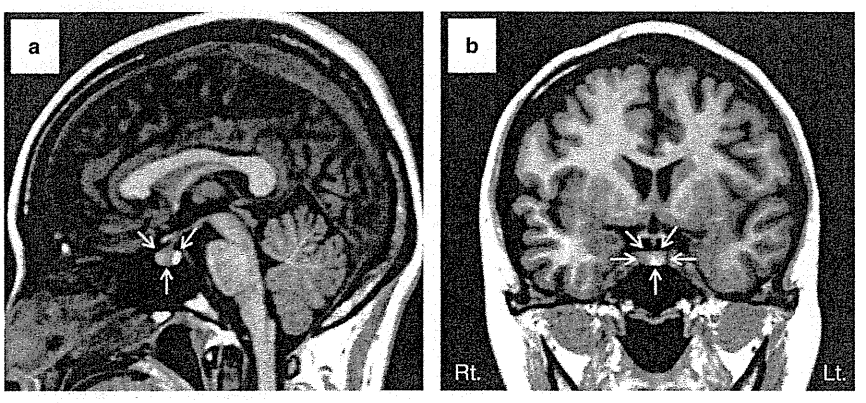


Figure 1. (a) Sagittal and (b) coronal views of the pituitary gland manually traced in this study. The pituitary stalk was excluded from the tracings, but a posterior bright spot was included.

covariance (ANCOVA) with age as a covariate and with diagnosis and gender as between-subject factors. The effect of medication type (typical vs atypical for FESz) and outcome (with vs without later transition for ARMS) on relative pituitary volume was also examined on ANCOVA. Post-hoc Scheffé's tests were carried out to follow up any significant main effects or interactions. Spearman's rank correlations were calculated to examine relationships between relative pituitary volume and the clinical variables. Statistical significance was defined as $P < 0.05$ (two-tailed).

RESULTS

Group comparisons of the pituitary volume

ANCOVAs of the pituitary volume showed significant main effects for diagnosis (ARMS vs their controls, $F(1,39) = 4.94$, $P = 0.032$; FESz vs their controls, $F(1,123) = 15.58$, $P < 0.001$) and gender (ARMS vs their controls, $F(1,39) = 26.39$, $P < 0.001$; FESz vs their controls, $F(1,123) = 113.58$, $P < 0.001$) but not diagnosis × gender interaction (ARMS vs their controls, $F(1,39) = 0.91$, $P = 0.346$; FESz vs their controls, $F(1,123) = 0.66$, $P = 0.417$). Post-hoc analyses showed that both the ARMS ($P = 0.030$) and FESz ($P < 0.001$) groups had a larger pituitary volume compared with matched controls, and female subjects had a larger volume than male subjects ($P < 0.001$; Table 2; Fig. 2). Direct comparison of the pituitary volume between ARMS and FESz showed no significant group difference ($F(1,81) = 1.58$, $P = 0.213$).

These results remained essentially the same even when we added medication dose and duration as covariates, and there was no difference in the pituitary volume between the FESz patients treated with typical ($n = 18$) and atypical ($n = 43$) antipsychotics

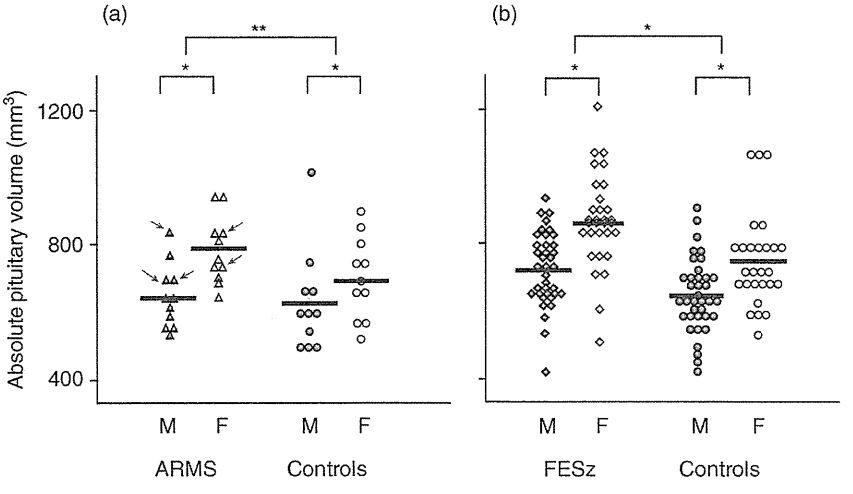


Figure 2. Absolute pituitary volume in the at-risk mental state (ARMS) individuals, controls for ARMS, first-episode schizophrenia (FESz) patients, and controls for FESz. Arrows, ARMS individuals with later transition into psychosis. Horizontal lines, mean. Post-hoc test: $*P < 0.01$, $**P = 0.03$ (statistical analysis for the pituitary gland was based on relative volume).

($F(1,58) = 0.30$, $P = 0.586$). Although the ARMS subjects who were taking antipsychotics at scanning ($n = 4$; pituitary volume, 843 ± 128 mm^3) had a larger pituitary volume than antipsychotic-naïve ARMS subjects ($n = 18$; pituitary volume, 746 ± 120 mm^3), the difference was not statistically significant ($F(1,19) = 1.73$, $P = 0.204$). The comparison of the pituitary volume between the antipsychotic-naïve ARMS and FESz subjects showed no significant group difference ($F(1,77) = 1.60$, $P = 0.209$). When we examined only antipsychotic-naïve ARMS subjects ($n = 18$) and 18 age- and gender-matched controls, pituitary expansion did not reach significance ($F(1,31) = 3.48$, $P = 0.072$). The pituitary volume did not differ significantly between the ARMS subjects who later developed schizophrenia ($n = 5$; pituitary volume, 803 ± 78 mm^3) and those who did not ($n = 17$; pituitary volume, 752 ± 134 mm^3; $F(1,19) = 0.87$, $P = 0.362$).

Correlation analysis

The relative pituitary volume did not correlate with age, education, or parental education in all groups. No significant correlation was found between the pituitary volume and the BDI or STAI (state, trait) score in the ARMS subjects. In the FESz group, the pituitary volume was not significantly correlated with onset age or illness duration. In both the ARMS and FESz groups, no significant correlation was found between the pituitary volume and the total scores for the SANS/SAPS or medication (daily dose at scanning, duration of antipsychotic treatment).

DISCUSSION

This MRI study identified an enlarged pituitary volume in both subjects with ARMS and patients with FESz compared with healthy controls. The effect of medication is an important consideration for pituitary findings,[7,9,10,37] but we found no significant effect of daily dose or duration of antipsychotic treatment on the pituitary volume. Consistent with previous reports,[4,17] the pituitary volume did not correlate with clinical symptoms in either clinical group. Despite the relatively small number of subjects with ARMS, the present findings suggest that these high-risk subjects may share HPA hyperactivity with FESz patients as a possible indicator of common stress vulnerability.

Pituitary volume in early psychosis

The present finding of enlarged pituitary volume in FESz is consistent with previous MRI studies,[5,6,8,20] supporting the role of HPA hyperactivity in the development of psychosis.[2,18] To our knowledge, however, there have been only two MRI studies of the pituitary volume in ARMS as compared with healthy controls, which have yielded partly inconsistent results. Garner *et al.* found no significant group difference in the pituitary volume between the ARMS and controls, but the ARMS subjects who later developed psychosis (ARMS-T) had a larger pituitary volume than those who did not (ARMS-NT).[4] The present results were similar to those of Büschlen *et al.*, who reported that the pituitary volume increased in the order of healthy controls to ARMS-NT to ARMS-T and first-episode psychosis, although the difference between the ARMS-T and -NT was not statistically significant.[20] As discussed by Büschlen *et al.*,[20] these inconsistencies may be partly due to different ascertainment strategies, as well as different characteristics, of regional psychiatric services. In fact, Garner *et al.*, who included ARMS subjects with a comorbid diagnosis of major depression or borderline personality disorder, suggested the role of the pituitary volume as a predictor of psychotic major depression,[4] whereas the ARMS-T subjects in the present study and those of Büschlen *et al.*,[20] neither of whom included ARMS subjects with those comorbidities, predominantly developed schizophrenic psychosis. Nevertheless, these MRI studies generally imply that these clinical high-risk subjects could exhibit pituitary expansion at least in some individuals, supporting the notion that an enhanced HPA axis response to stress appears to be part of the biological vulnerability to psychosis.[19] This notion may also be supported by hormone[38–40] and neuroimaging[35] findings in subjects with schizotypal personality disorder (SPD) who have a higher incidence of developing psychosis than the general population,[41] suggesting that distress related to social deficits or incipient psychotic experience could activate the stress response even without florid psychosis.

Possible underlying mechanism of the pituitary expansion

The present structural MRI study could not address the mechanism for pituitary volume changes, but a recent study by Habets *et al.* showed that higher pitu-

itary volume was associated with increased emotional stress reactivity especially in patients with psychotic disorder.[42] It may be possible that pituitary expansion in the present study reflects HPA axis hyperactivity and a subsequent increase in the size and number of corticotrophs (cells producing adrenocorticotropic hormone; ACTH), which can be explained by an activation of the hormonal stress response.[5,6] Estrogen treatment, hypothalamic tumor, pregnancy, and primary hypothyroidism also lead to pituitary expansion,[43,44] but these common causes of pituitary enlargement were excluded in the present subjects.

Antipsychotic medication could also influence HPA activation,[2,45,46] but the effect of medication on the pituitary volume remains controversial. Recent MRI studies suggested that atypical antipsychotics might reduce pituitary volume in the course of psychosis,[9,10,37] consistent with the notion that antipsychotic medication generally dampens HPA activity in schizophrenia.[1,2,46] In contrast, some antipsychotics may increase pituitary volume, possibly by activating prolactin-secreting cells.[5,7,47] Although we did not find a direct relation between the pituitary volume and medication (daily dose at scanning, duration of antipsychotic treatment), almost all of the present FESz patients had been taking antipsychotics for a substantial period at the time of scanning (mean, 8.3 months) and significant pituitary expansion of the ARMS subjects diminished when we investigated only antipsychotic-naïve ARMS subjects. Thus, the possibility still exists that the pituitary expansion in the present study was partly related to the effect of antipsychotic medication, which should be further examined.

Pituitary volume and clinical characteristics

In contrast to neuroendocrine observations demonstrating the association of plasma or salivary cortisol levels with prodromal (including depressive and anxiety) or psychotic symptoms in ARMS subjects,[14–17] this and previous MRI studies in ARMS found no significant correlation between the pituitary volume and global psychopathology, general functioning, or psychotic symptomatology.[4,17] Direct comparison of plasma and MRI findings in ARMS also showed that pituitary volume did not correlate with either plasma cortisol level or number of glucocorticoid receptors.[17] Our previous study, however, identified a significant relationship between ongoing pituitary expansion and treatment response or severity of positive psychotic symptoms in FESz,[8] suggesting that it is longitudinal pituitary changes during early phases of the illness that are relevant to clinical manifestations of psychosis. Interestingly, a recent study of cortisol level emphasized the role of longitudinal HPA changes in the development of psychosis.[18] Thus, further study of the association of longitudinal pituitary volume changes with HPA functioning and clinical characteristics (e.g. symptom severity, later transition into psychosis) is required to examine the potential role of HPA activity in the emergence of psychosis in vulnerable individuals.

Methodological considerations

A few possible methodological considerations in this study should be taken into account. First, the sample size of the present ARMS group (especially those who later developed psychosis) was relatively small and the clinical follow-up period was short for some individuals. Although we found no significant difference in pituitary volume between the ARMS subjects with and without later transition to psychosis, whether the baseline pituitary volume could predict onset of psychosis should be tested in a larger, well-defined high-risk cohort. Second, although the present findings of pituitary enlargement in early psychosis are thought to reflect state-related HPA axis dysregulation, we did not directly assess pituitary function. The pituitary gland is also considered to be sensitive to prolactin-elevating antipsychotics[7,47] and a recent study reported hyperprolactinemia in antipsychotic-naïve ARMS subjects.[48] The present findings replicated the sexual dimorphism of the pituitary gland volume (female > male),[32] potentially reflecting different endogenous estrogen levels.[49] We did not, however, assess prolactin or estrogen level in this study. Thus, additional assessment of both pituitary volume and hormone levels (e.g. cortisol, ACTH, prolactin, and estrogen) is required. The present study might be also limited by a lack of urine toxicology screening for substance use. Finally, given that HPA axis functioning also appears to be affected in major depressive disorders[50–52] and that Garner *et al.* found an enlarged pituitary volume prior to the onset of psychotic major depression,[4] further investigation of the disease specificity of pituitary findings is warranted.

Conclusion

Both the ARMS and FESz subjects had significant enlargement of the pituitary gland, presumably reflecting activation of the hormonal stress response during early psychosis. Given that the pituitary gland is a dynamic organ reflecting state-related HPA axis dysregulation, longitudinal study of the pituitary volume and its relation to clinical characteristics, as well as hormone levels, is required to further understand the role of HPA functioning in the emergence of psychosis.

ACKNOWLEDGMENTS

This research was supported in part by Grants-in-Aid for Scientific Research (C) (No. 22591275, 24591699) and Grants-in-Aid for Scientific Research (B) (No. 24390281) from the Japanese Society for the Promotion of Science, Health and Labour Sciences Research Grants (Comprehensive Research on Disability, Health and Welfare, H23-Seishin-Ippan-002 and H23-Seishin-Ippan-009), and a Research Grant from the JSPS Asian Core Program. The authors are grateful to Ms Valentina Lorenzetti for assistance with MRI analysis. The authors have no conflicts of interest to declare.

REFERENCES

1. Phillips LJ, McGorry PD, Garner B *et al.* Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: Implications for the development of psychotic disorders. *Aust. N. Z. J. Psychiatry* 2006; **40**: 725–741.
2. Walker E, Mittal V, Tessner K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu. Rev. Clin. Psychol.* 2008; **4**: 189–216.
3. Upadhyaya AR, El-Sheikh R, MacMaster FP, Diwadkar VA, Keshavan MS. Pituitary volume in neuroleptic-naïve schizophrenia: A structural MRI study. *Schizophr. Res.* 2007; **90**: 266–273.
4. Garner B, Pariante CM, Wood SJ *et al.* Pituitary volume predicts future transition to psychosis in individuals at ultra-high risk of developing psychosis. *Biol. Psychiatry* 2005; **58**: 417–423.
5. Pariante CM, Vassilopoulou K, Velakoulis D *et al.* Pituitary volume in psychosis. *Br. J. Psychiatry* 2004; **185**: 5–10.
6. Pariante CM, Dazzan P, Danese A *et al.* Increased pituitary volume in antipsychotic-free and antipsychotic-treated patients of the AEsop first-onset psychosis study. *Neuropsychopharmacology* 2005; **30**: 1923–1931.
7. MacMaster FP, El-Sheikh R, Upadhyaya AR, Nutche J, Rosenberg DR, Keshavan M. Effect of antipsychotics on pituitary gland volume in treatment-naïve first-episode schizophrenia: A pilot study. *Schizophr. Res.* 2007b; **92**: 207–210.
8. Takahashi T, Zhou SY, Nakamura K *et al.* Longitudinal volume changes of the pituitary gland in patients with schizotypal disorder and first-episode schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 2011; **35**: 177–183.
9. Klomp A, Koolschijn PC, Pol HE, Kahn RS, Haren NE. Hypothalamus and pituitary volume in schizophrenia: A structural MRI study. *Int. J. Neuropsychopharmacol.* 2012; **15**: 281–288.
10. Takahashi T, Kido M, Nakamura K *et al.* Longitudinal MRI study of the pituitary volume in chronic schizophrenia: A preliminary report. *Psychiatry Res.* 2012; **202**: 84–87.
11. Tournikioti K, Tansella M, Perlini C *et al.* Normal pituitary volumes in chronic schizophrenia. *Psychiatry Res.* 2007; **154**: 41–48.
12. Sassi RB, Nicoletti M, Brambilla P *et al.* Decreased pituitary volume in patients with bipolar disorder. *Biol. Psychiatry* 2001; **50**: 271–280.
13. Yung AR, Yuen HP, McGorry PD *et al.* Mapping the onset of psychosis: The Comprehensive Assessment of At-Risk Mental States. *Aust. N. Z. J. Psychiatry* 2005; **39**: 964–971.
14. Corcoran CM, Smith C, McLaughlin D, Auther A, Malaspina D, Cornblatt B. HPA axis function and symptoms in adolescents at clinical high risk for schizophrenia. *Schizophr. Res.* 2012; **135**: 170–174.
15. Mittal VA, Walker EF. Minor physical anomalies and vulnerability in prodromal youth. *Schizophr. Res.* 2011; **129**: 116–121.
16. Thompson KN, Berger G, Phillips LJ, Komesaroff P, Purcell R, McGorry PD. HPA axis functioning associated with transition to psychosis: Combined DEX/CRH test. *J. Psychiatr. Res.* 2007a; **41**: 446–450.
17. Thompson KN, Phillips LJ, Komesaroff P *et al.* Stress and HPA-axis functioning in young people at ultra high risk for psychosis. *J. Psychiatr. Res.* 2007b; **41**: 561–569.
18. Walker EF, Brennan PA, Esterberg M, Brasfield J, Pearce B, Compton MT. Longitudinal changes in cortisol secretion and conversion to psychosis in at-risk youth. *J. Abnorm. Psychol.* 2010; **119**: 401–408.
19. Aiello G, Horowitz M, Hepgul N, Pariante CM, Mondelli V. Stress abnormalities in individuals at risk for psychosis: A review of studies in subjects with familial risk or with 'at risk' mental state. *Psychoneuroendocrinology* 2012; **37**: 1600–1613.
20. Büschlen J, Berger GE, Borgwardt SJ *et al.* Pituitary volume increase during emerging psychosis. *Schizophr. Res.* 2011; **125**: 41–48.
21. Mizuno M, Suzuki M, Matsumoto K *et al.* Clinical practice and research activities for early psychiatric intervention at Japanese leading centres. *Early Interv. Psychiatry* 2009; **3**: 5–9.
22. Toru M. *Psychotropic Manual*, Third edn. Igaku-Shoin, Tokyo, 2008 (in Japanese).