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## References

Ahveninen J, Jaaskelainen IP, Osipova D, Huttunen MO, Ilmoniemi RJ, Kaprio J, et al. Inherited auditory-cortical dysfunction in twin pairs discordant for schizophrenia. Biol Psychiatry 2006;60:612–20.

Alho K, Winkler I, Escera C, Huotilainen M, Virtanen J, Jaaskelainen IP, et al. Processing of novel sounds and frequency changes in the human auditory cortex: magnetoencephalographic recordings. Psychophysiology 1998;35:211–24.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition. American Psychiatric Association; 1994.

Catts SV, Shelley AM, Ward PB, Liebert B, McConaghy N, Andrews S, et al. Brain potential evidence for an auditory sensory memory deficit in schizophrenia. Am J Psychiatry 1995;152:213–9.

Fisher DJ, Labelle A, Knott VJ. Auditory hallucinations and the mismatch negativity: processing speech and non-speech sounds in schizophrenia. Int J Psychophysiol 2008;70:3-15.

Giard MH, Perrin F, Pernier J, Bouchet P. Brain generators implicated in the processing of auditory stimulus deviance: a topographic event-related potential study. Psychophysiology 1990;27:627–40.

Hall MH, Rijsdijk F, Kalidindi S, Schulze K, Kravariti E, Kane F, et al. Genetic overlap between bipolar illness and event-related potentials. Psychol Med 2007;37: 667–78.

Hall MH, Schulze K, Rijsdijk F, Kalidindi S, McDonald C, Bramon E, et al. Are auditory P300 and duration MMN heritable and putative endophenotypes of psychotic bipolar disorder? A Maudsley Bipolar Twin and Family Study. Psychol Med 2009;39:1277–87.

Hamalainen M, Hari R, Ilmoniemi R, Knuutila J, Lounasmaa OV. Magnetoencephalography-theory, instrumentation, and applications to noninvasive studies of the working human brain. Rev Mod Phys 1993;65:413–97.

Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;23: 56–62.

Hari R, Hamalainen M, Ilmoniemi R, Kaukoranta E, Reinikainen K, Salminen J, et al. Responses of the primary auditory cortex to pitch changes in a sequence of tone pips: neuromagnetic recordings in man. Neurosci Lett 1984;50:127–32.

Hoddes E, Dement WC, Zarcone V. The history and use of the Stanford sleepiness scale. Psychophysiology 1971;9:150.

Kahkonen S, Ahveninen J, Pekkonen E, Kaakkola S, Huttunen J, Ilmoniemi RJ, et al. Dopamine modulates involuntary attention shifting and reorienting: an electromagnetic study. Clin Neurophysiol 2002;113:1894–902.

Kameyama M, Fukuda M, Yamagishi Y, Sato T, Uehara T, Ito M, et al. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 2006;29:172–84.

Kasai K, Yamada H, Kamio S, Nakagome K, Iwanami A, Fukuda M, et al. Do high or low doses of anxiolytics and hypnotics affect mismatch negativity in schizophrenic subjects? An EEG and MEG study. Clin Neurophysiol 2002;113:141–50.

Kasai K, Yamada H, Kamio S, Nakagome K, Iwanami A, Fukuda M, et al. Neuromagnetic correlates of impaired automatic categorical perception of speech sounds in schizophrenia. Schizophr Res 2003;59:159–72.

Knuutila J, Ahonen A, Hamalainen M, Kajola M, Laine P, Lounasmaa O, et al. A 122-channel whole-cortex SQUID system for measuring the brain's magnetic fields. IEEE Trans Magnetics; 1993;29:3315.

Kok A. On the utility of P3 amplitude as a measure of processing capacity. Psychophysiology 2001;38:557–77.

Konarski JZ, McIntyre RS, Kennedy SH, Rafi Tari S, Soczynska JK, Ketter TA. Volumetric neuroimaging investigations in mood disorders: bipolar disorder versus major depressive disorder. Bipolar Disord 2008;10:1-37.

Kreitschmann Andermahr I, Rosburg T, Meier T, Volz HP, Nowak H, et al. Impaired sensory processing in male patients with schizophrenia: a magnetoencephalographic study of auditory mismatch detection. Schizophr Res 1999;35:121–9.

Kujala T, Tervaniemi M, Schroger E. The mismatch negativity in cognitive and clinical neuroscience: theoretical and methodological considerations. Biol Psychol 2007;74:1-19.

Lehmann D, Skrandies W. Reference-free identification of components of checkerboard-evoked multichannel potential fields. Electroencephalogr Clin Neurophysiol 1980;48:609–21.

Leung S, Croft RJ, Guille V, Scholes K, O'Neill BV, Phan KL, et al. Acute dopamine and/or serotonin depletion does not modulate mismatch negativity (MMN) in healthy human participants. Psychopharmacology 2010;208:233–44.

Lyytinen H, Blomberg AP, Naatanen R. Event-related potentials and autonomic responses to a change in unattended auditory stimuli. Psychophysiology 1992;29:523–34.

Martinez Aran A, Vieta E, Reinares M, Colom F, Torrent C, Sanchez Moreno J, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. Am J Psychiatry 2004;161:262–70.

McGee TJ, King C, Tremblay K, Nicol TG, Cunningham J, Kraus N. Long-term habituation of the speech-elicited mismatch negativity. Psychophysiology 2001;38:653–8.

Näätänen R, Gaillard AW, Mantysalo S. Early selective-attention effect on evoked potential reinterpreted. Acta Psychol (Amst) 1978;42:313–39.

Oades RD, Wild Wall N, Juran SA, Sachsse J, Oknina LB, Ropcke B. Auditory change detection in schizophrenia: sources of activity, related neuropsychological function and symptoms in patients with a first episode in adolescence, and patients 14 years after an adolescent illness-onset. BMC Psychiatry 2006;6:7.

Ogura C, Nageishi Y, Fukao K, Shimoji Y, Hirano K, Hokama H, et al. Deviate N200 component of event-related potentials in Shuchaku–Seikaku, a premorbid personality of depression. Jpn J Psychiatry Neurol 1991;45:641–51.

Ogura C, Nageishi Y, Omura F. Reduced mismatch negativity and hyperactivation of N2b in depression. Electroencephalogr Clin Neurophysiol Suppl 1995;44:218–23.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97-113.

Oranje B, Jensen K, Wienberg M, Glenthoj BY. Divergent effects of increased serotonergic activity on psychophysiological parameters of human attention. Int J Neuropsychopharmacol 2008;11:453–63.

Pekkonen E, Katila H, Ahveninen J, Karhu J, Huotilainen M, Tiihonen J. Impaired temporal lobe processing of preattentive auditory discrimination in schizophrenia. Schizophr Bull 2002;28:467–74.

Quraishi S, Frangou S. Neuropsychology of bipolar disorder: a review. J Affect Disord 2002;72:209–26.

Reite M, Teale P, Rojas DC, Reite E, Asherin R, Hernandez O. MEG auditory evoked fields suggest altered structural/functional asymmetry in primary but not secondary auditory cortex in bipolar disorder. Bipolar Disorders 2009;11:371–81.

Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. J Affect Disord 2006;93:105–15.

Salisbury DF, Kuroki N, Kasai K, Shenton ME, McCarley RW. Progressive and interrelated functional and structural evidence of post-onset brain reduction in schizophrenia. Arch Gen Psychiatry 2007;64:521–9.

Savitz J, Solms M, Ramesar R. Neuropsychological dysfunction in bipolar affective disorder: a critical opinion. Bipolar Disord 2005;7:216–35.

Schulze KK, Hall MH, McDonald C, Marshall N, Walshe M, Murray RM, et al. Auditory P300 in patients with bipolar disorder and their unaffected relatives. Bipolar Disord 2008;10:377–86.

Takei Y, Kumano S, Hattori S, Uehara T, Kawakubo Y, Kasai K, et al. Preattentive dysfunction in major depression: a magnetoencephalography study using auditory mismatch negativity. Psychophysiology 2009;46:52–61.

Tervaniemi M, Kujala A, Alho K, Virtanen J, Ilmoniemi RJ, Naatanen R. Functional specialization of the human auditory cortex in processing phonetic and musical sounds: a magnetoencephalographic (MEG) study. Neuroimage 1999;9:330–6.

Thompson JM, Gallagher P, Hughes JH, Watson S, Gray JM, Ferrier IN, et al. Neurocognitive impairment in euthymic patients with bipolar affective disorder. Br J Psychiatry 2005;186:32–40.

Trejo LJ, Ryan Jones DL, Kramer AF. Attentional modulation of the mismatch negativity elicited by frequency differences between binaurally presented tone bursts. Psychophysiology 1995;32:319–28.

Umbricht D, Koller R, Schmid L, Skrabo A, Grubel C, Huber T, et al. How specific are deficits in mismatch negativity generation to schizophrenia? Biol Psychiatry 2003;53:1120–31.

Umbricht D, Krljes S. Mismatch negativity in schizophrenia: a meta-analysis. Schizophr Res 2005;76:1-23.

Wienberg M, Glenthoj B, Jensen K, Oranje B. A single high dose of escitalopram increases mismatch negativity without affecting processing negativity or P300 amplitude in healthy volunteers. J Psychopharmacol 2009;0 0269881109102606v1.

Woldorff MG, Hillyard SA, Gallen CC, Hampson SR, Bloom FE. Magnetoencephalographic recordings demonstrate attentional modulation of mismatch-related neural activity in human auditory cortex. Psychophysiology 1998;35:283–92.

Yabe H, Koyama S, Kakigi R, Gunji A, Tervaniemi M, Sato Y, et al. Automatic discriminative sensitivity inside temporal window of sensory memory as a function of time. Brain Res Cogn Brain Res 2001;12:39–48.

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ORIGINAL RESEARCH

# Serotonin-1A Receptor Gene Polymorphism and the Ability of Antipsychotic Drugs to Improve Attention in Schizophrenia

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## ABSTRACT

***Introduction:*** The purpose of this study was to determine if the functional single nucleotide polymorphisms of rs6259 C(-1019)G in the promoter region, which regulates serotonin 5-$HT_{1A}$ receptor transcription, affects the ability of antipsychotic drugs to improve attention in patients with schizophrenia. ***Methods:*** Subjects were neuroleptic-free and meeting DSM-IV-TR criteria for schizophrenia. Psychopathology and attention were evaluated with the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) at baseline and 3 months after treatment with atypical antipsychotic drugs (AAPDs). DNA was extracted from peripheral blood following standard procedures. Genotyping was performed with HS-Taq assay (LaboPass™). ***Results:*** Data were available from 30 subjects (male/female=19/11), in which 17 had the CC genotype, three had the GG genotype, and 10 were heterozygous. The 3-month treatment with AAPDs was associated with significant improvements in positive and negative symptoms, but not attention as measured by SANS-Attention subscale in the entire subject group. There were no significant differences in the degree of improvements of SAPS and SANS scores between the CC genotype group and the (C/G plus G/G) combined group. On the other hand, improvement of attention was significantly greater for the former group compared to the latter group ($P<0.016$), suggesting a detrimental influence of the G-allele. ***Conclusions:*** These results provide additional support to the role of 5-$HT_{1A}$ receptors in some of the cognitive disturbances of schizophrenia. Further studies with a larger number of subjects are warranted.

**Keywords:** atypical antipsychotic drugs; cognition; pharmacogenetics; schizophrenia; SNPs; 5-$HT_{1A}$

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## INTRODUCTION

Disturbances of cognitive function, such as verbal memory, attention, executive function, and verbal fluency, have been reported to determine outcome in patients with schizophrenia.[1,2] Among the domains of cognition, attention/vigilance has been widely studied as a measure of impaired frontal lobe function, which is characteristic of schizophrenia.[1-3] Therefore, efforts to address specific domains of cognition, such as attention, are needed in the treatment of schizophrenia.

A growing number of investigations have been directed to several types of serotonin (5-HT) receptors, such as 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors, in the pathophysiology and treatment of schizophrenia.[4-9] Postmortem[10,11] and positron emission tomography[12,13] studies report altered expression of 5-$HT_{1A}$ receptors in frontal and temporal cortical regions in patients with schizophrenia and related psychoses, while others[14] do not. Accordingly, ipsapirone (a 5-$HT_{1A}$ partial agonist)-induced plasma cortisol response has been found to be blunted in female patients with schizophrenia.[15] These findings are consistent with the concept that 5-$HT_{1A}$ receptors play an important role in the cognitive disturbances of schizophrenia.[6,16,17]

Based on these lines of evidence, we previously conducted a series of studies on the effects of the addition of 5-$HT_{1A}$ partial agonists (eg, buspirone[3] and tandospirone[18,19]) to ongoing treatment with typical or atypical antipsychotic drugs on cognitive function in patients with schizophrenia. In a randomly assigned, placebo-controlled, double-blind study, augmentation therapy with buspirone was found to selectively improve cognitive performance on the digit symbol substitution test, a measure of attention/speeded motor performance, but not other domains of cognition, in subjects with schizophrenia treated with atypical antipsychotic drugs (AAPDs).[3] However, positive and negative psychotic symptoms were not significantly affected.[3] As genetic variations have been suggested to affect cognitive performance, pharmacogenetic approaches may provide further insights into treatment development.[20]

Polymorphisms of genes encoding specific 5-HT receptor subtypes, such as 5-$HT_{1A}$, 5-$HT_{2A}$, and 5-$HT_{2C}$, have been suggested to predict response to treatment with antipsychotic drugs.[21-25] Among these polymorphisms, functional single nucleotide polymorphisms (SNPs) of 5-$HT_{1A}$ receptors, eg, rs6295 C(-1019)G in the promotor region of the 5-$HT_{1A}$ receptor, have been a focus of extensive research on psychiatric diseases.[7,9,22,25-27] C(-1019)G regulates 5-$HT_{1A}$ receptor transcription, and subjects with G-allele homozygotes show elevated 5-$HT_{1A}$ receptor density in presynaptic raphe neurons, which is associated with major depression and anxiety. On the other hand, C-allele carriers elicit a better response to treatment with AAPDs[22,25] or antidepressants,[28-31] although controversy exists.[32]

Intrinsic 5-$HT_{1A}$ agonist activity of the AAPDs, by causing an increase in dopamine (DA) and/or acetylcholine release, has been suggested to provide a basis for the ability of these compounds to improve specific domains of cognition,[33,34] such as attention.[7] In support of this view, the 5-$HT_{1A}$ antagonist WAY 100635 inhibits the increase in DA release produced by AAPDs, such as clozapine and ziprasidone, which are themselves 5-$HT_{1A}$ partial agonists,[33,35] as well as olanzapine and risperidone, which do not directly interact with 5-$HT_{1A}$ receptors.[33,36] These observations indicate that the ability of AAPDs to enhance cognition is dependent on the 5-$HT_{1A}$ receptor function, irrespective of type of compounds.

In view of accumulated evidence, discussed above, it was hypothesized that the C(-1019)G

polymorphism of the 5-$HT_{1A}$ receptor gene is associated with response to treatment with AAPDs in terms of cognitive function governed by frontal lobe function, such as attention. However, there has been no study of the relationship between the C(-1019)G polymorphism and the ability of antipsychotic drugs to treat this specific aspect of cognition. The purpose of this study, therefore, was to determine if AAPDs improve attention more effectively in patients with schizophrenia who have C/C genotype than those who carry the G-allele (C/G or G/G genotype).

## MATERIALS AND METHODS

### Subjects

Records were obtained from Japanese patients meeting the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revision*[37] (DSM-IV-TR) criteria for schizophrenia treated at the University Hospital of Toyama Outpatient Clinic. Diagnosis was made with a structured clinical interview by means of the Structured Clinical Interview for DSM-IV Axis I Disorders.[38] Subjects were diagnosed by a consensus of at least two experienced psychiatrists, as reported previously.[39,40] Patients known to be abusing alcohol or other illicit drugs, or those with epilepsy, brain damage, or neurologic disorders, were excluded from the study. This study was carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained after the explanation of the study. The protocol was approved by Ethical Committee of University of Toyama School of Medicine. At baseline, the subjects had not received any medication for more than 1 month, and were actively psychotic with the Scale for the Assessment of Positive Symptoms (SAPS)[41] (sum of the Global Scales) score of more than 4. SAPS and the Scale for Assessment of Negative symptoms (SANS)[42] were performed by experienced psychiatrists who were not informed of medication status. Interrater reliability was >80%.[39,40]

Immediately after the baseline assessment, antipsychotic medication with olanzapine (once daily) or perospirone (an atypical antipsychotic drug marketed in Japan[40]) (four times/day) was started and titrations were completed during the initial 6 weeks, based on previous reports[39,40] (see Table 1 for dosage). The choice of medication was at the discretion of treating psychiatrists, who adjusted the dose to optimize improvement in psychopathology, while attempting to keep the side effects of the drug tolerable. Concomitant medications were restricted to small doses of benzodiazepines (diazepam equivalent doses of <6 mg/day). Three months after the start of the treatment, clinical assessments were repeated.

Based on the a priori hypothesis, genotype effect on the change of the SANS-Attention subscale score, in addition to the SAPS-Total and SANS-Total scores, was analyzed.

### Genotyping

Genotyping was performed by technicians blinded to clinical status. DNA was extracted from peripheral blood samples following standard procedures. Polymerase chain reaction (PCR) was carried out using Taq DNA polymerase (LaboPass™ HS-Taq, LaboPass, Seoul, Korea). The C(-1019)G promotor region polymorphism of the 5-HT1A receptor gene was identified by following pair of primers (forward primer: 5'CCTCTCCTTGTCCTTTGA3'; reverse primer: 5'GTCAGACCAAGGTTGTAAC3'). PCR amplification for a total 30 μL reaction volume contained 20 ng genomic DNA, 0.2 μL HS-Taq (2.5 U/μL), 2.4 μL dNTPs, 1.0 μL of each primer.

The reaction product underwent electrophoresis on a 2.0% agarose gel, from which alleles were identified.

### Data Analysis

Statistical analysis was carried out using SPSS version 18 (IBM, Chicago, IL, USA). A *t*-test (two-tailed) was conducted to investigate the effects of genotype on changes of psychopathology and attention scores. Significance was considered when $P<0.05$. Data are expressed as mean±SD.

## RESULTS

Clinical data from 30 patients were available for analysis (Table 1). For these subjects, SAPS-Total (sum of the Global Scales), SANS-Total (sum of the Global Scales), and SANS-Attention subscale scores at baseline were 5.6±4.2, 11.6±3.7, and 1.8±1.3, respectively. Seventeen subjects had the CC genotype, three had the GG genotype, and 10 were heterozygous.

Owing to the small number of the GG group, statistical analyses were conducted between subjects with the CC genotype versus those with the GG genotype or heterozygous genotype.

In all subjects, 3-month treatment with antipsychotic drugs was found to significantly improve positive symptoms, as evaluated by the SAPS ($t=4.4$, $P<0.001$) and negative symptoms, as evaluated by SANS ($t=3.7$, $P=0.001$); while attention, as measured by the SANS-Attention subscale score, was not affected ($t=1.5$, $P$=nonsignificant). As shown in Table 1, there were no significant differences in the degree of change in SAPS and SANS-Total scores between the CC genotype group and (C/G plus G/G) combined group, although the former group showed a numerically better response to treatment. On the other hand, the CC genotype group showed a significantly better response in terms of attention, as compared to the (C/G plus G/G) group (Table 1).

**Table 1.** Demographic data and influence of the serotonin 5-$HT_{1A}$ receptor C(-1019)G polymorphism on the effect of antipsychotic drugs on symptom changes in patients with schizophrenia.

| | 5-$HT_{1A}$ receptor genotypes, mean±SD | | *P* value |
|---|---|---|---|
| | C/C | C/G or G/G | |
| Sex, male/female | 12/5 | 7/6 | NS |
| Age, year | 31.9±11.1 | 31.2 ±11.3 | NS |
| Onset of illness, year | 21.4±7.5 | 25.6±8.2 | NS |
| Neuroleptic dose* | 3.7±4.2 | 2.5±1.5 | NS |
| Score change | | | |
| ΔSAPS | −3.1±3.3 | −2.4±3.0 | 0.64 |
| ΔSANS | −3.2±3.7 | −2.1±3.4 | 0.22 |
| ΔAttention | −1.1±1.3 | 0.0±1.7 | 0.016 |

Attention=SANS-Attention subscale score; NS=nonsignificant; SANS=Scale for the Assessment of Negative Symptoms–Sum of the Global Scales; SAPS=Scale for the Assessment of Positive Symptoms–Sum of the Global Scales; Δ=change in.
*Risperidone equivalent dose (mg/day) at 3 months.

## DISCUSSION

Consistent with the a priori hypothesis, the results of this study suggest antipsychotic drugs ameliorate impaired attention of schizophrenia more effectively in patients having the C/C genotype at C(-1019)G of 5-$HT_{1A}$ receptors than those having the G/G or G/C genotype. The presence of the G-allele was associated with, on average, no improvement in attention.

To our knowledge, this study is the first to investigate the effect of the C(-1019)G polymorphism on the ability of AAPDs to improve a specific aspect of cognition in schizophrenia, and extends previous findings that the C(-1019)G SNP is associated with a response to treatment with various antipsychotic drugs in terms of negative[22,25] and depressive[22]

symptoms. Specifically, both of these studies[22,25] report that the presence of the G-allele, ie, G/G or G/C genotype, predicts a limited response to treatment with AAPDs. Thus, the results of the present study may provide a rational treatment strategy for attention impairments characteristic of schizophrenia.

It is possible that the increase in somatodendritic 5-$HT_{1A}$ receptor expression associated with the G-allele[43] may mediate decreased efficacy of AAPDs. Also, our findings are in concert with the suggestion that the C(-1019)G polymorphism in the 5-$HT_{1A}$ receptor predicts structural and functional characteristics in cortical regions receiving projection of 5-HT neurons, such as parahippocampal gyrus and prefrontal cortex, as these brain areas are responsible for some of the key domains of cognitive function, eg, verbal learning memory, working memory, attention/information processing, and social cognition.[7,44] Whether the C(-1019)G polymorphism is associated with the ability of antipsychotic drugs to treat other cognitive domains deserves further studies.

The limitations of the present study include a small sample number that might have caused a lack of robust level of significance and variability. For example, it might explain the absence of a significant effect of C(-1019)G SNPs on negative symptoms, in contrast to the results in previous studies.[22,25] Secondly, the measure of attention used here is "nonspecific" and not a cognitive test per se; the use of a specific neuropsychological test should enhance sensitivity and specificity to detect possible changes of attention. Furthermore, the two test drugs, perospirone and olanzapine, have different affinities for 5-$HT_{1A}$ receptors; olanzapine shows minimal direct affinity for these receptors.[45] However, the increase in DA release produced by it is blocked by 5-$HT_{1A}$ antagonists (eg, WAY 100635),[35,36] indicating this antipsychotic drug indirectly stimulates 5-$HT_{1A}$ receptors.[45,46] Pretreatment with WAY 100635 also suppresses the increase in DA concentrations induced by perospirone, an AAPD having a high affinity for 5-$HT_{1A}$ receptors.[47]

## CONCLUSION

In conclusion, these results provide additional support to the role of 5-$HT_{1A}$ receptors in some of the cognitive disturbances of schizophrenia, and facilitate the treatment strategy to effectively improve attention. Further studies with a larger number of subjects are warranted.

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## REFERENCES

1. Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: Are we measuring the "right stuff"? Schizophr Bull. 2000;26:119-136.

2. McGurk SR, Meltzer HY. The role of cognition in vocational functioning in schizophrenia. Schizophr Res. 2000;45:175-184.

3. Sumiyoshi T, Park S, Jayathilake K, Roy A, Ertugrul A, Meltzer HY. Effect of buspirone, a serotonin1A partial agonist, on cognitive function in schizophrenia: a randomized, double-blind, placebo-controlled study. Schizophr Res. 2007;95:158-168.

4. Millan MJ. Improving the treatment of schizophrenia: focus on serotonin (5-HT)(1A) receptors. J Pharmacol Exp Ther. 2000;295:853-861.

5. Bantick RA, Deakin JF, Grasby PM. The 5-HT1A receptor in schizophrenia: a promising target for novel atypical neuroleptics? J Psychopharmacol. 2001;15:37-46.

6. Meltzer HY, Sumiyoshi T. Does stimulation of 5-HT(1A) receptors improve cognition in schizophrenia? Behav Brain Res. 2008;195:98-102.

7. Sumiyoshi T, Bubenikova-Valesova V, Horacek J, Bert B. Serotonin1A receptors in the pathophysiology of schizophrenia: development of novel cognition-enhancing therapeutics. Adv Ther. 2008;25:1037-1056.

8. Lesch KP, Gutknecht L. Focus on The 5-HT1A receptor: emerging role of a gene regulatory variant in psychopathology and pharmacogenetics. Int J Neuropsychopharmacol. 2004;7:381-385.

9. Drago A, Ronchi DD, Serretti A. 5-HT1A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. Int J Neuropsychopharmacol. 2008;11:701-721.

10. Hashimoto T, Nishino N, Nakai H, Tanaka C. Increase in serotonin 5HT1A receptors in prefrontal and temporal cortices of brains from patients with chronic schizophrenia. Life Sci. 1991;48:355-363.

11. Sumiyoshi T, Stockmeier CA, Overholser JC, Dilley GE, Meltzer HY. Serotonin1A receptors are increased in postmortem prefrontal cortex in schizophrenia. Brain Res. 1996;708:209-214.

12. Kasper S, Tauscher J, Willeit M, et al. Receptor and transporter imaging studies in schizophrenia, depression, bulimia and Tourette's disorder--implications for psychopharmacology. World J Biol Psychiatry. 2002;3:133-146.

13. Tauscher J, Kapur S, Verhoeff NP, et al. Brain serotonin 5-HT1A receptor binding in schizophrenia measured by positron emission tomography and [11C]WAY-100635. Arch Gen Psychiatry. 2002;59:514-520.

14. Frankle WG, Lombardo I, Kegeles LS, et al. Serotonin 1A receptor availability in patients with schizophrenia and schizo-affective disorder: a positron emission tomography imaging study with [11C]WAY 100635. Psychopharmacology (Berl). 2006;189:155-164.

15. Lee MA, Meltzer HY. 5-HT(1A) receptor dysfunction in female patients with schizophrenia. Biol Psychiatry. 2001;50:758-766.

16. Sumiyoshi T, Meltzer HY. Serotonin1A receptors in memory function. Am J Psychiatry. 2004;161:1505.

17. Borg J. Molecular imaging of the 5-HT(1A) receptor in relation to human cognition. Behav Brain Res. 2008;195:103-111.

18. Sumiyoshi T, Matsui M, Nohara S, et al. Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. Am J Psychiatry. 2001;158:1722-1725.

19. Sumiyoshi T, Matsui M, Yamashita I, et al. Effect of adjunctive treatment with serotonin-1A agonist tandospirone on memory functions in schizophrenia. J Clin Psychopharmacol. 2000;20:386-388.

20. Harvey PD. Pharmacological cognitive enhancement in schizophrenia. Neuropsychol Rev. 2009;19:324-335.

21. Lane HY, Chang YC, Chiu CC, Chen ML, Hsieh MH, Chang WH. Association of risperidone treatment response with a polymorphism in the 5-HT(2A) receptor gene. Am J Psychiatry. 2002;159:1593-1595.

22. Reynolds GP, Arranz B, Templeman LA, Fertuzinhos S, San L. Effect of 5-HT1A receptor gene polymorphism on negative and depressive symptom response to antipsychotic treatment of drug-naive psychotic patients. Am J Psychiatry. 2006;163:1826-1829.

23. Reynolds GP, Yao Z, Zhang X, Sun J, Zhang Z. Pharmacogenetics of treatment in first-episode schizophrenia: D3 and 5-HT2C receptor polymorphisms separately associate with positive and negative symptom response. Eur Neuropsychopharmacol. 2005;15:143-151.

24. Richtand NM, Welge JA, Logue AD, Keck PE, Jr., Strakowski SM, McNamara RK. Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacol. 2007;32:1715-1726.

25. Wang L, Fang C, Zhang A, et al. The --1019 C/G polymorphism of the 5-HT1A receptor gene is associated with negative symptom response to risperidone treatment in schizophrenia patients. J Psychopharmacol. 2008;22:904-909.

26. Huang YY, Battistuzzi C, Oquendo MA, et al. Human 5-HT1A receptor C(-1019)G polymorphism and psychopathology. Int J Neuropsychopharmacol. 2004;7:441-451.

27. Kishi T, Tsunoka T, Ikeda M, et al. Serotonin1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients. Neuropharmacol. 2010;58:452-456.

28. Serretti A, Artioli P, Lorenzi C, Pirovano A, Tubazio V, Zanardi R. The C(-1019)G polymorphism of the 5-HT1A gene promoter and antidepressant response in mood disorders: preliminary findings. Int J Neuropsychopharmacol. 2004;7:453-460.

29. Lemonde S, Du L, Bakish D, Hrdina P, Albert PR. Association of the C(-1019)G 5-HT1A functional promoter polymorphism with antidepressant response. Int J Neuropsychopharmacol. 2004;7:501-506.

30. Hong CJ, Chen TJ, Yu YW, Tsai SJ. Response to fluoxetine and serotonin1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder. Pharmacogenomics J. 2006;6:27-33.

31. Yu YW, Tsai SJ, Liou YJ, Hong CJ, Chen TJ. Association study of two serotonin1A receptor gene polymorphisms and fluoxetine treatment response in Chinese major depressive disorders. Eur Neuropsychopharmacol. 2006;16:498-503.

32. Baune BT, Hohoff C, Roehrs T, Deckert J, Arolt V, Domschke K. Serotonin receptor 1A-1019C/G variant: impact on antidepressant pharmacoresponse in melancholic depression? Neurosci Lett. 2008;436:111-115.

33. Chung YC, Li Z, Dai J, Meltzer HY, Ichikawa J. Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT1A receptor agonism. Brain Res. 2004;1023:54-63.

34. Sato M, Ago Y, Koda K, et al. Role of postsynaptic serotonin1A receptors in risperidone-induced increase in acetylcholine release in rat prefrontal cortex. Eur J Pharmacol. 2007;559:155-160.

35. Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY. 5-HT(2A) and D(2) receptor blockade increases cortical DA release via 5- HT1A receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. J Neurochem. 2001;76:1521-1531.

36. Diaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, Celada P, Artigas F. Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. J Neurosci. 2005;25:10831-10843.

37. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (Text Revision) . Washington DC: APA; 2000.

38. First MB, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders. New York: New York State Psychiatric Institute; 1997.

39. Higuchi Y, Sumiyoshi T, Kawasaki Y, Matsui M, Arai H, Kurachi M. 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. 2008;101:320-330.

40. Sumiyoshi T, Higuchi Y, Itoh T, et al. Effect of perospirone on P300 electrophysiological activity and social cognition in schizophrenia: a three-dimensional analysis with sLORETA. Psychiatry Res Neuroimag 2009;172:180-183.

41. Andreasen NC. Scale for the Assessment of Positive Symptoms (SAPS). Iowa City: University of Iowa; 1983.

42. Andreasen NC. Scale for the Assessment of Negative Symptoms (SANS). Iowa City: University of Iowa; 1983.

43. Parsey RV, Oquendo MA, Ogden RT, et al. Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. Biol Psychiatry. 2006;59:106-113.

44. Erdmann J, Shimron-Abarbanell D, Rietschel M, et al. Systematic screening for mutations in the human serotonin-2A receptor gene: identification of two naturally occurring receptor variants and association analysis in schizophrenia. Hum Genet. 1996;97:614-619.

45. Sumiyoshi T. A possible dose-side effect relationship of antipsychotic drugs: Relevance to cognitive function in schizophrenia. Expert Rev Clin Pharmacol. 2008;1:791-802.

46. Sumiyoshi T, Matsui M, Yamashita I, et al. The effect of tandospirone, a serotonin1A agonist, on memory function in schizophrenia. Biol Psychiatry. 2001;49:861-868.

47. Yoshino T, Nishijima K, Shioda K, Yui K, Katoh S. Perospirone, a novel atypical antipsychotic drug, potentiates fluoxetine-induced increases in dopamine levels via multireceptor actions in the rat medical prefrontal cortex. Neurosci Lett. 2004;24:16-21.

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# Impaired ability to organize information in individuals with autism spectrum disorders and their siblings

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ABSTRACT

Despite rigorous research on disturbances of executive function and social cognition in autism spectrum disorders (ASD), little information has been available concerning higher cognitive functions, such as the ability to focus and associate relevant features to form categories, or 'organizing of information'. The purpose of this study was to investigate this issue by using the Wisconsin Card Sorting Test (WCST) and the Verbal Learning Task (VLT). Cognitive assessments were conducted in 22 individuals with ASD, 14 non-affected siblings, and 15 age-matched control subjects. Overall, individuals with ASD performed significantly worse on the WCST and VLT compared to their siblings and normal control subjects. Although siblings performed generally well on both tasks, they exhibited similar degree of perseverative responses in the WCST compared to the probands. A linear increase of the memory organization score in the VLT was also absent in siblings as well as the ASD group. These results suggest an impaired ability to organize information is one of the cognitive endophenotypes for ASD.

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## 1. Introduction

High-functioning autism (HFA) and Asperger syndrome (AS) are defined as a part of autism spectrum disorders (ASD), characterized by difficulties in establishing social relationships, poor communication skills, lack of imaginative behavior, and repetitive stereotypic behaviors (American Psychiatric Association, 1994; World Health Organization, 1992). Although subjects with HFA or AS do not show a significant delay in intelligence, they have been reported to elicit disturbances of some domains of cognitive function, e.g. social cognition (Baron-Cohen et al., 1985) and executive function (Hill, 2004). For example, they perform poorly on various types of Theory of Mind tasks, ranging from perceptual (e.g. The Eyes Task), verbal (e.g. The Strange Stories) (Kaland et al., 2008a) to emotional ones (identification of emotional states of others) (Shamay-Tsoory, 2008). These results suggest the inability to recognize thoughts and feelings to understand how others act. Also, subjects with ASD have been reported to show impaired executive function, specifically, cognitive flexibility (Geurts et al., 2004) and inhibition (Happe et al., 2006).

Although previous studies have identified some aspects of cognitive disturbance associated with ASD, more specific assessments of higher cognitive functions would help further understand the psychopathology of the disorder. Specifically, 'organizing infor-

*Abbreviations:* ASD, autistic spectrum disorders; WCST, Wisconsin Card Sorting Test; VLT, verbal learning task; SCR, stimulus category repetition.

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[1] The AQ is a self-report questionnaire consisting of five domains of questions regarding the psychopathology of ASD: social skills, attention switching, attention to detail, communication, and imagination.

[2] The CARS is a behavior rating scale completed by clinician or parents based on subjective observation. The scale contains 15 items (e.g. relationship to people, imitation, and so on) and each item is rated with 1 (normal for child's age) to 4 (severely abnormal).

[3] This BAP scale covers three domains of autism, i.e. 'communication impairment', 'social dysfunction', and 'stereotyped and repetitive behavior'. Each domain includes items coded as either 'presence' or 'absence' of autistic symptoms. A subject would be classified as BAP if his/her total score of each domain exceeds the designated cut-off point.

[4] There were two outliers in the %PEM, deviating 2SD from the average of the ASD group. We re-analyzed the data excluding these deviations but the main results have remained the same.

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mation', i.e. the process of focusing and associating relevant information to form categories, appears to be worth investigating, as they are assumed to be pertinent to some cardinal traits, such as inflexible and perseverated behavior or restricted interests (Kenworthy et al., 2005).

The Wisconsin Card Sorting Test (WCST; Heaton et al., 1993) has been used to provide a good measure to evaluate the focusing process. This test consists of four stimulus types, and requires subjects to detect sorting principles from stimulus cards and categorize a response card. The task proceeds through the shifts of sorting principles, i.e. color, form and number. Successful performance on the WCST depends on the ability to detect the correct sorting principle on the basis of feedback, and maintain the principle until it is replaced by a new one.

From 1980s onward, more than 30 studies have been conducted to examine the WCST performance in individual with ASD. The majority of them have reported the degradation in performance on some measures of the task (generally, the number of categories achieved and perseverative errors. For review, Hill (2004), Pennington and Ozonoff (1996), for recent studies, Ambery et al., 2006; Geurts et al., 2004; Hill, 2004; Hill and Bird, 2006; Kaland et al., 2008b; Lopez et al., 2005; Pennington and Ozonoff, 1996; Sergeant et al., 2002; Voelbel et al., 2006; Winsler et al., 2007). Studies targeting adults with ASD, however, have been relatively limited (Ambery et al., 2006; Ciesielski and Harris, 1997; Lopez et al., 2005; Rumsey, 1985; Rumsey and Hamburger, 1988, 1990). Most of these investigations have reported poor achievement of the task in adults with ASD, as has been observed in the studies for younger samples. Ambery et al. (2006), for example, has reported that adults with ASD produced substantially greater perseverative errors, indicating that the ability to find and utilize relevant features of in-coming information may not have been well developed in people with ASD.

There is also paucity of information as to the ability to associate information for category formation in individuals with ASD, besides the findings from free-recall task (Tager-Flusberg, 1991) and the California Verbal Learning Task (CVLT; Delis et al., 1987). Both tasks are similar in that they require subjects to learn orally presented word lists and let them recognize the association of presented stimulus; the association process is evaluated to calculate the difference in the number of recalled words between related (members of a particular category) and unrelated word lists in the free-recall task, or by the number of voluntarily re-organized category-wise responses in the CVLT. Subjects with ASD have been reported to fail to achieve better in the related lists compared to the unrelated lists in the free-recall paradigm, unlike IQ-matched normal controls (Bowler et al., 1997). On the other hand, a study with the CVLT (Minshew and Goldstein, 1993) has not detected clear differences in the semantic cluster ratio, a measure of category formation, between subjects with ASD and normal controls.

Given the mixed results from these verbal learning tasks, more sensitive methods to evaluate the ability to associate information for categorization is needed. The Verbal Learning Task (VLT; Gold et al., 1992; Yamashita et al., 2000), which has been used in the studies of schizophrenia, would be appropriate for this purpose. The VLT consists of three types of word lists: the Random, Blocked, and Semi-blocked lists. The Random list consists of semantically unrelated nouns while the Blocked and Semi-blocked lists contain category exemplars. In the Blocked list, the words are presented on a category-basis, while the words of the same category are never presented consecutively in the Semi-blocked list. One of the strength of the VLT is the inclusion of the SCR (Stimulus Category Repetition; Bousfield and Bousfield, 1966), which quantifies the category-wised responses in the Semi-blocked list. With this measure, the process of category-formation is directly evaluated.

To determine if the deficits of organizing information, discussed above, are cognitive traits specific to ASD, i.e. a cognitive endophenotype, it would be worthwhile to investigate this cognitive ability in biological relatives. A number of studies have reported that several domains of cognitive function are, to some extent, disturbed in first-degree relatives of individuals with ASD (Bailey et al., 1998; Dorris et al., 2004): executive function (Delorme et al., 2007; Hughes et al., 1999; Kawakubo et al., 2009), central coherence (Baron-Cohen and Hammer, 1997; Baron-Cohen et al., 2006; de Jonge et al., 2006; Fombonne et al., 1997), and Theory of Mind (Smalley and Asarnow, 1990; Szatmari et al., 1993). As to the processes of organizing information, however, clear results have not been obtained. For instance, Ozonoff et al. (1993) has reported that sibling of ASD exhibited no distinct impairments in the overall performance on the WCST. On the other hand, studies using the intradimensional/extradimensional (ID/ED) set-shifting task, a categorization task akin to the WCST, have reported that parents (Hughes et al., 1997) and siblings (Hughes et al., 1999) of ASD probands performed poorly compared with those of typically-developed children. Apart from those contradicted findings for the ability to focus on relevant information, little is known about the process for category formation, at least under the verbal leaning task paradigm, in siblings of individuals with ASD.

The purposes of the current study were two-fold: first, the ability to organize information, specifically focusing and associating the relevant features to form categories, were investigated in subjects with ASD using the WCST and the VLT. The two tasks have been typically used as the measure for executive function (flexibility) and verbal learning (or working memory), respectively. The simultaneous implementation of these two tests, however, would be useful to evaluate the two processes of organizing of information; the percentage of perseverative errors of Milner (%PEM) in the WCST provide the index for the focusing process while the SCR in the VLT represents the one for the association process. Second, the possibility that the deficits of the two cognitive processes are ones of the cognitive markers, i.e. endophenotypes, of ASD was examined by administrating these tasks to siblings of subjects with ASD.

## 2. Method

### 2.1. Subjects

Twenty-two individuals with ASD (M/F = 19/3), 14 non-affected siblings (M/F = 8/6), and 15 age-matched normal controls (M/F = 11/4) entered the study. Male/female ratio was not significantly different among the groups ($\chi^2 = 4.01$, $df = 2$, *n.s.*). Subjects in the ASD group met DSM-IV criteria for autistic disorder ($N = 8$), Asperger disorder ($N = 12$) or pervasive developmental disorder not otherwise specified (PDD-NOS) ($N = 2$).

Individuals with ASD and their siblings were recruited from outpatient clinics in the following institutions: Departments of Neuropsychiatry and Child Psychiatry, University of Tokyo Hospital and Mie Prefectural Asunaro Hospital for Children and Adolescent Psychiatry. Participants from public symposia on ASD which took place at The University of Tokyo were also included. Healthy controls were mainly recruited from hospital staff members, their acquaintances and children, and college students. Exclusion criteria were neurological illness, traumatic brain injury with any known cognitive consequences and loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and alcohol/substance abuse or addiction. Besides, based on the Structured Interview Schedule of DSM-IV Axis I Disorders Research Version Non-patient Edition (SCID-I/NP), normal controls were excluded if they or their first-degree relatives had a history of DSM-IV axis I disorders. IQs were evaluated with the WISC-III or WAIS-R (IQ range: normal controls; 87–120, siblings; 90–118, ASD; 58–114). The full

**Table 1**
Demographic and clinical data.

| | Controls | Siblings | ASD[a] |
|---|---|---|---|
| Male/female | 11/4 | 8/6 | 19/3 |
| Age | 29.7(6.4) | 24.5 (4.0) | 26.5(7.4) |
| Education | 15.9(2.1) | 15.3(1.6) | 12.5(1.8)** |
| CARS | – | 15.6(0.8) | 31.2 (6.1)‡ |
| AQ-J | 16.3 (5.6) | 22.7 (5.52) | 30.6 (8.6)† |
| Medication (mg/day)[b] | – | – | 220.8 (347.7) |
| IQ[c] | 99.7(8.6) | 101.2(10.4) | 94.1(17.8) |

*Note*: ASD, autism spectrum disorders; CARS, Childhood Autism Rating Scale; AQ-J, Autism-Spectrum Quotient Japanese version.

[a] Missing data in education = 7, CARS = 1, AQ-J = 10.
[b] Chlorpromazine equivalent dose.
[c] Estimated IQ, controls and siblings; Full IQ, ASD.
** $p < 0.01$; compared to controls and siblings.
‡ $p < 0.01$; compared to siblings.
† $p < 0.05$; compared to siblings.

version was administered to subjects with ASD and siblings while the abbreviated one (i.e. Information, Similarities, Picture Completion, and Digit Symbol-Coding) was applied to normal controls. Although 4 subjects with ASD had IQs of less than 70, they were included in the study as they had completed at least high school education (i.e. more than 12 years). In fact, the directions of principal results, presented later, did not change even if data from those cases were excluded. The Ethical Committee of The University of Tokyo Hospital approved this study (receipt No. 630-5). The Mie Prefectural Asunaro Hospital for Children and Adolescent Psychiatry delegated the ethical review to the ethical committee of The University of Tokyo Hospital because they did not have an institutional review board. Written informed consent was obtained from all participants.

The Autism-Spectrum Quotient Japanese version (AQ-J; Kurita and Koyama, 2006)[5] was administered to probands, siblings, and normal controls. In addition, subjects with ASD and siblings were assessed by the Childhood Autism Rating Scale-Tokyo Version (CARS-TV; Kurita et al., 1989)[6] by trained child psychiatrists. One-way ANOVA for AQ-J yielded a significant group difference ($F = 9.65$, $df = 2, 25$, $p < 0.01$). Multiple comparisons with the Tukey method revealed that the ASD group elicited a significantly higher score than other two groups ($p < 0.05$). As to CARS, $t$-test revealed a significantly higher score for the ASD group compared to siblings ($t = 9.23$, $df = 17$, $p < 0.01$). These results indicate that the ASD group and other two groups were clinically independent. In fact, no siblings were found to elicit autistic features as evaluated by the Broader Autism Phenotype (Le Couteur et al., 1996).[7] All but one on the ASD subjects received medication, with eight of them treated with antipsychotics (risperidone = 4; pimoizide = 2; haloperidol = 2). Other medications included mood stabilizers (e.g. valproate = 6, lithium = 3), benzodiazepines (e.g. bromazepam = 3, nitrazepam = 2, triazolam = 2), anti-depressants (e.g. fluvoxamine = 4, paroxetine = 3), and anti-parkinson drugs (e.g. biperiden = 5, trihexyphenidyl = 2). Demographic and clinical profiles of participants are summarized in Table 1.

[5] The AQ is a self-report questionnaire consisting of five domains of questions regarding the psychopathology of ASD: social skills, attention switching, attention to detail, communication, and imagination.

[6] The CARS is a behavior rating scale completed by clinician or parents based on subjective observation. The scale contains 15 items (e.g. relationship to people, imitation, and so on) and each item is rated with 1 (normal for child's age) to 4 (severely abnormal).

[7] This BAP scale covers three domains of autism, i.e. 'communication impairment', 'social dysfunction', and 'stereotyped and repetitive behavior'. Each domain includes items coded as either 'presence' or 'absence' of autistic symptoms. A subject would be classified as BAP if his/her total score of each domain exceeds the designated cut-off point.

### 2.2. Design and procedure

*WCST.* A computerized version of the WCST (WCST-64: Computer Version-2 Research Edition, Psychological Assessment Resources, Inc.) was used. Subjects were requested to sort cards according to one of the implicit principles (i.e. color, shape, and number), which is altered after 10 consecutive correct responses. A test session was terminated when 6 shifts had been completed. Three variables, (1) number of categories achieved (CA), (2) the percentage of perseverative errors of Milner (%PEM), and (3) reaction time (RT), were analyzed.

*VLT.* The Japanese version of the VLT (JVLT; Yamashita et al., 2000) was used. This task consists of three 16-word lists: Random list, Blocked list, and Semi-blocked list. The Random list consists of 16 unrelated nouns. Other two lists contain four exemplars from four taxonomic categories (the Blocked list: stationery, animal, musical instruments, and sports; the Semi-blocked list: vehicles, seasoning, flower, and countries). In the Blocked list, exemplars in the same category were presented consecutively, while they were never given serially in the Semi-blocked list. Thus, in the Semi-blocked list, the list items would be re-organized in a category-wise manner, if a subject voluntary formed categories to be utilized as recalling cues.

Three trials were conducted and each trial included the Random, Blocked, and Semi-blocked lists. The lists were presented in the fixed order of the Random, Blocked, and Semi-blocked lists. Every word in the lists was presented orally in 1 s basis, and subjects were instructed to learn them. The VLT scores were calculated for each type of list as the average of the three trials. In addition, for the Semi-blocked lists, Stimulus Category Repetition (SCR; Bousfield and Bousfield, 1966) was calculated. SCR is defined as the total number of exemplars in the same category consecutively recalled in each trial. Thus, it was considered to be an index of the degree to which a subject associates information based on implicitly given categories (Koh et al., 1976).

### 2.3. Statistical analyses

Multivariate analysis of variance (MANOVA) was conducted to examine group differences for demographic variables (age, education, IQ) and three measures of WCST (CA, %PEM, RT). The arcsine transformation and logarithmic transformation were applied for %PEM and RT, respectively. The VLT scores were analyzed by three-way ANOVA with Group (normal controls vs. siblings vs. ASD) as between-subject factor, and Block (Random vs. Blocked vs. Semi-blocked) and Trial (1st vs. 2nd vs. 3rd) as within-subjects factor. The SCR scores were examined by two-way ANOVA, with Group (normal controls vs. siblings vs. ASD) as between-subjects factor and Trial (1st vs. 2nd vs. 3rd) as within-subjects factor. The correlation analyses were conducted between the CARS Total scores and the measures of the WCST and VLT to examine the relationship between the severity of symptoms and cognitive performances.

## 3. Results

### 3.1. Demographic variables

Demographic and clinical data are shown in Table 1. MANOVA indicated an overall difference among the three groups (*Wilks' lambda* = 0.52, $F = 4.45$, $df = 6, 68$, $p < 0.01$). Subsequent univariate analyses revealed that the ASD group showed a significantly shorter education period than did other two groups ($F = 13.22$, $df = 2, 39$, $p < 0.01$), while Age ($F = 2.26$, $df = 2, 48$, *n.s.*) and IQ ($F = 2.29$, $df = 2, 44$, *n.s.*) did not differ among the groups.

**Table 2**
Performance on the Wisconsin Card Sorting Test (WCST) and Verbal Learning Task (VLT).

| | Controls | Siblings | ASD |
|---|---|---|---|
| WCST | | | |
| CA | 6.0(1.7) | 5.9(0.5) | 4.2 (2.2) |
| %PEM | 9.9 (4.5) | 12.1 (9.2) | 24.1 (19.5) |
| RT (min) | 1.6(0.3) | 1.8(0.8) | 2.1(1.1) |
| JVLT | | | |
| Random | 9.9(3.6) | 10.1 (3.0) | 8.6(3.6) |
| Semi-blocked | 11.8(3.3) | 12.9 (2.7) | 10.0 (4.0) |
| Blocked | 13.8 (2.9) | 13.8(2.5) | 11.2(4.3) |
| SCR1 | 3.0(2.4) | 4.6 (2.8) | 3.5 (3.2) |
| SCR2 | 6.7 (4.3) | 7.6(3.8) | 5.8 (4.0) |
| SCR3 | 8.9(4.3) | 8.9(4.5) | 5.9 (4.8) |

*Note*: ASD, autism spectrum disorders; WCST, Wisconsin Card Sorting Test; JVLT, Japanese Verbal Learning Task; CA, categories achieved; %PEM, percentages of Milnere-type perseverative errors; RT, reaction time; SCR, stimulus category repetition.

### 3.2. Performance on the WCST

Mean and SD for variables of the WCST are summarized in Table 2. MANOVA demonstrated significant overall group difference (*Wilks' lambda* = 0.71, $F = 2.70$, $df = 6, 86$, $p < 0.05$). Subsequent univariate analyses revealed main effects of CA ($F = 8.31$, $df = 2, 45$, $p < 0.01$) and %PEM ($F = 5.20$, $df = 2, 45$, $p < 0.01$),[8] but not RT ($F = 0.10$, $df = 2, 45$, *n.s.*). Multiple comparisons showed a significantly lower CA score for the ASD group compared to other two groups ($p < 0.01$). As for the %PEM, the difference was found only between the ASD group and normal controls. Siblings did not differ from either group.

### 3.3. Performance on the VLT

Mean and SD for variables of the VLT are shown in Table 2. ANOVA showed a significant Block × Group interaction effect ($F = 2.54$, $df = 4, 96$, $p < 0.05$) on the VLT scores. Simple main effects of Group were significant for the Blocked ($F = 4.137$, $df = 2, 144$, $p < 0.05$) and Semi-blocked ($F = 5.87$, $df = 2, 144$, $p < 0.01$), but not the Random ($F = 1.72$, $df = 2, 144$, *n.s.*) lists condition. Multiple comparisons yield significant differences in the Blocked lists condition in the order of controls = siblings > ASD ($p < 0.05$). For the Semi-blocked lists condition, on the other hand, the difference was only found in siblings > ASD ($p < 0.01$).

ANOVA revealed a significant Trial × Group interaction effect ($F = 3.14$, $df = 4, 86$ $p < 0.05$) on the SCR score. Therefore, simple main effect for the Trial condition was examined, which yielded significant results for all groups (normal controls: $F = 49.86$, $df = 2, 129$, $p < 0.01$; siblings; $F = 27.23$, $df = 2, 129$, $p < 0.01$; ASD; $F = 10.22$, $df = 2, 129$, $p < 0.01$). Multiple comparisons revealed a stepwise enhancement on the SCR score (3rd trial > 2nd trial > 1st trial) in normal controls ($p < 0.01$), while the difference was detected only between the first two trials (2nd trial > 1st trial) in siblings ($p < 0.01$) and ASD patients ($p < 0.01$).

### 3.4. Correlation analyses

No measures in the WCST were significantly correlated with the CARS total score (CA: $r = -0.34$, $df = 19$, *n.s.*; %PEM: $r = -0.12$; RT: $r = 0.15$ $df = 18$, *n.s.*). The same result was obtained in the VLT (Random: $r = -2.66$; Blocked: $r = -0.14$; Semi-blocked: $r = -0.21$, $df = 20$, *n.s.*; SCR1: $r = -0.06$; SCR2: $r = -0.23$; SCR3: $r = -0.16$, $df = 15$, *n.s.*).

[8] There were two outliers in the %PEM, deviating 2SD from the average of the ASD group. We re-analyzed the data excluding these deviations but the main results have remained the same.

## 4. Discussion

The purpose of the current study was to investigate the ability to organize information in individuals with ASD and their siblings. Specifically, the two cognitive processes in that ability, i.e. focusing on relevant features and associating them to form categories, were examined using the WCST and VLT.

Overall, individuals with ASD performed poorly on the WCST and VLT, as shown by the significantly worse scores on most measures of the tasks (Table 3). Specifically, greater %PEM in the ASD group is in accordance with a previous study (Kenworthy et al., 2005), representing the poor ability to focus on the relevant information in subjects with ASD using other tasks (i.e. the Object Assembly, the Rey-Osterrieth Complex Figure, the Story Sentence Memory, and letter fluency with "F, A, S". Similarly, the lack of an increase in the SCR supports the result from the free-recall task (Bowler et al., 1997), reporting the failure to associate category members in subjects with ASD. Both %PEM and the SCR scores did not correlate with the CARS score in the ASD group. Thus, it is possible that degradation in the cognitive process, such as focusing and associating relevant information, is intrinsic to the symptomatology of ASD irrespective of severity of the illness.

The poor cognitive performance in subjects with ASD cannot be attributed entirely to their shorter education period. First, the IQ level in subjects with ASD on the whole was not significantly different from those in siblings and normal controls, as shown by the analysis of the demographic variables. In addition, the RT in the WCST and performance on the Random List condition in the VLT were equivalent to those for normal controls and siblings. In all, these results suggest that general intelligence, visuo-motor skills, attention, and memory capacity, were relatively uniform among the three groups studied here.

Generally, the sibling group performed almost as equally well as the normal control group; however, there were some noticeable similarities between siblings and subjects with ASD (Table 3, highlighted in bold). First, siblings have failed to show the linear increase in the SCR score in the VLT. Second, the %PEM for siblings was not significantly smaller than that for the ASD group, although it did not differ from that of normal controls, either (Table 3). This result is worth noting as siblings performed better than individuals with ASD on other measures, such as the CA. In all, the results from the two tasks are likely to reflect the limited ability of siblings to focus and conceptualize categories using updated information.

The tendency of 'weak central coherence' may explain the current results for the subjects with ASD. 'Central coherence' is a cognitive trait to extract meaning, gist, and gestalt from given stimuli (Frith and Happe, 1994). Impairment of this ability, or 'weak central coherence', leads to an inclination to focus on individual parts of information without integrating them (Happe and Frith, 2006). This may be related to increased perseverative responses in subjects with ASD; once their attention has been captured by a particular dimension of features, shifting it according to negative feedback would become difficult.

The weak central coherence trait also seems to be related to higher cognitive function such as category formation and organization (Plaisted, 2001); in order to form a category, it is necessary to extract a commonality from each piece of information and ignore idiosyncratic features. This cognitive process may be disturbed in people with ASD, leading to the restriction in forming categories. The lack of an increase in the SCR scores may be accounted for by this cognitive deficit.

Interestingly, the tendency related to weak central coherence has also been reported in first-degree relatives of patients with ASD, especially their fathers (Happe et al., 2001). This seems to be consistent with our observations that siblings of subjects with ASD failed to show a steady increase of the SCR in the VLT, suggesting

**Table 3**
Summary of performances on the WCST and JVLT.

| WCST | | JVLT | | | |
|---|---|---|---|---|---|
| | | SCORE | | SCR | |
| **CA** | Controls = Siblings > ASD | **Blocked** | Controls = Siblings > ASD | **Normal controls** | SCR1 > SCR2 > SCR3 |
| **%PEM** | Controls < ASD | **Semi-** | Siblings > ASD | **ASD** | **SCR1 = SCR2 > SCR3** |
| | Controls = siblings | **blocked** | Controls = Siblings | | |
| | **Siblings = ASD** | | | | |
| **RT** | Controls = Siblings = ASD | **Random** | Controls = Siblings = ASD | **Siblings** | **SCR1 = SCR2 > SCR3** |

*Note*: ASD, autism spectrum disorders WCST; Wisconsin Card Sorting Test; JVLT, Japanese Verbal Learning Task; SCR, stimulus category repetition.

that degradation in the ability to associate information is one of the cognitive markers, i.e. endophenotypes for people vulnerable to ASD.

It is hypothesized that attenuated neural activities in parahippocampal regions may be responsible for the difficulties in organizing information in subjects with ASD. The left parahippocampal regions, including parahippocampal gyrus, has been suggested to play an important role in sorting, relating, and sending information to hippocampus (Eichenbaum, 1997; Sumiyoshi et al., 2006; Vargha-Khadem et al., 1997). Thus, dysfunction of these regions is assumed to impair the ability to systemize incoming information. In fact, several idiosyncrasies around parahippocampal regions have been reported in individuals with ASD. For example, Boucher et al. (2005) found a smaller parahippocampal volume in patients with ASD compared to normal controls, as well as weaker correlational activities between this region and the hippocampal region.

A key issue for future research is the developmental course of the knowledge structure, that is, semantic association in the long-term memory, in individuals with ASD. Given that the ability to organize information is impaired, knowledge structure would suffer from insufficient maturation in subjects with ASD, as has been reported in children with William's syndrome (Johnson and Carey, 1998). Although several attempts have been already made to address this issue (Dunn et al., 1996), further research is expected to clarify the link between the limited ability to organize information and the development of knowledge structure in individuals with ASD and those who are vulnerable to the disorder.

Finally, several limitations for the present study should be mentioned. First, it would be worthwhile to relate some cognitive measures studied here, e.g. %PEM and SCR, to endophenotypes of ASD in replication studies with a larger sample of ASD probands and their siblings. Second, although clinical variables such as intelligence did not significantly affect current findings, evaluation with more adjusted samples would be desirable in future studies.

## 5. Conclusion

The current study has identified the limited ability to organize information in individuals with ASD, extending the previous observations that they performed poorly on most of the measures of the WCST and verbal learning compared with normal controls. In addition, their siblings showed similar impairments on the measures of organizing of information derived from these tasks, suggesting that the deficits of this cognitive ability is one of the cognitive endophenotypes of ASD.

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## References

Ambery, F.Z., Russell, A.J., Perry, K., Morris, R., Murphy, D.G., 2006. Neuropsychological functioning in adults with Asperger syndrome. Autism 10, 551–564.
American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Rev. ed. American Psychiatric Press, Washington, DC.
Bailey, A., Palferman, S., Heavey, L., Le Couteur, A., 1998. Autism: the phenotype in relatives. J. Autism Dev. Disord. 28, 369–392.
Baron-Cohen, S., Hammer, J., 1997. Is autsim an extreme form of the "male brain"? Adv. Infancy Res. 11, 193–217.
Baron-Cohen, S., Leslie, A.M., Frith, U., 1985. Does the autistic child has a "theory of mind"? Cognition 21, 37–46.
Baron-Cohen, S., Ring, H., Chitnis, X., Wheelwright, S., Gregory, L., Williams, S., Brammer, M., Bullmore, E., 2006. fMRI of parents of children with Asperger Syndrome: a pilot study. Brain Cogn. 61, 122–130.
Boucher, J., Cowell, P., Howard, M., Broks, P., Farrant, A., Roberts, N., Mayes, A., 2005. A combined clinical, neuropsychological, and neuroanatomical study of adults with high functioning autism. Cognit. Neuropsychiatry 10, 165–213.
Bousfield, A.K., Bousfield, W.A., 1966. Measurment of cluster organizational strategies in free recall following frontal lobing and of sequential constancies in repeated free recall. Psychol. Rep. 19, 935–942.
Bowler, D.M., Matthews, N.J., Gardiner, J.M., 1997. Asperger's syndrome and memory: similarity to autism but not amnesia. Neuropsychologia 35, 65–70.
Ciesielski, K.T., Harris, R.J., 1997. Factors related to performance failure on executive tasks in autism. Child Neuropsychol. 3, 1–12.
de Jonge, M.V., Kemner, C., van Engeland, H., 2006. Superior disembedding performance of high-functioning individuals with autism spectrum disorders and their parents: the need for subtle measures. J. Autism Dev. Disord. 36, 677–683.
Delis, D.C., Kramer, J.H., Kaplan, E., Ober, B.A., 1987. California Verbal Learning Test: Adult Version Mannual. The Psychological Corporation, San Antonio, TX.
Delorme, R., Gousse, V., Roy, I., Trandafir, A., Mathieu, F., Mouren-Simeoni, M.C., Betancur, C., Leboyer, M., 2007. Shared executive dysfunctions in unaffected relatives of patients with autism and obsessive-compulsive disorder. Eur. Psychiatry 22, 32–38.
Dorris, L., Espie, C.A., Knott, F., Salt, J., 2004. Mind-reading difficulties in the siblings of people with Asperger's syndrome: evidence for a genetic influence in the abnormal development of a specific cognitive domain. J. Child Psychol. Psychiatry 45, 412–418.
Dunn, M., Gomes, H., Sebastian, M.J., 1996. Prototypicality of responses of autistic, language disordered, and normal children in word fluency task. Child Neuropsychol. 2, 99–108.
Eichenbaum, H., 1997. How does the brain organize memories? Science 277, 330–332.
Fombonne, E., Bolton, P., Prior, J., Jordan, H., Rutter, M., 1997. A family study of autism: cognitive patterns and levels in parents and siblings. J. Child Psychol. Psychiatry 38, 667–683.
Frith, U., Happe, F., 1994. Autism: beyond "theory of mind". Cognition 50, 115–132.
Geurts, H.M., Verte, S., Oosterlaan, J., Roeyers, H., Sergeant, J.A., 2004. How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism? J. Child Psychol. Psychiatry 45, 836–854.
Gold, J.M., Randolph, C., Carpenter, C.J., Goldberg, T.E., Weinberger, D.R., 1992. Forms of memory failure in schizophrenia. J. Abnorm. Psychol. 101, 487–494.

Happe, F., Booth, R., Charlton, R., Hughes, C., 2006. Executive function deficits in autism spectrum disorders and attention-deficit/hyperactivity disorder: examining profiles across domains and ages. Brain Cogn. 61, 25–39.
Happe, F., Briskman, J., Frith, U., 2001. Exploring the cognitive phenotype of autism: weak "central coherence" in parents and siblings of children with autism. I. Experimental tests. J. Child Psychol. Psychiatry 42, 299–307.
Happe, F., Frith, U., 2006. The weak coherence account: detail-focused cognitive style in autism spectrum disorders. J. Autism Dev. Disord. 36, 5–25.
Heaton, R.K., Chelune, G.J., Talley, J.L., Kay, G.G., Curtiss, G., 1993. Wisconsin Card Sorting Test Manual: Revised and Expanded. Psychological Assessment Resources, Odessa, FL.
Hill, E.L., 2004. Executive dysfunction in autism. Trends Cognit. Sci. 8, 26–32.
Hill, E.L., Bird, C.M., 2006. Executive processes in Asperger syndrome: patterns of performance in a multiple case series. Neuropsychologia 44, 2822–2835.
Hughes, C., Leboyer, M., Bouvard, M., 1997. Executive function in parents of children with autism. Psychol. Med. 27, 209–220.
Hughes, C., Plumet, M.H., Leboyer, M., 1999. Towards a cognitive phenotype for autism: increased prevalence of executive dysfunction and superior spatial span amongst siblings of children with autism. J. Child Psychol. Psychiatry 40, 705–718.
Johnson, S.C., Carey, S., 1998. Knowledge enrichment and conceptual change in folkbiology: evidence from Williams syndrome. Cognit. Psychol. 37, 156–200.
Kaland, N., Callesen, K., Moller-Nielsen, A., Mortensen, E.L., Smith, L., 2008a. Performance of children and adolescents with Asperger syndrome or high-functioning autism on advanced theory of mind tasks. J. Autism Dev. Disord. 38, 1112–1123.
Kaland, N., Smith, L., Mortensen, E.L., 2008b. Brief report: cognitive flexibility and focused attention in children and adolescents with Asperger syndrome or high-functioning autism as measured on the computerized version of the Wisconsin Card Sorting Test. J. Autism Dev. Disord. 38, 1161–1165.
Kawakubo, Y., Kuwabara, H., Watanabe, K., Minowa, M., Someya, T., Minowa, I., Kono, T., Nishida, H., Sugiyama, T., Kato, N., Kasai, K., 2009. Impaired prefrontal hemodynamic maturation in autism and unaffected siblings. PLoS One 4, e6881.
Kenworthy, L.E., Black, D.O., Wallace, G.L., Ahluvalia, T., Wagner, A.E., Sirian, L.M., 2005. Disorganization: the forgotten executive dysfunction in high-functioning autism (HFA) spectrum disorders. Dev. Neuropsychol. 28, 809–827.
Koh, S.D., Kayton, L., Peterson, R.A., 1976. Affective encoding and consequent remembering in schizophrenic young adults. J. Abnorm. Psychol. 85, 156–166.
Kurita, H., Koyama, T., 2006. Autism-spectrum quotient Japanese version measures mental health problems other than autistic traits. Psychiatry Clin. Neurosci. 60, 373–378.
Kurita, H., Miyake, Y., Katsuno, K., 1989. Reliability and validity of the Childhood Autism Rating Scale—Tokyo version (CARS-TV). J. Autism Dev. Disord. 19, 389–396.
Le Couteur, A., Bailey, A., Goode, S., Pickles, A., Robertson, S., Gottesman, I., Rutter, M., 1996. A broader phenotype of autism: the clinical spectrum in twins. J. Child Psychol. Psychiatry 37, 785–801.
Lopez, B.R., Lincoln, A.J., Ozonoff, S., Lai, Z., 2005. Examining the relationship between executive functions and restricted, repetitive symptoms of Autistic Disorder. J. Autism Dev. Disord. 35, 445–460.
Minshew, N.J., Goldstein, G., 1993. Is autism amnestic disorder? Evidence from the California verbal learning test. Neuropsychology 7, 209–216.
Ozonoff, S., Rogers, S.J., Farnham, J.M., Pennington, B.F., 1993. Can standard measures identify subclinical markers of autism? J. Autism Dev. Disord. 23, 429–441.
Pennington, B.F., Ozonoff, S., 1996. Executive functions and developmental psychopathology. J. Child Psychol. Psychiatry 37, 51–87.
Plaisted, K.C., 2001. Reduced generalization in autism: an alternative to weak central coherence. In: Burack, J.A., Charman, T., Yirmiya, N., Zelazo, P.R. (Eds.), The Development of Autism: Perspective from Theory and Research. LEA, New Jersey, pp 149–169.
Rumsey, J.M., 1985. Conceptual problem-solving in highly verbal, nonretarded autistic men. J. Autism Dev. Disord. 15, 23–36.
Rumsey, J.M., Hamburger, S.D., 1988. Neuropsychological findings in high-functioning men with infantile autism, residual state. J. Clin. Exp. Neuropsychol. 10, 201–221.
Rumsey, J.M., Hamburger, S.D., 1990. Neuropsychological divergence of high-level autism and severe dyslexia. J. Autism Dev. Disord. 20, 155–168.
Sergeant, J.A., Geurts, H., Oosterlaan, J., 2002. How specific is a deficit of executive functioning for attention-deficit/hyperactivity disorder? Behav. Brain Res. 130, 3–28.
Shamay-Tsoory, S.G., 2008. Recognition of 'fortune of others' emotions in Asperger syndrome and high functioning autism. J. Autism Dev. Disord. 38, 1451–1461.
Smalley, S.L., Asarnow, R.F., 1990. Cognitive subclinical markers in autism. J. Autism Dev. Disord. 20, 271–278.
Sumiyoshi, T., Sumiyoshi, C., Nohara, S., Hagino, H., Hasegawa, S., Kuwayama, N., Endo, S., Kurachi, M., 2006. Verbal memory deficits in a preadolescent case of lesions of the left parahippocampal gyrus associated with a benign tumor. Prog. Neuropsychopharmacol. Biol. Psychiatry 30, 733–736.
Szatmari, P., Jones, M.B., Tuff, L., Bartolucci, G., Fisman, S., Mahoney, W., 1993. Lack of cognitive impairment in first-degree relatives of children with pervasive developmental disorders. J. Am. Acad. Child Adolesc. Psychiatry 32, 1264–1273.
Tager-Flusberg, H., 1991. Semantic proceesing in the free recall of autistic children: further evidence for a cognitive deficit. Br. J. Psychol. 9, 417–430.
Vargha-Khadem, F., Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., Mishkin, M., 1997. Differential effects of early hippocampal pathology on episodic and semantic memory. Science 277, 376–380.
Voelbel, G.T., Bates, M.E., Buckman, J.F., Pandina, G., Hendren, R.L., 2006. Caudate nucleus volume and cognitive performance: are they related in childhood psychopathology? Biol. Psychiatry 60, 942–950.
Winsler, A., Abar, B., Feder, M.A., Schunn, C.D., Rubio, D.A., 2007. Private Speech and executive functioning among high-functioning children with autistic spectrum disorders. J. Autism Dev. Disord. 37, 1617–1635.
World Health Organization, 1992. International Classification of Diseases and Related Health Problems. 10th revision. WHO, Geneva.
Yamashita, I., Matsui, M., Kurachi, M., 2000. Development of word memory test measuring memory organization. Seishin Igaku 42, 1279–1283.

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# Association between severe dorsolateral prefrontal dysfunction during random number generation and earlier onset in schizophrenia

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ABSTRACT

*Objectives:* Schizophrenia involves impairment in attention, working memory and executive processes associated with prefrontal cortical function, an essential contributor of social functioning. Age at onset is a major factor for predicting social outcome in schizophrenia. In clinical settings, we need an objective assessment tool for evaluating prefrontal function and social outcome.
*Methods:* Participants included 22 right-handed patients with schizophrenia and 40 gender- and age-matched healthy controls. We used a 52-channel near-infrared spectroscopy (NIRS) instrument to measure oxygenated haemoglobin ([oxy-Hb]) changes over the prefrontal cortex during a random number generation (RNG) task.
*Results:* In healthy controls, we found significant [oxy-Hb] increase in the bilateral dorsolateral (DLPFC; BA9 and BA46) and ventrolateral prefrontal cortex (VLPFC; BA44, 45 and 47). The patients with schizophrenia showed significantly smaller activation than the healthy controls in the same approximate regions. In the patient group, a smaller [oxy-Hb] increase in the right DLPFC region (BA9) was significantly correlated with earlier age at onset.
*Conclusions:* NIRS can detect prefrontal cortical dysfunction associated with an executive task, which was coupled with earlier age at onset in schizophrenia.
*Significance:* Multichannel NIRS, a non-invasive and user-friendly instrument, may be useful in evaluating cognitive function and social outcome in clinical settings in psychiatry.
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## 1. Introduction

Age at onset of schizophrenia is assumed to be one of the major risk factors that worsen symptoms and cognitions, and consequently influences occupational and social deficits (Eggers and Bunk, 1997; Hafner, 2000). In recent years, therefore, some research groups have tried to delay and finally prevent the onset of schizophrenia in adolescents (Brewer et al., 2005; Hafner, 2000; McGlashan et al., 2006; McGorry et al., 2002). However, it has been difficult to predict the onset of schizophrenia with clinical assessment, and neuropsychological or neuroimaging tools that could be easily applied in a practical clinical setting have never been developed.

The random number generation (RNG) task is one of neuropsychological tasks used to assess functional abnormalities in the frontotemporal cortex, which requires participants to generate random digits at equal intervals. The RNG task requires the ability not only to inhibit competing or habitual responses, but also requires attention, working memory and executive processes, all of which are related to prefrontal cortical functions (Baddeley and Wilson, 1988; Ginsburg and Karpiuk, 1994). The RNG task requires less specificity for a certain function than other neurocognitive tasks, such as n-back task for working memory, but rather requires coordinating these functions related in a major way to the prefrontal cortex (PFC). Furthermore, the RNG task can measure prefrontal dysfunction easily in clinical settings. Patients with schizophrenia showed lower performance than controls (Hoshi et al., 2006; Peters et al., 2007; Shinba et al., 2004), but showed performance similar to patients with other frontal dysfunctions, such as Alzheimer's disease (Brugger et al., 1996), and frontal injuries (Spatt and Goldenberg, 1993).

A functional neuroimaging study using positron emission tomography (PET) showed that healthy participants had increased activation in the right inferior frontal cortex, the left dorsolateral prefrontal cortex (DLPFC) and the bilateral cerebellum during the

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RNG task (Jahanshahi et al., 2000). A functional magnetic resonance imaging (fMRI) study also showed that healthy participants had increased activation bilaterally in the DLPFC, the lateral premotor cortex, the anterior cingulate, the inferior and superior parietal cortex and in the cerebellar hemispheres (Daniels et al., 2003).

A near-infrared spectroscopy (NIRS) technique can measure the signals that reflect haemodynamic oxygenated haemoglobin ([oxy-Hb]) and deoxygenated haemoglobin ([deoxy-Hb]) changes in the cerebral cortex, while functional brain imaging methodologies such as fMRI and PET instruments are limited because of their large apparatuses, which prevent their use at a bedside setting for diagnostic and treatment purposes. The advantages of NIRS are its non-invasiveness, easy set-up, minimal constraint, rather small machinery and quietness. In addition, NIRS instruments with multichannels can evaluate the spatio-temporal characteristics of cortical function, and have considerable replicability of signal changes over the PFC in repetitive measurements (Kakimoto et al., 2009; Kono et al., 2007). Accordingly, NIRS has been used to assess brain functions in many psychiatric disorders, including schizophrenia (Fallgatter and Strik, 2000; Hoshi et al., 2006; Lee et al., 2008; Shinba et al., 2004; Suto et al., 2004; Takizawa et al., 2008, 2009), bipolar disorder (Matsuo et al., 2007), depression (Matsuo et al., 2005), post-traumatic stress disorder (Matsuo et al., 2003), panic disorder (Nishimura et al., 2007) and pervasive developmental disorders (Kuwabara et al., 2006). Even though it has also been indicated that patients with schizophrenia had reduced activation in the PFC using a two-channel NIRS instrument during the RNG task (Hoshi et al., 2006; Shinba et al., 2004), these studies had difficulty in detecting differential spatio-temporal characteristics. Our multichannel NIRS study showed reduced activations of the frontopolar region, rather than other prefrontal regions, and showed significant positive correlations with lower global assessment of functioning (GAF) scores in the schizophrenia group (Takizawa et al., 2008). Therefore, multichannel NIRS instruments could be useful in evaluating the spatio-temporal activation patterns in the prefrontal sub-regions.

In this study, we measured the change in prefrontal activation during the RNG task in patients with schizophrenia and healthy controls by using a 52 multichannel NIRS instrument with a wide coverage over the prefrontal cortical surface area. Furthermore, we sought to explore the relationship between haemodynamic prefrontal responses and age at onset in patients with schizophrenia.

## 2. Methods

### 2.1. Participants

Participants included 22 patients with schizophrenia and 40 gender- and age-matched healthy controls. All participants were right handed (>70 in the Edinburgh handedness scale (Oldfield, 1971)). They gave written informed consent to the ethical committee of the Faculty of Medicine, University of Tokyo (approval number 630-5), according to the Declaration of Helsinki, after a complete explanation of this study. The patients with schizophrenia were recruited among outpatients and inpatients at the University of Tokyo Hospital. One experienced psychiatrist, K.K., diagnosed with the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 2003). The exclusion criteria for both groups were neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, low premorbid Intelligence Quotient (IQ) (below 70) and previous alcohol/substance abuse or addiction. The exclusion criterion for the control group was a previous psychiatric disorder in themselves or a family history of psychotic disorders in their first-degree relatives. To rule out psychiatric disorders in healthy controls, we used the modified Mini-International Neuropsychiatric Interview for all control participants (Otsubo et al., 2005).

**Table 1**
Demographic characteristics and performance of random number generation task in patients with schizophrenia (SZ) and healthy controls (HC).

| | HC ($n$ = 40) | | SZ ($n$ = 22) | | $p$-Value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Male | 20 | N.A. | 11 | N.A. | 1.0 |
| Age (years) | 36.8 | 15.3 | 41.0 | 11.6 | 0.238 |
| Education (year) | 15.3 | 1.6 | 14.5 | 2.8 | 0.200 |
| Participant's SES[a] | 2.0 | 0.6 | 3.6 | 1.1 | <0.001 |
| PANSS scores[b] | | | | | |
| Positive | N.A. | N.A. | 16.3 | 4.2 | N.A. |
| Negative | N.A. | N.A. | 23.0 | 6.3 | N.A. |
| General | N.A. | N.A. | 38.8 | 7.6 | N.A. |
| GAF[c] | N.A. | N.A. | 46.7 | 11.9 | N.A. |
| Age at onset (years) | N.A. | N.A. | 26.3 | 8.8 | N.A. |
| DUP (weeks)[d] | N.A. | N.A. | 44.5 | 49.2 | N.A. |
| DOI (years)[e] | N.A. | N.A. | 14.7 | 8.8 | N.A. |
| Chlorpromazine eq. (mg) | N.A. | N.A. | 809 | 674 | N.A. |
| Diazepam eq. (mg) | N.A. | N.A. | 10.5 | 13.1 | N.A. |
| Biperiden eq. (mg) | N.A. | N.A. | 4.1 | 2.1 | N.A. |
| RNG index | 0.112 | 0.034 | 0.194 | 0.184 | 0.0497 |

N.A., not applicable.
[a] SES, socioeconomic status.
[b] PANSS, Positive and Negative Syndrome Scale.
[c] GAF, the global assessment of functioning.
[d] DUP, duration of untreated psychosis.
[e] DOI, duration of illness.

On the same day as the NIRS measurement, all participants were assessed using the GAF (American Psychiatric Association, 1994) and the socioeconomic status (SES) and parental SES using the Hollingshead scale (Hollingshead, 1965) (Table 1). In the patients with schizophrenia, we evaluated their psychiatric symptoms by using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). To verify the age at onset of schizophrenia, we interviewed the patients and one of their family members in detail to obtain a history of their symptoms around their initial psychotic periods, and retrospectively estimated the age at onset in accordance with Addington et al. (2004). The onset of schizophrenia is defined by the presence of first positive symptoms (hallucinations, delusions or thought disorder) rated as above 4 on the PANSS and lasting throughout the day for several days or several times a week, not being limited to a few brief moments (Addington et al., 2004). At the time of the experiment, all patients received antipsychotics and/or anxiolytics and/or antiparkinsonian agents; therefore, we assessed their dose of medication and calculated equivalent doses of chlorpromazine, diazepam and biperiden, respectively (American Psychiatric Association, 1997).

### 2.2. RNG task

We divided the RNG task into three periods; counting, activation and post-activation periods. During the 60-s activation period, we instructed the participants to generate and call out random digits from 1 to 9 by using 1-Hz pacing, and noted their generated numbers correctly. During the 30-s counting and 70-s post-activation periods, they were asked to repeat aloud a sequence of digits from 1 to 9, guided by 1-Hz pacing. The pacing rate (every 1 s) was controlled with a metronome sound throughout the task. All participants performed a short training version of this task at least twice and then performed the RNG task. In this study, we defined 'valid response' as saying a correct digit aloud along with the metronome sound, and adopted those participants who could achieve a greater than 90% valid response during the activation period.

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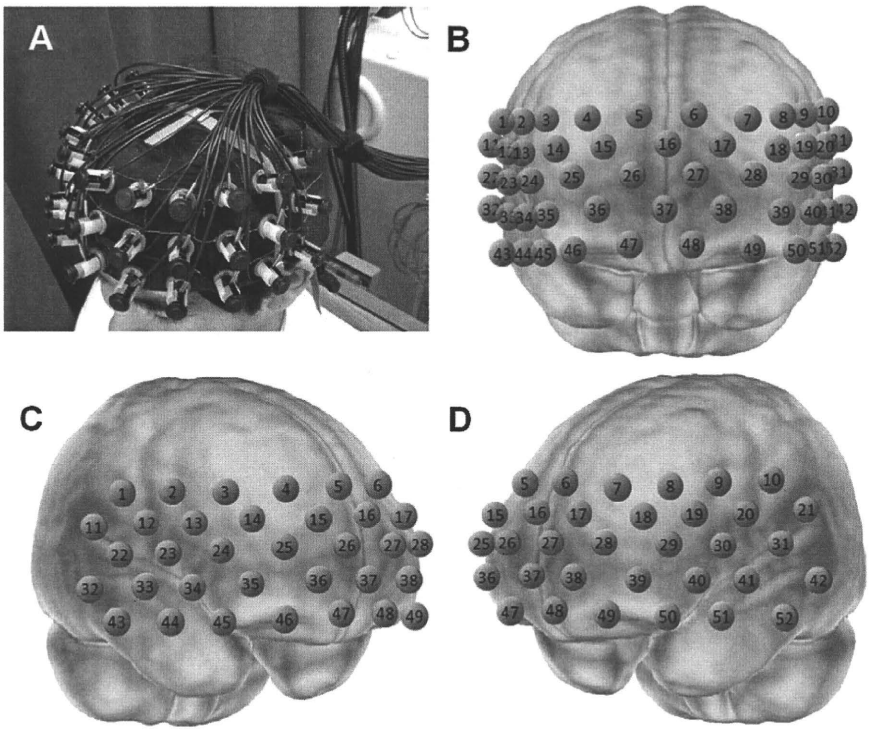


**Fig. 1.** The probe setting and measurement points of 52-channel near-infrared spectroscopy (NIRS). (A) The probes with thermoplastic 3 × 11 shells were placed over a subject's bilateral frontal regions. (B–D) The 52 measuring positions of the NIRS machine are superimposed on a 3D-reconstructed cerebral cortical surface from the Montreal Neurological Institute (MNI) Colin27 average MRI image (B, frontal view; C, right anterior oblique view; D, left anterior oblique view). The channel numbers are indicated above the measuring points.

We calculated the RNG index for analysing their performance in generating random numbers (Evans, 1978) because the RNG index has a high test–retest stability and has been preferentially used in previous studies in schizophrenia. The RNG index measures the difference between expected and observed probabilities of pairs of consecutive digits. For example, if a participant selects more specific patterns (mostly '3' following '7'), the number of pairs of consecutive digits (the pair '37') increases so that the difference between ideal and actual frequencies of the pairs becomes wider. Ideal randomness is measured as 0 and pure non-randomness would be 1 in the RNG index (Evans, 1978).

### 2.3. NIRS measurement

We used a 52-channel NIRS instrument (ETG-4000, Hitachi Medical Co.). The participants only needed to sit in a chair in a relaxed state with their eyes open, and cap the thermoplastic attachment of the NIRS probes on their head. To minimise motion artefacts, we instructed them to avoid physical motions, such as head movement and strong biting, during the measurement. The NIRS probe attachment was a thermoplastic $3 * 11$ shell and set with 52 fixed channels (Fig. 1). The lowest probe line was set along the Fp1–Fp2 line defined by the international 10–20 system used in electroencephalography. The 52 measuring areas are labelled ch1–ch52 from the right posterior to the left anterior. This arrangement of probes can measure [Hb] from the bilateral prefrontal (approximately dorsolateral (Brodmann's area (BA) 9, 46), ventrolateral (BA 44, 45 and 47), and frontopolar (BA 10)) and superior temporal cortical surface regions (Fig. 1).

The theoretical methodology of haemoglobin concentration measurement using NIRS instruments has been described in details elsewhere (Takizawa et al., 2008), but is briefly described as follows. The NIRS instrument measures relative changes in [oxy-Hb] and [deoxy-Hb] using two wavelengths (695 and 830 nm) of infrared light, based on the Beer–Lambert law (Watanabe et al., 1996; Yamashita et al., 1996). In this continuous-wave NIRS system, the [Hb] values include a differential path-length factor (DPF). The distance between pairs of source-detector probes was set at 3.0 cm, and we defined each measurement area between pairs of source-detector probes as one 'channel'. It is assumed that an NIRS system, in which the source-detector spacing is 3.0 cm, measures points at a 2–3 cm depth from the scalp, that is, the surface of the cerebral cortex (Okada and Delpy, 2003).

We set the time resolution of NIRS signals at 0.1 s. Because the NIRS signal was sometimes unstable at the start of the measurement due to technical issues and/or some effects of participant's tense state and thoughts before starting the task, the pre-task baseline was determined as the mean across the last 10 s of the pre-task period and the post-task baseline was determined as the mean across the last 5 s of the post-task period, and a linear fitting was performed on the basis of the data between the two baselines (Fig. 2). Moving average methods were applied to remove short-term motion artefacts in the analysed data (moving average window: 5 s). Grand mean waveforms averaged across subjects were created separately for each type of [Hb] and for each group. Even when we used these artefact rejection methods, the visible artefact waveforms remained. Thus, we used a computer program, which rejected a channel when there was a visible artefact waveform (Takizawa et al., 2008, 2009). The valid channels varied among participants (schizophrenia: number of channels = 33–52 (mean, 44.7; SD, 10.2); healthy subjects: $n = 28$–52 (mean, 48.1; SD, 5.1); percentage: schizophrenia, 85.9%; healthy controls, 92.5%, n.s.).

### 2.4. Statistical analysis

All analyses were performed using Statistical Package for Social Sciences (SPSS) 10.1J (SPSS Inc., Chicago, USA). The recorded haemoglobin data from the counting and activation periods were

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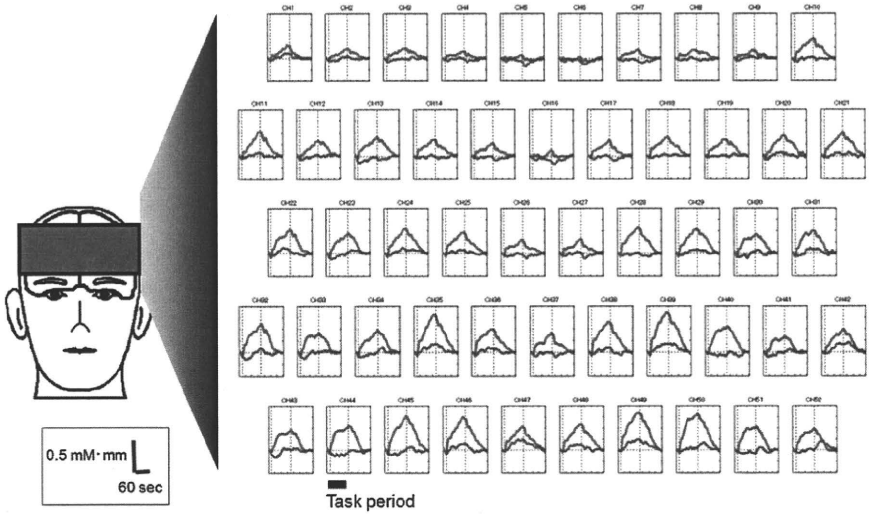


**Fig. 2.** Grand average waveforms of NIRS signals in patients with schizophrenia and healthy controls during the RNG task. The 52 measuring areas were labelled ch1–ch52 from right-superior to left-inferior sides. This arrangement of the probes can measure haemoglobin concentration changes from the bilateral prefrontal (approximately dorsolateral [Brodmann's area (BA) 9, 46], ventrolateral [BA 44, 45, 47], and frontopolar [BA 10]), and superior temporal cortical surface regions. Oxygenated haemoglobin changes in healthy controls and the patients with schizophrenia during the RNG task were represented as grand average waveforms in 52 channels with red and green lines, respectively. As we got relative waveforms related to task induced activation, we determined pre-task baseline as the mean across last 10 s of the pre-task period and post-task baseline as the mean across last 5 s of the post-task period, and we performed linear fitting on the basis of data between the two baselines. Task period showed between vertical dash lines in boxes, and also indicated a black bar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

averaged for each period. In this study, we focussed on [oxy-Hb] because [oxy-Hb] changes can reflect more direct cortical activation than [deoxy-Hb] (Kennan et al., 2002), and is well correlated with Blood-oxygen-level dependence (BOLD) signals of fMRI (Lee et al., 2008; Strangman et al., 2002).

First, for every channel in each group, we compared the mean [oxy-Hb] changes from pre-task baseline to activation period using paired Student's *t*-test. As we performed 52 paired *t*-tests, we adopted the false discovery rate (FDR) correction method for correcting the multiple comparisons (two-tailed; we set the value specifying the maximum FDR to 0.05 so that there were no more than 5% false positives on average) (Singh and Dan, 2006). Then, we calculated the effect size (Cohen's *d*) of the mean [oxy-Hb] changes during the activation period in every channel, for inter-channel comparison to validate the differences among prefrontal cortical sub-regions (Cohen, 1988). Second, we compared the differences and effect size between the patients with schizophrenia and healthy controls during the activation period using Student's *t*-test (FDR correction method). Finally, we measured the correlation coefficient between the age at onset and [oxy-Hb] changes by Spearman's rank correlation coefficient. We set a significant $\rho$ level to 0.05.

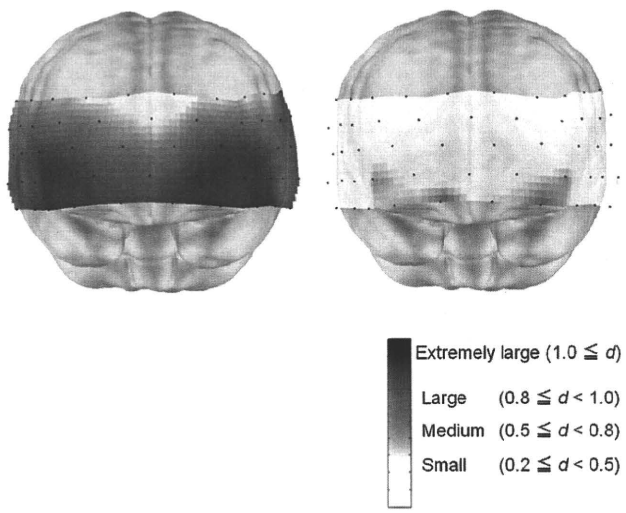


**Fig. 3.** Topographical 3D mapping of the effect size of [oxy-Hb] changes during the activation period relative to a baseline in control subjects, (left) and patients with schizophrenia (right).

## 3. Results

RNG task performance (RNG index) was significantly lower in patients with schizophrenia than in healthy controls ($p = 0.0497$; Table 1).

### *3.1. [oxy-Hb] changes from counting period to RNG activation period (Fig. 2)*

In healthy controls, we found a significant [oxy-Hb] increase in response to performing the RNG task at 44 channels (ch3, 7–8, 10–15, 17–40, 42–50; FDR-corrected $p = 0.001$–$0.038$, Fig. 3, left), which confirmed significant cognitive activation during the RNG task. The interchannel comparison between counting and activation periods showed the large effect size at 31 channels (ch13–15, 17–19, 21–22, 24–25, 28–32, 34–40, 42, 44–51; *d*'s >0.8, Fig. 3, left), and these channels were located bilaterally in the lateral side of prefrontal regions, compared with those in the frontopolar PFC region. These regions approximately corresponded to the bilateral dorsolateral (DLPFC; BA9 and BA46) and the bilateral ventrolateral prefrontal cortex (VLPFC; BA44, 45, 47). On the other hand, we did not find any significant activation channel in patients with schizophrenia, except for one channel of activation trend (ch49; uncorrected $p = 0.021$, Fig. 3, right).

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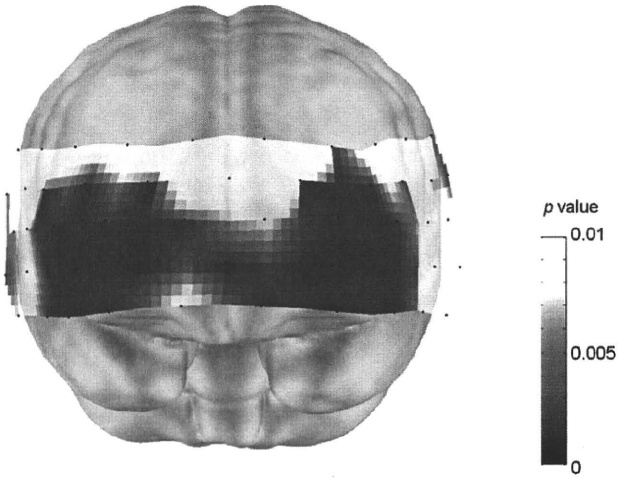


**Fig. 4.** Topographical 3D mapping of $p$ values from the difference between [oxy-Hb] changes in control and schizophrenia groups. Significant differences are indicated with dark red dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.2. Comparison between groups during activation period

The patients with schizophrenia showed significantly smaller activation than the healthy controls during the activation period at 35 channels (ch7, 10–11, 13–15, 17–19, 21, 23–26, 28–40, 43–46, 48–51; FDR-corrected $p = 0.001$–$0.029$, Fig. 4). The interchannel comparisons of the differences between groups showed large effect size at 23 channels (ch13, 15, 17–19, 21, 24, 28–30, 32, 34–36, 38–40, 44–46, 48–50; $d$'s >0.8), which were approximately located in the bilateral DLPFC and VLPFC regions.

### 3.3. RNG performance comparison and performance-matched analysis

RNG index was significantly lower in patients with schizophrenia than in healthy controls ($p = 0.0497$; Table 1). Therefore, in a confirmatory analysis, we intended to detect specific haemoglobin changes regardless of the cognitive performances. As we matched RNG task performance between the two groups, we performed a confirmatory analysis in 18 patients with schizophrenia and 33 healthy controls (Schizophrenia: mean RNG index = 0.131 (SD = 0.032); controls: mean = 0.124 (SD = 0.027); $p = 0.42$). The demographic characteristics between the groups remained to be matched for age and gender. In the same manner as the original analysis, the patients with schizophrenia showed significantly reduced activation compared with the healthy controls during the activation period at 32 channels (ch7, 11, 13–15, 17–19, 21–26, 28–29, 32–40, 43–48, 50–51; FDR-corrected $p = 0.001$–$0.020$).

### 3.4. Correlation coefficients between NIRS signal changes and onset age

In patients with schizophrenia, four channels in the right DLPFC regions (BA9) showed a significantly positive correlation coefficient with age at onset (ch5: $\rho = 0.607$, $p = 0.003$; ch15: $\rho = 0.584$, $p = 0.005$; ch26: $\rho = 0.544$, $p = 0.013$; ch36: $\rho = 0.522$, $p = 0.015$; Fig. 5), that is, the patients with an earlier onset had smaller [oxy-Hb] changes in the right DLPFC. Their ages at onset were not correlated with any other demographic or clinical variables (age, education, RNG index, any PANSS subscores, GAF score, duration of illness, duration of untreated psychosis and dose of medication). In a confirmatory analysis, even when we divided the schizophrenia group into two subgroups on the basis of median age at onset (median age at onset = 26.0 years; early onset: $n = 10$, mean age at onset = 19.0 years, SD = 3.3; late onset: $n = 12$, mean age at onset = 32.3 years, SD = 7.2), two out of four channels were still significantly different (Ch 15, $p = 0.004$; Ch26, $p = 0.00052$).

## 4. Discussion

In this study, we investigated haemodynamic changes over PFC during the RNG task using a multichannel NIRS instrument in healthy controls and patients with schizophrenia. Our results showed that bilateral DLPFC and VLPFC activation in healthy controls significantly increased during the RNG task, but did not increase in patients with schizophrenia, even when task performances were matched between groups. Furthermore, the present study showed a significant positive correlation between the age at onset of schizophrenia and their [oxy-Hb] increase in the right DLPFC region (BA 9). In other words, the right DLPFC activations in patients with schizophrenia were poorer when the age at onset was earlier.

### 4.1. [oxy-Hb] changes due to RNG task

To replicate the previous two-channel NIRS findings, we confirmed a significant [oxy-Hb] increase over the wide area of the PFC in healthy controls associated with the RNG task. In particular, we observed large effect sizes in the bilateral DLPFC and VLPFC regions, suggesting that DLPFC and VLPFC function, rather than other prefrontal cortical function was preferentially recruited when generating random numbers. A previous PET study showed increased activity in the left DLPFC and right inferior frontal cortex (Jahanshahi et al., 2000), and an fMRI study showed increased activation in the bilateral DLPFC in healthy controls during the RNG task (Daniels et al., 2003). It was also implicated in an event-related potential study where the negative component related to random number generation resulted in a maximum negative peak over the left DLPFC region using a low-resolution brain electromagnetic tomography (LORETA) analysis (Joppich et al., 2004). Generating random numbers at a certain time interval demands highly executive processes, attention shifting and inhibition of repetitive attitudes; these functions were mostly related to part of the central executive component included in a working memory model (Artiges et al., 2000; Baddeley and Wilson, 1988; Jahanshahi et al., 2000). Our results support the theory that DLPFC is a major component of the working memory system, and that an NIRS instrument can measure haemodynamic activities in the DLPFC related to the use of working memory.

Our results also showed increased activation in the bilateral VLPFC regions. It has been assumed that VLPFC is also involved in the working memory system (Christ et al., 2009; Dolcos et al., 2007). A previous fMRI study using n-back tasks showed that VLPFC activation, in addition to the DLPFC regions, was increased when the participants underwent tasks that demand the maintenance of working memory (Schneider et al., 2007). Therefore, the bilateral VLPFC activation in this study might reflect the need for executive control, inhibition and maintenance of working memory in generating random numbers.

### 4.2. Group comparison during the RNG task

This study showed that the patients with schizophrenia had significantly smaller activation than the healthy controls over the wide prefrontal cortical regions (35 out of 52 channels), especially in the bilateral DLPFC and VLPFC regions (Fig. 4). Previous two-channel NIRS studies showed significantly reduced [oxy-Hb] changes of the bilateral prefrontal regions during the RNG task in

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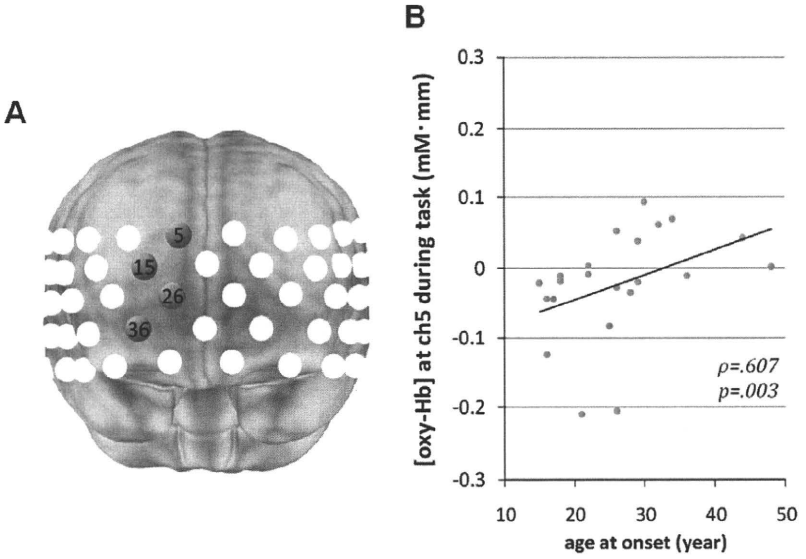


**Fig. 5.** Relationship between [oxy-Hb] change and age at onset of schizophrenia. (A) The channels which showed significant correlation ($p < 0.05$) are indicated in red. (B) Scatter diagram at ch5 (Spearman's rank correlation coefficient; $\rho = 0.607$, $p = 0.003$). *X* axis shows ages at onset of schizophrenia and *Y* axis shows [oxy-Hb] changes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

patients with schizophrenia (Hoshi et al., 2006; Shinba et al., 2004). Our multichannel NIRS results replicated the reduced prefrontal activation shown by these two-channel studies and further confirmed the spatial characteristics of prefrontal abnormality in schizophrenia. This reduced activation in schizophrenia may involve decreased capacity of working memory. Recent fMRI studies using working memory tasks have shown that there is an inverted U-curve relationship between DLPFC activation and task difficulty, that is, the activation increased as the task became more difficult up to a certain level, but it decreased when the task level was beyond the capacity of the working memory (Manoach, 2003). An fMRI study in non-psychiatric participants using an RNG task showed that the left DLPFC activation reduced and the right DLPFC activation was absent when the task became difficult (from 1-Hz to faster 2-Hz pacing) (Daniels et al., 2003). In a PET study, healthy volunteers had increased activations in the bilateral DLPFC during slower rate of an RNG task such as 0.5-Hz pacing, and the activation decreased when the pace of response was faster, such as 1-Hz and 2-Hz pacing (Jahanshahi et al., 2000). Furthermore, the task demanded a larger working memory load on patients with schizophrenia than on healthy controls during the same task. Therefore, when comparing the DLPFC activation between patients with schizophrenia and healthy controls, patients with schizophrenia show a relatively larger activation than healthy controls for easy tasks, while showing smaller activation in more difficult tasks (Manoach, 2003). These results suggest that the RNG task using 10 digits and 1-Hz pacing in the present study appeared to be relatively more demanding, even for healthy participants than other working memory tasks, such as Stroop or Sternberg tasks (Daniels et al., 2003). Therefore, our result was in line with previous studies that showed that RNG task with 1-Hz pacing required much working memory load in the healthy controls and resulted in decreased activation in the patients with schizophrenia assuming over their working memory capacity.

### *4.3. Relationship between oxy-Hb concentration and task performance*

The present study showed that the RNG performance in patients with schizophrenia was significantly, although marginally, worse than that of healthy controls, which replicates previous neuropsychological studies (Hoshi et al., 2006; Peters et al., 2007; Shinba et al., 2004). Furthermore, the prefrontal activity (32 out of 52 channels) in the patients with schizophrenia was still significantly reduced compared with healthy controls even when RNG performance was matched. These results suggest that the subtle prefrontal functional abnormality in schizophrenia can be sensitively detected with NIRS, regardless of task performance.

This study did not show any correlation between [oxy-Hb] changes and task performance, while some neuroimaging studies using an RNG task showed a relationship between PFC activation and task performance (Jahanshahi et al., 2000; Shinba et al., 2004). A previous two-channel NIRS study showed that the [oxy-Hb] changes of bilateral frontal regions in healthy controls were positively correlated with task performance during 'written' RNG tasks, but not during 'oral' RNG tasks (Shinba et al., 2004). It has been also shown that activations in the left DLPFC region were positively correlated with task performance in a PET study (Jahanshahi et al., 2000). These studies had a rather small number of participants (13 patients and six healthy controls, respectively), and adopted task procedures and indices of calculating randomness different from our study. Our results limited the high-performance participants who could perform better than 90% accuracy during activation period, which might also have affected the discrepant results. Therefore, the relationship between prefrontal haemodynamic response and task performance during RNG tasks remains controversial, and further studies using a more sophisticated design and a larger sample may be needed to clarify this point.

### *4.4. Relationship between age at onset and oxy-Hb concentration changes*

The present study using multichannel NIRS showed a significant positive correlation between the age at onset and the [oxy-Hb] changes in four channels (ch5, 16, 26 and 36) located mainly over the right DLPFC region (BA9), regardless of gender, symptom severity, duration of illness or duration of untreated psychosis. Some long-term follow-up studies have suggested that patients with early-onset schizophrenia were associated with severe symptoms and poorer social outcomes (Eggers and Bunk, 1997; Hafner, 2000). A previous neuropsychological study using a verbal

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