sunlit room with the eyes open throughout the measurement. During the task, subjects were instructed orally to vocally generate as many nouns as possible beginning with the Japanese syllables "a", "ki", "ha", "to", "se", "o", "i", "no" or "ta", without repetitions or resorting to proper nouns. Stimulus syllables were counterbalanced for each treatment condition. The subject performed a verbal fluency task consisting of a 30-s pre-task, 60-s verbal fluency task, and 60-s post-task. The number of words generated during the verbal fluency task was determined as a measure of task performance. Subjects were instructed to repeat the vowels "a", "i", "u", "e" and "o" during the pre-task and post-task periods as the Japanese counterparts of A, B, and C in English (Kameyama et al. 2006). Prior to the main examination when the NIRS response was recorded, practice sessions were conducted until the experimenter judged that the subjects understood the procedure.

NIRS measurements

Relative changes in oxy-Hb and deoxy-Hb were measured using a FOIRE-3000 functional NIRS system (Shimadzu, Kyoto, Japan) at three wavelengths (780, 805, and 830 nm). A NIRS shell with 3×5 arrays of light emitters and detectors were used (distance between probes, 3 cm). This apparatus could measure the relative concentrations of oxy-Hb and deoxy-Hb at 22 measurement points in a 9×15 cm area (Fig. 2). The NIRS shell was placed over the frontal region. The location of the shell was determined according to the International 10–20 system used in electroencephalography, with the lowest probes positioned along the Fp1-2 line (Okamoto et al. 2004).

Data analysis and statistics

We analyzed oxy-Hb values in the 22 channels located above the prefrontal cortex. We focused on oxy-Hb concentrations, since oxy-Hb change is assumed to more directly reflect cognitive activation than deoxy-Hb change as shown by a stronger correlation with blood oxygenation level-dependent signal measured by fMRI (Strangman et al. 2002). Near-infrared light absorption was measured with a temporal resolution of 0.1 s. Waveforms of oxy-Hb changes were acquired from all subjects in all of the 22 channels during the task. NIRS data that clearly contained motion artifacts as determined by close observation of the subject were excluded from further statistical analysis (one subject in total). A low-pass filter with a high cutoff of 0.1 Hz was used to exclude short-term motion artifacts from the data for analysis. In addition, oxy-Hb data in the following channels showing low signal-to-noise (S/N) ratios were excluded from further analysis: channels 1, 5, 14, 18, 19, 20, 21, and 22. Oxy-Hb concentrations were averaged during the 60-s verbal fluency task period. The pretask baseline was determined by employing the mean across the last 10 s of the whole 30-s pretask period. Oxy-Hb concentration changes during the verbal fluency task observed on days 2 and 9 were statistically tested after subtracting changes between the task period and pre-task period from day-pre measurements. We utilized repeated-measures analysis of variance (ANOVA) for the drugs (mirtazapine, trazodone, and placebo) on days 2 and 9, followed by post hoc multiple comparison with Fisher's protected least significant difference.

The number of words correctly generated was statistically tested using repeated-measures ANOVA for the drugs (mirtazapine, trazodone, and placebo) on days 2 and 9.

SSS scores were statistically tested using Friedman's test followed by multiple comparison adjustment using the Bonferroni method. Exploratory correlational analysis between oxy-Hb concentration change and SSS was performed for each channel with Spearman's ρ. Values of $p<0.05$ were considered statistically significant for all analyses.

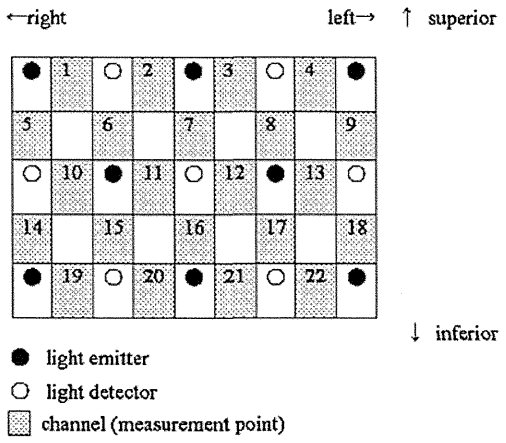

Fig. 2 Placement of NIRS shells

Results

Behavioral performance

The numbers of words correctly generated during the 60-s verbal fluency task period for each administration condition are summarized in Table 1. Repeated-measures ANOVA revealed no significant difference between day-pre, day 2, and day 9.

SSS

Mean SSS for mirtazapine (Table 1) on day 2 was significantly higher than any other scores ($p=0.00$). Changes in

Table 1 Number of words correctly generated and subjective sleepiness (mean ± SD)

	Pre	Mirtazapine		Trazodone		Placebo	
		Day 2	Day 9	Day 2	Day 9	Day 2	Day 9
BP	15.3±4.2	15.9±3.6	14.7±4.2	16.3±3.7	14.9±4.2	16.4±3.8	14.8±4.5
SSS	2.3±0.5	3.7±1.2	2.7±0.7	2.3±0.5	2.4±0.6	2.4±0.8	2.4±0.5

BP behavioral performance, *SSS* Stanford Sleepiness Scale

oxy-Hb concentration did not correlate with SSS in any channels.

NIRS response

For oxy-Hb, repeated-measures ANOVA for day 2 revealed no significant difference between drugs. However, repeated-measures ANOVA for day 9 revealed significant differences in ch8 (F=4.50, p=0.02), ch12 (F=4.59, p=0.02), ch13 (F=2.80, p=0.08), ch15 (F=3.04, p=0.06), ch16 (F=3.46, p=0.04), and ch17 (F=3.03, p=0.06). Post hoc testing for day 9 showed that oxy-Hb increases with mirtazapine were larger than those with trazodone in ch8 (p=0.02), ch12 (p=0.02), ch13 (p=0.06), ch15 (p=0.02), ch16 (p<0.01), and ch17 (p=0.04) and were larger than those with placebo in ch8 (p=0.05) and ch12 (p=0.03) (Fig. 3).

Discussion

We examined the effects of two types of sedative antidepressants on brain activity, performed the verbal fluency task, and used NIRS to measure changes in oxy-Hb concentration. No significant differences were detected between mirtazapine, trazodone, and placebo on day 2 for oxy-Hb. However, activation was significantly increased with mirtazapine in comparison to the other drugs in 6 of 22 channels.

Results for the SSS indicated that sleepiness on day 2 was significantly increased with mirtazapine compared to other conditions. No significant difference between drugs was seen for the number of words correctly uttered during the task, representing behavioral performance.

We assumed in this study that the superior frontal gyrus was the area activated in oxy-Hb concentration change, as

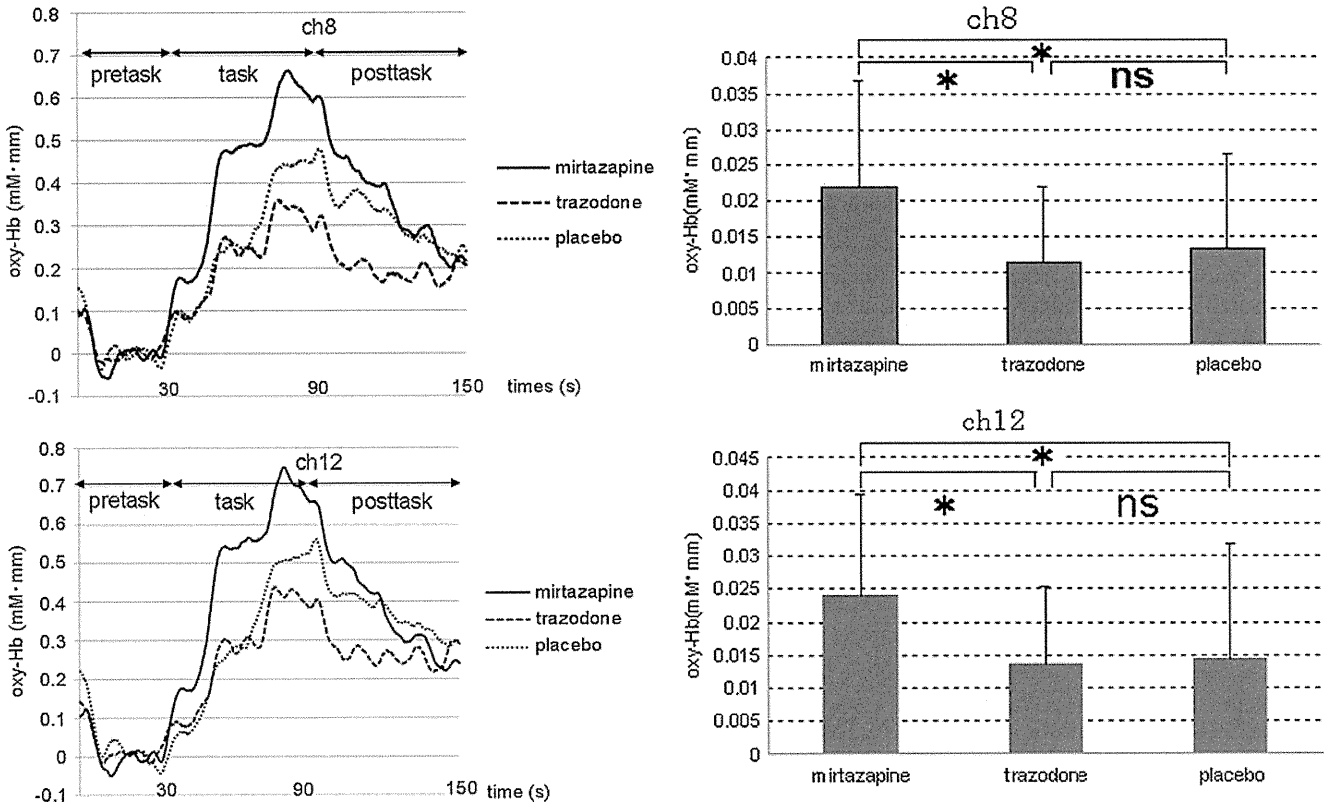

Fig. 3 Oxy-Hb concentration change during the whole 150-s period on day9 s (*left*) and averaged oxy-Hb concentration change during the 60-s task period on day9 s (*right*). The *asterisk* indicates p<0.05 (post hoc test; protected least significant difference)

measured by NIRS (Tsuzuki et al. 2007). A previous study revealed that activation in the left prefrontal cortex and right premotor cortex was decreased with paroxetine, as measured by fMRI during a linguistic task, similar to our task (Peran et al. 2008). To the best of our knowledge, no previous studies have examined the effects of antidepressants on brain blood flow during verbal fluency tasks. However, we did identify several studies that examined the effects of antidepressants on brain activity (frontal lobe function) during cognitive tasks. In a study using the Go/No-Go task during fMRI, activity in bilateral prefrontal cortices was increased with mitrazapine compared to placebo (Vollm et al. 2006). Another fMRI study revealed that activity in the frontal area was decreased with escitalopram during the Mackworth Clock Test (Wingen et al. 2008).

According to these studies, brain response during cognitive tasks (which measure frontal lobe function) differs according to the type of drug used. This suggests that activity in the frontal area is decreased in a cognitive task with selective serotonin reuptake inhibitors (SSRIs) such as paroxetine and escitalopram, whereas activity in the area can be increased with mirtazapine, which was also examined in this study. SSRI is known to have serotonergic effect. By contrast, mirtazapine has adrenergic and dopaminergic in addition to serotonergic effect (Millan et al. 2000; Nakayama et al. 2004). As for antihistamines, NIRS studies revealed that ketotifen significantly decreased cortical activation in the lateral prefrontal cortex (Tsujii et al. 2009; 2007). Studies using PET also clarified that D-chlorpheniramine decreased regional cerebral blood flow in the frontal area (Mochizuki et al. 2002; Okamura et al. 2000). In the present study, antihistaminic effect of mirtazapine significantly increased sleepiness on day 2 as is the case in antihistamines. The increase of oxy-Hb concentration change in the frontal cortex with mirtazapine may attribute to other pharmacological effects such as adrenergic, dopaminergic, and serotonergic effects in addition to antihistaminic effect. Differences in pharmacological properties can thus influence brain activity, manifesting as different responses (i.e., blood flow as measured by NIRS). Moreover, outcomes can change with the dose and duration of drug administration. Further examination of the effects of different factors that may influence brain activity is thus needed using other neuroimaging techniques (e.g., fMRI).

According to some previous studies, the increased oxy-Hb concentration change during cognitive tasks implies that mirtazapine can intensify brain activity (Hock et al. 1997; Kleinschmidt et al. 1996; Mehagnoul-Schipper et al. 2002; Toronov et al. 2001). Oxy-Hb change is reportedly decreased in patients with major depressive disorder, suggesting reduced brain activity (Suto et al. 2004; Herrmann et al. 2004). We therefore speculate that mirtazapine may have some potential to restore deteriorated function of brain activity in depression. On the other hand, continuous administration of mirtazapine did not change the scores for behavioral performance. We can therefore also assume that increased brain activation might be needed to achieve the same performance during mirtazapine administration, which was indicated in the study of Alzheimer's disease using NIRS (Tomioka et al. 2009).

Several limitations must be considered when interpreting the results of this study. First of all, NIRS measurement has been suggested to have insufficient spatial resolution, although the temporal resolution is high (Kameyama et al. 2006). NIRS probes in this study could measure limited cerebral regions. We had a considerable area that was not measured between the probes and outside of the NIRS shell. In addition, we had to exclude 8 of 22 channels from detailed analysis because of low S/N ratios. Second, initial doses for both mirtazapine and trazodone were applied to the subjects to carefully examine drug effects, side effects, and influences on brain function at a low dose. Nevertheless, we have to acknowledge that these doses hold a methodological problem because the initial doses may not be sufficient to influence brain activity. Third, we have not measured oxy-Hb concentration changes after the previous drug was washed out. This might interfere with assessment of the exact difference between the pretreatment baseline and the periods treated with the second and the third drugs. However, we assume that this problem could be resolved to some extent because the order of the drugs administered was randomized and counterbalanced. Fourth, the validity of NIRS measurement should be considered. Further investigations are needed to determine whether the results of NIRS measurement are consistent with the results of other neuroimaging methods such as fMRI. Finally, all subjects in this study were healthy men who were not taking any medications. In general, most patients are taking other pharmacotherapies, and extracting exact responses to a single specific administered drug in such patients would be difficult. Hormonal changes resulting from the menstrual cycle might also affect cognitive function, thus influencing the results of cognitive tasks (Hampson 1990; Maki et al. 2002; Phillips and Sherwin 1992). Changes to brain function in a patient could differ from those in a healthy man, and sensitivity and response to a drug might not be the same in women and the elderly. The results from healthy subjects in this study thus might not be fully applicable to patients.

Conclusion

We observed that continuous administration of an antidepressant would affect brain function in this study by examining using a NIRS recorder for functional brain imaging. In addition, influences on brain function differed between the

drugs used in our experimental protocol. Medication and the type of antidepressant a subject takes appear to represent factors affecting Hb concentration change in NIRS, along with age, sex, and sleepiness; all of which should be considered when assessing brain activity in a patient with a psychiatric disorder. In addition, differences between each antidepressant in terms of the response of the brain need to be determined to allow easy evaluation of whether a drug will be effective for a patient. These techniques are expected to prove beneficial in future personalization of therapy.

Acknowledgments We wish to offer our sincere thanks to the healthy volunteers who participated in our study. This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Health, Labor and Welfare of Japan, the Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University, the Yokoyama Foundation for Clinical Pharmacology, and the Japan Research Foundation for Clinical Pharmacology.

Conflict of interest None of the authors have any conflicts of interest directly relevant to the content of this study.

References

Hampson E (1990) Variations in sex-related cognitive abilities across the menstrual cycle. Brain Cogn 14:26–43

Herrmann MJ, Ehlis AC, Fallgatter AJ (2004) Bilaterally reduced frontal activation during a verbal fluency task in depressed patients as measured by near-infrared spectroscopy. J Neuropsychiatry Clin Neurosci 16(2):170–175

Hock C, Villringer K, Muller-Spahn F, Wenzel R, Heekeren H, Schuh-Hofer S, Hofmann M, Minoshima S, Schwaiger M, Dirnagl U, Villringer A (1997) Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)—correlation with simultaneous rCBF-PET measurements. Brain Res 755:293–303

Kameyama M, Fukuda M, Uehara T, Mikuni M (2004) Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. Neuroimage 22:1715–1721

Kameyama M, Fukuda M, Yamagishi Y, Sato T, Uehara T, Ito M, Suto T, Mikuni M (2006) Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 29:172–184

Kleinschmidt A, Obrig H, Requardt M, Merboldt KD, Dirnagl U, Villringer A, Frahm J (1996) Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. J Cereb Blood Flow Metab 16:817–826

Maki PM, Rich JB, Rosenbaum RS (2002) Implicit memory varies across the menstrual cycle: estrogen effects in young women. Neuropsychologia 40:518–529

Mehagnoul-Schipper DJ, van der Kallen BF, Colier WN, van der Sluijs MC, van Erning LJ, Thijssen HO, Oeseburg B, Hoefnagels WH, Jansen RW (2002) Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. Hum Brain Mapp 16:14–23

Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A, Dekeyne A, Nicolas JP, Lejeune F (2000) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of alpha2-adrenergic and serotonin2C receptors: a comparison with citalopram. Eur J Neurosci 12:1079–1095

Miyata S, Noda A, Ozaki N, Hara Y, Minoshima M, Iwamoto K, Takahashi M, Iidaka T, Koike Y (2010) Insufficient sleep impairs driving performance and cognitive function. Neurosci Lett 469:229–233

Mochizuki H, Tashiro M, Tagawa M, Kano M, Itoh M, Okamura N, Watanabe T, Yanai K (2002) The effects of a sedative antihistamine, D-chlorpheniramine, on visuomotor spatial discrimination and regional brain activity as measured by positron emission tomography (PET). Hum Psychopharmacol 17:413–418

Nakayama K, Sakurai T, Katsu H (2004) Mirtazapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. Brain Res Bull 63:237–241

Okamoto M, Dan H, Sakamoto K, Takeo K, Shimizu K, Kohno S, Oda I, Isobe S, Suzuki T, Kohyama K, Dan I (2004) Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21:99–111

Okamura N, Yanai K, Higuchi M, Sakai J, Iwata R, Ido T, Sasaki H, Watanabe T, Itoh M (2000) Functional neuroimaging of cognition impaired by a classical antihistamine, D-chlorpheniramine. Br J Pharmacol 129:115–123

Peran P, Demonet JF, Cardebat D (2008) Paroxetine-induced modulation of cortical activity supporting language representations of action. Psychopharmacology (Berl) 195:487–496

Phillips SM, Sherwin BB (1992) Variations in memory function and sex steroid hormones across the menstrual cycle. Psychoneuroendocrinology 17:497–506

Sadock BJ, Sadock VA, Sussman N, Cancro R (2005) Kaplan and Sadock's pocket handbook of psychiatric drug treatment. Lippincott Williams & Wilkins, Philadelphia

Schecklmann M, Ehlis AC, Plichta MM, Boutter HK, Metzger FG, Fallgatter AJ (2007) Altered frontal brain oxygenation in detoxified alcohol dependent patients with unaffected verbal fluency performance. Psychiatry Res 156:129–138

Strangman G, Culver JP, Thompson JH, Boas DA (2002) A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. Neuroimage 17:719–731

Suda M, Sato T, Kameyama M, Ito M, Suto T, Yamagishi Y, Uehara T, Fukuda M, Mikuni M (2008) Decreased cortical reactivity underlies subjective daytime light sleepiness in healthy subjects: a multichannel near-infrared spectroscopy study. Neurosci Res 60:319–326

Suda M, Uehara T, Fukuda M, Sato T, Kameyama M, Mikuni M (2010) Dieting tendency and eating behavior problems in eating disorder correlate with right frontotemporal and left orbitofrontal cortex: a near-infrared spectroscopy study. J Psychiatr Res 44:547–555

Suto T, Fukuda M, Ito M, Uehara T, Mikuni M (2004) Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol Psychiatry 55:501–511

Tomioka H, Yamagata B, Takahashi T, Yano M, Isomura AJ, Kobayashi H, Mimura M (2009) Detection of hypofrontality in drivers with Alzheimer's disease by near-infrared spectroscopy. Neurosci Lett 451:252–256

Toronov V, Webb A, Choi JH, Wolf M, Michalos A, Gratton E, Hueber D (2001) Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. Med Phys 28:521–527

Tsujii T, Yamamoto E, Ohira T, Saito N, Watanabe S (2007) Effects of sedative and non-sedative H1 antagonists on cognitive tasks: behavioral and near-infrared spectroscopy (NIRS) examinations. Psychopharmacology (Berl) 194:83–91

Tsujii T, Masuda S, Yamamoto E, Ohira T, Akiyama T, Takahashi T, Watanabe S (2009) Effects of sedative and nonsedative antihistamines on prefrontal activity during verbal fluency task in young children: a near-infrared spectroscopy (NIRS) study. Psychopharmacology (Berl) 207:127–132

Tsuzuki D, Jurcak V, Singh AK, Okamoto M, Watanabe E, Dan I (2007) Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 34:1506–1518

Vollm B, Richardson P, McKie S, Elliott R, Deakin JF, Anderson IM (2006) Serotonergic modulation of neuronal responses to behavioural inhibition and reinforcing stimuli: an fMRI study in healthy volunteers. Eur J Neurosci 23:552–560

Wingen M, Kuypers KP, van de Ven V, Formisano E, Ramaekers JG (2008) Sustained attention and serotonin: a pharmaco-fMRI study. Hum Psychopharmacol 23:221–230

Geriatrics Gerontology INTERNATIONAL Medline Indexed

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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Necessity of normative data on the Japanese version of the Wechsler Memory Scale-Revised Logical Memory subtest for old-old people

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Aim: Episodic memory is vulnerable to deterioration in people suffering from Alzheimer's disease. Currently, the Logical Memory (LM) subtest of the Wechsler Memory Scale-Revised (WMS-R) is used internationally as an operational definition to identify people with mild cognitive impairment (MCI). However, the Japanese version of the LM has not been adequately normalized for old-old people. Therefore, norms of the LM for people aged 75 years and over are required, and the effects of sex, age and education on performance were evaluated.

Methods: A total of 50 (27 female and 23 male) participants without a history of dementia and symptomatic stroke events recruited from the community and hospital populations were investigated using the Mini-Mental State Examination, the LM and some interference tasks.

Results: The mean scores (standard deviations) of the sample were 15.5 (5.4) on LM-I and 9.9 (6.6) on LM-II. The distributions of the LM-I and -II scores satisfied the normality assumption. The LM-I and LM-II scores correlated with age and the LM-I score correlated with educational background.

Conclusions: For the Japanese version of the LM, the means, standard deviations and distribution features of the old-old sample are presented. Although the normal sample was chosen to closely match the demographic profile of the Japanese population, the present sample might have had a higher educational background than the age-matched population, especially the males. Further study is required to standardize the Japanese version of the LM subtest for each 5-year interval for latter-stage elderly people. **Geriatr Gerontol Int 2013; 13: 726–730.**

Keywords: episodic memory, Logical Memory, mild cognitive impairment, normative data, old-old people.

Introduction

A mild cognitive impairment (MCI) as a result of Alzheimer's disease (AD) is seen as memory impairment, and this symptom is the key early marker in the prodromal stages of AD.[1] Although the memory deficits in individuals with MCI are clinically discernible, in order to make a diagnosis of MCI, amnesia that does not interfere notably with activities of daily life (ADL) must be identified. It is operationally defined as performance 1.0–1.5 standard deviations (SD) below age- and education-adjusted norms on an episodic memory measure of delayed verbal recall.[2]

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The Logical Memory (LM) subtest of the Wechsler Memory Scale (WMS), which includes immediate (LM-I) and 30-min delayed (LM-II) trials of prose recall, is a large contributor to discriminating between healthy older adults and individuals with very mild AD. Guillozet *et al.* reported that AD pathology is more numerous in medial temporal lobe regions associated with the LM scores of the revised version of WMS (WMS-R),[3] and shows a relationship with LM performance on the WMS-R in individuals in the non-demented stage.[4] Although the relative ability of memory tests to discriminate between the AD converter type of MCI and normal aging has not been well characterized, a previous study reported that the LM-II was one of the best predictors for detecting progression from MCI to AD over a 4-year period.[1] The LM of the WMS-R is one of the standard memory criteria for MCI clinical and research; for example, in the Alzheimer's Disease Neuroimaging Initiative study.[5]

Various factors have been associated with LM test score differences. Abikoff *et al.* have already reported that age and education norms are generated for immediate, 30-min delayed and 24-h delayed recall in the LM of the WMS, and performance is more closely related to educational background.[6] Their sample ranged in age from 18 to 81 years, with a mean educational level of 13.96 years (range 6–18 years). The LM performance increase is somewhat more common with higher levels of education.[6,7] However, Abikoff *et al.* noted that "Although education was more highly related to scores than was age, small but significant relationships between age and verbal recall remained over and above the influence of education.[6]" The impact of age is most obvious in 24-h delayed recall, and drop-off in performance occurs over the age of 60 years. Therefore, the latest version of the WMS has paid attention to elderly participants in the form of advancing an elderly battery.

However, the WMS-R version is the only LM task that has been standardized for Japanese people, and the normative sample has been limited to the ages of between 16 and 74 years. The incidences of AD, combined dementia and other types of dementia rise with increasing age, particularly after the age of 85 years.[8] Although not only for young-old people, but also for old-old or oldest-old people, an amnestic state examination of high accuracy is required, because the Japanese versions of the WMS-R, LM-I and LM-II have not been adequately normalized for latter-stage elderly people. In the current study, normative data for the LM in Japanese elderly people aged 75 years and older were gathered.

Methods

Participants

A total of 50 (27 female and 23 male) participants without a history of dementia and symptomatic stroke events were recruited from the community and hospital populations living in two urban areas. All participants could attend the trial sites alone. The sample size determination was based on the original version of the WMS-R and the general recommendation on statistics in psychology and education, taking a sample of 50 and over per age group interval.[9] A total of 30 participants (60%) had no history of psychiatric problem as assessed by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disoriders, Fourth Edition revised (SCID),[10] and they did not report clinical evidence of amnesia and ADL impairment. There is nothing to suggest that participants did not hear something at the time of auditory stimulus presentation in the study. They ranged in age between 75 and 87 years (mean ± SD: 79.3 ± 3.6 years), and in educational background between 6 and 18 years (mean ± SD: 11.7 ± 3.1 years).

The ethics committee of the Tokyo Metropolitan Institute of Gerontology and the Nagoya University School of Medicine approved the present study, and each participant signed a consent form after being sufficiently informed about the outline of the study by the principal investigator.

Tasks

Logical Memory (LM)-I and -II from the Japanese version of the WMS-R[11] were carried out. In the LM-I, participants were asked to immediately recall from the number of prose units twice: the first trial presented story A verbally, and the second trial presented story B verbally. In the LM-II, participants were asked to recall words from the two stories 30 min later. During the time delay, participants were asked to carry out the Mini-Mental State Examination (MMSE)[12] and some interference tasks.

In the present study, not all of the participants carried out every task item, other than LM-I, II and MMSE. As the purpose of the present study was to provide normative data for LM-I and -II, the sample size was kept the same; hence, missing data were not substituted. The results are based on the eight task scales.

Statistical analysis

All statistical analyses were carried out using SPSS 17.0 J. for Windows (SPSS, Chicago. IL, USA). Normative data are provided in the form of means and standard deviations (SD) broken down by sex, age and educational background. Correlation analyses between LM scores and various factors were carried out using the Pearson product-moment correlation coefficient. A *P*-value of less than 0.05 was considered significant. The percentile rank of each LM-I or -II score was calculated, after the Shapiro–Wilk test was carried out to check the normality of the sampling distribution.

Results

Sample characteristics

In the present sample, the mean ± SD score of MMSE (27.3 ± 2.2) reflected the expected distribution of general cognitive status for aged groups. The normative sample was confirmed to match closely the demographic profile of this population as reported in a recent census. Table 1 shows the percentiles of the normalization sample by age, sex and educational background compared with these population averages in Japan (Statistics Bureau 2010: Ministry of Internal Affairs and Communications). The results showed that the sample might have had a higher educational background than Japan's age-matched population.

Table 1 Percentiles of the normalization sample by age, sex and educational background

Age (years)	Sex	Education 0–11 (years) Sample	Population (Japan)	12 (years) Sample	Population (Japan)	>13 (years) Sample	Population (Japan)
>75	Male	25.9	45.6	18.5	37.6	40.7	16.8
	Female	63.0	53.3	18.5	41.3	18.5	5.4
	Total	48.0	50.3	20.0	39.9	32.0	9.8

The estimated population was calculated excluding active students and unknown individuals of education backgrounds from total number.

Table 2 Performance of the sample aged 75 years and older

		Mean SD (min–max)
LM-I		15.5 ± 5.4 (5–8)
	Story A	8.3 ± 3.2 (3–16)
	Story B	7.4 ± 2.8 (2–14)
LM-II		9.9 ± 6.6 (0–25)
	Story A	5.0 ± 3.8 (0–13)
	Story B	4.9 ± 3.2 (0–12)

I, immediate recall; II, delayed recall; LM, Wechsler Memory Scale-Revised Logical Memory subtest.

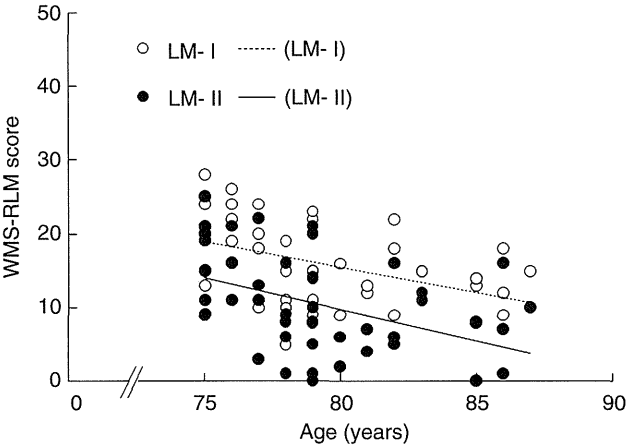

Figure 1 The scatter plot of Wechsler Memory Scale-Revised Logical Memory (WMS-RLM) subtest scores (y-axis) and age in years (x-axis). I, immediate recall; II, delayed recall; LM, Logical Memory subtest.

Reference data of the normal group

Mean scores (SD) of the sample were 15.5 (5.4) on LM-I and 9.9 (6.6) on LM-II. Table 2 summarizes the performance of the sample. To check the normality of the sampling distribution, coefficients of skewness and kurtosis were calculated for each trial. In the LM-I, the skewness value was 0.19 and the kurtosis value was –0.89; in the LM-II, the skewness value was 0.32 and the kurtosis value was –0.82. The distributions of the LM-I and -II scores satisfied the normality assumption using the Shapiro–Wilk test ($P > 0.05$).

Characteristics and performances

To examine the effect of sex on performance, unpaired t-tests comparing the LM-I and -II scores in male and female participants were carried out. In both the LM-I and -II, no significant difference was found. The mean scores (SD) of the male group were 15.6 (5.5) on the LM-I and 10.8 (6.4) on the LM-II, compared with 16.3 (5.5) on the LM-I and 10.0 (6.8) on the LM-II in the female group.

To examine associations between age (years) or educational background (years) and LM scores, correlation analyses were carried out. The LM-I and LM-II scores were moderately correlated with age ($r = -0.44$, $P < 0.01$; $r = -0.45$, $P < 0.01$), and the LM-I score was moderately correlated with educational background ($r = 0.36$, $P < 0.05$). There was no significant correlation between the LM-II score and educational background ($r = 0.23$, not significant.). The figures show scatter plots of the WMS-R LM scores and age in years (Fig. 1), or years of education (Fig. 2). Considering that the sample had a moderate to high education, partial correlation analyses between age (years) and LM scores were carried out. The LM-I and LM-II scores were moderately correlated with age ($r = -0.36$, $P < 0.05$; $r = -0.40$, $P < 0.01$).

Discussion

In the current study, LM normal performances of healthy Japanese people aged 75 years and older were surveyed, and the effects of sex, age and education on performance were identified. The means, SD and distribution features of the LM-I and -II of the WMS-R are presented for Japanese old-old people.

The sample had mean (SD) scores of 15.5 (3.2) on the LM-I and 9.9 (6.6) on the LM-II. According to

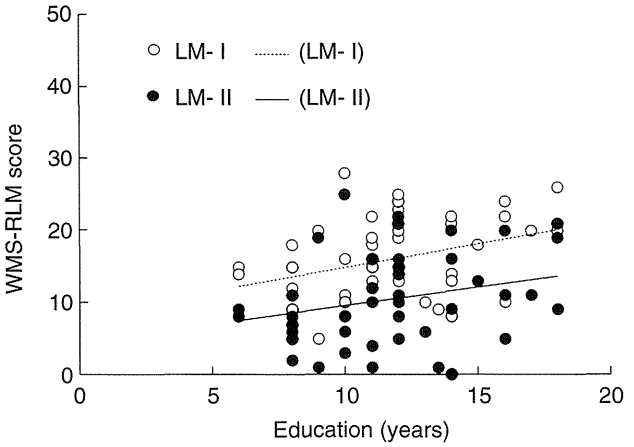

Figure 2 The scatter plot of Wechsler Memory Scale-Revised Logical Memory (WMS-RLM) scores (*y*-axis) and years of education (*x*-axis). I, immediate recall; II, delayed recall; LM, Logical Memory subtest.

Sugishita,[11] people in each age group (16–17 years ($n = 50$), 20–24 years ($n = 54$), 35–44 years ($n = 56$), 55–64 years ($n = 50$), 65–69 years ($n = 52$) and 70–74 years ($n = 54$)) had the following scores: on the LM-I, 27.7 (7.2), 26.6 (6.4), 25.1 (7.5), 22.0 (7.1), 19.5 (6.8) and 18.5 (7.5), respectively; and on the LM-II, 24.9 (7.7), 22.8 (6.7), 20.7 (7.6), 16.8 (7.0), 15.3 (7.0) and 13.2 (6.8), respectively. These results are consistent with previous data, and indicate an age-related decrease in LM task performance.[6,13] Considering the Flynn effect, the present data should not be compared directly with the previous data by Sugishita.[11] It is recommended that a larger study for the normalization of LM in older people be carried out.

Furthermore, the study showed that the LM-I and -II scores were moderately negatively correlated with age in a healthy sample aged 75 years and older. In particular, the LM-II score reflected the individual difference associated with age, independent of educational background. The result also confirms the age-related changes in memory functions. This finding, that the LM-II was *not* correlated with education leaves room for interpretation. Although the present sample from among community-dwelling older adults had generally better health and education, high-risk MCI persons might have been present in definite proportions, or the normal population might have individuals who, despite educational levels, may have been less able in cognitive abilities throughout their life.[14,15] According to the Mayo clinic's team, the LM-II data were *not* correlated with education in a community-based healthy sample.[15] They noted that the education-WMS performance association in the restricted age range of their older sample did not reflect true underlying relationships between the intelligence quotient (IQ) and task performance, and they recommended that WMS norms be stratified by IQ. The education-LM performance association might reflect these confounding factors.

Although the present sample was chosen to closely match the demographic profile of the Japanese population, the sample might have had a higher educational background than the age-matched population, especially among males. Community-based surveys in rural areas should also be carried out at the same time as surveys in urban areas. Thus, the sample bias is inappropriate for determining the "range of normal" memory functioning in an older population.[15] Norms stratified to be representative of the general population have great diagnostic value. However, the present result showed that the aging-related memory decline was observed in highly educated people, who had a greater likelihood of preserving cognitive function than people with low educational achievement. The result suggests that normalization of LM must be carried out for latter-stage elderly Japanese people. To establish the norms for the Japanese version of the LM, a further community-based study using the Intelligence Scale in parallel will be necessary. In addition, it will be necessary to compare between the LM norms based on the separately-carried out condition and that based on the completely-carried out WMS-R condition, and to normalize the latest version of WMS in Japanese people, because the latest version has a short battery for ages 65–90 years (the Older Adult Battery), including the new LM composed of the 14-paragraph-story (story A) and the 25-paragraph-story (story B).

The present estimated values based on LM scores of people aged 75 years and older, which are currently based on the population aged less than 75 years, show that current percentile ranks underestimate the memory ability of people aged 75 years and older. Furthermore, the present study obviously showed that the LM-I and LM-II scores were correlated with age. These results suggest the necessity of normative data on the Japanese version of the WMS-R LM subtest for each 5-year interval for the population aged 75 years and older, like the original version. In the future, for old-old people, it will be necessary to carry out a survey to establish norms of the WMS-R LM for each 5-year interval.

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Disclosure statement

No potential conflicts of interest were disclosed.

References

1 Rabin LA, Paré N, Saykin AJ *et al.* Differential memory test sensitivity for diagnosing amnestic mild cognitive impairment and predicting conversion to Alzheimer's disease. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2009; **16**: 357–376.

2 Jack CR Jr, Lowe VJ, Senjem ML *et al.* 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain* 2008; **131** (Pt 3): 665–880.

3 Wecheler D. *Wechsler Memory Scale-reviced: Manual.* San Antonio, TX: The Psychological Corporation, 1987.

4 Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol* 2003; **60**: 729–736.

5 Petersen RC, Aisen PS, Beckett LA *et al.* Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. *Neurology* 2010; **74**: 201–209.

6 Abikoff H, Alvir J, Hong G *et al.* Logical Memory subtest of the Wechsler Memory Scale: age and education norms and alternate-form reliability of two scoring systems. *J Clin Exp Neuropsychol* 1987; **9**: 435–448.

7 Economou A. Memory score discrepancies by healthy middle-aged and older individuals: the contributions of age and education. *J Int Neuropsychol Soc* 2009; **15**: 963–972.

8 Matsui Y, Tanizaki Y, Arima H *et al.* Incidence and survival of dementia in a general population of Japanese elderly: the Hisayama Study. *J Neurol Neurosurg Psychiatry* 2009; **80**: 366–370.

9 First MB, Spitzer RL, Gibbon M, Williams JBW. *User's Guide for the Structured Clinical Interview for DSM-IV Axis I Disorders-clinival Version (SCID-CV).* Washington, DC: American Psychiatric Press, 1997.

10 D'elia L, Satz P, Schretlen D. Wechsler Memory Scale: a critical appraisal of the normative studies. *J Clin Exp Neuropsychol* 1989; **11**: 551–568.

11 Sugishita M. *Wechsler Memory Scale-Revised (Japanese).* Tokyo: Nihonbunkakagakusya, 2001.

12 Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.

13 Drozdick LW, Holdnack JA, Hilsabeck RC. *Essentials of WMS-IV Assessment: Essentials of Psychological Assessment.* Hoboken, NJ: John Wiley & Sons, Inc., 2009.

14 Ivnik RJ, Smith GE, Tangalos EG, Petersen RC, Kokmen E, Kurland LT. Wechsler Memory Scale: IQ-dependent norms for persons age 65 to 97 years. *Psychol Assess* 1991; **3**: 156–161.

15 Malec JF, Smith GE, Ivnik RJ, Petersen RC, Tangalos EG. Clusters of impaired normal elderly do not decline cognitively in 3 to 5 years. *Neuropsychology* 1996; **10**: 66–73.

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SHORT COMMUNICATION

Effects of low-dose mirtazapine on driving performance in healthy volunteers

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Objective This study aimed to assess whether a lower initial dose of mirtazapine can lessen the harmful effect on driving performance or not in a double-blinded, placebo-controlled crossover trial.
Methods Thirteen healthy men received 8 days of continuous nocturnal doses of mirtazapine at 7.5 mg or 15 mg, or placebo. At baseline and on days 2 and 9, subjects performed three driving tasks (road-tracking, car-following, and harsh-braking tasks) using a driving simulator and a Continuous Performance Test. Stanford Sleepiness Scale (SSS) scores were also assessed. In the mirtazapine 7.5 mg series, 15 mg of mirtazapine was additionally administered on day 9, followed by all the same assessments on day 10.
Results Mirtazapine 7.5 mg had no significant effects on any tasks except for SSS compared with placebo. Mirtazapine 15 mg impaired road-tracking task and SSS. The increase in mirtazapine dose also had no significant effects on any tasks compared with those before dose increase.
Conclusions Mirtazapine 7.5 mg did not cause driving impairment compared with mirtazapine 15 mg, while both doses of mirtazapine produced subjective somnolence. The increase in mirtazapine had no detrimental effects on psychomotor performance. Initial low-dose mirtazapine may be safer for automobile driving than the normal starting dose.

KEY WORDS—mirtazapine; sedation; driving performance; cognitive function; starting dose

INTRODUCTION

Mirtazapine is a noradrenergic and specific serotonergic antidepressant with a unique pharmacologic profile that differs from currently available antidepressants. The therapeutic effects are derived by blockade of the alpha$_2$-adrenoreceptors and by indirect stimulation of serotonin (5-HT)$_1$ receptors, via blockade of 5-HT$_2$ and 5-HT$_3$ receptors (de Boer, 1995). Efficacy of mirtazapine has been established in a systematic review and meta-analysis of randomized controlled trials (Cipriani *et al.*, 2009; Watanabe *et al.*, 2008). Mirtazapine is also one of the most commonly used drugs for chronic insomnia in the US because of safety and lower dependence.

Despite the efficacy of mirtazapine, a key clinical problem is tolerability, and the most commonly reported adverse event is somnolence (Watanabe *et al.*, 2010). Sedation and somnolence are considered as the most important causes of driving impairment in patients being treated with antidepressants (Ramaekers, 2003). In fact, previous studies have suggested that acute administration of mirtazapine could impair road-tracking performance (Ramaekers *et al.*, 1998; Ridout *et al.*, 2003; Wingen *et al.*, 2005). Therefore, administration methods that can reduce driving impairment of mirtazapine are needed for patients' social lives and public safety.

It is considered that a lower initial dose of mirtazapine provides potent histaminergic blockade inducing prominent somnolence, whereas a higher initial dose of mirtazapine is associated with reduced

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sedating antihistaminergic activity through increased noradrenergic transmission (Stahl *et al.*, 1997; Stimmel *et al.*, 1997). Generally, dose reduction may be used to relieve antidepressants' detrimental effects, but little is known regarding the effects of mirtazapine, especially at lower doses, on driving performance. The aim of the present study was thus to evaluate the effects of a lower initial dose of mirtazapine on driving performance and cognitive function. By measuring the effects of different low doses of mirtazapine on driving performance, we evaluated the driving safety of an initial low dose of mirtazapine.

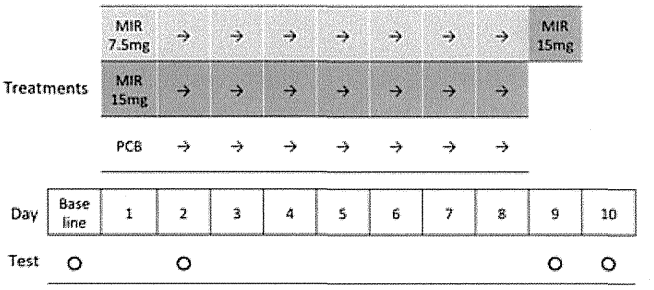

Figure 1. Summary of treatments and schedules in this study. Each subject received nocturnal dosing with mirtazapine (MIR) 7.5 mg, MIR 15 mg, or matched placebo (PCB) for 8 days in a double-blind, crossover design. In the MIR 7.5 mg series, MIR 15 mg was also administered on day 9. A washout period of ≥7 days was provided between each treatment session. Assessments were performed at baseline (once before treatment) and on days 2, 9, and 10 (only in the MIR 7.5 mg series) of each treatment series

MATERIAL AND METHODS

Thirteen healthy male volunteers (32–49 years old, mean ± SD, 39.2 ± 6.2 years) were included through health interviews and the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. All applicants had had a driving license for ≥10 years and had been driving a car daily (minimum 5000 km/year). The study was approved by the ethics review committees of the Nagoya University Graduate School of Medicine and Nagoya University Hospital, and written informed consent was obtained from each subject before participation.

The present study used a double-blind, placebo-controlled, three-way crossover design. Each subject received 8 days of continuous bedtime dosing with either 7.5 or 15 mg of mirtazapine, or matched placebo in identical capsules across three different treatment series. Under the mirtazapine 7.5 mg series, 15 mg of mirtazapine was additionally administered on day 9. Dosing started at bedtime on day 1, preceding the first test day (day 2). A washout period of ≥7 days was provided between each treatment series. All subjects received substantial training in both driving and cognitive tests 1–2 weeks before the first testing until reaching a plateau level. After baseline assessments without treatment, subsequent assessments were performed on days 2 and 9 at 0930 for each treatment series. In addition, the same assessments were performed on day 10 (dose increase from 7.5 to 15 mg) only for the mirtazapine 7.5 mg series. The study schedule is shown in Figure 1.

A driving simulator (DS) (Toyota Central R&D Labs, Nagakute, Japan) was used to examine three driving skills that have been associated with traffic accidents. The details of DS configuration and tasks have been described previously (Iwamoto *et al.*, 2008). The road-tracking test measures standard deviation of lateral position (SDLP) on a gently winding road at a constant speed of 100 km/h. The car-following test measures coefficient of variation of the distance between preceding car and subject's own (Uchiyama *et al.*, 2003). Subject was required to maintain a constant distance between cars. The harsh-braking test measures mean brake reaction time in seven braking trials to avoid crashing into the humanoid models that randomly ran into the road. Each test was recorded every 20 ms and lasted for 5 min. As for the cognitive test, the Continuous Performance Test—Identical Pairs version (Cornblatt *et al.*, 1988) was used to measure sustained attention. A series of four-digit stimuli was used, and performance was measured by the signal detection index d-prime, a measure of discriminability computed from "hits" and "false alarms". The Stanford Sleepiness Scale (SSS) (Hoddes *et al.*, 1973) is also used to examine the level of alertness at the beginning of each test day.

To compare the conditions following the administration of the three drugs, the differences between the baseline values and each evaluation point values were analyzed. Two-way repeated-measures analysis of variance with time and drug as factors was used to analyze the outcome variables over 8 days. Post-hoc tests were examined with one-way repeated-measures analysis of variance followed by the Bonferroni test at each evaluation point. Outcome variables on day 10 in the mirtazapine 7.5 mg series were compared with those on day 9 in the same series using paired *t*-tests. All tests were two-tailed, with the alpha level set at 0.05.

RESULTS

In the road-tracking test, 1 subject failed to complete the test on day 2 for both the mirtazapine 7.5 and 15 mg series, as he was sliding off the track. No other subjects were stopped prematurely and crashed during driving test. Because of technical malfunctions, road-tracking test, harsh-braking test, and Continuous Performance Test data were incomplete for 1 subject. Only complete data sets were included in analyses.

A summary of the results is shown in Table 1. There is a significant *main drug effect* in the road-tracking test ($F=10.2$, $df=1$, 13, $p=0.004$). SDLP in the mirtazapine 15 mg series was significantly greater than that observed in the mirtazapine 7.5 mg or placebo series on day 2 ($p=0.004$, both). There is no significant drug × time interaction or *main drug effect* in other driving and cognitive tests. There is a significant drug × time interaction in sleepiness ($F=6.46$, $df=2$, 24, $p=0.006$). SSS scores in the mirtazapine 7.5 and 15 mg series were significantly greater than that observed in the placebo series on day 2 ($p=0.028$ and $p=0.027$, respectively). The results for SDLP and SSS on days 2 and 9 are presented in Figure 2. With regard to increased mirtazapine on day 9, any variables on day 10 did not significantly changed compared with those on day 9.

DISCUSSION

The present results indicate that mirtazapine 7.5 mg did not significantly affect driving performances and sustained attention, although mirtazapine 15 mg had a significantly deleterious effect on road-tracking performance. However, mirtazapine 7.5 mg, like 15 mg, significantly increased subjective sleepiness compared with placebo in acute dosing. Mirtazapine-induced sleepiness decreased over time and was no longer clinically relevant after repeated dosing. Furthermore, the increase in mirtazapine from 7.5 to 15 mg did not impair any performance. This study examines the effects of an initial lower dose of mirtazapine on both driving performance and cognitive function.

Mirtazapine-induced sedation is considered attributable in large part to potent blockade of histamine$_1$ receptors. Antihistamine activity is thought to be offset by increased noradrenergic transmission at higher doses (Stahl *et al.*, 1997; Stimmel *et al.*, 1997). Radhakishun *et al.* (2000) showed that initial mirtazapine doses of 15 and 30 mg had similar impacts on subjective alertness, but few data have been accumulated to confirm this theory, particularly at lower doses. In the case of antihistamines, dose-dependent effects on psychomotor performance including driving performance (Theunissen *et al.*, 2004) and brain

Table 1. Summary of the results of driving tests, cognitive test, and subjective measurement in healthy subjects enrolled in a crossover trial of mirtazapine 7.5 mg, mirtazapine 15 mg and placebo ($N=13$)

Measure	Test time	Mean (SD)		
		Placebo	Mirtazapine 7.5 mg	Mirtazapine 15 mg
Driving test	Baseline		42.9 (12.6)	
SDLP* (cm)	Day 2	40.7 (10.6)	41.3 (9.8)	48.3 (11.2)
	Day 9	42.5 (11.5)	40.3 (10.6)	44.8 (12.3)
	Day 10	...	40.8 (10.5)	...
DCV	Baseline		37.4 (25.0)	
	Day 2	39.3 (40.4)	57.3 (85.7)	67.0 (86.2)
	Day 9	27.9 (18.5)	24.5 (21.1)	27.1 (24.8)
	Day 10	...	26.3 (21.8)	...
BRT** (ms)	Baseline		542.3 (43.9)	
	Day 2	533.3 (70.8)	521.2 (41.1)	538.6 (44.7)
	Day 9	525.2 (43.5)	526.8 (41.0)	538.9 (52.9)
	Day 10	...	527.0 (38.8)	...
Cognitive test	Baseline		3.0 (0.8)	
CPT (d')**	Day 2	3.4 (0.7)	3.4 (0.5)	3.1 (0.8)
	Day 9	3.4 (0.7)	3.6 (0.5)	3.4 (0.7)
	Day 10	...	3.6 (0.6)	...
Subjective measurement	Baseline		2.4 (0.5)	
SSS	Day 2	2.5 (0.7)	3.3 (0.9)	3.8 (1.3)
	Day 9	2.5 (0.6)	2.3 (0.5)	2.6 (0.6)
	Day 10	...	2.5 (0.6)	...

SDLP, standard deviation of lateral position; DCV, distance coefficient of variation; BRT, brake reaction time; CPT, Continuous Performance Test; SSS, Stanford Sleepiness Scale.
Baseline data were assessed once before treatment.
*$N=11$, **$N=12$,

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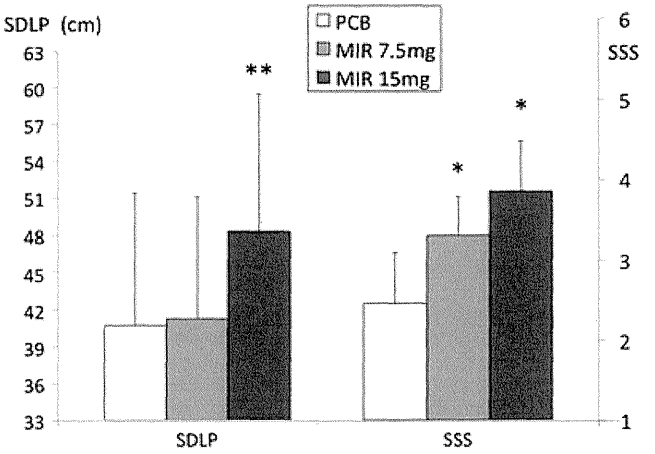

Figure 2. Mean standard deviation of lateral position (SDLP) (left) and Stanford Sleepiness Scale (SSS) (right) on days 2 of the crossover treatment with mirtazapine (MIR) 7.5 mg, MIR 15 mg, or placebo (PCB). **Post-hoc Bonferroni test demonstrated that SDLP under the MIR 15 mg series was significantly greater than that observed under the MIR 7.5 mg series or PCB series ($p < 0.01$, both). *Post-hoc Bonferroni test demonstrated that SSS in the MIR 7.5 and 15 mg series were significantly greater than that observed in the PCB series ($p < 0.05$, both). All statistics were corrected for baseline values

histamine$_1$ receptor occupancy have been confirmed using positron emission tomography (Tashiro *et al.*, 2009). The same effect may also be applicable to mirtazapine only at single low dose. In fact, the present study showed that SDLP after single dose of mirtazapine 7.5 mg was significantly lower than that of 15 mg and SSS after single dose of mirtazapine 7.5 mg was nonsignificantly lower than that of 15 mg. In addition, this impairing effect of mirtazapine disappeared after repeated dosing because of tolerance (Ramaekers, 2003) as with antihistamines. Moreover, the sensitivity of road-tracking test for histamine$_1$ antagonism may be related to the difference in driving impairment between mirtazapine 7.5 and 15 mg doses. Further studies should investigate the dose-dependene of mirtazapine effects using subjective and objective measures of sedation, including neuroimaging.

Mirtazapine 7.5 mg did not impair road-tracking performance in acute dosing, but significantly increased subjective sleepiness. This discrepancy between objective performance and subjective sedation may be attributable to different level and mechanisms of sedation (Hindmarch, 1998). Wezenberg *et al.* (2007) showed that objective sedation tests helped uncover differences in sedative effects, whereas subjective testing or use of a visual analogue scale could not discriminate between drugs and dosages. In the present study, mirtazapine 7.5 mg may result in less sedation as measured by driving performance than mirtazapine 15 mg, whereas SSS did not discriminate between sedation with different dosage regimens. Furthermore, evening dose of mirtazapine produced somnolence, but its effect on driving performance was mild in the next day (Ramaekers, 2003). On the contrary, the predictive validity of the alertness for driving performance was low (Verster and Roth, 2012). Thus, the examinations of both objective and subjective measures are important when considering psychotrophics' effects on driving performance.

Previous study examined low-dose effects of esmirtazapine on actual driving (Ramaekers *et al.*, 2011). Esmirtazapine 4.5 mg, unlike 1.5 mg, impaired actual road-tracking performance, and its' acute effect on driving impairment is suggested to be dose-dependent. It is difficult to clearly explain that esmirtazapine 4.5 mg caused significant driving impairment and mirtazapine 7.5 mg did not. Esmirtazapine has approximately the same affinity to histamine$_1$ receptors as mirtazapine and is believed to be responsible for alpha$_2$ heteroreceptor blockade and the 5-HT$_3$ receptor antagonism (de Boer *et al.*, 1988; Kooyman *et al.*, 1994; Haddjeri *et al.*, 1996). This discrepancy in driving impairment cannot be accounted for by the difference in receptor binding profiles. Instead, sample size, sex of subjects (Timmer *et al.*, 2000; Borobia *et al.*, 2009), CYP2D6 genotype (Timmer *et al.*, 2000; Brockmoller *et al.*, 2007; Borobia *et al.*, 2009; Ramaekers *et al.*, 2011), and the sensitivity of driving test may explain the different results. Future study needs to draw a comparison between mirtazapine and esmirtazapine in the same low dose. Meanwhile, dose-dependent influence of mirtazapine may be consistent with that of esmirtazapine at low dosage.

The present study has several limitations. First, participation was restricted to a small number of healthy adult male volunteers. Female, elderly, and depressed patients were not included. Mirtazapine could both impair driving performance (Wingen *et al.*, 2005) and improve driving ability in depressed patients (Brunnauer *et al.*, 2008; Shen *et al.*, 2009). Meanwhile, depressed patients' psychomotor impairments related to driving abilities were influenced by different classes of antidepressants (Brunnauer *et al.*, 2006). Because of many confounding factors such as antidepressant treatment and the depression itself, it is important to examine the effect of antidepressant on driving performance in healthy subject to find the inherent influences of antidepressants for driving impairment. Future study needs to elucidate the impact of same antidepressants in depressed patients in same experimental line and make a comparison with depressed patients. Second, the validity and sensitivity of DS need to be considered. This

DS has not been validated against real car driving; however, our past results using same DS are roughly consistent with preceding results using actual driving test (Iwamoto *et al.*, 2008; Takahashi *et al.*, 2010). In future studies, we are aiming to verify the validity of DS for real car driving in cooperation with Toyota Central R&D Labs to return the results of research to society. Third, we need to evaluate dose–response relationships within the range of up to 30 mg, to clarify the impact of a lower initial dose of mirtazapine on driving performance.

Finally, mirtazapine 7.5 mg did not impair road-tracking performance compared with mirtazapine 15 mg. An initial lower dose of mirtazapine may have less harmful effect on driving performance and be more suitable for some patients as a starting dosage.

CONFLICT OF INTEREST

There is no conflict of interest that is directly relevant to the content of this study.

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REFERENCES

de Boer T. 1995. The effects of mirtazapine on central noradrenergic and serotonergic neurotransmission. *Int Clin Psychopharmacol* **10** (Suppl 4): 19–23.

de Boer TH, Maura G, Raiteri M, de Vos CJ, WieringaI J, Pinder RM. 1988. Neurochemical and autonomic pharmacological profiles of the 6-aza-analogue of mianserin, Org 3770 and its enantiomers. *Neuropharmacology* **27**: 399–408.

Borobia AM, Novalbos J, Guerra-Lopez P, *et al.* 2009. Influence of sex and CYP2D6 genotype on mirtazapine disposition, evaluated in Spanish healthy volunteers. *Pharmacol Res* **59**: 393–398.

Brockmoller J, Meineke I, KirchheinerI J. 2007. Pharmacokinetics of mirtazapine: enantioselective effects of the CYP2D6 ultra rapid metabolizer genotype and correlation with adverse effects. *Clin Pharmacol Ther* **81**: 699–707.

Brunnauer A, Laux G, Geiger E, Soyka M, Moller HJ. 2006. Antidepressants and driving ability: results from a clinical study. *J Clin Psychiatry* **67**: 1776–1781.

Brunnauer A, Laux G, David I, Fric M, Hermisson I, Moller HJ. 2008. The impact of reboxetine and mirtazapine on driving simulator performance and psychomotor function in depressed patients. *J Clin Psychiatry* **69**: 1880–1886.

Cipriani A, Furukawa TA, Salanti G, *et al.* 2009. Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. *Lancet* **373**: 746–758.

Cornblatt BA, Risch NJ, Faris G, Friedman D, Erlenmeyer-Kimling L. 1988. The Continuous Performance Test, Identical Pairs version (CPT-IP): I. New findings about sustained attention in normal families. *Psychiatry Res* **26**: 223–238.

Haddjeri N, Blier P, de Montigny C. 1996. Effect of the alpha-2 adrenoceptor antagonist mirtazapine on the 5-hydroxytryptamine system in the rat brain. *J Pharmacol Exp Ther* **277**: 861–871.

Hindmarch I. 1998. It's all in the mind: measuring somnolence and CNS drug side effects. *Hum Psychopharmacol* **13**: 385–387.

Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. 1973. Quantification of sleepiness: a new approach. *Psychophysiology* **10**: 431–436.

Iwamoto K, Takahashi M, Nakamura Y, *et al.* 2008. The effects of acute treatment with paroxetine, amitriptyline, and placebo on driving performance and cognitive function in healthy Japanese subjects: a double-blind crossover trial. *Hum Psychopharmacol* **23**: 399–407.

Kooyman AR, Zwart R, Vanderheijden PM, Van Hooft JA, VijverbergI HP. 1994. Interaction between enantiomers of mianserin and ORG3770 at 5-HT3 receptors in cultured mouse neuroblastoma cells. *Neuropharmacology* **33**: 501–507.

Radhakishun FS, van den Bos J, van der Heijden BC, Roes KC, O'Hanlon JF. 2000. Mirtazapine effects on alertness and sleep in patients as recorded by interactive telecommunication during treatment with different dosing regimens. *J Clin Psychopharmacol* **20**: 531–537.

Ramaekers JG. 2003. Antidepressants and driver impairment: empirical evidence from a standard on-the-road test. *J Clin Psychiatry* **64**: 20–29.

Ramaekers JG, Muntjewerff ND, van Veggel LMA, Uiterwijk MMC, O'Hanlon JF. 1998. Effects of nocturnal doses of mirtazapine and mianserin on sleep and on daytime psychomotor and driving performance in young, healthy volunteers. *Hum Psychopharmacol* **13**: S87–S97.

Ramaekers JG, Conen S, de Kam PJ, *et al.* 2011. Residual effects of esmirtazapine on actual driving performance: overall findings and an exploratory analysis into the role of CYP2D6 phenotype. *Psychopharmacology (Berl)* **215**: 321–332.

Ridout F, Meadows R, Johnsen S, Hindmarch I. 2003. A placebo controlled investigation into the effects of paroxetine and mirtazapine on measures related to car driving performance. *Hum Psychopharmacol* **18**: 261–269.

Shen J, Moller HJ, Wang X, *et al.* 2009. Mirtazapine, a sedating antidepressant, and improved driving safety in patients with major depressive disorder: a prospective, randomized trial of 28 patients. *J Clin Psychiatry* **70**: 370–377.

Stahl S, Kremer C, Pinder R. 1997. Tolerability of mirtazapine used in high or low initial dose. *J Psychopharmacol* **11**: A53.

Stimmel G, Sussman N, Wingard P. 1997. Mirtazapine safety and tolerability: analysis of the clinical traials database. *Prim Psychiatry* **6**: 82–96.

Takahashi M, Iwamoto K, Kawamura Y, *et al.* 2010. The effects of acute treatment with tandospirone, diazepam, and placebo on driving performance and cognitive function in healthy volunteers. *Hum Psychopharmacol* **25**: 260–267.

Tashiro M, Kato M, Miyake M, *et al.* 2009. Dose dependency of brain histamine H(1) receptor occupancy following oral administration of cetirizine hydrochloride measured using PET with [11C]doxepin. *Hum Psychopharmacol* **24**: 540–548.

Theunissen EL, Vermeeren A, van Oers AC, van Maris I, Ramaekers JG. 2004. A dose-ranging study of the effects of mequitazine on actual driving, memory and psychomotor performance as compared to dexchlorpheniramine, cetirizine and placebo. *Clin Exp Allergy* **34**: 250–258.

Timmer CJ, Sitsen JM, Delbressine LP. 2000. Clinical pharmacokinetics of mirtazapine. *Clin Pharmacokinet* **38**: 461–474.

Uchiyama Y, Ebe K, Kozato A, Okada T, Sadato N. 2003. The neural substrates of driving at a safe distance: a functional MRI study. *Neurosci Lett* **352**: 199–202.

Verster JC, Roth T. 2012. Drivers can poorly predict their own driving impairment: a comparison between measurements of subjective and objective driving quality. *Psychopharmacology (Berl)* **219**: 775–781.

Watanabe N, Omori IM, Nakagawa A, *et al.* 2008. Mirtazapine versus other antidepressants in the acute-phase treatment of adults with major depression: systematic review and meta-analysis. *J Clin Psychiatry* **69**: 1404–1415.

Watanabe N, Omori IM, Nakagawa A, *et al.* 2010. Safety reporting and adverse-event profile of mirtazapine described in randomized controlled trials in comparison with other classes of antidepressants in the acute-phase treatment of adults with depression: systematic review and meta-analysis. *CNS Drugs* **24**: 35–53.

Wezenberg E, Sabbe BG, Hulstijn W, Ruigt GS, Verkes RJ. 2007. The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related impairments from memory dysfunctions. *J Psychopharmacol* **21**: 579–587.

Wingen M, Bothmer J, Langer S, Ramaekers JG. 2005. Actual driving performance and psychomotor function in healthy subjects after acute and subchronic treatment with escitalopram, mirtazapine, and placebo: a crossover trial. *J Clin Psychiatry* **66**: 436–443.

Hum. Psychopharmacol Clin Exp 2013; **28**: 523–528.
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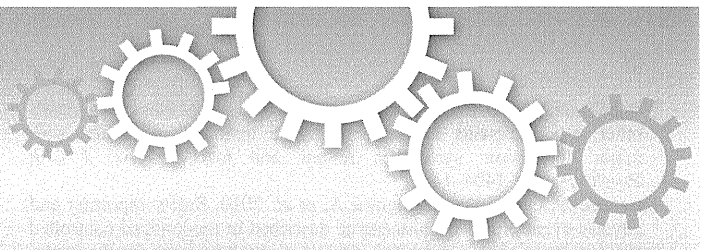

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Definition and refinement of the 7q36.3 duplication region associated with schizophrenia

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Using a very high-resolution oligonucleotide array for copy number variant (CNV) screening of samples comprising schizophrenic patients, we detected a novel CNV within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) previously shown to be associated with schizophrenia. We investigated the association between the novel CNV identified in the current study and schizophrenia. Three independent samples were used: (1) *Screening set*, 300 Japanese schizophrenic patients (53.28 ± 14.66 years); (2) *Confirmation set*, 531 schizophrenic patients (46.03 ± 12.15 years); and (3) 711 healthy controls (47.12 ± 11.03 years). All subjects enrolled in the study were Japanese. Chromosomal position was determined using fluorescence in situ hybridization. We identified a novel duplication within the region associated with schizophrenia identified on 7q36.3 that is adjacent to *VIPR2* and is not associated with schizophrenia. In the Japanese population, the 35-kb region that harbors the common, novel CNV should be excluded from the region associated with schizophrenia on 7q36.3.

Schizophrenia is a chronic, debilitating illness characterized by impairments in cognition, affect and behaviour[1]. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV-TR)[2] defines the essential features of schizophrenia as a mixture of characteristic signs and symptoms (both positive and negative) that have been present for a significant portion of time during a 1-month period (or for a shorter time if successfully treated), with some signs of the disorder persisting for at least 6 months. In this regard, positive refers to the presence of active symptoms including delusions and hallucinations. Negative symptoms refer to a loss, typically of emotions, speech, or motivation. Schizophrenic disorders exist on a continuum from mild to severe. The DSM IV-TR[2] recognizes a number of different types, which include disorganized, catatonic, paranoid, schizophreniform, residual, schizoaffective, undifferentiated and not otherwise specified[2]. Schizophrenia is a relatively common disorder, with a lifetime prevalence of about 1%[1]. Although the overall sex ratio is almost equal, males tend to have an earlier onset than females, a finding accounted for by the later age of onset in those females who lack a family history of the disease[3]. Family history is the most important risk factor for schizophrenia, consistent with a genetic contribution to its etiology[4]. However, as with most mental disorders, the origins and mechanisms of schizophrenia are not fully understood.

Genetic factors influence human disorders by determining disease susceptibility or resistance[5]. Therefore, genetic studies can help pinpoint the exact molecular mechanism of a disease. Recent successes in the genetic mapping and molecular mechanism of the Mendelian traits have been remarkable, owning to the development of genome wide screening techniques[6]. As such, attention has been gradually shifting towards more complex, common, genetic disorders and traits that involve multiple genes and environmental effects, such as celiac disease[7], diabetes[8], rheumatoid arthritis[9] and psychiatric disorders[10]. In this context, recurrent microdeletions at 1q21.1[11], 15q13.3[12], and 15q11.2[12], microduplications at 16p11.2[13], and copy number variations (CNVs) at other genomic loci[14] have been shown to be associated with schizophrenia in large cohorts examined by CNV analyses and other molecular studies. Furthermore, duplication at chromosome 7q36.3, encompassing *VIPR2*, was implicated in schizophrenia for the first time in a recent report[15]. In a specific genome-wide association study of 8,290 patients with schizophrenia performed by Vacic et al.[15], the authors found that 0.35% of these patients carry rare CNVs in the chromosomal locus 7q36.3. In contrast, these microduplications were much less frequent

(0.03%) among the 7,431 healthy controls. All variants overlap with *VIPR2* or lie within the noncoding subtelomeric region, <89 kb (NCBI36/hg18, Chr7: 158,630,410–158,719,410) from the transcriptional start site of *VIPR2*. This gene encodes the vasoactive intestinal peptide (VIP) receptor VPAC2, which is a G-protein-coupled receptor that is expressed in the suprachiasmatic nucleus, hippocampus, amygdala and hypothalamus[16]. VPAC2 binds VIP, activates cyclic AMP (cAMP)-signalling and PKA, regulates synaptic transmission in the hippocampus, and promotes the proliferation of neural progenitor cells in the dentate gyrus[17]. Moreover, it has been shown that alteration in synaptic plasticity of hippocampal neurons may contribute to the symptoms observed in schizophrenic patients[18]. The aforementioned lines of evidence provide support for the role of *VIPR2* as a candidate gene for schizophrenia from a biological point of view.

In the present study, by using a very high-resolution oligonucleotide array for CNV screening of samples from schizophrenic patients, we were able to detect CNV within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) on 7q36.3 that was shown to be associated with schizophrenia by the Vacic et al. study[15]. Thus, the goal of the present study was to follow-up on the novel CNV that was previously detected in schizophrenic patients and further investigate any association between this CNV and schizophrenia.

Results

In the present study, we detected a smaller (35 kb) duplication (NCBI36/hg18, Chr7: 158,658,128–158,693,128) within the critical region identified by Vacic et al.[15] (Figure 1). The observed frequency of the CNVs was ~2% and we did not detect any statistically significant difference between the patients and controls (Table 1). There was a 100% concordance rate between the custom NimbleGen 12 × 135,000 CGH arrays and the NimbleGen 3 × 720,000 CGH arrays or custom TaqMan copy number assay for the detection of the smaller (35 kb) duplication (NCBI36/hg18, Chr7: 158,658,128–158,693,128) within the critical region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) previously identified by Vacic et al.[15].

In metaphase cells, all duplication-specific FISH signals localize to the subtelomeric region of 7q, confirming that the duplications lie adjacent to each other in the 7q36.3 region (Figure 2). In addition, NS102 exhibited two signals, one of which had a higher intensity compared to that of the other. This suggests that there is unilocus duplication in the *VIPR2* promoter region. During the orientation analysis, an amplicon was detected by electrophoresis only in samples with duplication, which indicates that there is a head-to-tail orientation of the repeated DNA fragment (Supplementary Figure 1). Additionally, sequence analysis of the repeat junction revealed that all samples with duplication shared exactly the same sequence within the junction region (Supplementary Figure 2). Based on the

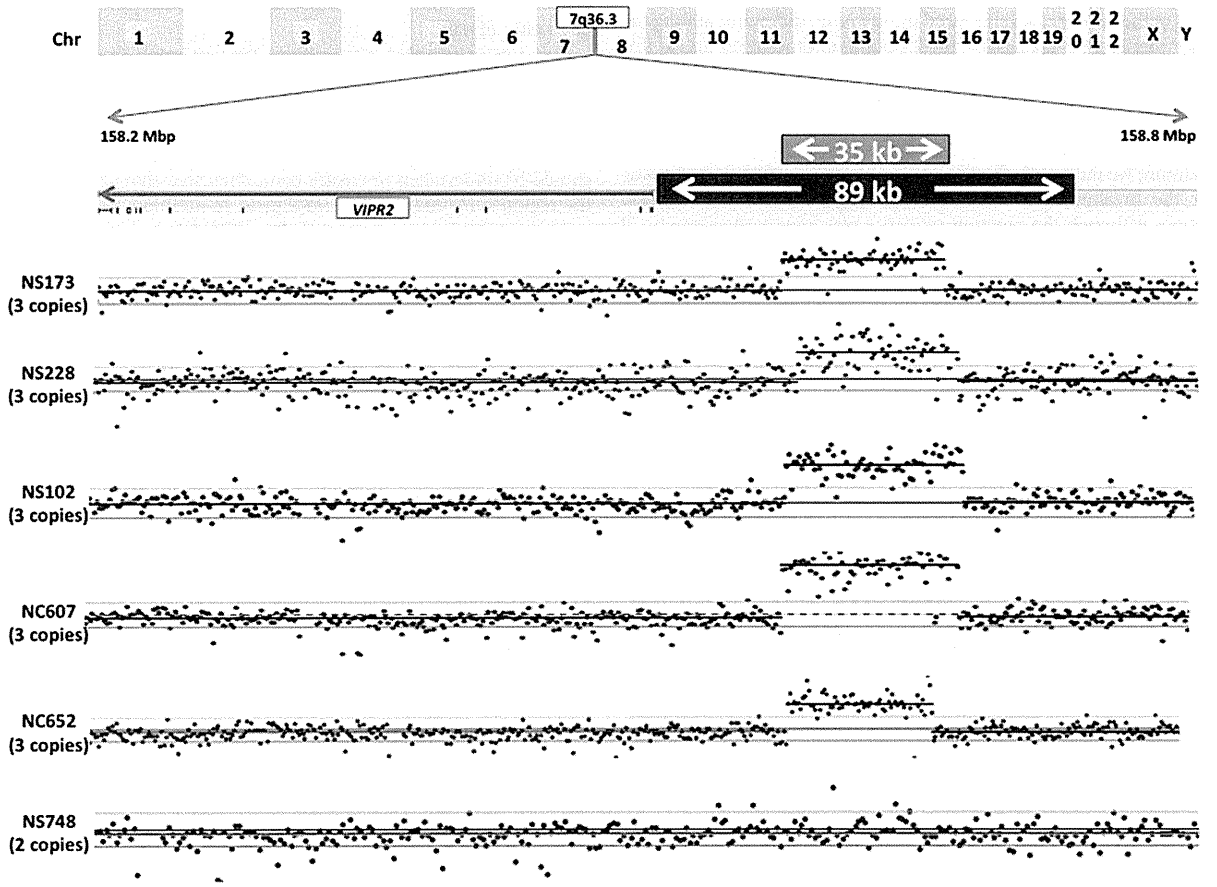

Figure 1 | High-resolution aCGH data. Probe intensity ratios. The orange box (NCBI36/hg18, Chr7: 158,658,128–158,693,128) represents CNVs detected in the present study and the purple box (NCBI36/hg18, Chr7: 158,630,410–158,719,410) represents the region revealed by Vacic et al. to show an association peak in a schizophrenia group. Coordinates are based on the NCBI36 build.

Table 1 | Frequency distribution (confirmation set)

		Number of copies										Aggregated data		
		0		1		2		3		4		>2 copies		P-Value
Set	Sample size	N	%	N	%	N	%	N	%	N	%	N	%	(Fisher exact test)
Cases	531	0	0.0%	0	0.0%	520	97.9%	10	1.9%	1	0.2%	11	2.1%	0.96
Controls	711	0	0.0%	0	0.0%	696	97.9%	13	1.8%	2	0.3%	15	2.1%	

panel of 8 SNPs in the CNV region that were detected in the current study, we did not observe the existence of any common haplotype (Supplementary Table 2).

Discussion

Using samples from only Japanese subjects, we identified a smaller 35-kb (NCBI36/hg18, Chr7: 158,658,128–158,693,128) common (>1%) duplication within the region that Vacic et al.[15] previously showed was associated with schizophrenia (NCBI36/hg18, Chr7: 158,630,410–158,719,410). However, the common duplication that we detected in the present study was not found to be associated with schizophrenia. It is of note that these results may be specific to Japanese subjects, and therefore further studies involving other population groups will need to be undertaken. We have experimentally confirmed that the common CNV detected in the current study was located adjacent to *VIPR2*. Our analysis of the breakpoint junctions at the sequence level showed there was no difference among the CNV carriers. On the basis of the 2-bp microhomology found at this junction (Supplementary Figure 2), we speculate that duplication formation occurs by the FoSTeS (fork stalling and template switching)/MMBIR (microhomology mediated break-induced replication) mechanism previously proposed by Lupski's research group[23]. In addition, we did not find any sequence motif that was characteristic for the breakpoint of recurrent rearrangements at the junction region. Although the junction sequence was exactly the same in all of the subjects in whom the 35-kb CNV was detected, we could not confirm that these subjects shared any common haplotype. Differences in the haplotype among the duplication carriers were likely due to the high recombination rate that occurs at the subtelomeric region[24]. Regarding the origin of the CNV that was identified in the current study, our observation of the same breakpoint junction sequence that was observed in the CNV carriers is highly suggestive of a common ancestral founder.

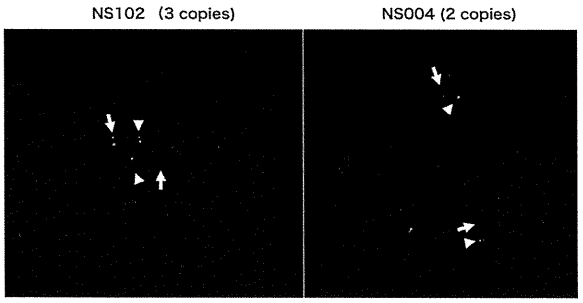

Figure 2 | Tandem duplications of 7q36.3 confirmed in two patients by fluorescence in situ hybridization (FISH). 7p-green (arrowheads) and 7q-red (arrows, CNV specific). Left NS102 (3 copies); Right NS004 (2 copies). Cytogenetic confirmation was obtained for two samples with and without duplication of VIPR2. Probes for duplicated region were produced by long range PCR. The subtelomeric probe, 7p-green (Abbott Molecular), was used as a reference. Hybridizations were performed according to the manufacturer's protocols.

The main limitation of the current study was that we examined a much smaller number of samples as compared to the Vacic et al. study[15]. It is of note that the frequency of the common 35-kb CNV detected in the current study was 2%, and thus with our current sample size of 300 schizophrenic patients, it was large enough to capture the variation. Regarding the individual with 4 copies, we do not have any data indicating whether the individual is a homozygote of duplication or is a carrier of triplication. This point should be considered as another limitation of the current study. The results of the current study do suggest that in case of a duplication event on 7q36.3, the relevant region is not the *VIPR2* promoter (as has been suggested by Vacic et al.[15]), but rather suggest that it is the *VIPR2* gene region. In addition, the 2-bp microhomology in the promoter region of VIPR2 may be associated with the relative meiotic instability of the region harboring the common CNV that is adjacent to the *VIPR2* gene[25]. This in turn may give rise to the larger *VIPR2* duplications that were shown to be associated with schizophrenia with an odds ratio of 4.0[14].

It is interesting that our findings demonstrated that CNV was detected in our study in contrast to the previous reports by both Vacic et al.[15] and Beri et al.[26]. Moreover, CNV has not been listed in the database of genomic variants[27]. Although the CNV detected by our group may be specific to Japanese populations, further studies should be undertaken to ensure comprehensive characterization of the region surrounding the *VIPR2* gene. In addition, to address the question regarding the origin of the CNV detected in the current study, it is necessary to perform family analysis of the carriers and determine whether CNV is a *de novo* event, or if it is transmitted from the parents. In conclusion, the 35-kb region that harbors the common CNV in the Japanese population should be excluded from the region of the association peak in the schizophrenia group reported in the Vacic et al. study[15].

Methods

Three independent samples were used in the current study: (1) *Screening set*, 300 Japanese patients suffering from schizophrenia (53.28 ± 14.66 years); (2) *Confirmation set*, 531 patients suffering from schizophrenia (46.03 ± 12.15 years); and (3) 711 healthy control subjects (47.12 ± 11.03 years). All schizophrenic patients met the current DSM IV-TR criteria[2], which was reflected by consensual diagnosis of two experienced psychiatrists. Prior to inclusion in the control set, subjects were screened on the basis of a brief diagnostic interview. Detailed characterization and psychiatric assessment of the subjects is available elsewhere[19]. All subjects enrolled in the study were Japanese and provided written informed consent prior to the study. Venous blood was drawn from each subject and genomic DNA was extracted according to the standard phenol/chloroform method. Comparative genomic hybridization of DNA was performed using the high-resolution NimbleGen (Roche NimbleGen, Inc., U.S.) CGH array (3 × 720,000 or 12 × 135,000). Labeling and hybridization of patient (test) and sex-matched commercial (Promega Corporation, U.S.) reference DNA was performed according to the manufacturer's protocols. Test and reference DNA were labeled by Cy3- and Cy5-labeled random primers, respectively, and were combined and hybridized to the array for 40–72 h. Arrays were washed in four steps, as indicated in the protocol. Two-color scanning was performed using a NimbleGen MS 200 microarray scanner. Acquisition of the microarray images was performed with NimbleGen MS 200 software. Data extraction, analysis and visualization were done using NimbleScan version 2.4 software. CNV calling was performed using NEXUS software. The FASST2 Segmentation Algorithm, a Hidden Markov Model (HMM) based approach, was used to make copy number calls. The FASST2 algorithm, unlike other common HMM methods for copy number estimation, does not aim to estimate the copy number state at each probe, but uses many states to cover more possibilities, such as mosaic events. These state values are then used to make calls based on a log ratio threshold. The significance threshold for

segmentation was set at 10^{-6} and also required a minimum of three probes per segment. The log ratio thresholds for single copy gain and single copy loss were set at 0.3 and −0.3, respectively. The log ratio thresholds for the gain of two or more copies and homozygous loss were set at 0.9 and −0.9 respectively.

Custom TaqMan copy number assay was specifically designed to interrogate a duplication region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) without interspersed repeats, low complexity or a homologous DNA sequence. A TaqMan copy number assay for RNase P was used as a reference. Experiments were carried out on four technical replicates according to the manufacturer's protocol. CNV typing of the screening sample was performed using Roche NimbleGen, Inc. CGH array 3 × 720,000, while confirmation of the sample was performed using the TaqMan copy number assay. Sixteen randomly selected duplication events (both in the screening and confirmation samples) were validated using custom NimbleGen 12 × 135,000 CGH arrays (Roche NimbleGen, Inc., U.S.) covering the region (NCBI36/hg18, Chr7: 158,630,410–158,719,410) implicated in the Vacic et al. study[15], with an average of one probe per 500 bp. P values derived from association analysis were based on Fisher's exact test.

We performed PCR based analysis to determine the orientation of the detected duplications. We designed forward and reversed primers to align with the region of the duplication junction (F: 5′-TGTGGATTCCCTTCAGAGGCGAC-3′, R: 5′-CATTCTTCAGCCCATGGAGTCATC-3′) (Supplementary Figure 1). Cytogenetic confirmation was obtained for two samples with and without duplication of VIPR2. Probes for duplicated region were produced by long range PCR (NCBI36/hg18, Chr7: 158,658,128–158,693,128). Subtelomeric probe, 7p-green (Abbott Molecular, U.S.), was used as a reference. Hybridizations were performed according to the manufacturer's protocols.

Haplotypes were estimated using the statistical software package PHASE version 3.4.1 (http://www.stat.washington.edu/stephens/)[20–22]. This program is based on a Bayesian statistical method using coalescent-based models that infers phases at loci from unphased genotype data for a sample of unrelated individuals[20]. The algorithm uses a flexible model for the decay of linkage disequilibrium with distance and explicitly incorporates an assumption about the recombination rate variation. PHASE uses Gibbs sampling, a Markov-Chain Monte Carlo algorithm for the estimation of the posterior distribution. Hence, the individual haplotype can be estimated from the posterior distribution by choosing the most likely haplotype reconstruction for each individual. Using the extension for unrelated individuals, we used the default settings to infer the haplotypes from the genotype data[19] of the 8 SNPs (Supplementary Table 1) surrounding the duplication in sample that comprised 517 subjects (7 with a structural variant detected in the current study). Estimates of the sample haplotype frequencies together with their standard deviation, a list of the most likely pairs of haplotypes for each individual together with their probability, and the estimates of recombination parameters in the region, were calculated using the same software.

1. van Os, J. & Kapur, S. Schizophrenia. *Lancet* **374**, 635–45 (2009).
2. Author. *Diagnostic and statistical manual of mental disorders (4th ed., text rev.).* (American Psychiatric Association., Washington, DC, 2000).
3. Loranger, A. W. Sex difference in age at onset of schizophrenia. *Arch Gen Psychiatry* **41**, 157–61 (1984).
4. Sullivan, P. F. The genetics of schizophrenia. *PLoS Med* **2**, e212 (2005).
5. Manolio, T. A. *et al.* Finding the missing heritability of complex diseases. *Nature* **461**, 747–53 (2009).
6. Chakravarti, A. Genomic contributions to Mendelian disease. *Genome Res* **21**, 643–4 (2011).
7. Trynka, G. *et al.* Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. *Nat Genet* **43**, 1193–201 (2011).
8. Todd, J. A. *et al.* Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* **39**, 857–64 (2007).
9. Thomson, W. *et al.* Rheumatoid arthritis association at 6q23. *Nat Genet* **39**, 1431–3 (2007).
10. Hamshere, M. L. *et al.* Genome-wide significant associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the Schizophrenia PGC. *Mol Psychiatry* **18**, 708–712 (2013).
11. Mefford, H. C. *et al.* A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease. *Genome Res* **19**, 1579–85 (2009).
12. Stefansson, H. *et al.* Large recurrent microdeletions associated with schizophrenia. *Nature* **455**, 232–6 (2008).
13. McCarthy, S. E. *et al.* Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* **41**, 1223–7 (2009).
14. Levinson, D. F. *et al.* Copy number variants in schizophrenia: confirmation of five previous findings and new evidence for 3q29 microdeletions and VIPR2 duplications. *Am J Psychiatry* **168**, 302–16 (2011).
15. Vacic, V. *et al.* Duplications of the neuropeptide receptor gene VIPR2 confer significant risk for schizophrenia. *Nature* **471**, 499–503 (2011).
16. Sheward, W. J., Lutz, E. M. & Harmar, A. J. The distribution of vasoactive intestinal peptide2 receptor messenger RNA in the rat brain and pituitary gland as assessed by in situ hybridization. *Neuroscience* **67**, 409–18 (1995).
17. Zaben, M. *et al.* The neurotransmitter VIP expands the pool of symmetrically dividing postnatal dentate gyrus precursors via VPAC2 receptors or directs them toward a neuronal fate via VPAC1 receptors. *Stem Cells* **27**, 2539–51 (2009).
18. Sanderson, T. M. *et al.* Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia. *Neuropharmacology* **62**, 1349–58 (2012).
19. Ikeda, M. *et al.* Genome-wide association study of schizophrenia in a Japanese population. *Biol Psychiatry* **69**, 472–8 (2011).
20. Stephens, M. & Donnelly, P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* **73**, 1162–9 (2003).
21. Stephens, M. & Scheet, P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am J Hum Genet* **76**, 449–62 (2005).
22. Stephens, M., Smith, N. J. & Donnelly, P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* **68**, 978–89 (2001).
23. Hastings, P. J., Ira, G. & Lupski, J. R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet* **5**, e1000327 (2009).
24. Nachman, M. W. Variation in recombination rate across the genome: evidence and implications. *Curr Opin Genet Dev* **12**, 657–63 (2002).
25. Warren, S. T. & Nelson, D. L. Trinucleotide repeat expansions in neurological disease. *Curr Opin Neurobiol* **3**, 752–9 (1993).
26. Beri, S., Bonaglia, M. C. & Giorda, R. Low-copy repeats at the human VIPR2 gene predispose to recurrent and nonrecurrent rearrangements. *Eur J Hum Genet* **21**, 757–61 (2013).
27. Zhang, J., Feuk, L., Duggan, G. E., Khaja, R. & Scherer, S. W. Development of bioinformatics resources for display and analysis of copy number and other structural variants in the human genome. *Cytogenet Genome Res* **115**, 205–14 (2006).

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Author contributions

B.A., I.K., T.O. and N.O. designed the study and wrote the protocol. B.A., I.K., T.O., N.I., H.K. and N.O. performed the literature review. B.A., I.K. and T.O. made and managed the sample database. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K., S.I., H.K., N.I. and N.O. collected and managed the genome samples. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K. and S.I. conducted the statistical analysis. B.A., I.K., T.O., M.I., S.K., Y.N., A.Y., T.K., S.I., H.K., N.I. and N.O. interpreted and discussed the results. B.A., I.K., T.O., M.I., H.K., N.I. and N.O. wrote the manuscript and edited the final manuscript.

Additional information

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