analyzed, and the *CADPS2* SNP was found to be associated with temporal lobe volume, the region especially crucial for memory.

There is also a link between *CADPS2* expression and human brain disorders. Aberrant splicing of *CADPS2* mRNA was reported in autism; an exon-3 skipped isoform, *CADPS2ΔExon3* was detected in the peripheral blood samples of several autistic patients but not in those of healthy controls (Sadakata et al., 2007b). The authors showed that CADPS2ΔExon3 protein was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. While the relationship of *CADPS2ΔExon3* expression in the brains and autism is unclear, the aberrant splicing of *CADPS2* could contribute to autism susceptibility by affecting neurotrophin and/or monoamine release.

Previously, we found that both *CADPS2* and *CADPS2ΔExon3* expression were increased in the post-mortem brains of schizophrenic patients (Hattori et al., 2011). We also detected *CADPS2ΔExon3* in the blood of both schizophrenic patients and control subjects. There were more *CADPS2ΔExon3* positive subjects in the schizophrenic patients than in the controls, although the difference was not statistically significant.

To get more insight into CADPS2's role in human brain function, the present study examined the possible association between the blood expression levels of *CADPS2/CADPS2ΔExon3* and intelligence/memory in healthy subjects. Considering the continuity between developmental disorders and healthy state (Bishop, 1989; Volkmar et al., 2004), intermediate phenotypes related to the developmental disorders should also be expressed in "healthy" subjects and might be associated with *CADPS2/CADPS2ΔExon3* expression levels. We applied quantitative PCR, a more reliable method, to detect each transcript rather than evaluating electrophoresis bands, applied in the past studies (Eran et al., 2009; Sadakata et al., 2007b). As a result, we found that *CADPS2ΔExon3* expression was associated with lower intelligence and memory. To our knowledge, this is a novel finding, which is likely to have relevance to the susceptibility to autism and learning disorders.

2. Subjects and methods

2.1. Participants

Subjects were 271 healthy volunteers [67 males and 204 females; age range 20–74 years; mean age 43.3 ± 15.3 (standard deviation: SD) years] recruited through advertisements in free local magazines and our website announcement. All subjects were biologically unrelated healthy Japanese from the same geographical area (Western part of Tokyo Metropolitan). They were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and those who had a current history of psychiatric disorder were not enrolled in the study. In addition, those individuals who demonstrated one or more of the following conditions in a non-structured interview performed by an experienced psychiatrist were excluded from this study: past or current regular contact to psychiatric services, having a history of regular use of psychotropics or substance abuse/dependence, presenting other obvious self-reported signs of past primary psychotic and mood disorders, and having a prior medical history of central nervous system disease or severe head injury. After the nature of the study procedures had been fully explained, written informed consent was obtained from every subject. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Sample preparation

Blood collection and RNA isolation was performed using the PAXgene blood RNA system (Qiagen, Valencia, CA) as described previously (Hattori et al., 2011). Blood samples were collected around 11 A.M. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA) as described previously (Hattori et al., 2011). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. *CADPS2* (Hs01095968_m1 at Exons 4–5, on NM_017954.9), *CADPS2ΔExon3* (forward primer: GTAGCTGACGAAGCATTTTGCA, reverse Primer: TGATCTGGGCTGCTTGTTCAT, reporter: CTGCGTTATCCAGCTCAT) and a primer of the housekeeping gene, Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (4326317E), all purchased from Applied Biosystems. Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, 95 °C for 10 min and in 40 (for *CADPS2* and *GAPDH*) or 45 (for *CADPS2ΔExon3*) cycles changing between 95 °C for 15 s and 60 °C for 1 min. Data were analyzed using the Sequence Detection System (SDS) 2.0 software (Applied Biosystems) as follows. A standard amplification curve was made by serial dilution of a "standard" pooled cDNA sample in each plate. The mean value of triplicate of each sample was normalized to the standard curve. Then the values of *CADPS2* from each sample were normalized to those of *GAPDH*. With respect to *CADPS2ΔExon3*, we counted the number of tubes in which signals were detected, among triplicates, as reported previously (Hattori et al., 2011). In brief, for each tube, we defined 'detected' if the signal reached a threshold automatically set by the SDS 2.0 software within 45 cycles, and a threshold cycle (Ct) value was obtained. Second, we counted the number of 'detected' tubes of each triplicate (Supplemental Fig. S1). Third, we defined 'positive' when 2 or 3 tubes in triplicate analysis of each sample were detected as we assumed that the 'detection' should be repeated at least once. We defined 'negative' when no tube was detected. The samples with only one-tube detection were excluded from the statistical comparison between individuals with *CADPS2ΔExon3* positive and those with *CADPS2ΔExon3* negative. To avoid an arbitrary interpretation, we also performed statistical analyses including one-tube detection and dividing subjects into 3 groups (negative, one-tube detection, and positive).

Table 1
Demographic information and *CADPS2ΔExon3* expression levels of participants.

	N	Age (SD)	Number of tubes with *CADPS2ΔExon3* detection		
			0	1	2–3
WAIS-R					
Male	54	43.5 (15.6)	36	12	6
Female	185	45.8 (14.6)	114	52	19
Total	239	45.2 (14.8)	150	64	25
WMS-R					
Male	67	40.6 (15.5)	46	13	8
Female	199	43.9 (15.2)	122	56	21
Total	266	43.1 (15.3)	168	69	29

2.4. Neuropsychological test measures

To assess memory and intelligence, the Japanese full versions of the Wechsler Memory Scale-Revised (WMS-R) (Sugishita, 2001) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Shinagawa et al., 1990) respectively, were administered.

2.5. Statistical analyses

CADPS2 expression levels were converted to a -10 logarithmic scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005) as reported previously (Hattori et al., 2011). One extremely high value of *CADPS2* expression was excluded. The relationship between *CADPS2* expression and each score was analyzed by Spearman correlation test. The effect of *CADPS2* or *CADPS2ΔExon3* expression on intelligence or memory was assessed by multiple analysis of covariance (MANCOVA), controlling for age, sex, and education years. These analyses were performed by SPSS software version 11 (SPSS Japan, Tokyo, Japan).

3. Results

First, we analyzed the association between blood *CADPS2* expression and IQ and memory indices. Spearman correlation analyses did not detect any significant correlation between blood *CADPS2* expression levels and IQ scores (Supplemental Table S1). Among WMS-R scores, verbal memory and general memory tended to correlate with *CADPS2* expression levels (Supplemental Table S1). However, no significant effect of *CADPS2* expression was detected on those scores when age, sex and education years were controlled for ($p = 0.15$ for verbal memory and $p = 0.21$ for general memory).

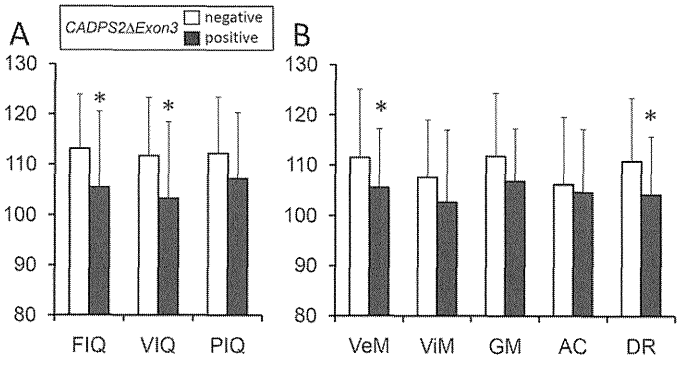

Fig. 1. Association between IQ/memory and *CADPS2 Δ Exon3* expression. WAIS-R scores and WMS-R scores were compared between those who did not (open-bar) and did (filled-bar) express *CADPS2ΔExon3* in the blood. (A) IQ and *CADPS2ΔExon3* expression. FIQ, full-scale IQ; VIQ, verbal IQ; PIQ, performance IQ. (B) Memory and *CADPS2ΔExon3* expression. VeM, verbal memory; ViM, visual memory; GM, general memory; AC, attention and concentration; DR, delayed recall. Data are mean ± SD; $^{*}p < 0.05$, MANCOVA controlled for sex, age, and education years.

Then, we analyzed the possible association of *CADPS2ΔExon3* expression with IQ and memory. As reported previously (Hattori et al., 2011), the expression level was very low and no expression was detected for the majority of samples. Thus, we counted the number of signal-detected tubes among triplicate analyses of each sample (Supplemental Fig. S1, Table 1).

With respect to WAIS-R, full-scale IQ (FIQ) was significantly lower in the *CADPS2ΔExon3* positive group, compared with that of the negative group ($F = 5.3$, df = 1, $p = 0.022$, $\eta_p^2 = 0.031$, Fig. 1A). When verbal IQ (VIQ) and performance IQ (PIQ) were examined separately, VIQ ($F = 5.6$, df = 1, $p = 0.019$, $\eta_p^2 = 0.032$) was significantly lower in the positive group.

With respect to WMS-R, verbal memory ($F = 5.0$, df = 1, $p = 0.026$, $\eta_p^2 = 0.026$) and delayed recall ($F = 4.2$, df = 1, $p = 0.042$, $\eta_p^2 = 0.021$) were significantly lower in the positive group compared with the negative group (Fig. 1B).

Even if one-tube detection was included in the analysis, the results were essentially the same. With respect to WAIS-R, there were marginal effects of *CADPS2ΔExon3* expression levels on FIQ ($F = 2.33$, df = 2, $p = 0.099$, $\eta_p^2 = 0.020$) and VIQ ($F = 2.57$, df = 2, $p = 0.079$, $\eta_p^2 = 0.022$) and the *post hoc* tests detected significant reduction of FIQ ($p = 0.036$) and VIQ ($p = 0.026$) in the positive group compared to negative group (Supplemental Fig. S2A). With respect to WMS-R, significant effects of expression level were detected on verbal memory ($F = 4.5$, df = 2, $p = 0.012$, $\eta_p^2 = 0.034$) and delayed recall ($F = 5.8$, df = 2, $p = 0.003$, $\eta_p^2 = 0.043$). A marginal effect on general memory ($F = 3.0$, df = 2, $p = 0.051$, $\eta_p^2 = 0.023$) was also detected. The *post hoc* tests detected significant reduction of verbal memory ($p = 0.028$) and delayed recall ($p = 0.001$) in the positive group compared to the negative group (Supplemental Fig. S2B).

When males and females were analyzed separately, statistically significant differences were detected only in females with respect to FIQ, VIQ, visual memory, general memory and delayed recall (Supplemental Fig. S3). Nonetheless, average scores of these tests were lower in the *CADPS2ΔExon3* positive group of the male subjects than in the negative group. The failure to reach statistical significance is likely to be ascribed to the lack of statistical power due to the small number of male subjects.

It is possible that we might have removed cognitive ability variance when education years were controlled for. To examine this possibility, we performed an additional analysis in which education was not controlled for. However, the results were essentially unchanged; *CADPS2ΔExon3* expression levels were significantly associated with FIQ ($F = 6.3$, df = 1, $p = 0.013$, $\eta_p^2 = 0.036$), VIQ ($F = 6.7$, df = 1, $p = 0.011$, $\eta_p^2 = 0.038$), verbal memory ($F = 5.1$, df = 1, $p = 0.025$, $\eta_p^2 = 0.026$) and delayed recall ($F = 4.5$, df = 1, $p = 0.035$, $\eta_p^2 = 0.023$).

4. Discussion

In the present study, we examined the possible association between the expression of *CADPS2* transcripts (*CADPS2* and *CADPS2ΔExon3*) in the peripheral blood and higher brain functions such as intelligence and memory in healthy subjects. While *CADPS2* expression levels were not associated with the scores of these measurements, *CADPS2ΔExon3* expression was significantly associated with lower IQ, lower verbal memory and delayed recall of WMS-R.

4.1. Evaluation of CADPSΔExon3 expression levels

Because there were relatively large number of 1-tube detection samples, we suppose that there are continuity between negative and positive samples, and 1-tube detection might stochastically reflects the expression levels between negative (0) and positive (>1). Since inclusion or exclusion of 1-tube detection samples in the criteria did not affect the results essentially, our conclusion; the expression of *CADPS2Δ3* was associated with cognition and memory, was supported. However, because the expression levels might continuous rather than qualitative values, future studies should improve the sensitivity of analyses, i.e. by using larger sample volume.

4.2. Did the participants include autism?

It has been reported that *CADPS2ΔExon3* was present in individuals with autism but not in controls (Sadakata et al., 2007b). In the present study, all participants were screened for current and past psychiatric histories by experienced psychiatrists using structured (M.I.N.I.) and unstructured interviews. As the M.I.N.I. is not designed to diagnose autism, there remains the possibility that some patients with mild, high functioning autism could have been included. However, interviews by experienced psychiatrists did not detect any subject who could be diagnosed as autism or other pervasive developmental disorders. Thus, our results suggest that *CADPS2ΔExon3* may be positive even in non-autistic individuals. Rather, *CADPS2ΔExon3* is likely to be present in individuals with lower VIQ and lower memory function which may be intermediate phenotypes of autism (see below).

4.3. Autism and intelligence

Approximately three-quarters of individuals with autism have low (<70) full-IQ scores (Yeargin-Allsopp et al., 2003). With respect to profiles of IQ, Lincoln et al. reported depressed verbal IQ relative to performance IQ (VIQ < PIQ) in autism (Lincoln et al., 1988), although inconsistent findings (no difference or VIQ < PIQ) have also been reported (Ehlers et al., 1997; Siegel et al., 1996; Williams et al., 2008). Thus, *CADPS2ΔExon3* positive subjects partly share similar cognitive deficits with autism.

4.4. Autism and memory

Similar to *CADPS2ΔExon3* positive subjects, adults with high functioning autism were reported to have impaired memory functions (Bennetto et al., 1996; Minshew and Goldstein, 2001; Steele et al., 2007; Williams et al., 2005b). Not all but several studies have shown that the impairments were prominent in visual memories especially for human faces rather than verbal memories (Hillier et al., 2007; Williams et al., 2005a, 2006). In the case of *CADPS2ΔExon3* positive subjects in the present study,

although visual memory also tended to be lower, the significant reduction was rather detected in verbal memory. The different memory profiles between previous findings in autistic adults and our *CADPS2ΔExon3*-positive subjects might be partly due to the reduced IQ in our *CADPS2ΔExon3* positive subjects, while the previous studies compared memory between patients with high functioning autism and IQ-matched controls.

4.5. Implications on schizophrenia susceptibility

Previously, we analyzed *CADPS2/CADPS2ΔExon3* expression both in the post-mortem brains of psychiatric patients from the Stanley neuropathology consortium, consisting of 15 patients with schizophrenia, 15 with depression, 15 with bipolar disorder and 15 control subjects and bloods of 121 schizophrenic patients and 318 controls (Hattori et al., 2011). In the brain samples, we found that not only *CADPS2* but also *CADPS2ΔExon3* was significantly increased in the schizophrenic group. These changes were not observed in other psychiatric disorders. We also found that the ratio of blood *CADPS2ΔExon3* positive subjects was higher in the schizophrenic group (21 out of 121 patients, ratio = 0.17) compared to the control group (36 out of 318, ratio= 0.11), although the difference in the ratio was not statistically significant.

Of all cognitive domains, verbal memory is one of the most frequently and severely affected in schizophrenia (Aleman et al., 1999; Heinrichs and Zakzanis, 1998; Leeson et al., 2009). Thus, *CADPS2ΔExon3* positive subjects and schizophrenic patients share a similar phenotype. On the other hand, with respect to IQ, many studies showed PIQ is lower than VIQ in schizophrenic patients (Amminger et al., 2000; Aylward et al., 1984), suggesting that *CADPS2ΔExon3* positive subjects also have a different feature to schizophrenia.

Because autism and schizophrenia are supposed to be heterogeneous disorders, *CADPS2ΔExon3* could be a susceptibility marker for a sub-type, especially the patients with affected verbal functions. Alternatively, *CADPS2ΔExon3* expression might be merely related to verbal functions or associated with verbal learning disorder.

4.6. How does blood CADPS2ΔExon3 affect brain function?

The mechanism of how peripheral *CADPS2ΔExon3* expression affects brain functions is unclear. Although *CADPS2ΔExon3* was shown to express in the human brain (Eran et al., 2009; Hattori et al., 2011), there is no evidence that blood *CADPS2ΔExon3* expression reflects its high expression in the brain. In case of autism, high *CADPS2ΔExon3* expression might have genetic basis as *CADPS2* has been suggested to be a susceptibility gene for autism (Cisternas et al., 2003). Sadakata et al. (2007b) found several non-synonymous SNPs in *CADPS2* from autistic patients but such SNPs were not detected in healthy subjects. Although CADPS2ΔExon3 retains BDNF releasing activity, it lacks ability to be transported to axons, which would result in the loss of local synaptic BDNF release (Sadakata et al., 2007b). Therefore, higher *CADPS2ΔExon3* expression in the brain, may affect BDNF release through the dominant-negative effect.

CADPS2 mediates the release of monoamine neurotransmission as well. CADPS2 promotes monoamine uptakes and storage (Brunk et al., 2009; Liu et al., 2008) and mediates priming process of monoamine-containing dense core vesicles, so that it facilitates Ca^{2+}-triggered release of neurotransmitters (Jockusch et al., 2007). Therefore, it is also plausible that *CADPS2ΔExon3* expression affects brain function through altered monoamine transmission.

Among monoamines, dopamine's roles on cognition and learning have been established by both animal and human studies (Kehagia et al., 2010). The dopamine neurotransmission in the hippocampus and the prefrontal cortex plays an essential role in working memory (Goldman-Rakic, 1998) and that in striatum also mediates reinforcement and reversal learning (Kehagia et al., 2010). Several studies suggest a link between human verbal function and dopamine D2 receptor. Striatal dopamine D2/D3 receptor availability was reported to correlate with VIQ assessed by WAIS-R (Guo et al., 2006). Hippocampal D2/D3 receptor availability was also related to verbal memory (Takahashi et al., 2007). In addition, dopamine release was enhanced in the frontal cortex, the amygdala and the hippocampus during verbal working memory task (Aalto et al., 2005). Thus, the features of *CADPS2ΔExon3* positive subjects have some similarity with deficits in the D2 receptor function. Because CADPS2 is highly expressed in the dopamine-rich brain areas such as ventral tegmental area and substantia nigra of mice brain (Sadakata et al., 2006), and it is reported to interact with dopamine D2 receptor (Binda et al., 2005), the features of *CADPS2ΔExon3* positive subjects could be ascribed, at least in part, to impaired dopamine transmission.

Although no significant association was detected between wild-type *CADPS2* expression levels and WMS-R scores, *CADPS2* expression level tended to correlate with verbal/general memory. Thus, CADPS2 and CADPS2ΔExon3 might have opposite effects on memory, which is consistent with the above discussion about CADPS2/CADPS2ΔExon3 functions. On the other hand, with respect to intelligence, the mechanism of CADPS2/CADPS2ΔExon3 effects might be more complicated or CADPS2ΔExon3 might have functions independent from wild-type CADPS2.

4.7. Limitation

A limitation is that the mean IQ of our sample was relatively high (mean full-scale IQ: 111.7 ± 12.1). This may have arisen by the geographic area of the sample (i.e. Western part of Tokyo Metropolitan) because average income in Tokyo is approximately 20% higher than national average (Ministry of Health Labour and Welfare, 2009) and income correlates with education level. Therefore, our sample was overrepresented by individuals with higher IQ relative to the Japanese population as a whole. This may have missed the possible effects of *CADPS2* and *CADPS2ΔExon3* on brain functions in people with relatively low IQ.

5. Conclusion

We found that expression of the splice variant of *CADPS2*, *CADPS2ΔExon3*, in the peripheral blood is associated with human brain functions such as intelligence and memory. Those individuals who expressed *CADPS2ΔExon3* had lower IQ and memory. Because CADPS2 mediates the release of BDNF and monoamines, impaired function of CADPS2 due to CADPS2ΔExon3 might exert detrimental effects on BDNF and monoamine functions which are crucial in the brain development and higher brain functions. Further studies to elucidate the mechanisms of production of CADPS2ΔExon3 and its effects at the molecular level are warranted.

Acknowledgements

This work was supported by a Health and Labor Science Research Grant (H21-KOKORO-WAKATE-20 and H21-KOKORO-001), CREST of JST, Grant-in-Aid for Scientific Research (KAKENHI, 22591269), Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP, Takeda Science Foundation, and Mitsubishi Pharma Research Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biopsycho.2011.09.017.

References

Aalto, S., Bruck, A., Laine, M., Nagren, K., Rinne, J.O., 2005. Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. Journal of Neuroscience 25, 2471–2477.

Aleman, A., Hijman, R., de Haan, E.H., Kahn, R.S., 1999. Memory impairment in schizophrenia: a meta-analysis. American Journal of Psychiatry 156, 1358–1366.

Amminger, G.P., Schlogelhofer, M., Lehner, T., Looser Ott, S., Friedrich, M.H., Aschauer, H.N., 2000. Premorbid performance IQ deficit in schizophrenia. Acta Psychiatrica Scandinavica 102, 414–422.

Aylward, E., Walker, E., Bettes, B., 1984. Intelligence in schizophrenia: meta-analysis of the research. Schizophrenia Bulletin 10, 430–459.

Bennetto, L., Pennington, B.F., Rogers, S.J., 1996. Intact and impaired memory functions in autism. Child Development 67, 1816–1835.

Binda, A.V., Kabbani, N., Levenson, R., 2005. Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). Biochemical Pharmacology 69, 1451–1461.

Bishop, D.V., 1989. Autism Asperger's syndrome and semantic-pragmatic disorder: where are the boundaries? British Journal of Disorders of Communication 24, 107–121.

Brunk, I., Blex, C., Speidel, D., Brose, N., Ahnert-Hilger, G., 2009. Ca2+-dependent activator proteins of secretion promote vesicular monoamine uptake. Journal of Biological Chemistry 284, 1050–1056.

Castensson, A., Aberg, K., McCarthy, S., Saetre, P., Andersson, B., Jazin, E., 2005. Serotonin receptor 2C (HTR2C) and schizophrenia: examination of possible medication and genetic influences on expression levels. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 134B, 84–89.

Cisternas, F.A., Vincent, J.B., Scherer, S.W., Ray, P.N., 2003. Cloning and characterization of human CADPS and CADPS2, new members of the Ca^{2+}-dependent activator for secretion protein family. Genomics 81, 279–291.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.

Ehlers, S., Nyden, A., Gillberg, C., Sandberg, A.D., Dahlgren, S.O., Hjelmquist, E., Oden, A., 1997. Asperger syndrome, autism and attention disorders: a comparative study of the cognitive profiles of 120 children. Journal of Child Psychology and Psychiatry and Allied Disciplines 38, 207–217.

Eran, A., Graham, K.R., Vatalaro, K., McCarthy, J., Collins, C., Peters, H., Brewster, S.J., Hanson, E., Hundley, R., Rappaport, L., Holm, I.A., Kohane, I.S., Kunkel, L.M., 2009. Comment on "Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients". Journal of Clinical Investigation 119, 671–680, 679–680; author reply.

Fukumoto, N., Fujii, T., Combarros, O., Kamboh, M.I., Tsai, S.J., Matsushita, S., Nacmias, B., Comings, D.E., Arboleda, H., Ingelsson, M., Hyman, B.T., Akatsu, H., Grupe, A., Nishimura, A.L., Zatz, M., Mattila, K.M., Rinne, J., Goto, Y., Asada, T., Nakamura, S., Kunugi, H., 2010. Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: new data and meta-analysis. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 153B, 235–242.

Goldman-Rakic, P.S., 1998. The cortical dopamine system: role in memory and cognition. Advances in Pharmacology 42, 707–711.

Guo, J.F., Kuang Yang, Y., Tsing Chiu, N., Lieh Yeh, T., See Chen, P., Lee, I.H., Lin Chu, C., 2006. The correlation between striatal dopamine D2/D3 receptor availability and verbal intelligence quotient in healthy volunteers. Psychological Medicine 36, 547–554.

Hattori, K., Tanaka, H., Wakabayashi, C., Yamamoto, N., Uchiyama, H., Teraishi, T., Hori, H., Arima, K., Kunugi, H., 2011. Expression of Ca(2+)-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 1738–1743.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12, 426–445.

Hillier, A., Campbell, H., Keillor, J., Phillips, N., Beversdorf, D.Q., 2007. Decreased false memory for visually presented shapes and symbols among adults on the autism spectrum. Journal of Clinical and Experimental Neuropsychology 29, 610–616.

Houlihan, L.M., Harris, S.E., Luciano, M., Gow, A.J., Starr, J.M., Visscher, P.M., Deary, I.J., 2009. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. Genes, Brain and Behaviour 8, 238–247.

Jockusch, W.J., Speidel, D., Sigler, A., Sorensen, J.B., Varoqueaux, F., Rhee, J.S., Brose, N., 2007. CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell 131, 796–808.

Kehagia, A.A., Murray, G.K., Robbins, T.W., 2010. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. Current Opinion in Neurobiology 20, 199–204.

Leeson, V.C., Barnes, T.R., Hutton, S.B., Ron, M.A., Joyce, E.M., 2009. IQ as a predictor of functional outcome in schizophrenia: a longitudinal, four-year study of first-episode psychosis. Schizophrenia Research 107, 55–60.

Lincoln, A.J., Courchesne, E., Kilman, B.A., Elmasian, R., Allen, M., 1988. A study of intellectual abilities in high-functioning people with autism. Journal of Autism and Developmental Disorders 18, 505–524.

Liu, Y., Schirra, C., Stevens, D.R., Matti, U., Speidel, D., Hof, D., Bruns, D., Brose, N., Rettig, J., 2008. CAPS facilitates filling of the rapidly releasable pool of large dense-core vesicles. Journal of Neuroscience 28, 5594–5601.

Ministry of Health, Labour and Welfare, 2009. Basic Survey on Wage Structure, http://www.e-stat.go.jp/SG1/estat/List.do?bid=000001025026&cycode=0.

Minshew, N.J., Goldstein, G., 2001. The pattern of intact and impaired memory functions in autism. Journal of Child Psychology and Psychiatry and Allied Disciplines 42, 1095–1101.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., Nabeshima, T., 2000. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. Journal of Neuroscience 20, 7116–7121.

Monteggia, L.M., Barrot, M., Powell, C.M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R.W., Nestler, E.J., 2004. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proceedings of the National Academy of Sciences of the United States of America 101, 10827–10832.

Naoe, Y., Shinkai, T., Hori, H., Fukunaka, Y., Utsunomiya, K., Sakata, S., Matsumoto, C., Shimizu, K., Hwang, R., Ohmori, O., Nakamura, J., 2007. No association between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and schizophrenia in Asian populations: evidence from a case-control study and meta-analysis. Neuroscience Letters 415, 108–112.

Otsubo, T., Miyaoka, H., Kamijima, K. (Eds.), 2005. M.I.N.I. Mini International Neuropsychiatric Interview. Seiwa Shoten Publishers, Tokyo.

Robbins, T.W., Arnsten, A.F., 2009. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annual Review of Neuroscience 32, 267–287.

Rybakowski, J.K., 2008. BDNF gene: functional Val66Met polymorphism in mood disorders and schizophrenia. Pharmacogenomics 9, 1589–1593.

Sadakata, T., Itakura, M., Kozaki, S., Sekine, Y., Takahashi, M., Furuichi, T., 2006. Differential distributions of the Ca^{2+}-dependent activator protein for secretion family proteins (CAPS2 and CAPS1) in the mouse brain. Journal of Comparative Neurology 495, 735–753.

Sadakata, T., Kakegawa, W., Mizoguchi, A., Washida, M., Katoh-Semba, R., Shutoh, F., Okamoto, T., Nakashima, H., Kimura, K., Tanaka, M., Sekine, Y., Itohara, S., Yuzaki, M., Nagao, S., Furuichi, T., 2007a. Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. Journal of Neuroscience 27, 2472–2482.

Sadakata, T., Mizoguchi, A., Sato, Y., Katoh-Semba, R., Fukuda, M., Mikoshiba, K., Furuichi, T., 2004. The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. Journal of Neuroscience 24, 43–52.

Sadakata, T., Washida, M., Iwayama, Y., Shoji, S., Sato, Y., Ohkura, T., Katoh-Semba, R., Nakajima, M., Sekine, Y., Tanaka, M., Nakamura, K., Iwata, Y., Tsuchiya, K.J., Mori, N., Detera-Wadleigh, S.D., Ichikawa, H., Itohara, S., Yoshikawa, T., Furuichi, T., 2007b. Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. Journal of Clinical Investigation 117, 931–943.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 59 (Suppl. 20), 34–57, 22–33; quiz.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihonbunkakagakusha, Tokyo.

Siegel, D.J., Minshew, N.J., Goldstein, G., 1996. Wechsler IQ profiles in diagnosis of high-functioning autism. Journal of Autism and Developmental Disorders 26, 389–406.

Steele, S.D., Minshew, N.J., Luna, B., Sweeney, J.A., 2007. Spatial working memory deficits in autism. Journal of Autism and Developmental Disorders 37, 605–612.

Stein, J.L., Hua, X., Lee, S., Ho, A.J., Leow, A.D., Toga, A.W., Saykin, A.J., Shen, L., Foroud, T., Pankratz, N., Huentelman, M.J., Craig, D.W., Gerber, J.D., Allen, A.N., Corneveaux, J.J., Dechairo, B.M., Potkin, S.G., Weiner, M.W., Thompson, P., 2010. Voxelwise genome-wide association study (vGWAS). Neuroimage 53, 1160–1174.

Strauss, J., Barr, C.L., George, C.J., Ryan, C.M., King, N., Shaikh, S., Kovacs, M., Kennedy, J.L., 2004. BDNF and COMT polymorphisms: relation to memory phenotypes in young adults with childhood-onset mood disorder. Neuromolecular Medicine 5, 181–192.

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

Takahashi, H., Kato, M., Hayashi, M., Okubo, Y., Takano, A., Ito, H., Suhara, T., 2007. Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. Neuroimage 34, 1643–1649.

Tsai, S.J., Hong, C.J., Yu, Y.W., Chen, T.J., 2004. Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females. Neuropsychobiology 49, 13–16.

Volkmar, F.R., Lord, C., Bailey, A., Schultz, R.T., Klin, A., 2004. Autism and pervasive developmental disorders. Journal of Child Psychology and Psychiatry and Allied Disciplines 45, 135–170.
Williams, D.L., Goldstein, G., Carpenter, P.A., Minshew, N.J., 2005a. Verbal and spatial working memory in autism. Journal of Autism and Developmental Disorders 35, 747–756.
Williams, D.L., Goldstein, G., Kojkowski, N., Minshew, N.J., 2008. Do individuals with high functioning autism have the IQ profile associated with nonverbal learning disability? Research in Autism Spectrrm Disorders 2, 353–361.
Williams, D.L., Goldstein, G., Minshew, N.J., 2005b. Impaired memory for faces and social scenes in autism: clinical implications of memory dysfunction. Archives of Clinical Neuropsychology 20, 1–15.
Williams, D.L., Goldstein, G., Minshew, N.J., 2006. The profile of memory function in children with autism. Neuropsychology 20, 21–29.
Yeargin-Allsopp, M., Rice, C., Karapurkar, T., Doernberg, N., Boyle, C., Murphy, C., 2003. Prevalence of autism in a US metropolitan area. Journal of the American Medical Association 289, 49–55.
Zhang, H., Ozbay, F., Lappalainen, J., Kranzler, H.R., van Dyck, C.H., Charney, D.S., Price, L.H., Southwick, S., Yang, B.Z., Rasmussen, A., Gelernter, J., 2006. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 141B, 387–393.

Journal of Psychiatric Research 46 (2012) 555–559

Contents lists available at SciVerse ScienceDirect

Journal of Psychiatric Research

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

Association between the functional polymorphism (C3435T) of the gene encoding P-glycoprotein (*ABCB1*) and major depressive disorder in the Japanese population

Takashi Fujii [a], Miho Ota [a], Hiroaki Hori [a,b], Daimei Sasayama [a], Kotaro Hattori [a], Toshiya Teraishi [a], Noriko Yamamoto [a], Miyako Hashikura [a], Masahiko Tatsumi [c], Teruhiko Higuchi [d], Hiroshi Kunugi [a,b,*]

[a] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187 8502, Japan*
[b] *Core Research of Evolutional Science & Technology (CREST), Japan Science and Technology Agency (JST), Tokyo 102 0075, Japan*
[c] *Yokohama Shinryo Clinic, Yamamotobiru 2F, 3-28-5 Tsuruyacho, Kanagawaku, Yokohama 221 0835, Japan*
[d] *National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187 8502, Japan*

ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form
28 December 2011
Accepted 9 January 2012

Keywords:
P-glycoprotein
ABCB1
Major depressive disorder
Functional polymorphism
Genetic association

ABSTRACT

Human P-glycoprotein (P-gp), which is encoded by *ABCB1* (ATP-binding cassette, sub-family B member 1), is expressed in the blood brain barrier and protects the brain from many kinds of drugs and toxins including glucocorticoids by acting as an efflux pump. We examined whether functional polymorphisms of *ABCB1* give susceptibility to major depressive disorder (MDD). The five functional single nucleotide polymorphisms (SNPs), A-41G (rs2188524), T-129C (rs3213619), C1236T (Gly412Gly: rs1128503), G2677A/T (Ala893Ser/Thr: rs2032582), and C3435T (Ile1145Ile: rs1045642) were genotyped in 631 MDD patients and 1100 controls in the Japanese population. A tri-allelic SNP, G2677A/T, was genotyped by pyrosequencing and the remaining SNPs were genotyped by the TaqMan 5′-exonuclease allelic discrimination assay. The minor T3435 allele was significantly increased in MDD patients than in the controls ($\chi^2 = 4.5$, df = 1, $p = 0.034$, odds ratio [OR] 1.16, 95% confidential interval [CI] 1.01–1.34). Homozygotes for the T3435 allele was significantly more common in patients than in the controls ($\chi^2 = 7.5$, df = 1, $p = 0.0062$, OR 1.43, 95%CI 1.11–1.85). With respect to the other 4 SNPs, there was no significant difference in genotype or allele distribution. In the haplotype-based analysis, the proportion of individuals with the TT1236-TT3435 haploid genotype was significantly increased in patients than in controls ($\chi^2 = 8.5$, df = 1, $p = 0.0037$, OR 1.50, 95%CI 1.14–1.98). Our results suggest that the T3435 allele or carrying two copies of this allele confers susceptibility to MDD in the Japanese population.

1. Introduction

Major depressive disorder (MDD) is a stress-related disorder, and there is mounting evidence for an important role of hypothalamus-pituitary-adrenal axis abnormalities in the pathophysiology of MDD (Kunugi et al., 2010). In the hypothalamus-pituitary-adrenal axis, glucocorticoids are end products and central to the stress response. At the first step, stress activates the hypothalamus-pituitary-adrenal axis and increases glucocorticoid levels in blood. Next, regulated by the blood brain barrier, these hormones penetrate into the brain. Because excessive glucocorticoids are toxic to cells including neurons, high levels are considered to damage the brain and cause depression (Kunugi et al., 2010).

Human P-glycoprotein (P-gp) is encoded by *ABCB1* (ATP-binding cassette, sub-family B member 1) (Chen et al., 1986) or alternatively referred to as multidrug resistance polypeptide 1 (*MDR1*). P-gp is a 1280 amino acid transporter expressed in the blood brain barrier and protects the brain from many drugs or neurotoxic substances such as glucocorticoids and amyloid-beta as an efflux pump.

The most extensively studied *ABCB1* polymorphisms are the G2677A/T (Ala893Ser/Thr: rs2032582) and the C3435T (Ile1145Ile: rs1045642), which are located on exon 21 and exon 26, respectively. The G2677A/T polymorphism is a tri-allelic nonsynonymous polymorphism, Ala893 to Ser/Thr893. This replacement results in a change from a lipophilic residue to a hydrophilic one (Cascorbi et al., 2001). Alanine is a structurally neutral amino acid that does not introduce a constraint into the polypeptide backbone. Therefore, it is possible that the substitution of Ser or Thr for Ala would affect the geometric precision of the interaction site and the secondary structure. The G to T transversion was initially isolated from a full-length *ABCB-1* cDNA from the human adrenal gland

* Corresponding author. Department of Mental Disorder Research, National Institute of Neuroscience, National, Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187 8502, Japan. Tel./fax: +81 42 346 1714.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

0022-3956/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2012.01.012

(Kioka et al., 1989). Although the C3435T does not result in an amino acid change, several lines of evidence have indicated that this polymorphism affects the expression and function of P-gp (Hoffmeyer et al., 2000; Goto et al., 2002; Wang et al., 2005; Kimchi-Sarfaty et al., 2007). The C1236T (rs1128503) is closely linked to G2677A/T and C3435T and the combinations of these single nucleotide polymorphisms (SNPs) were shown to be associated with P-gp activity (Wong et al., 2005).

In a previous study, genetic associations of functional polymorphisms of *ABCB1* have been reported for mood disorders including depression in a Japanese sample (62 patients and 160 controls) (Qian et al., 2006). In patients with mood disorders, the G-41 and C-129 alleles were significantly less common and the A2677 allele was more common compared with the controls (Qian et al., 2006). A-41G (rs2188524) and T-129C (rs3213619) are located in the promoter region of *ABCB1* and reported to be associated with P-gp expression levels (Tanabe et al., 2001; Koyama et al., 2006).

In the present study, we focused on the above-mentioned functional polymorphisms of *ABCB1* (A-41G, T-129C, C1236T, G2677A/T, and C3435T) and examined whether *ABCB1* is associated with MDD, using a relatively large Japanese sample. Because specific genotype combinations may play an important role, we also performed haplotype-based association analyses.

2. Methods

2.1. Subjects

A total of 631 patients with MDD (262 males and 369 females, mean age of 49.5 years [SD 15.9], range 17–89) and 1100 healthy controls (371 males and 729 females, 45.6 [SD 16.1], range 18–86) were used in the study. All participants were biologically unrelated Japanese subjects recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records (American Psychiatric Association, 1994). Among the total 631 patients, 49% had a single episode, 49% recurrent episodes, and the remaining patients were unclassified. With regard to history of admission, 19% had a history of admission to a psychiatric hospital and the remaining 81% did not have such a history. The controls were healthy volunteers recruited from the same geographical area. They were interviewed using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist to rule out any axis I psychiatric disorders, and individuals with a current or past history of psychiatric treatment were excluded. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Genotyping

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNPs, except for rs2032582, were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA); the assay ID was C__26907787_10 for rs2188524, C__27487486_10 for rs3213619, C___7586662_10 for rs1128503, and C___7586657_20 for rs1045642. Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95 °C for 10 min followed by 50 cycles at 92 °C for 15 s and 60 °C for 1 min. Genotype data were read blind to the case-control status.

Because rs2032582 is a tri-allelic SNP, we used pyrosequencing for genotyping (Supplemental Fig. S1). The region including this SNP was amplified by PCR with primers of Biotin-ABCB1_G2677TA_F137 (5′- GAATATAGCAAATCTTGGGACAGGAA-TAA-3′) and ABCB1_G2677TA_R544L (5′- AATGGCCTGAAAACT-GAAAAAGTCTGT-3′) for the reverse direction. These primers were designed with the PSQ Assay Design software ver. 1.0.6. (Biotage AB, Uppsala, Sweden). Pyrosequencing was performed with the PSQ96MA System and PSQ96 SNP Reagent Kit (Pyrosequencing, AB, Uppsala, Sweden). Sequencing primers for the reverse direction (ABCB1_G2677TA_SR333; 5′-ATCAAT-CATATTTAGTTTGACTCACCTTCC-3′) were designed with the software supplied by the PSQ Assay Design software ver. 1.0.6. (Biotage AB). For sequencing in the reverse direction, 'the sequence to analyze' and 'the dispensation order' were set as 5′-CAGA/C/TACCTTC-3′ and 5′-GCAGCTAGCT-3′, respectively. For ambiguous genotypic data, we repeated experiments and determined the genotype for every subject. Genotypes were read blind to the case-control status.

2.3. Haplotype and statistical analysis

Deviations of genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. These tests were performed with the SPSS software ver.11 (SPSS Japan, Tokyo, Japan). Haplotype-based association analyses were performed with the SNPAlyze software ver.6.6 (http://www.dynacom.co.jp/e/products/package/snpalyze/about.html) . Measures of linkage disequilibrium (LD), denoted as D′ and r^2, were calculated from the haplotype frequency using the expectation–maximization (EM) algorithm. Haplotypes with an estimated frequency of less than 1% were considered to be rare and excluded from the analyses. All *p*-values reported are two-tailed. We performed 10,000 permutations. Odds ratios (ORs) and 95% confidence intervals (CI) were also calculated. To correct the critical *p* value for multiple testing, we used the spectral decomposition method of SNPSpD software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004; Li and Ji, 2005), which considers marker linkage disequilibrium information and generates an experiment-wide significance threshold required to keep the type I error rate at 5%.

3. Results

Genotype and allele distributions of the examined SNPs of *ABCB1* in patients and controls are shown in Table 1. LD estimates of pairwise SNPs, expressed in D′ and r^2, are presented in Fig. 1. The genotype distributions did not significantly deviate from the HWE in patients and controls for any of the examined SNPs. For the C3435T polymorphism, the minor T allele was significantly increased in patients than in controls ($\chi^2 = 4.5$, df = 1, $p = 0.034$, odds ratio [OR] 1.16, 95% confidential interval [CI] 1.01–1.34). There was a significant difference in the genotype distribution between patients and controls ($\chi^2 = 7.5$, df = 2, $p = 0.024$) (Table 1). The proportion of individuals carrying the TT genotype was significantly increased in patients than in controls ($\chi^2 = 7.5$, df = 1, $p = 0.0062$, OR 1.43, 95% CI 1.11–1.85) (Table 2). To correct for multiple testing, we calculated the experiment-wide significance threshold required to keep the type I error rate at 5%. As a result, the corrected *p* value

Table 1
Genotype and allelic distribution of *ABCB1* SNPs in Japanese patients with MDD and controls.

SNP	position		Total N.	Genotype count (frequency)						Allele count (frequency)				HWE	
								$\chi 2$	GF-P			$\chi 2$	AF-P	$\chi 2$	HWE-P
A-41G	87230435	MDD	631	A/A	G/A	G/G				A	G				
rs2188524	5'UTR	Control	1100	508 (0.81)	112 (0.18)	11 (0.02)		3.27	0.19	1128 (0.89)	134 (0.11)	2.24	0.13	2.66	0.10
				847 (0.77)	235 (0.21)	18 (0.02)				1929 (0.88)	271 (0.12)			0.13	0.71
T-129C	87230193	MDD	631	T/T	T/C	C/C				T	C				
rs3213619	5'UTR	Control	1100	549 (0.87)	78 (0.12)	4 (0.01)		1.72	0.42	1176 (0.93)	86 (0.07)	1.73	0.19	0.45	0.50
				934 (0.85)	155 (0.14)	11 (0.01)				2023 (0.92)	177 (0.08)			2.50	0.11
C1236T	87179601	MDD	631	C/C	C/T	T/T				C	T				
rs1128503	exon 12	Control	1100	93 (0.15)	304 (0.48)	234 (0.37)		0.78	0.68	490 (0.39)	772 (0.61)	0.22	0.64	0.13	0.72
				179 (0.16)	514 (0.47)	407 (0.37)				872 (0.40)	1328 (0.60)			0.61	0.44
G2677A/T	87160618	MDD	631	G/G	G/T	G/A	T/T	T/A	A/A	G	T	A			
rs2032582	exon 21	Control	1100	121(0.19)	211(0.33)	86(0.14)	110(0.17)	83(0.13)	20(0.03)	539 (0.427)	514 (0.407)	209 (0.166)	1.85	0.40	1.50	0.68
				193(0.18)	385(0.35)	175(0.16)	156(0.14)	158(0.14)	33(0.03)	946 (0.43)	855 (0.39)	399 (0.18)			2.37	0.50
C3435T	87138645	MDD	631	C/C	C/T	T/T				C	T				
rs1045642	exon 26	Control	1100	207 (0.33)	299 (0.47)	125 (0.20)		7.50	0.024	713 (0.56)	549 (0.44)	4.49	0.034	0.82	0.37
				386 (0.35)	552 (0.50)	162 (0.15)				1324 (0.60)	876 (0.40)			2.44	0.12

was calculated as 0.011. The recessive genotypic association (CC3435, CT3435 vs TT3435) remained significant after this correction (Table 2). However, with respect to the other 4 SNPs (A-41G, T-129C, C1236T, and G2677 A/T), there was no significant difference in genotype or allele distribution between patients and controls (Table 1).

The results of haplotype-based analyses are shown in Table 3. There was no significant haplotypic association of the SNPs in *ABCB1* when comparing the patients and controls. However, the proportion of individuals with the TT1236 - TT3435 haploid genotype was significantly increased in patients than in controls ($\chi^2 = 8.5$, df = 1, $P = 0.0037$, OR 1.50, 95% CI 1.14–1.98) (Table 2).

4. Discussion

We performed a genetic association study on 5 functional SNPs of *ABCB1* with MDD in a fairly large Japanese sample. Among the 5 SNPs, only one SNP (C3435T polymorphism) differed in genotype and allele frequencies between patients with MDD and controls. In particular, the TT genotype of this SNP was significantly more common in patients with MDD than in controls. The frequency of the TT haplotype (C1236T-C3435T) homozygote was also significantly higher in patients when compared to controls. These results suggest that the homozygosity of the T3435 allele or the T1236-T3435 haplotype is a risk factor for MDD. The C3435T may play a key role in the development of MDD. It is also possible that unknown functional polymorphisms, which are in linkage disequilibrium to the C3435T may give susceptibility to MDD. To reveal other polymorphisms responsible for the susceptibility, full sequencing of the *ABCB1* gene would be more desirable.

On the other hand, Qian et al. (2006) found no significant association between the C3435T polymorphism and mood disorders in a relatively small Japanese sample (62 cases and 160 controls) (Qian et al., 2006). This negative result may have arisen by inadequate statistical power due to the sample size. Furthermore, the subjects in the Qian et al. (2006) study were composed of patients with MDD, bipolar disorder, and dysthymia, whereas all our patients were diagnosed as MDD. This difference may explain in part the inconsistent results between the two studies. To our knowledge, there have been only two studies (Qian et al., 2006 and ours) that investigated the genetic association between the C3435T polymorphism and the development of MDD in a case-control design. Although several studies examined the C3435T polymorphism for association with response to antidepressants in MDD patients, they did not compare genotype/allele distributions between patients and controls (Kato et al., 2008; Menu et al., 2010; Mihaljevic-Peles et al., 2007; Mihaljevic Peles et al., 2008; Peters et al., 2008; Roberts et al., 2002; Uhr et al., 2008). Future replication studies in various genetic backgrounds are necessary.

Many kinds of antidepressants have been identified as P-gp substrates. For example, amitriptyline and its metabolites (including nortriptyline) are P-gp substrates and have been shown to have enhanced penetration into the brain in P-gp deficient mice compared to wild type (Uhr et al., 2000, 2007; Grauer and Uhr, 2004; Ejsing et al., 2006). Roberts et al. found an association between the C3435T polymorphism and frequency of side effects, and reported that homozygosity of the T3435 allele is a risk factor for nortriptyline-induced postural hypotension (Roberts et al., 2002). They hypothesized that this may be due to a relative increase in the accumulation of nortriptyline, or its metabolites, in the brain due to reduced P-gp function. Similarly, we could discuss cortisol, the C3435T polymorphism, and the development of MDD. However, clinical response to nortriptyline was not associated with the C3435T in their study (Roberts et al., 2002). Citalopram, paroxetine, venlafaxine, sertraline and trimipramine have also been

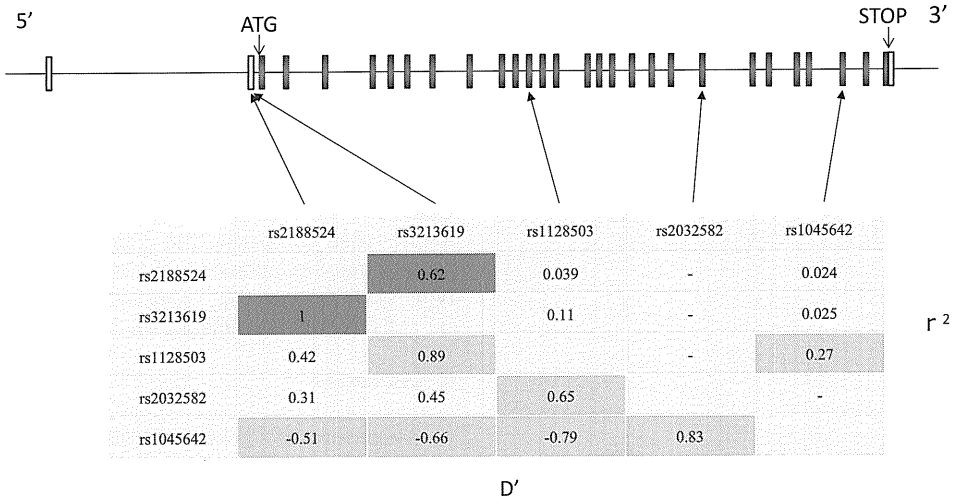

Fig. 1. The genomic structure of *ABCB1* and location of the examined SNPs. The D′ and r2 values between paired SNPs are shown in the diagram. The exons are gray squares. The intensity of the box color corresponds to the strength of D′ and r2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Recessive model genotype distribution in Japanese patients with MDD and controls.

SNP or haplotype		Total N.	Recessive model (frequency)			
					$\chi 2$	*P* value
			C/C, C/T	T/T		
C3435T	MDD	631	506 (0.80)	125 (0.20)	7.5	0.0062
	Control	1100	938 (0.85)	162 (0.15)		
			non T-T/T-T	T-T/T-T		
C1236T-C3435T	MDD	631	523 (0.83)	108 (0.17)	8.5	0.0037
	Control	1100	967 (0.88)	133 (0.12)		

shown to be substrates (Ejsing et al., 2006; Uhr et al., 2003, 2000, 2008; Wang et al., 2008). However, conflicting results have raised queries as to whether the C3435T polymorphism influences the response to antidepressants in patients with MDD (Roberts et al., 2002; Peters et al., 2008; Kato et al., 2008; Mihaljevic Peles et al., 2008; Uhr et al., 2008 et al., Gex-Fabry et al., 2008). These results may be caused by the fact that P-gp is a transporter for both drugs and toxins.

We hypothesized that decreased P-gp function at the blood brain barrier may induce the increased accumulation of toxins, such as glucocorticoids, in the brain, contributing to the development of MDD. Accumulating reports support that the TT3435 genotype could be associated with the low level of expression and/or the activity of P-gp, except for some discrepancies (Hoffmeyer et al., 2000; Goto et al., 2002; Wang et al., 2005). With this in mind, the present result showing that the proportion of individuals with the TT3435 genotype was significantly increased in patients than in controls agrees with our hypothesis. Our results suggest that the C3435T polymorphism plays a role in the development of MDD.

Table 3
Pairewise linkage disequilibrium and association with MDD of the 5 SNPs and haplotypes in *ABCB1*.

dbSNP ID	Allele model *p* value	Haplotype *p*[a]			
		2 Locus	3 Locus	4 Locus	5 Locus
rs2188524	0.13				
		0.32			
rs3213619	0.19		0.55		
		0.64		0.51	
rs1128503	0.64		0.47		0.27
		0.54		0.30	
rs2032582	0.40		0.11		
		0.18			
rs1045642	0.034				

[a] global *p* value.

Role of funding source

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (H.K., T.F.), Japan Human Sciences Foundation (T.F.), JST, CREST (H.K.), and the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health) (H.K.). These agencies had no role in study design, acquisition and interpretation of data or writing the report.

Contributors

T.F. designed the study, performed genotyping of *ABCB1*, made statistical analysis, managed literature search, interpreted the data, and wrote the manuscript. N.Y. took part in genotyping. M.O., H.H., D.S., K.H., T.T., M.H., M.T., and T.H. collected samples and gave comments to the manuscript. H.K. organized recruitment and genotyping of patients and control subjects, and took part in analyzing the data and writing the manuscript.

Conflict of interest

All authors declare no conflict of interest that could influence their work.

Acknowledgements

We thank the patients and the healthy volunteers for their participation.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpsychires.2012.01.012.

References

American Psychiatric Association. DSM-IV: diagnostic and statistical manual of mental disorders. 4th ed. Washington D.C: American Psychiatric Press; 1994.

Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clinical Pharmacology & Therapeutics 2001;69:169–74.

Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, et al. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell 1986;47:381–9.

Ejsing TB, Hasselstrom J, Linnet K. The influence of P-glycoprotein on cerebral and hepatic concentrations of nortriptyline and its metabolites. Drug Metabolism and Drug Interactions 2006;21:139–62.

Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry JM, Bondolfi G, et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Therapeutic Drug Monitoring 2008;30:474–82.

Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, et al. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. Pharmacogenetics 2002;12:451–7.

Grauer MT, Uhr M. P-glycoprotein reduces the ability of amitriptyline metabolites to cross the blood brain barrier in mice after a 10-day administration of amitriptyline. Journal of Psychopharmacology 2004;18:66–74.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proceedings of the National Academy of Sciences USA 2000;97:3473–8.

Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2008;32:398–404.

Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525–8.

Kioka N, Tsubota J, Kakehi Y, Komano T, Gottesman MM, Pastan I, et al. P-glycoprotein gene (MDR1) cDNA from human adrenal: normal P-glycoprotein carries Gly185 with an altered pattern of multidrug resistance. Biochemical and Biophysical Research Communications 1989;162:224–31.

Koyama T, Nakamura T, Komoto C, Sakaeda T, Taniguchi M, Okamura N, et al. MDR1 T-129C polymorphism can be predictive of differentiation, and thereby prognosis of colorectal adenocarcinomas in Japanese. Biological & Pharmaceutical Bulletin 2006;29:1449–53.

Kunugi H, Hori H, Adachi N, Numakawa T. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. Psychiatry and Clinical Neurosciences 2010;64:447–59.

Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 2005;95:221–7.

Menu P, Gressier F, Verstuyft C, Hardy P, Becquemont L, Corruble E. Antidepressants and ABCB1 gene C3435T functional polymorphism: a naturalistic study. Neuropsychobiology 2010;62:193–7.

Mihaljevic-Peles A, Bozina N, Sagud M. Pharmacogenetics in modern psychiatry. Psychiatria Danubina 2007;19:231–3.

Mihaljevic Peles A, Bozina N, Sagud M, Rojnic Kuzman M, Lovric M. MDR1 gene polymorphism: therapeutic response to paroxetine among patients with major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2008;32:1439–44.

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American Journal of Human Genetics 2004;74:765–9.

Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, et al. Reliability and validity of Japanese version of the mini-international neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 2005;59:517–26.

Peters EJ, Slager SL, Kraft JB, Jenkins GD, Reinalda MS, McGrath PJ, et al. Pharmacokinetic genes do not influence response or tolerance to citalopram in the STAR*D sample. PLoS One 2008;3:e1872.

Qian W, Homma M, Itagaki F, Tachikawa H, Kawanishi Y, Mizukami K, et al. MDR1 gene polymorphism in Japanese patients with schizophrenia and mood disorders including depression. Biological & Pharmaceutical Bulletin 2006;29:2446–50.

Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. Pharmacogenomics Journal 2002;2:191–6.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 1998;59(Suppl. 20):22–33. quiz 34–57.

Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. Journal of Pharmacology and Experimental Therapeutics 2001;297:1137–43.

Uhr M, Grauer MT, Holsboer F. Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. Biological Psychiatry 2003;54:840–6.

Uhr M, Grauer MT, Yassouridis A, Ebinger M. Blood-brain barrier penetration and pharmacokinetics of amitriptyline and its metabolites in p-glycoprotein (abcb1ab) knock-out mice and controls. J Psychiatry Research 2007;41:179–88.

Uhr M, Steckler T, Yassouridis A, Holsboer F. Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. Neuropsychopharmacology 2000;22:380–7.

Uhr M, Tontsch A, Namendorf C, Ripke S, Lucae S, Ising M, et al. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. Neuron 2008;57:203–9.

Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435Cgt;T affects mRNA stability. Pharmacogenet Genomics 2005;15:693–704.

Wang JS, Zhu HJ, Gibson BB, Markowitz JS, Donovan JL, DeVane CL. Sertraline and its metabolite desmethylsertraline, but not bupropion or its three major metabolites, have high affinity for P-glycoprotein. Biological & Pharmaceutical Bulletin 2008;31:231–4.

Wong M, Evans S, Rivory LP, Hoskins JM, Mann GJ, Farlow D, et al. Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clinical Pharmacology & Therapeutics 2005;77:33–42.

Journal of Affective Disorders 136 (2012) 1047–1052

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

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

Preliminary communication

More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression

Daimei Sasayama [a,b,*], Hiroaki Hori [a], Toshiya Teraishi [a], Kotaro Hattori [a], Miho Ota [a], Junko Matsuo [a], Yumiko Kawamoto [a], Yukiko Kinoshita [a], Miyako Hashikura [a], Naoji Amano [b], Teruhiko Higuchi [c], Hiroshi Kunugi [a,d]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan
[b] Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, 390-8621, Japan
[c] National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan
[d] CREST (Core Research of Evolutional Science & Technology), Japan Science and Technology Agency (JST) , Tokyo, 102-0075, Japan

ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form 26 October 2011
Accepted 14 November 2011
Available online 12 December 2011

Keywords:
Unipolar depression
Bipolar disorder
Dexterity
Lithium
Antipsychotics

ABSTRACT

Background: Mood disorders are associated with various neurocognitive deficits. However, few studies have reported the impairment of motor dexterity in unipolar depression and bipolar disorder. In the present study, manual dexterity was compared between unipolar major depression, bipolar disorder, and healthy controls.

Methods: Manual dexterity was assessed by the Purdue pegboard test in 98 patients with unipolar major depression, 48 euthymic or depressed patients with bipolar disorder, and 158 healthy controls, matched for age and gender.

Results: Compared to healthy controls, sum of the scores of right, left, and both hands subtests (R+L+B) was significantly lower in both patients with unipolar depression and bipolar disorder ($P=0.0034$ and $P<0.0001$, respectively). Furthermore, R+L+B was significantly lower in bipolar disorder compared to unipolar depression ($P=0.0016$). Lithium dose and chlorpromazine equivalent dose of antipsychotics were significantly negatively correlated with some of the subtest scores. On the other hand, depression severity did not significantly correlate with any of the subtest scores. Difference in R+L+B between unipolar depression and bipolar disorder remained statistically significant even after controlling for gender, age, lithium dose, and chlorpromazine equivalent dose ($P=0.0028$).

Limitations

Bipolar patients during manic episode were not included in the study.

Conclusions: Gross movement dexterity was impaired in both patients with unipolar depression and bipolar disorder. The severity of impairment was significantly greater in patients with bipolar disorder. The functional difference between unipolar and bipolar patients may suggest different pathological conditions between the two depressive disorders.

1. Introduction

Classifications of mood disorders are based on the polarity of episodes. However, similar depressive symptomatology between unipolar and bipolar disorders makes the differentiation difficult in depressed patients without a history of manic episode. Although several clinical characteristics such

* Corresponding author at: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan. Tel.: +81 42 341 2712x5132; fax: +81 42 346 1744.
E-mail address: sasayama@shinshu-u.ac.jp (D. Sasayama).

0165-0327/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jad.2011.11.031

as hypersomnia and psychotic symptoms have been suggested to be helpful in distinguishing bipolar depression from unipolar depression (Forty et al., 2008; Mitchell et al., 2001), the lack of clear-cut clinical features distinguishing the two disorders has prompted researchers to seek genetic markers and endophenotypes. A few studies have found differences in personality profiles between unipolar and bipolar depression (Akiskal et al., 2006; Mendlowicz et al., 2005; Nowakowska et al., 2005; Sasayama et al., 2011). Recent evidence suggests that several neurocognitive deficits may also serve as endophenotypes of bipolar disorder (Bora et al., 2009; Langenecker et al., 2010). However, neurocognitive impairment is observed in unipolar depression as well (Han et al., in press; Schrijvers et al., 2009), and thus, whether there are characteristic neurocognitive deficits in bipolar disorder needs to be investigated.

Impaired dexterity is one of the neurocognitive phenotypes reported in patients with bipolar disorder (Langenecker et al., 2010; Wilder-Willis et al., 2001). On the other hand, some studies have also reported impaired fine motor movement in unipolar depression compared to healthy controls (Pier et al., 2004b; Swann et al., 1999). However, it remains to be elucidated whether the factors affecting dexterity and the severity of the impairment are similar in unipolar and bipolar disorder. In the present study, the Purdue pegboard test (Tiffin and Asher, 1948) was used to assess the manual dexterity in unipolar depression and bipolar disorder. The influence of depression severity and antipsychotics and lithium medications on dexterity was also examined.

2. Methods

2.1. Subjects

Subjects were 98 patients with unipolar major depressive disorder (50 patients with recurrent depression), 48 euthymic or depressive patients with bipolar disorder (8 patients with bipolar I and 40 with bipolar II disorder), and 158 healthy volunteers, matched for gender and age distributions. Participants were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in local free magazines, website announcement, notices posted in the hospital, flyers, and word of mouth. Only self-reported right-handed subjects were included in the study. Consensus diagnoses by at least two research psychiatrists were made according to the DSM-IV criteria (American Psychiatric Association, 1994) for unipolar major depressive disorder and bipolar disorder for enrollment in the study. Healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and only those who demonstrated no history of psychiatric illness or contact to psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. All subjects were biologically unrelated Japanese individuals who resided in the Western part of Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Measures

2.2.1. Purdue pegboard test

All participants were administered the Purdue pegboard test (Model 32030, manufactured by Lafayette Instrument Company USA) for evaluation of manual dexterity. The pegboard contains two vertical arrays of 25 holes in which pegs are placed one hand at a time and then with both hands simultaneously under timed conditions (30 s per trial). Scores for these measures (right, left, and both hands subtests) were derived for each trial according to how many pegs were placed within the time limit. The sum of the right, left, and both hands subtest scores (R + L + B) was used as the representation of gross dexterity of the fingers, hands, and arms. Fine fingertip dexterity was assessed by the assembly subtest, which involves using both hands alternately to construct assemblies consisting of a pin, a washer, a collar and another washer. This subtest requires participants to complete as many assemblies as possible within 60 s. The total number of pieces assembled was recorded as the score of the assembly subtest.

2.2.2. Handgrip force

Handgrip force was measured using a digital handgrip dynamometer (T.K.K.5401; Takei Co., Tokyo, Japan) to record the muscle strength of each hand. Participants were instructed to exert maximum grip force while standing upright, keeping their active arm stretched down vertically close to the body. The average of the two trials for each hand was defined as the maximal handgrip force.

2.2.3. Hamilton Depression Rating Scale

Depressive symptoms were assessed by an experienced research psychiatrist using the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HDRS) (Hamilton, 1967), which has been demonstrated to show excellent inter-rater reliability (Tabuse et al., 2007).

2.3. Statistical analyses

Statistical differences of demographic data among groups were evaluated by the chi-squared test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Student t-test was used for the post hoc analysis and for the comparisons of clinical variables between unipolar and bipolar patients. Due to the non-normal distribution of the Purdue pegboard scores and handgrip force, these data were compared between the three diagnostic groups using Kruskal–Wallis test, and thereafter, pairwise comparisons between two groups were done using Mann–Whitney test. Because antipsychotics and lithium, which are often prescribed for bipolar disorder, could cause tremors that may impair manual dexterity, correlations of the pegboard scores with the dose of these medications as well as with age, gender, antidepressant and anxiolytic prescription status, and HDRS scores were assessed using stepwise linear regression analysis (entry criteria $P<0.05$, removal criteria

$P>0.2$). Furthermore, analysis of covariance (ANCOVA) was performed to compare R + L + B scores between diagnostic groups while controlling for gender, age, antidepressant prescription status, lithium dose, and antipsychotic dose. R + L + B scores were squared before the ANCOVA to obtain normal distribution (Shapiro–Wilk test: $P>0.1$). The antipsychotic dose was calculated as chlorpromazine equivalent in mg/day according to published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999). Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the SPSS version 11.0 (SPSS Japan, Tokyo).

3. Results

Table 1 shows the demographic and clinical characteristics, Purdue pegboard scores, and handgrip force test results. Age distribution did not differ across the three diagnostic groups. Although the average years of education were highest in the controls, there was no significant difference between unipolar and bipolar patients. Over 35% of unipolar patients and 60% of bipolar patients were prescribed lithium and/or antipsychotics. Antidepressants and anxiolytics were also prescribed in 69% and 59% of unipolar patients and 54% and 63% of bipolar patients, respectively. Patients with unipolar depression and bipolar disorder did not differ significantly in age at onset or in HDRS scores. The mean score of every subtest of the Purdue Pegboard was highest in the control group and lowest in the bipolar disorder group. Post hoc pairwise comparisons with Bonferroni corrections revealed that R + L + B scores were significantly higher in control subjects compared to unipolar and bipolar disorders and were significantly lower in bipolar disorder compared to unipolar depression. Patients with bipolar disorder also scored significantly lower in assembly subtest scores compared to control subjects, although the difference with unipolar depression did not reach statistical significance. No significant

Table 1
Clinical characteristics and Purdue pegboard and handgrip force test results.

	Healthy controls (N = 158)	Unipolar depression (N = 98)	Bipolar disorder (N = 48)	Statistical difference	Post hoc pairwise comparisons		
					Unipolar depression vs controls	Bipolar disorder vs controls	Unipolar depression vs bipolar disorder
Demographic characteristics							
Gender (male/female)	79/79	49/49	24/24	$\chi^2 = 0.00$, $P = 1.00$			
Average age (years)	44.6 (14.8)	44.4 (13.5)	44.5 (14.5)	$F = 0.004$, $P = 1.00$			
Education years	15.4 (2.4)	14.4 (2.5)	14.6 (2.8)	$F = 5.36$, $P = 0.0052$	**$t = 3.17$, $P = 0.0017$**	$t = 1.94$, $P = 0.054$	$t = 0.98$, $P = 0.67$
Age at onset	na	35.0 (13.1)	31.7 (13.3)	$t = 1.39$, $P = 0.17$			
HDRS-17	na	10.9 (7.0)	11.5 (7.0)	$t = 0.502$, $P = 0.62$			
Medication status							
Antipsychotics without lithium (%)	0.0	25.1	25.0				
Antipsychotics with lithium (%)	0.0	6.6	27.1				
Lithium without antipsychotics (%)	0.0	4.0	8.3				
Other psychotropics only (%)	0.0	41.0	18.8				
No psychotropic medication (%)	100.0	13.2	20.8				
Purdue pegboard							
Right hand	14.9 (2.1)	13.9 (2.0)	13.0 (2.2)	$\chi^2 = 30.3$, $P<0.0001$	**$U = 5751$, $P = 0.0005$**	**$U = 2022$, $P<0.0001$**	**$U = 1719$, $P = 0.0075$**
Left hand	14.1 (2.0)	13.3 (2.1)	11.9 (2.6)	$\chi^2 = 27.8$, $P<0.0001$	**$U = 6256$, $P = 0.0090$**	**$U = 1978$, $P<0.0001$**	**$U = 1614$, $P = 0.0019$**
Both hands	11.6 (1.9)	11.3 (2.1)	10.1 (2.2)	$\chi^2 = 27.9$, $P = 0.0001$	$U = 7160$, $P = 0.31$	**$U = 2273$, $P<0.0001$**	**$U = 1609$, $P = 0.0017$**
Right + left + both hands	40.6 (5.1)	38.4 (5.5)	35.0 (6.2)	$\chi^2 = 31.6$, $P<0.0001$	**$U = 6059$, $P = 0.0034$**	**$U = 1852$, $P<0.0001$**	**$U = 1594$, $P = 0.0016$**
Assembly	35.4 (8.0)	33.9 (8.6)	30.7 (9.3)	$\chi^2 = 12.4$, $P = 0.0020$	$U = 6576$, $P = 0.043$	**$U = 2617$, $P = 0.0011$**	$U = 1889$, $P = 0.053$
Handgrip force test							
Right hand	33.1 (9.2)	31.9 (10.7)	31.2 (8.4)	$\chi^2 = 1.95$, $P = 0.38$			
Left hand	31.3 (8.6)	29.6 (10.1)	29.5 (8.1)	$\chi^2 = 2.73$, $P = 0.26$			

na: not applicable.
Bold indicates Bonferroni corrected significance of $P<0.017$ in the post hoc analysis.

difference was observed between groups in the results of the handgrip force test. Comparison between bipolar I and bipolar II disorders did not result in significant difference in the pegboard scores. However, each bipolar subtype showed significantly lower scores in R+L+B compared to healthy controls and unipolar depression (bipolar I vs controls: $P=0.0046$, bipolar II vs controls: $P<0.0001$, bipolar I vs unipolar: $P=0.045$, bipolar II vs unipolar: $P=0.0054$; Mann–Whitney test).

Table 2 shows the results of the stepwise linear regression analyses with R+L+B or assembly scores as the dependent variable. Age and gender, as well as lithium and antipsychotic chlorpromazine equivalent dose, antidepressant and anxiolytic prescription status (i.e., 0=non-prescribed and 1=prescribed), and HDRS scores in patient groups, were included as predictor variables. Age was negatively correlated with R+L+B and assembly scores in all diagnostic groups. Lithium dose showed significant positive correlation with assembly scores in the unipolar depression group. On the other hand, antipsychotic dose was significantly negatively correlated with R+L+B and assembly scores in bipolar disorder group and with R+L+B score in unipolar depression group. Significant negative correlation between lithium dose and R+L+B in patients with bipolar disorder was also observed.

Table 3 shows the results of the ANCOVA comparing square-transformed R+L+B scores between diagnostic groups. Each pairwise comparison yielded a statistically significant result.

Table 2
The results of the stepwise regression analyses.

	Right + left + both			Assembly		
	β	t	P value	β	t	P value
Healthy controls						
Age	−0.14	−5.81	<0.0001	−0.24	−6.35	<0.0001
Gender	2.79	3.92	0.0001	2.71	2.38	0.018
Patients with unipolar depression						
Age	−0.10	−2.09	0.040	−0.25	−3.28	0.0015
Gender	na	na	na	na	na	na
Lithium dose	na	na	na	0.01	2.27	0.026
Antipsychotic (CP equivalent) dose	−0.01	−2.09	0.039	na	na	na
Antidepressant medication use	na	na	na	na	na	na
Anxiolytic medication use	na	na	na	na	na	na
HDRS score	na	na	na	na	na	na
Patients with bipolar disorder						
Age	−0.19	−3.68	0.0007	−0.36	−4.51	<0.0001
Gender	na	na	na	na	na	na
Lithium dose	−0.01	−2.27	0.028	na	na	na
Antipsychotic (CP equivalent) dose	−0.02	−3.01	0.0045	−0.02	−2.56	0.014
Antidepressant medication use	na	na	na	na	na	na
Anxiolytic medication use	na	na	na	na	na	na
HDRS score	na	na	na	na	na	na

CP: chlorpromazine; HDRS: Hamilton depression rating scale; na: not applicable (not included in the stepwise model).

Table 3
The ANCOVA pairwise comparisons of the transformed R+L+B scores of the Purdue pegboard between unipolar and bipolar patients and healthy controls.

	Unipolar depression vs controls		Bipolar disorder vs controls		Unipolar depression vs bipolar disorder	
	F value	P value	F value	P value	F value	P value
Intercept	257.8	**<0.0001**	185.7	**<0.0001**	111.2	**<0.0001**
Gender	21.0	**<0.0001**	15.0	**0.0001**	5.1	0.026
Age	33.2	**<0.0001**	42.0	**<0.0001**	9.1	**0.0030**
Lithium dose	0.0	0.87	1.8	0.18	1.0	0.32
Antipsychotic (CP equivalent) dose	4.6	0.032	8.2	**0.0046**	10.1	**0.0018**
Diagnosis	7.2	**0.0077**	15.4	**0.0001**	9.3	**0.0028**

ANCOVA was performed with the square-transformed R+L+B scores as the dependent variable, diagnosis as the independent variable, and gender, age, lithium dose, and chlorpromazine equivalent dose as covariates. Bold indicates Bonferroni corrected significance of $P<0.017$.
CP: chlorpromazine; ANCOVA: analysis of covariance.

4. Discussion

Comparison with healthy controls revealed that the gross movement dexterity assessed by the R+L+B score was impaired in both unipolar and bipolar disorder patients. Furthermore, the severity of impairment was significantly greater in patients with bipolar disorder compared to patients with unipolar depression. No significant difference in handgrip force across diagnostic groups suggested that poor performance in the pegboard test in patients groups was not due to reduced muscle strength. Antipsychotic medications had significant negative influence on the gross movement dexterity. However, the impairment of gross movement dexterity in unipolar and bipolar disorder patients remained significant even after controlling for the effects of antipsychotic and lithium medications. Fine fingertip dexterity assessed by the assembly subtest was significantly impaired in patients with bipolar disorder.

Previous studies reported fine motor dysfunction in bipolar patients even when they were euthymic (Langenecker et al., 2010; Wilder-Willis et al., 2001). Although the patients in the present study included those in depressive states, the depression severity assessed by HDRS was not significantly correlated with the outcome of the pegboard scores. Furthermore, patients with bipolar disorder showed more severely impaired dexterity compared to patients with unipolar depression, despite the similar severity of depressive symptoms. Therefore, our results also suggest that the motor dexterity in bipolar disorder patients is impaired regardless of the presence of depressive symptoms.

Some studies have also reported fine motor slowing in patients with unipolar depression (Pier et al., 2004a, b; Schrijvers et al., 2009), consistent with our results. However, studies comparing the fine motor function between unipolar and bipolar patients are scarce. Swann et al. (Swann et al., 1999) examined dexterity assessed by continuous tapping of the right index finger in patients with unipolar depression and bipolar disorder. Their results showed that depressed

patients with unipolar depression and bipolar disorder showed equally reduced tapping speed compared to healthy controls; however, bipolar disorder patients during manic state did not show significant difference compared to the controls. On the contrary, our results suggested that patients with bipolar disorder showed more severe impairment of motor dexterity compared to patients with unipolar depression irrespective of the severity of the depressive symptoms. The different results in the study by Swann et al. (Swann et al., 1999) may be due to the sample selection and the method of evaluating motor function. Participants of the study by Swann et al. were inpatients while our study included only outpatients with relatively low HDRS scores. Thus, more severe depressive symptoms may have influenced the dexterity test outcomes. Also, the use of Purdue pegboard allowed us to evaluate the gross movement dexterity of fingers, hands, and arms instead of the fine motor speed of a finger assessed by the finger tapping test.

The most interesting finding of the present study was that patients with bipolar disorder were more severely impaired in motor dexterity compared to unipolar patients with similar severity of depressive symptoms. Both bipolar I and bipolar II patients, despite the small number of patients with each subtype, showed significantly lower scores in R + L + B compared to unipolar depression. Although bipolar patients were more likely to be prescribed with antipsychotics and/or lithium, the difference between unipolar and bipolar depression remained statistically significant even when these medications were controlled for.

The functional difference strongly suggests different pathological conditions between the two disorders. Swann et al. (Swann et al., 1999) reported that the relationship between psychomotor impairment and catecholamine function may be stronger in bipolar depression than in unipolar depression. Thus, the severer impairment of dexterity observed in bipolar depression may be etiologically different from that of the unipolar depression. There are other possibilities that could explain the difference in impaired dexterity between unipolar and bipolar depression. First, some of the patients with unipolar depression in this study may go on to experience a manic/hypomanic episode and be rediagnosed as bipolar disorder. Such patients may have been the cause of decreased R + L + B scores in the unipolar depression group. Secondly, unipolar depression may lie on a continuum with bipolar disorder (Akiskal and Benazzi, 2006), and thus, may show slightly impaired dexterity compared to healthy controls. Future studies should assess the motor dexterity in bipolar spectrum conditions (Akiskal et al., 2000) to examine these possibilities.

Another finding from the study worth noting is that antipsychotic medication had significantly negative influence on motor dexterity, which was consistent with findings in a recent study of schizophrenic subjects (Sponheim et al., 2010). Physicians should keep in mind that antipsychotics, often prescribed for those with bipolar disorder as well as unipolar depression, may enhance the disability caused by the impairment of dexterity.

There are several limitations to this study. First, the cross-sectional design did not allow any definitive conclusions as to whether the impairment of the motor dexterity preceded or resulted from illness onset. Furthermore, some patients with unipolar depression in this study may be rediagnosed as bipolar disorder in the future, and thus follow-ups are necessary for accurate diagnosis. Secondly, the number of patients with bipolar disorder was small. Larger studies are needed to compare bipolar I and II disorders. Thirdly, as the patients were limited to those receiving outpatient treatments, our subjects might have been overrepresented by milder forms of illness. Moreover, we did not include bipolar patients during the manic episode. Further studies are necessary to determine whether dexterity is dependent on the phase of the disorder. Fourthly, the self-reported handedness was not verified using a validated hand preference questionnaire. A previous study has shown that non-right handedness is associated with soft bipolarity in mood disorders (Fasmer et al., 2008). Therefore, different rates of mixed-handed persons in each diagnostic group may have confounded the results of the pegboard test. However, since significant impairment of dexterity in bipolar disorder was observed in both the right hand and the left hand subtests, our conclusion that dexterity is impaired in bipolar disorder is not weakened by the possible inaccuracy of the handedness. Finally, the effects of medication could not be fully controlled due to the variability in types and doses. However, analyses examining the influence of antipsychotics and lithium on the outcome of the Purdue pegboard test indicated that these medications were not the only explanatory variable to the impaired dexterity.

In conclusion, we assessed manual motor dexterity in patients with unipolar depression and bipolar disorder and confirmed that both unipolar and bipolar patients were impaired in gross motor dexterity when compared to healthy controls. However, the severity of impairment was significantly greater in bipolar disorder compared to unipolar depression, despite the similar severity of depressive symptoms. The functional difference between unipolar and bipolar depression may suggest different pathological conditions between the two depressive disorders.

Role of funding source

Funding for this study was provided by Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare; H21-kokoro-002) (H.K.), JST, CREST (H.K.), and Intramural Research Grant (21-9) for Neurological and Psychiatric Disorders of NCNP. They had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgment

The authors would like to thank the participants for taking part in the study.

References

Akiskal, H.S., Benazzi, F., 2006. The DSM-IV and ICD-10 categories of recurrent [major] depressive and bipolar II disorders: evidence that they lie on a dimensional spectrum. Journal of Affective Disorders 92, 45–54.

Akiskal, H.S., Bourgeois, M.L., Angst, J., Post, R., Moller, H., Hirschfeld, R., 2000. Re-evaluating the prevalence of and diagnostic composition within the broad clinical spectrum of bipolar disorders. Journal of Affective Disorders 59 (Suppl. 1), S5–S30.

Akiskal, H.S., Kilzieh, N., Maser, J.D., Clayton, P.J., Schettler, P.J., Traci Shea, M., Endicott, J., Scheftner, W., Hirschfeld, R.M., Keller, M.B., 2006. The

distinct temperament profiles of bipolar I, bipolar II and unipolar patients. Journal of Affective Disorders 92, 19–33.
American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition. . Washington D.C.
American Psychiatric Association, 1997. Practice Guidelines for the Treatment of Patients with Schizophrenia. American Psychiatric Press, Washington D.C.
Bora, E., Yucel, M., Pantelis, C., 2009. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. Journal of Affective Disorders 113, 1–20.
Fasmer, O.B., Akiskal, H.S., Hugdahl, K., Oedegaard, K.J., 2008. Non-right-handedness is associated with migraine and soft bipolarity in patients with mood disorders. Journal of Affective Disorders 108, 217–224.
Forty, L., Smith, D., Jones, L., Jones, I., Caesar, S., Cooper, C., Fraser, C., Gordon-Smith, K., Hyde, S., Farmer, A., McGuffin, P., Craddock, N., 2008. Clinical differences between bipolar and unipolar depression. The British Journal of Psychiatry 192, 388–389.
Hamilton, M., 1967. Development of a rating scale for primary depressive illness. The British Journal of Social and Clinical Psychology 6, 278–296.
Han, G., Klimes-Dougan, B., Jepsen, S., Ballard, K., Nelson, M., Houri, A., Kumra, S., Cullen, K., in press. Selective neurocognitive impairments in adolescents with major depressive disorder. Journal of Adolescence. doi:10.1016/j.adolescence.2011.06.009.
Inagaki, A., Inada, T., Fujii, Y., Yagi, G. (Eds.), 1999. Equivalent Dose of Psychotropics. Seiwa Shoten, Tokyo.
Langenecker, S.A., Saunders, E.F., Kade, A.M., Ransom, M.T., McInnis, M.G., 2010. Intermediate: cognitive phenotypes in bipolar disorder. Journal of Affective Disorders 122, 285–293.
Mendlowicz, M.V., Akiskal, H.S., Kelsoe, J.R., Rapaport, M.H., Jean-Louis, G., Gillin, J.C., 2005. Temperament in the clinical differentiation of depressed bipolar and unipolar major depressive patients. Journal of Affective Disorders 84, 219–223.
Mitchell, P.B., Wilhelm, K., Parker, G., Austin, M.P., Rutgers, P., Malhi, G.S., 2001. The clinical features of bipolar depression: a comparison with matched major depressive disorder patients. The Journal of Clinical Psychiatry 62, 212–216 [quiz 217].
Nowakowska, C., Strong, C.M., Santosa, C.M., Wang, P.W., Ketter, T.A., 2005. Temperamental commonalities and differences in euthymic mood disorder patients, creative controls, and healthy controls. Journal of Affective Disorders 85, 207–215.
Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 59, 517–526.
Pier, M.P., Hulstijn, W., Sabbe, B.G., 2004a. No psychomotor slowing in fine motor tasks in dysthymia. Journal of Affective Disorders 83, 109–120.
Pier, M.P., Hulstijn, W., Sabbe, B.G., 2004b. Psychomotor retardation in elderly depressed patients. Journal of Affective Disorders 81, 73–77.
Sasayama, D., Hori, H., Teraishi, T., Hattori, K., Ota, M., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Hashikura, M., Koga, N., Okamoto, N., Sakamoto, K., Higuchi, T., Amano, N., Kunugi, H., 2011. Difference in Temperament and Character Inventory scores between depressed patients with bipolar II and unipolar major depressive disorders. Journal of Affective Disorders 132, 319–324.
Schrijvers, D., Van Den Eede, F., Maas, Y., Cosyns, P., Hulstijn, W., Sabbe, B.G., 2009. Psychomotor functioning in chronic fatigue syndrome and major depressive disorder: a comparative study. Journal of Affective Disorders 115, 46–53.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of Clinical Psychiatry 59 (Suppl. 20), 22–33 [quiz 34–57].
Sponheim, S.R., Jung, R.E., Seidman, L.J., Mesholam-Gately, R.I., Manoach, D.S., O'Leary, D.S., Ho, B.C., Andreasen, N.C., Lauriello, J., Schulz, S.C., 2010. Cognitive deficits in recent-onset and chronic schizophrenia. Journal of Psychiatric Research 44, 421–428.
Swann, A.C., Katz, M.M., Bowden, C.L., Berman, N.G., Stokes, P.E., 1999. Psychomotor performance and monoamine function in bipolar and unipolar affective disorders. Biological Psychiatry 45, 979–988.
Tabuse, H., Kalali, A., Azuma, H., Ozaki, N., Iwata, N., Naitoh, H., Higuchi, T., Kanba, S., Shioe, K., Akechi, T., Furukawa, T.A., 2007. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Research 153, 61–67.
Tiffin, J., Asher, E.J., 1948. The Purdue pegboard; norms and studies of reliability and validity. The Journal of Applied Psychology 32, 234–247.
Wilder-Willis, K.E., Sax, K.W., Rosenberg, H.L., Fleck, D.E., Shear, P.K., Strakowski, S.M., 2001. Persistent attentional dysfunction in remitted bipolar disorder. Bipolar Disorders 3, 58–62.