those of their peers (Fuller et al., 2002; Reichenberg et al., 2005). Impairments in memory, working memory, and attention in patients with schizophrenia are well documented (Aleman et al., 1999; Silver et al., 2003; Hori et al., 2006), but the relationship of these cognitive deficits to the possible decline in IQ has not been established. Here we assessed cognitive functions including intellectual and wide-ranging memory functioning in patients with chronic schizophrenia in relation to age- and premorbid IQ-matched healthy controls.

2. Materials and methods

Eighty-two patients who met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia participated in this study. All patients were receiving antipsychotic drugs at the National Center of Neurology and Psychiatry (NCNP), Musashi Hospital and were clinically stable at the time of the neuropsychological tests. Eighty-two age- and premorbid IQ-matched healthy volunteers were recruited from hospital staff and their associates and also from the community. Healthy participants were interviewed by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Sheehan et al., 1998) to confirm the absence of any psychiatric illnesses. A portion of the subjects were from our previous sample (Hori et al., 2006). Written informed consent was obtained from all subjects prior to their inclusion in the study. The study was approved by the ethics committee of the NCNP.

Premorbid IQ was estimated with the Japanese Adult Reading Test (JART, Matsuoka et al., 2002; 2006), a Japanese version of the National Adult Reading Test (NART, Nelson and Wilson, 1991). This test is considered to provide an estimate of premorbid IQ in schizophrenia patients (Uetsuki et al., 2006), which is consistent with the original NART (Crawford et al., 1992; O'Carroll et al., 1992). In this test, subjects were required to read out 100 idioms of Han-Chinese characters (Japanese kanji characters). JART-estimated premorbid IQ was calculated for each subject according to previous reports (Matsuoka et al., 2002, 2006). The full version of the Wechsler Memory Scale-Revised (WMS-R, Wechsler, 1987; Sugishita, 2001) was administered to all participants. Outcome measures of the WMS-R were verbal memory, visual memory, delayed recall, auditory attention, visual attention, verbal working memory, and visual working memory. To precisely assess subjects' current intellectual function, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Wechsler, 1981; Shinagawa et al., 1990) was administered, yielding age-corrected indices of verbal, performance, and full-scale IQs.

Schizophrenic symptoms were assessed by an experienced research psychiatrist in 46 of the 82 patients using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Daily doses of antipsychotics and anticholinergic antiparkinsonian drugs were converted to chlorpromazine equivalents (CPZeq) and biperiden equivalents (BPDeq), respectively, using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999; Minzenberg et al., 2004).

Results are reported as mean ± standard deviation (S.D.). Demographic characteristics and cognitive test results were compared between groups. We used *t*-test or analysis of variance (ANOVA) to compare mean scores and the χ^2 tests to compare categorical variables. Analysis of covariance (ANCOVA) was used to compare means between groups, controlling for confounding variables. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

Male/female ratios of patients and controls were 48/34 and 25/57, respectively, indicating that the patient group had a greater representation of males ($\chi^2(1)=13.06$, $P<0.001$). The mean ages of the patients and controls were 44.3±13.8 and 44.2±14.9, respectively ($t=0.05$, $df=162$, $P=0.96$). The mean years of education of the patients and controls were 13.4±2.5 and 14.1±2.2, respectively ($t=1.88$, $df=162$, $P=0.06$). The JART-predicted premorbid IQ scores of patients and controls were 102.2±11.6 and 102.3±7.4, respectively ($t=0.46$, $df=137.8$, $P=0.96$). Of the 82 patients, 56 were outpatients and 26 were inpatients. The mean age of illness onset was 24.7±8.8. Illness duration was 19.6±13.7 years, demonstrating that our patients were in the chronic phase of schizophrenia. CPZeq and BPDeq were 781.7±710.1 and 2.2±2.0, respectively. PANSS positive, negative, and total scores were 13.9±6.7, 19.1±7.1, and 62.1±17.9, respectively.

Verbal, performance, and full-scale IQs of patients with schizophrenia and healthy controls are presented in Supplementary Table 1. ANOVA showed that these three IQ indices in patients were significantly lower than those in controls (all $P<0.001$). The VIQ/PIQ ratios of patients and controls were 1.08±0.18 and 0.95±0.11, respectively ($F=22.5$, $df=1,160$, $P<0.001$, by ANCOVA with gender as a covariate). Scores of 13 subscales of the WMS-R in patients and controls are also shown in Supplementary Table 1. Patients performed significantly

more poorly than controls on all these cognitive domains (all $P<0.001$), except for auditory attention ($P=0.15$). Fig. 1(a) shows mean scores of the patients and controls on JART-estimated IQ, WAIS-R full-scale IQ, and the main three memory indices of the WMS-R. Dips of current IQ and all memory domains in patients are apparent, although the two groups are matched for the JART-estimated premorbid IQ.

To control for the current IQ and gender effects on these test results, ANCOVA was used with full-scale IQ and gender as covariates. It revealed that patients performed significantly more poorly than controls on verbal memory, visual memory, delayed recall, visual attention, and verbal working memory, even after controlling for full-scale IQ and gender (Supplementary Table 1). To confirm these results, additional comparisons were made between patients whose current IQ scores were within normal limit (IQ-WNL patients, defined as WAIS-R full-scale IQ ≥ equal to or greater than 85; $n=46$) and total controls ($n=82$). Fig. 1(b) summarizes the results, showing that there was no difference in current IQ between IQ-WNL patients (mean IQ: 98.85±8.55) and controls (mean IQ: 101.95±11.30), while these patients still showed significantly lower scores on all three memory indices compared with controls. On the other hand, the JART-estimated premorbid IQ of IQ-WNL patients was significantly higher than that of controls.

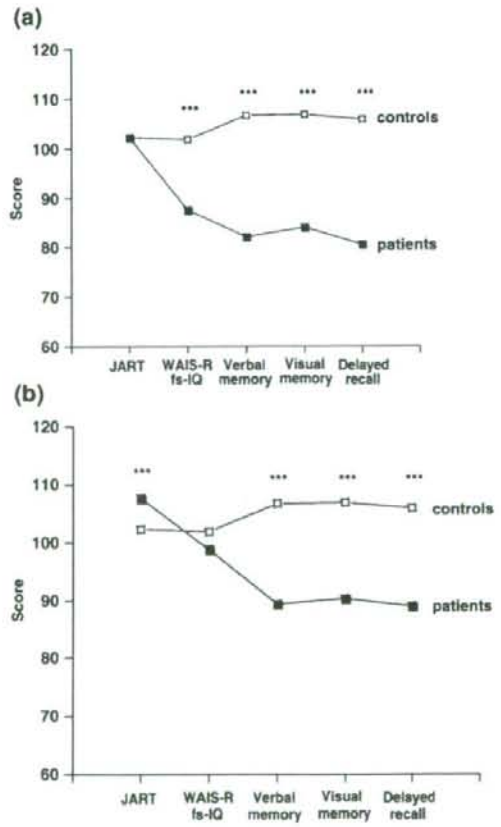

Fig. 1. Mean scores of patients and controls on JART IQ, WAIS-R full-scale IQ, Verbal memory, Visual memory, and Delayed recall indices (WMS-R). (a) total patients ($n=82$) vs. total controls ($n=82$) and (b) IQ-WNL patients (defined as WAIS-R full-scale IQ ≥ 85, $n=46$) vs. total controls ($n=82$). ***$P<0.001$.

4. Discussion

In the present study we examined intellectual and memory functions in patients with chronic schizophrenia relative to age- and premorbid IQ-matched healthy controls. Our results confirmed that patients with chronic schizophrenia have wide-ranging cognitive impairments, consistent with the literature on schizophrenia.

The relationship of the development of schizophrenia to declining IQ scores has been confounded by findings that premorbid intelligence itself is likely to be lower in persons who later develop schizophrenia than in their peers (Fuller et al., 2002; Reichenberg et al., 2005). To address this issue, we employed a premorbid IQ-matched case-control sample. Although the cross-sectional nature of the present study does not allow any definite conclusions to be drawn concerning the time when the IQ decline actually occurred (i.e., during the prodromal stage, immediately after illness onset, or during the chronic course of illness), the observed differences in current IQs between patients and controls provide evidence for marked IQ decline due to the development of schizophrenia. Means of estimated premorbid IQ and current full-scale IQ in patients were 102.20 and 87.68, respectively, suggesting an approximate 1 S.D. decline in IQ score related to the development of illness. On the other hand, the subgroup of patients whose current IQ was within normal limits (and thus similar to that of controls) showed significantly higher premorbid IQ as estimated by the JART than controls (Fig. 1(b)), which favors the view that even neuropsychologically normal patients with chronic schizophrenia have compromised cognitive functioning relative to their presumed premorbid level of intellectual function (Kremen et al., 2000). Furthermore, in the present study performance IQ of the patients was more severely impaired than verbal IQ, congruent with prior reports (Heinrichs and Zakzanis, 1998).

Pervasive memory impairment in patients with schizophrenia relative to premorbid IQ-matched controls was

found, and most deficits remained significant even after current IQ was controlled for, supporting that memory impairment is a core feature of schizophrenia (Saykin et al., 1991; Heinrichs and Zakzanis, 1998; Aleman et al., 1999). The marked impairment in verbal memory is consistent with numerous studies (e.g., Saykin et al., 1991; Heinrichs and Zakzanis, 1998). Although visual memory deficits in schizophrenia have attracted less attention from researchers than verbal memory, several studies have reported substantial impairment of visual memory (Saykin et al., 1991; Aleman et al., 1999), consistent with the present study. The pronounced impairment in delayed recall observed here is also in line with prior reports (Aleman et al., 1999; Dickinson et al., 2004). Deficits of verbal and spatial working memory in schizophrenia tapped by the Wechsler digit span backward and spatial span backward subtests, respectively, are fairly consistent findings (Conklin et al., 2000; Silver et al., 2003; Dickinson et al., 2004), which were replicated in the current study. Previous studies have reported that the performance on the forward digit span task of schizophrenia patients is significantly poorer than that of healthy people, indicating impaired attentional function in schizophrenia (Conklin et al., 2000; Silver et al., 2003). The findings of the present study, by contrast, suggest that auditory attention as measured by the forward digit span subtest is preserved in schizophrenia. The discrepant findings regarding auditory attention in the present study relative to previous ones might be due in part to the distinct matching status between patients and controls regarding education and premorbid IQ.

In conclusion, our results suggest that patients with chronic schizophrenia have substantially lower intellectual function relative to their presumed premorbid level and that their memory impairment is even more severe than the IQ decline. To definitively delineate the lifetime course of cognitive decline in schizophrenia, longitudinal studies that range from childhood to the chronic phase are needed.

Acknowledgements

This study was supported by Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health), a Grant from the Japan Foundation for Neuroscience and Mental Health, and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (H.K.). We thank Miho Tanaka, Sayaka Matsunaga, Tomoe Mori, Yuri Hiroi, Akifumi Yamashita and Mitsuo Kuno for helping with the neuropsychological tests and recruitment of participants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2007.11.002.

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Neuropsychopharmacology (2008) 33, 3237–3245

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Genetic Variations of Human Neuropsin Gene and Psychiatric Disorders: Polymorphism Screening and Possible Association with Bipolar Disorder and Cognitive Functions

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Human neuropsin (NP) (hNP) has been implicated in the progressive change of cognitive abilities during primate evolution. The hNP gene maps to chromosome 19q13, a region reportedly linked to schizophrenia and bipolar disorder. Therefore, hNP is a functional and positional candidate gene for association with schizophrenia, mood disorders, and cognitive ability. Polymorphism screening was performed for the entire hNP gene. The core promoter region was determined and whether or not transcriptional activity alters in an allele-dependent manner was examined by using the dual-luciferase system. Allelic and genotypic distributions of five single-nucleotide polymorphisms (SNPs) were compared between patients with schizophrenia ($n = 439$), major depression ($n = 409$), bipolar disorder ($n = 207$), and controls ($n = 727$). A possible association of the hNP genotype with memory index (assessed with Wechsler Memory Scale, revised; WMS-R) and intelligence quotient (IQ assessed with Wechsler Adult Intelligence Scale, revised; WAIS-R) was examined in healthy controls ($n = 166$). A total of 28 SNPs, including nine novel SNPs, were identified. No significant effects on transcriptional activity were observed for SNPs in the promoter region. A significant allelic association was found between several SNPs and bipolar disorder (for SNP23 at the 3′ regulatory region; odds ratio 1.48, 95% confidential interval 1.16–1.88, $P = 0.0015$). However, such an association was not detected for schizophrenia or depression. Significant differences were observed between SNP23 and attention/concentration sub-scale score of WMS-R ($P = 0.016$) and verbal IQ ($P < 0.001$). Genetic variation of the hNP gene may contribute to molecular mechanisms of bipolar disorder and some aspects of memory and intelligence.
Neuropsychopharmacology (2008) **33,** 3237–3245; doi:10.1038/npp.2008.29; published online 19 March 2008

Keywords: neuropsin; polymorphism screening; association study; bipolar disorder; memory; intelligence

INTRODUCTION

Neuropsin (NP, MIM: 605644), also called as kallikrein 8 (KLK8), is one of the secreted-type serine proteases, which was first cloned by our group in mice (Chen *et al*, 1995). NP mRNA is expressed specifically in the limbic system of mouse brain and is localized at the highest concentration in pyramidal neurons of the hippocampal CA1-3 sub-fields. Direct hippocampal stimulation and kindling induced by amygdaloid stimulation caused a significant bilateral change in NP mRNA level in the hippocampal pyramidal neurons. The activity-dependent changes and the specific localization indicate that NP is involved in hippocampal plasticity (Chen *et al*, 1995). Indeed, NP has a regulatory effect on Schaffer-collateral at the early phase of long-term potentiation (LTP) (Komai *et al*, 2000). Mice lacking NP were significantly impaired in the Morris water maze and Y maze, suggesting that NP has an important role in learning and memory (Tamura *et al*, 2006). The human NP (hNP) gene was cloned by Yoshida *et al* (1998), and then localized to chromosome 19q13.3–q13.4 (Gan *et al*, 2000; Harvey *et al*, 2000). It consists of six exons and the first exon is non-translational. Four alternative splicing variants have been identified (Mitsui *et al*, 1999; Magklara *et al*, 2001). The regular form is called type1, and type 2 contains a 135-bp

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Received 7 September 2007; revised 10 January 2008; accepted 7 February 2008

insertion of 5′ upstream region of exon 3 (Mitsui *et al*, 1999). Interestingly, type 2 is a hominoid-specific splicing form (Li *et al*, 2004) and is expressed as abundantly as the type 1 in human brain (Mitsui *et al*, 1999). These findings points to the possibility that type 2 hNP may contribute to progressive change of cognitive abilities during primate evolution. Moreover, dysfunctions in hNP may be involved in psychiatric diseases of cognitive abilities, including schizophrenia and mood disorders.

Family, twin, and adoption studies clearly suggest that genetic components play an important role in the pathogenesis of schizophrenia and mood disorders (reviewed by Shih *et al*, 2004). These psychiatric diseases demonstrate substantial cognitive deficits such as learning and memory (reviewed by Sharma and Antonova, 2003; Robinson *et al*, 2006; Green, 2006). A genome screen of linkage with bipolar disorder pedigrees provided evidence for susceptibility locus on chromosome 19q13 (Badenhop *et al*, 2002). Another genome scan in schizophrenia and bipolar pedigrees obtained an LOD ratio score of 1.5 at 19q13 in schizophrenic families (Macgregor *et al*, 2004). Therefore, the hNP gene is a good candidate gene for association with schizophrenia and mood disorders. Here we performed, for the first time, a polymorphism screening and association analysis of the hNP gene with schizophrenia, major depression, and bipolar disorder in a Japanese sample. A possible association of hNP with memory and intelligence in healthy subjects was also examined. In addition, we determined a core promoter region of the hNP gene and examined whether transcriptional activity varies in an allele-dependent manner.

MATERIALS AND METHODS

Subjects

Subjects for the association study were 439 patients with schizophrenia (240 males, mean age of 44.6 years (SD 14.0)), 409 patients with major depression (136 males, 53.3 years (15.9)), 207 patients with bipolar disorder (80 males, 50.2 years (14.7)), and 727 healthy controls (324 males, 43.5 years (16.4)). Among these, 104 patients with bipolar disorder and 108 controls were recruited around Shiga prefecture, approximately 350 km to the west of Tokyo, while the remaining 1570 subjects were recruited around Tokyo. Consensus diagnosis by at least two psychiatrists, one of whom was in charge of the patients, 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. Control subjects were healthy volunteers who had no current or past contact to psychiatric services. Among them 213 controls were screened by the Japanese version of the Mini-International Neuropsychiatric Interview (Sheehan *et al*, 1998; Otsubo *et al*, 2005) by a research psychiatrist, whereas the remaining controls were not screened by such a structured interview. Participants were excluded if they had prior medical histories of central nervous system disease or severe head injury, or if they met the criteria for substance abuse or dependence, or mental retardation. All subjects were biologically unrelated Japanese. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethics committees.

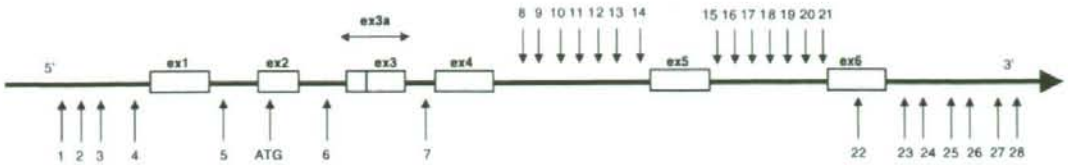
Neuropsychological Test Measures

Among controls, 166 (53 males, 37.6 years (12.4)) were subject to memory and intelligence tests to detect possible association with the hNP genotype. These individuals were all screened by the Mini-International Neuropsychiatric Interview with respect to their psychiatric history and confirmed that they had no current or past history of psychiatric illness. To assess memory and intelligence, Japanese full versions of the Wechsler Memory Scale-Revised (WMS-R) (Sugishita, 2001; Wechsler, 1987) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (WAIS-R, Shinagawa *et al*, 1990; Wechsler, 1981), respectively, were administered. Testing and scoring were performed by psychologists who were blind to genotypic data.

Polymorphism Screening and Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The genomic structure of hNP was determined from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and the University of California at Santa Cruz (UCSC) database (http://genome.ucsc.edu/cgi-bin/hgBlat). To screen for polymorphisms, we used direct sequencing with the Genome Lab-DTCS (Dye Terminator Cycle Sequencing) kit and CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). The entire 7428-bp genomic region containing all the exons, introns, the 1078-bp 5′ flanking region upstream to exon 1, and the 655-bp 3′ flanking region downstream to exon 6 were amplified from the genomic DNA of 24 randomly selected schizophrenic subjects. Sequences of 24 sets of primers for the polymorphism screening are listed in Supplementary Table S1.

The examined 7428-bp region seemed to constitute of single haplotype blocks (Supplementary Figure S1). We genotyped five single-nucleotide polymorphisms (SNPs) using TaqMan 5′-exonuclease allelic discrimination assay. They were A-807>G (ss73688625, SNP3), G-658>C (rs1722550, SNP4), IVS2-101T>A (rs1701947, SNP6), IVS3-10A>G (rs1701946, SNP7), and A5229>G (rs1612902, SNP23) (Figure 1). SNPs 3 and 4 were chosen from the 5′ regulatory region since they may have some effects on transcriptional activity, and SNPs 1, 2, 4, and 5 were in absolute linkage disequilibrium (LD) (ie, genotypes were completely the same) with each other. SNPs 6 and 7 were chosen because they were SNPs located close to the splicing sites of exon 3a (ie, an exon specific to type 2 hNP) and exon 4, respectively, and may have some effects on splicing. SNP23 was chosen from the 3′ region, since SNPs15, 16, 17, 19, 21, 23, 24, 25, 27, and 28 were in absolute LD with each other. TaqMan probes and Universal PCR master mix were obtained from Applied Biosystems (Foster City, CA). Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95°C for 10 min followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. After



1. rs2569456	g.G-1302>T	15. rs1654539	g. IVS5+106G>A
2. rs6509509	g.C-948>T	16. rs1722559	g. IVS5+184C>T
3. ss73688625	g.A-807>G	17. rs1701914	g. IVS5+187T>C
4. rs1722550	g.G-658>C	18. rs1722560	g. IVS5+673T>G
5. ss73688633	g.IVS1+25,26 ins/del CA	19. rs1722561	g. IVS5+764T>C
6. rs1701947	g.IVS2-236T>A (exon3)	20. rs1701915	g. IVS5-442A>G
7. rs1701946	g.IVS3-10A>G	21. rs1627239	g. IVS5-355G>T
8. ss73688628	g.IVS4+596G>A	22. ss73688626	g. G5047>A (Val 286 Ile)
9. rs7245714	g.IVS4+866C>T	23. rs1612902	g. A5229>G
10. ss73688629	g.IVS4+1034C>T	24. rs1654542	g. T5308>C
11. ss73688630	g.IVS4-951A>G	25. rs1654543	g. A5435>G
12. rs12610313	g.IVS4-624C>A	26. ss73688627	g.A5579>G
13. ss73688631	g.IVS4-585C>T	27. rs1897605	g. T5646>C
14. ss73688632	g.IVS4-306T>C	28. rs1897606	g. C5777>T

Figure 1 Genomic structure and identified polymorphisms in the human NP gene. A total of 28 SNPs, including one insertion/deletion (ins/del) polymorphism, were identified. The A of the translational start ATG is designated + 1. Nine SNPs were novel and have been registered in the dbSNP (ss-tagged numbers). ex, exon; ex3a, exon3 in hNP type2.

amplification, the allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems). Genotype data were read blind to the case–control status. Ambiguous genotype data were not included in the analysis.

Promoter Assay in Primary Cultured Neurons

Primary dissociated cultures were prepared from the brain cortex of postnatal 2-day-old rats (SLC, Shizuoka, Japan) as described previously (Numakawa *et al*, 2002). To generate plasmids for luciferase gene reporter assay, two differentially sized (964 and 128 bp) fragments of the 5′ flanking region of hNP were amplified by PCR with primers 5′-CGA CGCGTGCGTGTGCTGGGTTTGAA-3′ (forward) and 5′-GA AGATCTCTAGAGCCTGGGGAGCTTCT-3′ (reverse) for the 964-bp fragment, and 5′-CGACGCGTCCTCCTCTCCCTAGC CTCAG-3′ (forward) and 5′-GAAGATCTCTAGAGCCTGGG GAGCTTCT 3′ (reverse) for the 128-bp fragment. These primers were designed to incorporate *Mlu*I (forward) and *Bgl*II (reverse) restriction sites, and the PCR product was inserted into the multiple cloning site upstream of the luciferase coding region in the pGL3-Basic vector (Promega, Madison, WI). The inserted sequence was confirmed with the auto sequencer CEQ8000 in both directions using primers 5′-TCTCCATCAAAACAAAACGAA-3′and 5′-TTCC ATCTTCCAGCGGATA-3′.

Among the four SNPs (SNPs 1–4; see Figure 1) in the 5′ upstream region (ie, putative promoter region) of the hNP gene, the genotypes of SNPs 1, 2, and 4 were completely the same for all the 24 schizophrenic subjects, and we found a significant association of bipolar disorder with SNP4 but not SNP3 (see results). In addition, haplotypes containing the A allele (the major allele), but not the G-allele, of SNP3 showed some evidence for association with bipolar disorder in haplotype analysis (see Table 2). We therefore made two allele-specific promoter fragments (haplotypes consisting of SNPs 1-2-3-4 were G-C-A-G and T-T-A-C) of 964- and 128 bp upstream from the transcription initiation site, which were subject to the luciferase reporter gene assay. The plasmid constructs were transfected into cultured neurons at 5 days *in vitro*. Cells on 24-well plates were co-transfected with 3200 ng of pGL3-Basic firefly luciferase reporter vectors, which included allele-specific promoter fragments of 964 and 128 bp, and 100 ng of phRL-TK *Renilla* luciferase vector (Promega, Tokyo, Japan) as an internal control using Lipofectamine 2000 regent (Invitrogen, Tokyo, Japan). As negative control, an empty pGL3-Basic vector was simultaneously transfected in all the experiments. At 24 h after transfection, luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega) and a Lumat LB 9507 luminometer (Berthold, Bad Wildbad, Germany), as described previously (Tadokoro *et al*, 2004; Okada *et al*, 2006). Firefly and *Renilla* luciferase activities were quantified sequentially as relative light unit (RLU) by addition of their respective substrates according to the protocol of the supplier. The ratio of firefly RLU to *Renilla* RLU of each sample was automatically computed. The activity of each construct was expressed at the relative value compared with that of pGL3-Promoter (as a positive control), and these relative values were computed by *t*-test. Primary cultured cells were prepared six times and transfection was performed quadruplicate for each cell culture.

Statistical Analysis

Deviations of genotype distributions from Hardy–Weinberg equilibrium were assessed with χ^2-test for goodness of fit. Genotype and allele distributions of each SNP were compared between patients and controls using χ^2-test for independence. The association of the hNP genotype with

memory and intelligence was examined by multiple analysis of variance (MANOVA) controlling for possible confounders (age, sex, and education years). These tests were performed with the SPSS software version 11 (SPSS Japan, Tokyo, Japan). The LD (D') between polymorphisms was examined using the Haploview program (http://www.broad.mit.edu/mpg/haploview/) (Barrett *et al*, 2005) and haplotype-based association analyses were performed with COCAPHASE software version 2.4 (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/; Dudbridge *et al*, 2000). The expectation-maximization (EM) and 'droprare' options were used. Haplotypes with frequencies less than 3% were considered to be rare. We examined associations by permutation procedure (10 000 replications) to determine the empirical significance. All *P*-values reported are two-tailed. Statistical significance was considered when $P<0.05$.

RESULTS

Polymorphism Identification and Genotyping

The 7428-bp genomic region containing all the exons, introns, 5′ flanking, and 3′ flanking regions of hNP were screened for polymorphisms in 24 schizophrenic patients. A total of 28 SNPs, including one insertion/deletion (ins/del) polymorphism, were identified (Figure 1). Among them 19 SNPs had already been listed in the NCBI dbSNP database, whereas nine SNPs were novel. Four SNPs were located in 5′ upstream, one SNP in exon 6, six SNPs in 3′ downstream, and the remaining 17 polymorphisms in introns. There was only one SNP that resulted in an amino-acid change, SNP22 (G5047>A; Val286Ile: the number of the amino acid is according to NP_653088), which gave rise to a restriction site for *Acy*I and was located at an evolutionarily conserved (rodents through humans) residue. This non-synonymous polymorphism was found in only one schizophrenic patient. Additional genotyping was performed for 178 individuals with schizophrenia; however, there was no individual carrying the 286Ile allele, indicating that this amino-acid change is a rare mutation. The LD between SNPs is shown in Supplementary Figure S1, indicating that the entire genomic region consists of single haplotype block. Genotypes for SNPs 1, 2, 4, and 5, those for SNPs 8 and 9, those for SNPs 10 and 13, and those for SNPs15, 16, 17, 19, 21, 23, 24, 25, 27, and 28, respectively, were completely the same as each other for the 24 individuals.

Promoter Assay

We identified four SNPs in the 1078-bp 5′ upstream region of the hNP gene, and SNPs 1, 2, and 4 were found to be associated with bipolar disorder, memory, and intelligence quotient (IQ) (see below). Furthermore, to our knowledge, there is no information in the literature on the location of core promoter of the hNP gene. We therefore performed a promoter assay using the dual-luciferase system (Promega) in rat cultured cortical neurons and examined whether transcriptional activity alters in an allele-dependent manner. As shown in Figure 2a, pGL3-Basic vectors containing 128- and 964-bp fragments, which consisted of major alleles for the four SNPs, demonstrated substantially higher RLEs

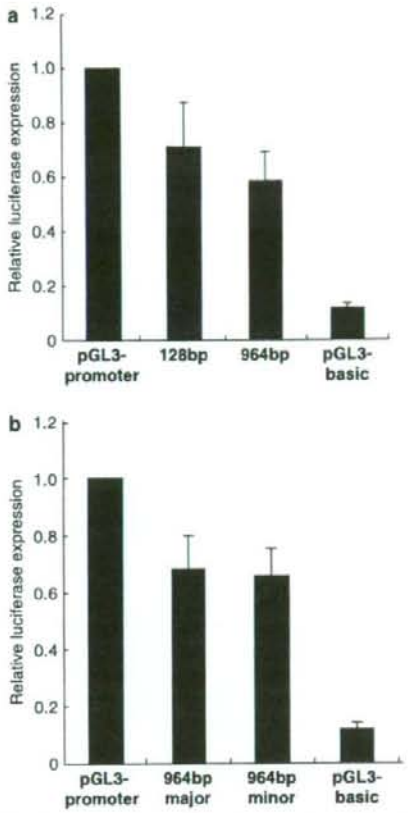


Figure 2 Promoter assay. (a) RLE for pGL3-Basic vector with insertion of 128 and 964 bp of the hNP 5′ flanking regions in comparison with pGL3-Basic vector, which dose not contain a promoter sequence. The RLE for pGL3-promoter vector containing SV40 promoter (positive control vector) was assigned a value of 1. Both 128- and 964-bp fragments showed substantially higher RLE compared with pGL3-basic vector without promoter sequence. (b) Comparison of RLE between the major (G-C-A-G for SNPs 1-2-3-4) and minor (T-T-A-C) alleles. No significant difference was found between the two alleles.

(relative luciferase expression) than that of pGL3-basic empty vector, suggesting that the core promoter region is located within the 128-bp fragment. We then cloned the 964-bp allele-specific promoter fragments (SNP1-2-3-4; major allele: G-C-A-G, minor allele: T-T-A-C) and compared RLEs between the two alleles (Figure 2b); however, we found no significant difference in the RLE between the two haplotype fragments. These results suggest that SNPs 1, 2, and 4 might not influence the transcriptional activity of the hNP gene.

Association with Psychiatric Diseases

We genotyped five SNPs (SNPs 3, 4, 6, 7, and 23) to examine possible association with schizophrenia, major depression, and bipolar disorder. Genotype and allele distributions in the diagnostic groups are shown in Table 1. Genotype

Table 1 Genotype and Allele Distributions of the five SNPs of the hNP Gene in Patients with Schizophrenia, those with Major Depression, those with Bipolar Disorder, and the Controls

SNP	Diagnosis	N	Genotype frequency (GF)			Allele frequency (AF)		Odds ratio (95% CI)	χ^2-Test vs controls		
									GF vs HW	GF (df = 2)	AF (df = 1)
SNP3			A/A	A/G	G/G	A	G				
	Controls	696	462 (0.66)	208 (0.30)	26 (0.04)	1132 (0.81)	260 (0.19)		0.67		
	SZ	421	277 (0.66)	126 (0.30)	18 (0.04)	680 (0.81)	162 (0.19)	1.06 (0.85–1.33)	0.45	$\chi^2 = 0.21$ $P = 0.90$	$\chi^2 = 0.11$ $P = 0.74$
	MD	382	276 (0.72)	90 (0.24)	16 (0.04)	642 (0.84)	122 (0.16)	1.18 (0.93–1.51)	0.02	$\chi^2 = 4.94$ $P = 0.08$	$\chi^2 = 2.48$ $P = 0.12$
	BD	202	139 (0.69)	56 (0.28)	7 (0.03)	334 (0.83)	70 (0.17)	1.09 (0.82–1.46)	0.65	$\chi^2 = 0.4$ $P = 0.81$	$\chi^2 = 0.36$ $P = 0.54$
SNP4			G/G	G/C	C/C	G	C				
	Controls	683	388 (0.57)	243 (0.36)	52 (0.08)	1019 (0.75)	347 (0.25)		0.11		
	SZ	406	234 (0.58)	150 (0.37)	22 (0.05)	618 (0.76)	194 (0.24)	1.1 (0.90–1.36)	0.75	$\chi^2 = 1.97$ $P = 0.37$	$\chi^2 = 0.62$ $P = 0.43$
	MD	371	219 (0.59)	126 (0.34)	26 (0.07)	564 (0.76)	178 (0.24)	1.1 (0.89–1.36)	0.19	$\chi^2 = 0.5$ $P = 0.78$	$\chi^2 = 0.58$ $P = 0.47$
	BD	198	91 (0.46)	90 (0.45)	17 (0.09)	272 (0.69)	124 (0.31)	1.33 (1.04–1.7)	0.43	$\chi^2 = 7.47$ $P = 0.023$	$\chi^2 = 5.35$ $P = 0.019$
SNP6			T/T	T/A	A/A	T	A				
	Controls	711	316 (0.44)	306 (0.43)	89 (0.13)	938 (0.66)	484 (0.34)		0.27		
	SZ	422	195 (0.46)	192 (0.45)	35 (0.08)	582 (0.69)	262 (0.31)	1.17 (0.97–1.41)	0.20	$\chi^2 = 4.86$ $P = 0.09$	$\chi^2 = 2.15$ $P = 0.14$
	MD	378	171 (0.45)	164 (0.43)	43 (0.11)	506 (0.67)	250 (0.33)	1.06 (0.88–1.29)	0.70	$\chi^2 = 0.31$ $P = 0.86$	$\chi^2 = 0.21$ $P = 0.65$
	BD	197	70 (0.36)	99 (0.50)	28 (0.14)	239 (0.61)	155 (0.39)	1.25 (0.99–1.58)	0.46	$\chi^2 = 5.03$ $P = 0.08$	$\chi^2 = 3.8$ $P = 0.051$
SNP7			A/A	A/G	G/G	A	G				
	Controls	718	325 (0.45)	314 (0.44)	79 (0.11)	964 (0.67)	472 (0.33)		0.81		
	SZ	433	209 (0.48)	190 (0.44)	34 (0.08)	608 (0.70)	258 (0.30)	1.16 (0.96–1.40)	0.31	$\chi^2 = 3.26$ $P = 0.20$	$\chi^2 = 2.36$ $P = 0.12$
	MD	387	182 (0.47)	163 (0.42)	42 (0.11)	527 (0.68)	247 (0.32)	1.05 (0.87–1.28)	0.55	$\chi^2 = 0.33$ $P = 0.85$	$\chi^2 = 0.21$ $P = 0.65$
	BD	203	72 (0.35)	103 (0.51)	28 (0.14)	247 (0.61)	159 (0.39)	1.31 (1.04–1.65)	0.36	$\chi^2 = 6.3$ $P = 0.042$	$\chi^2 = 5.56$ $P = 0.018$
SNP23			A/A	A/G	G/G	A	G				
	Controls	714	428 (0.60)	241 (0.34)	45 (0.06)	1097 (0.77)	331 (0.23)		0.16		
	SZ	421	267 (0.63)	135 (0.32)	19 (0.05)	669 (0.79)	173 (0.21)	1.17 (0.94–1.44)	0.71	$\chi^2 = 2.25$ $P = 0.32$	$\chi^2 = 2.13$ $P = 0.14$
	MD	388	240 (0.62)	127 (0.33)	21 (0.05)	607 (0.78)	169 (0.22)	1.08 (0.87–1.34)	0.44	$\chi^2 = 0.56$ $P = 0.75$	$\chi^2 = 0.56$ $P = 0.45$
	BD	204	98 (0.48)	86 (0.42)	20 (0.10)	282 (0.69)	126 (0.31)	1.48 (1.16–1.88)	0.86	$\chi^2 = 9.82$ $P = 0.0073$	$\chi^2 = 10.07$ $P = 0.0015$

Abbreviations: 95% CI, 95% confidence interval; BD, bipolar disorder; df, degrees of freedom; hNP, human neuropsin; HW, Hardy–Weinberg; MD, major depression; SNP, single-nucleotide polymorphism; SZ, schizophrenia.Significant *p*-values are gray colored.

distributions of these SNPs did not deviate significantly from Hardy–Weinberg equilibrium, except for SNP3 in patients with major depression ($P = 0.02$). There was no significant difference in genotype or allele distribution for any SNP between patients and controls for schizophrenia or major depression. However, there was a significant difference in genotype distributions between patients with bipolar disorder and controls for three SNPs, that is, SNPs 4, 7, and 23. Allele frequencies for these SNPs also differed significantly between the two groups. *P*-values, odds ratios, and their 95% confidence interval (CI) are shown in Table 1. Then we performed haplotype-based analysis with a two-marker sliding window method. We obtained no evidence of a significant association for schizophrenia or major depression (data not shown). With respect to bipolar disorder, we obtained significant individual *P*-values for all combinations of two markers; however, significant global *P*-value (0.0068) was obtained only when haplotype consisted of SNPs 7 and 23 (Table 2). Furthermore, overall global *P*-value ($P = 0.083$), considering all multiple testing for all the combinations of two-marker haplotypes, just failed to reach statistical significance. Thus, we did not obtain any stronger evidence for association in the haplotype-based analysis than in the single-marker analysis of SNP23 ($P = 0.0015$).

Association with Memory and IQ

Among the 166 controls whose memory scale and IQ were measured, SNP23 (A/G) was successfully genotyped in 163 individuals. Mean (SD) index scores of verbal memory, visual memory, general memory, attention and concentration, and delayed recall in the 163 controls were 110.9 (13.7), 109.9 (9.0), 112.2 (12.1), 103.9 (13.5), and 112.1

Table 2 Two-Marker Haplotype Analysis in Patients with Bipolar Disorder and Controls.

Markers					Haplotype frequency			P-value	
SNP3	SNP4	SNP6	SNP7	SNP23	BD	Controls	Individual	Global[a]	Overall global[a]
A	C				0.31	0.26	0.028	0.073	
	C	A			0.30	0.24	0.030	0.10	
		A	G		0.39	0.33	0.028	0.11	
			G	G	0.30	0.23	0.0068	0.014	0.083

Abbreviations: BD, bipolar disorder; SNP, single-nucleotide polymorphism.
[a]Global *P*-value for each combination of two markers and overall global significance for all combinations of two markers were calculated by permutation of 10 000 simulations.

(12.0), respectively. Mean (SD) full-scale IQ, verbal IQ, and performance IQ were 109.3 (11.6), 107.3 (12.9), and 110.3 (11.7), respectively. Since SNP23 showed the strongest association with bipolar disorder (G-allele was the risk allele) among the 5 SNPs examined, memory and IQ were compared between those who carried the G-allele (carrier, G/G or A/G, $N=64$) and those who did not (non-carrier, A/A, $N=99$) (Figure 3). Since the number of individuals with G/G genotype was very small ($N=8$), they were combined with those with the A/G genotype. With respect to sub-scales of WMS-R, the mean score of attention/concentration was significantly lower in carriers than in non-carriers ($P=0.016$); however, there were no significant differences between the two groups for the remaining sub-scales (verbal memory, visual memory, general memory, and delayed recall). With respect to WAIS-R, there was a significant difference in full-scale IQ ($P=0.018$) between the two groups. When verbal and performance IQ were examined separately, there was a highly significant difference in verbal IQ ($P<0.001$), but not in performance IQ, between the two groups. The mean verbal IQ (SD) for carriers and non-carriers was 103.4 (12.9) and 109.8 (12.3), respectively. As for the other SNPs, similar results are obtained (data not shown) because of the tight LD across the SNPs.

DISCUSSION

In the present study, we performed polymorphism screening and identified 28 SNPs, including nine novel SNPs, in the 7428-bp region of the whole hNP gene, including the 5′ and 3′ flanking regions. Then we performed promoter assay and determined a core promoter region of the hNP gene, although failing to find significant effects of SNPs on transcriptional activity. Association analysis using five SNPs as markers revealed significant difference in genotype and allele distributions for some of the SNPs between patients and controls for bipolar disorder, but not for schizophrenia or major depression. When a possible association of the SNPs with memory and IQ was examined in healthy control subjects, we found significant differences in attention/concentration sub-scale score of the WMS-R and verbal IQ between genotypes.

Among the 28 SNPs identified, there was only one SNP in the exons; SNP22 was a Val286Ile missense mutation in exon 6, which was detected in a patient with schizophrenia. Additional genotyping for 178 schizophrenic subjects did not find anyone carrying this variant, indicating that this is a rare mutation. Thus, whether this mutation is pathogenic or not is unclear. Since we examined only 24 individuals for polymorphism screening, we may have missed some rare mutations as the SNP22.

Our promoter assay in rat primary cultured neurons suggested that the core promoter is present in the 128-bp 5′ upstream region of the hNP gene. Since the RLE of pGL3-vector containing the 964-bp fragment was somewhat lower than that of pGL3-vector containing 128-bp fragment, a silencer-like region may be present between 128- and 964-bp positions upstream of the hNP gene. Then we examined whether transcriptional activity differs in an allele-dependent manner; however, we found no significant difference between alleles. These results suggest that SNPs 1, 2, and 4 might not influence the transcriptional activity of the hNP gene. According to the TFSEARCH database (http://mbs.cbrc.jp/research/db/TFSEARCHJ.html), these SNPs are not located on any of the binding sites of transcriptional factors, which is in line with our finding of no significant difference between the alleles.

In our association study with psychiatric diseases, we found, for the first time, significant differences in genotype and allele distributions between patients with bipolar disorder and controls. The best *P*-value was obtained for SNP23 in allele distribution ($P=0.0015$, odds ratio 1.48, 95% CI 1.16–1.88). This *P*-value remained significant even after correcting the critical *P*-value for Bonferroni's multiple testing (15 comparisons: 5 SNPs × 3 diseases). Haplotype-based analysis also yielded nominally significant results particularly when SNP23 was included in markers of analysis. These results suggest that SNP23 or other unknown SNPs in LD with SNP23 confers susceptibility to bipolar disorder. Since hNP is a part of a gene cluster (kallikreins), there remains a possibility that variations of some other kallikrein gene might be truly responsible to giving susceptibility to bipolar disorder. The results are in line with a previous study reporting a susceptibility locus for bipolar disorder on chromosome 19q13 (Badenhop *et al*, 2002). A possible limitation is that a portion of patients with bipolar disorder and controls were recruited in a geographically different area (ie, Shiga prefecture but not in Tokyo), which may have resulted in a population stratification; however, the minor allele frequency of SNP23 was very similar in controls from Shiga and those from Tokyo (0.233 in Shiga and 0.232 in Tokyo), suggesting that the effect of stratification is unlikely. Another limitation might be that

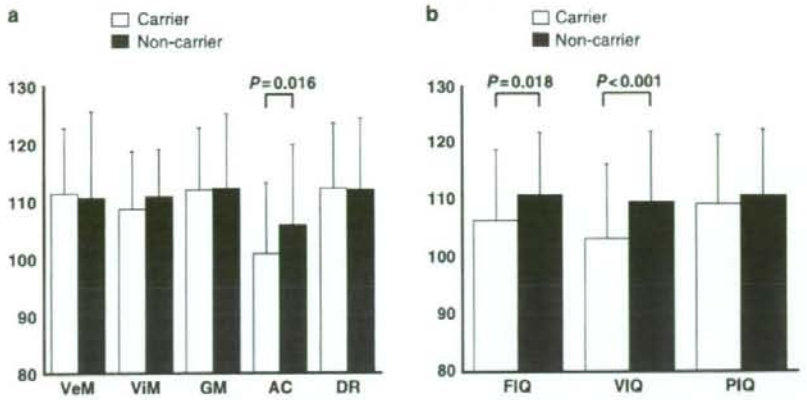


Figure 3 Relationship of memory and IQ with genotype of SNP23. Memory and IQ were compared between those who carried the G allele (carrier: G/G or A/G) of SNP23 and those who did not (non-carrier: A/A). (a) Memory and genotype. VeM, verbal memory; ViM, visual memory; GM, general memory; AC, attention and concentration; DR, delayed recall. (b) IQ and genotype. FIQ, full-scale IQ; VIQ, verbal IQ; PIQ, performance IQ.

we did not conduct structured interview for diagnosis of the patients. However, consensus diagnosis was made by at least two psychiatrists one of whom was in charge of the patients; thus, the possibility of misdiagnosis might be minimal. In addition, our sample size (207 bipolar disorder subjects and 727 controls) was not very large, and thus further investigations in other samples are required to draw any conclusion. With respect to schizophrenia or major depression, we did not obtain any evidence for association with hNP.

Interestingly, we found significant association of memory and IQ with the hNP gene in healthy subjects. Carrying the G-allele of SNP23, the risk allele for bipolar disorder, was associated with lower score in attention/concentration assessed with the WMS-R ($P=0.016$) and lower verbal IQ assessed with WAIS-R ($P<0.001$). The evidence for the former association (with attention/concentration) was weak and it would not be significant any more after correcting for multiple testing; however, the latter association (with verbal IQ) was highly significant and remained significant even when multiple testing was taken into consideration. Since bipolar disorder shows a wide range of cognitive deficits, including memory and IQ (Schretlen *et al*, 2007; Daban *et al*, 2006), the observed impact on intelligence may have some relevance to susceptibility to bipolar disorder. However, given that deficits in intelligence and memory are generally worse in schizophrenia than in bipolar disorder, alterations in hNP may have some effects specific to molecular mechanisms of bipolar disorder.

NP is a secretory serine protease that degrades cell adhesion molecule L1 (CAM-L1) (Matsumoto-Miyai *et al*, 2003) and is possibly involved in the synaptogenesis and maturation of orphan and small synapses (Nakamura *et al*, 2006). Furthermore, NP has been shown to be involved in activity-dependent synaptic plasticity, that is, LTP and kindling epileptogenesis (Komai *et al*, 2000; Okabe *et al*, 1996). As mentioned above, the type 2 splice variant has been shown to be expressed as abundant as the type 1 in human brain (Mitsui *et al*, 1999) and the hominoid-specific form (Li *et al*, 2004), which occurred through a human-specific T-to-A mutation (c.71–127T>A) during primate evolution (Lu *et al*, 2007). Taken together, NP is involved in synaptic plasticity via modulation of synaptic structure, and may play an important role in brain function of higher order such as learning, memory, and mental disorders. With respect to psychiatric diseases, indeed, altered expression levels of CAM-L1 mRNA and protein have been reported in postmortem brains of depressed patients (Laifenfeld *et al*, 2005). In line with this, chronic antidepressants increase expression levels of CAM-LI in rats (Sairanen *et al*, 2007; Laifenfeld *et al*, 2002). It would be intriguing to examine the expression levels of NP in postmortem brains of psychiatric patients.

We found that SNP23 is most associated with bipolar disorder among the examined SNPs. Haplotype-based analysis did not yield any stronger results, suggesting that SNP23 may be responsible for giving susceptibility to bipolar disorder. In addition, SNP23 showed strong impact on verbal IQ in healthy subjects. SNP23 is located 69 bp downstream to the 3′ end of exon 6 (the final exon). Thus SNP23 is on the 3′ regulatory region of the hNP gene. Growing evidence has shown that 3′ regulatory regions of human genes play an important role in regulating mRNA 3′ end formation, stability/degradation, nuclear export, subcellular localization, and translation, and are consequently rich in regulatory elements. Indeed, several diseases have been reported to be associated with variants in the 3′ regulatory region (Chen *et al*, 2006). Notably, the major allele (A) of SNP23 differs from the corresponding base (G) in monkeys or apes (ie, rhesus macaques or chimpanzees), according to the UCSC database, and thus it is not evolutionally conserved. It is interesting that carriers of the G allele were found to be poorer in memory and IQ subscales than individuals with A/A genotype in the present study. Although SNP23 is not located on obvious motifs or conserved sequence elements, it is also possible that this human-specific mutation may contribute to the higher memory and intelligence functions in humans. If our results

are replicated in other samples, it is important to elucidate the possible functional effects of SNP23 on regulation of hNP mRNA, which may contribute to understanding of the pathogenesis of bipolar disorder and brain function of higher order specific to human beings.

ACKNOWLEDGEMENTS

This study was supported by Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health; Research on Human Genome and Tailor-made medicine), the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug innovation), Grant-in-Aid for Scientific Research on Priority Areas of Applied Genomics from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank Ms Tomoko Shizuno for technical assistance.

DISCLOSURE/CONFLICT OF INTEREST

All authors declare that they have no conflict of interests to disclose.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)

Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 1944–1951

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Functional near-infrared spectroscopy reveals altered hemispheric laterality in relation to schizotypy during verbal fluency task

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ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 17 September 2008
Accepted 17 September 2008
Available online 1 October 2008

Keywords:
Laterality
Near-infrared spectroscopy
Prefrontal cortex
Schizotypal personality
Verbal fluency

ABSTRACT

Previous functional neuroimaging studies have demonstrated that patients with schizophrenia and those with schizotypal personality disorder (SPD) show reduced laterality, or relative right hemispheric dominance, during the performance of cognitive activation tasks; however, neuroimaging studies looking at non-clinical schizotypy have been few. We have recently reported that schizotypal traits at a non-clinical level are associated with right prefrontal dominance during a letter version of the verbal fluency task (VFT), but it is unknown whether such relationship between schizotypy and functional laterality would be observed across various cognitive tasks. Here we examined the relationships of schizotypal traits as measured by the Schizotypal Personality Questionnaire (SPQ) in healthy adults with hemispheric lateralization of prefrontal activation during letter and category VFTs, using near-infrared spectroscopy. Thirty-two participants were divided into high- ($n=16$) and low- ($n=16$) SPQ groups by the median split of the total SPQ score. The high-SPQ group, but not low-SPQ group, showed significantly right-greater-than-left asymmetry of prefrontal activation during letter VFT, whereas such pronounced hemispheric asymmetry in relation to schizotypy was not found during category VFT. These results indicate that non-clinical schizotypy is related to right prefrontal preference during the letter version of VFT in particular, suggesting that the association between schizotypal traits and functional laterality may vary depending on cognitive activation tasks.

1. Introduction

Schizotypy can be viewed as a dimensional trait ranging from non-clinical people to schizophrenic spectrum patients (Claridge, 1985) and the extent to which individuals in the general population exhibit schizotypal traits also varies on a continuum (Kendler et al., 1991). Although this dimensional model of schizotypy seems plausible, the notion of dimensionality is not entirely unequivocal such that some investigators have argued for the competing theory, namely the categorical approach to schizotypy. For example, Meehl (1962) proposed the concept called "schizotypy taxon", i.e., a bimodal distribution of two taxa, those with and without schizotypy. This Meehl's dichotomous approach to schizotypy has been supported and further explored by other workers (reviewed in Lenzenweger and Korfine, 1995). Still, however, a large number of schizophrenia/schizotypy researchers have substantiated the dimensional model of schizotypy (Claridge and Beech, 1995; Siever and Davis, 2004). This dimensional model of schizotypy assumes that non-clinical schizotypy, schizotypal personality disorder (SPD) and schizophrenia qualitatively resemble each other but quantitatively differ in the manner that the latter, particularly chronic schizophrenia, lies at the extreme end of the continuum. Among these, non-clinical schizotypy, which by definition is not confounded by potential consequences of psychiatric diagnoses such as antipsychotic treatment and institutionalization, offers a unique opportunity to disentangle and isolate the pathophysiology intrinsic to schizophrenia. In accord with this view, patients with SPD and those with schizophrenia have been demonstrated to share similar, but not completely identical, neurophysiological (Siever and Davis, 2004), neurocognitive (Mitropoulou et al., 2005; Siever and Davis, 2004) and neuroimaging (Dickey et al., 2002; Siever and Davis, 2004), impairments. Non-clinical schizotype has also been shown to display neurophysiological (Kiang and Kutas, 2005; O'Driscoll et al., 1998) and neurocognitive (Gooding et al., 2006; Lenzenweger and Korfine, 1994; Noguchi et al., in press; Park and McTigue, 1997) deficits which are similar to, albeit less severe than, those in schizophrenia

Abbreviations: ANCOVA, Analysis of covariance; CFT, Category version of verbal fluency task; deoxy-Hb, Deoxygenated hemoglobin; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, third edition, revised; IQ, Intelligence quotient; LFT, Letter version of verbal fluency task; LT, Left; NIRS, Near-infrared spectroscopy; oxy-Hb, Oxygenated hemoglobin; PFC, Prefrontal cortex; RT, Right; SPD, Schizotypal personality disorder; SPQ, Schizotypal personality questionnaire; VFT, Verbal fluency task.

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doi:10.1016/j.pnpbp.2008.09.019

patients and in patients with SPD; however, neuroimaging studies looking at non-clinical schizotypes have been scarce.

One of the repeatedly reported findings in functional neuroimaging studies of schizophrenia spectrum disorders including schizophrenia is the reduced hemispheric laterality, or relative right hemispheric dominance. Using a variety of cognitive activation tasks, this reduced laterality has been well documented in schizophrenia patients (Fallgatter and Strik, 2000; Razafimandimby et al., 2007; Sommer et al., 2003) as well as in patients with SPD (Buchsbaum et al., 1997; Folley and Park, 2005). Since the left hemisphere is normally specialized for processing verbal material (Hellige, 1993), it has been proposed that schizophrenia could be seen as an anomaly of language function due to a failure of hemispheric lateralization for language (Crow, 1997).

A verbal fluency task (VFT), in which subjects are asked to generate as many words as possible within a designated time period by either a phonological cue (i.e., letter VFT) or a semantic cue (i.e., category VFT), is a widely used neuropsychological test known to activate a distribute set of brain regions including the prefrontal cortex (PFC); however, letter and category VFTs are considered to demand somewhat different cognitive processing. The former requires unfamiliar lexical search based on phonology whereas the latter necessitates semantic search based on organization of semantic memory networks. In patients with schizophrenia, findings on overall extent of frontal activation during VFT are somewhat controversial such that some researchers have found attenuated frontal activity (Ehlis et al., 2007; Takizawa et al., 2008; Yurgelun-Todd et al., 1996), whereas others have observed overall preserved activation with altered distribution of activated regions (Artiges et al., 2000; Weiss et al., 2006). On the other hand, reduced hemispheric lateralization during this task has been consistently reported (Artiges et al., 2000; Sommer et al., 2001,2003; Weiss et al., 2006). Moreover, VFT is considered to tap not only the frontally-mediated executive function, which is found to be substantially impaired in schizophrenia, but also a certain aspect of creativity or divergent thinking (Nemoto et al., 2005; Weinstein and Graves, 2001), which may in turn relate to a relatively benign facet of schizotypal traits (Folley and Park, 2005; Weinstein and Graves, 2002). In this context, VFT would be suited to investigate the possible relationship between schizotypy and functional laterality.

Near-infrared spectroscopy (NIRS), a noninvasive optical neuroimaging technique which measures changes of the hemoglobin concentration in the human brain with a high time resolution, has been extensively used to assess brain function in healthy subjects as well as in various medical conditions including psychiatric diseases (Ehlis et al., 2007; Fallgatter and Strik, 2000; Folley and Park, 2005; Kubota et al., 2005; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008). The majority of these studies employed VFT as a cognitive activation task (Ehlis et al., 2007; Kubota et al., 2005; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008), partly because NIRS enables investigators to monitor brain activity during overt word generation. The most consistently reported finding in functional NIRS studies is task-related activation of PFC with an increase in oxygenated hemoglobin (oxy-Hb) and a small decrease in deoxygenated hemoglobin (deoxy-Hb).

Two previous NIRS studies administered both letter and category VFTs to patients with schizophrenia and healthy controls and found that patients exhibited more pronounced attenuation in prefrontal activation during letter VFT than during category VFT (Ehlis et al., 2007; Kubota et al., 2005); while on a behavioral level as indexed by the number of words produced, schizophrenia patients have been found to be disproportionately deficient in category vis-à-vis letter VFT (Bokat and Goldberg, 2003). As we have recently shown that schizotypal traits in healthy women are related to the relative right prefrontal dominance during performance of letter VFT (Hori et al., 2008a), it would be of interest to further examine whether the two VFTs are distinctively associated with the functional laterality in relation to schizotypy.

Moreover, another line of research has found non-clinical schizotypal traits to be associated with abnormal typicality of responses in category VFT (Kiang and Kutas, 2006), pointing to the alteration in semantic memory organization in non-clinical schizotypes. Given the putative relations of creativity to schizotypy and to reduced hemispheric laterality (Folley and Park, 2005; Weinstein and Graves, 2001,2002), this finding of an association between non-clinical schizotypy and abnormal typicality may account for a potential route whereby schizotypy is linked to reduced laterality. A further question may therefore be raised as to what the relationships between schizotypy, functional laterality, and typicality of responses in category VFT are.

The present study aimed 1) to investigate whether letter and category VFTs would induce similar or different patterns of functional laterality in relation to schizotypal traits as assessed with the Schizotypal Personality Questionnaire (SPQ; Raine, 1991) and 2) to examine the possible relationships between schizotypy, functional laterality and typicality of responses in category VFT, by using NIRS.

2. Methods

2.1. Subjects

Thirty-two healthy volunteers (age range, 22–59 years; male/female: 10/22) participated in this study. Of the volunteers, 14 were employees of a small company who were engaged in intellectual occupations. For these subjects, the whole experimental procedure was carried out in their office. The remaining 18 participants were recruited through flyers and advertisements and by word of mouth. They were administered the experimental procedure at our laboratory. Assessment of intelligence quotient (IQ) of the former participants was left out, while IQ of the latter was estimated with the Japanese Adult Reading Test (Hori et al., 2008b; Matsuoka et al., 2002), a Japanese version of the National Adult Reading Test (Nelson and Wilson, 1991), and those whose estimated-IQ score was less than 85 were not enrolled in the study. All participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by research psychiatrists (H.H. and Y.O.), and only those who demonstrated no history of psychiatric illness were enrolled in this study. Participants were excluded if they had a prior medical history of central nervous system disease or severe head injury. Participants who had one or more first-degree relatives with schizophrenia spectrum disorders or bipolar disorder were also excluded. All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). After the nature of the procedures had been fully explained, written informed consent was obtained from all subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Schizotypy measure

The SPQ (Raine, 1991) is a 74-item validated self-report questionnaire with a "yes/no" response format that incorporates DSM-III-R (American Psychiatric Association, 1987) criteria for a diagnosis of SPD. All items endorsed "yes" are scored 1 point. The questionnaire consists of 9 subscales, which have been found to load onto 3 factors: cognitive–perceptual (comprising the ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, and suspiciousness/paranoid ideation subscales), interpersonal (social anxiety, no close friends, constricted affect, and suspiciousness/paranoid ideation), and disorganized (eccentric/odd behavior and odd speech) factors (Raine et al., 1994).

The Japanese version of the SPQ translated by Fujiwara (1993) was used in the present study. The questionnaire had been administered to 258 Japanese college students in a validation study (Someya et al.,

1994), and the reliability and validity of this Japanese version of SPQ were demonstrated to be similar to those of the original version of Raine (1991), whereas this validation study showed a lower mean total SPQ score of around 10 (Someya et al., 1994). Our study sample was split by median of the total SPQ score (median = 11; range, 1–40) into high- (n = 16) and low- (n = 16) SPQ groups. The company employees/other participants ratios of the high- and low-SPQ groups were 6/10 and 8/8, respectively (χ^2 = 0.51, p = 0.48). All comparisons in this study, including comparisons of NIRS data, were made between the two groups unless otherwise specified.

2.3. Task procedure

First, participants sat on a chair near the NIRS machine with their eyes open. Then they were given practice with mimic tasks using a certain syllable (/a/) for letter VFT and a certain semantic category (fruit) for category VFT. Both VFTs consisted of a 30-s rest, a 30-s pretask, a 60-s task condition and a 60-s posttask. During the task condition, participants were required to verbally generate as many words as possible beginning with given syllables (/ki/, /nu/, /ra/, each for 20-s) for the letter VFT and belonging to given semantic categories (four-footed animals, vegetables, vehicles, each for 20-s) for the category VFT. These phonological and semantic cues were visually presented on a computer screen. During the pretask and posttask periods in both VFTs, participants were asked to repeatedly pronounce the syllables /a/,/i/, /u/, /e/, and /o/ at approximately the same speed as an example provided by an examiner during the practice time. The order of the two VFTs was counterbalanced among subjects. To prevent artifacts subjects were asked to avoid body movements, particularly head movements, during the NIRS measurements. The movements of subjects were monitored throughout the examination by an experimenter (H.H.). The phonological cues employed in letter VFT were different from those used in the majority of previous studies conducted in Japan including ours (e.g., Hori et al., 2008a; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008), while the semantic cues in category VFT were determined according to prior studies (Ehlis et al., 2007; Kiang and Kutas, 2006; Kubota et al., 2005). The verbal responses during the two VFTs were recorded using an integrated circuit recorder.

2.4. Analyses of behavioral data

For both letter and category VFTs, the number of correct words (no repetitions) generated during the task period was used as a measure of behavioral performance. In addition, the typicality of responses, a measure indicative of the extent to which the each individual's response set corresponds to the ranking in the category production norms, was calculated only for category VFT. We used the category production norms for Japanese compiled by Ogawa (1972) that lists the ranking of the frequencies of occurrence for words which belong to each of 52 categories including the four-footed animal, vegetable and vehicle (e.g., for vegetable, the ranking in the norms is the following order: *carrot*, *Chinese cabbage*, *radish*, *cabbage*, *tomato*, *cucumber*, *spinach*, and so on). Based on the study by Kiang and Kutas (2006), overall typicality of responses (typicality index t) for each of the three categories employed herein was calculated using the following equation:

$$t = \frac{\sum_{i=1}^{n} [f_i/i]}{n}$$

where n = the total number of responses for each category, i = the ordinal position of each response, and f = its position in the ranking in the norms of Ogawa (1972). For instance, as for vegetable, f ranged from 1 for *carrot* to 37 for *bamboo shoot*. If an individual's response was not found in the norms but actually belonged to the designated category, its position (i.e., f) was treated as one greater than the total number of items listed for the category in the norms-for example, as for vegetables, since the total number of items in the norms is 37, f of an individual's response unlisted in the norms (e.g., *butterbur*) was treated as 38. Thus, lower values of t represent higher typicality, with the minimum possible value of t being 1 where an individual's responses exactly match the ranking in the norms. In addition to the t of each category, the average of these three typicality indices ("average t") was calculated. When examining the possible association between typicality and hemispheric laterality, total subjects were split into two groups by the median value of "average t" and these two groups were compared for the laterality.

2.5. NIRS measurements

NIRS was performed using a 31-channel spectrometer (FOIRE-3000; Shimadzu Corporation, Japan) at three wavelengths of near-infrared light (780, 805 and 830 nm), with the distance between pairs of emission and detector probes set at 3.0 cm. Probes on a 4×5 probe holder were placed on the forehead of each subject, with the lowest probes being symmetrically positioned along the Fp1–Fp2 line according to the International 10–20 system of electroencephalogram electrode placement. The measurement channel was defined as each area between pairs of emission-detector probes (Fig. 1). While NIRS data include three measures of hemoglobin concentration, namely oxy-Hb, deoxy-Hb and total-Hb, we employed concentration change (mM×cm) in oxy-Hb for main statistical analyses because it is considered the most reliable indicator of changes in regional cerebral blood flow (Hoshi et al., 2001, 2003; Kono et al., 2007); however, data of deoxy-Hb were also provided where appropriate. The time resolution was set at 0.1-s. An average of 10 right prefrontal channels (i.e. channels #1, 5, 6, 10, 14, 15, 19, 23, 24 and 28) and that of the 10 left prefrontal channels (i.e. channels #4, 8, 9, 13, 17, 18, 22, 26, 27 and 31) were designated as two representative measures for NIRS data, namely "RTmean" and "LTmean", respectively; those 20 channels included Brodmann's areas 10 and 46 (Hori et al., 2008a; Kuwabara et al., 2006). Thus, outcomes for NIRS data used in the statistical analyses were limited to 8 summary measures, i.e., "LFT-RTmean-oxy", "LFT-RTmean-deoxy", "LFT-LTmean-oxy", "LFT-LTmean-deoxy", "CFT-RTmean-oxy", "CFT-RTmean-deoxy", "CFT-LTmean-oxy" and "CFT-LTmean-deoxy" (i.e., the first 4 were the measures for letter VFT and the last 4 for category VFT). In addition, taking into account that channels on the upper area of a head covered by hair were subject to artifact, the main statistical analyses were repeated with the RTmean and LTmean being limited to an average of 4 right prefrontal channels (i.e. channels #19, 23, 24 and 28) and that of the 4 left prefrontal channels (i.e. channels #22, 26, 27 and 31), respectively, which were on a lower forehead. This criterion of channel reduction was according to previous studies (Hori et al., 2008a; Kuwabara et al., 2006).

By referring to prior studies (Hori et al., 2008a; Kubota et al., 2005; Suto et al., 2004), a period of 10-s for the pretask condition (excluding first 20-s) and a period of 50-s for the task condition (excluding first 10-s) were sampled for the calculation of the average concentration change in oxy- and deoxy-Hb. Then, analyses were performed on the change in oxy- and deoxy-Hb (from pretask baseline) during the task condition.

2.6. Statistical analyses

Averages are reported as means ± S.D. for the variables which satisfied the assumptions for parametrical testing and as medians with ranges in parentheses for those which did not. Demographic characteristics, SPQ scores, and NIRS data (i.e. LFT-RTmean, LFT-LTmean, CFT-RTmean and CFT-LTmean, each for oxy-Hb and deoxy-Hb) were compared between the high- and low-SPQ groups. Kolmogorov–Smirnov test was used to

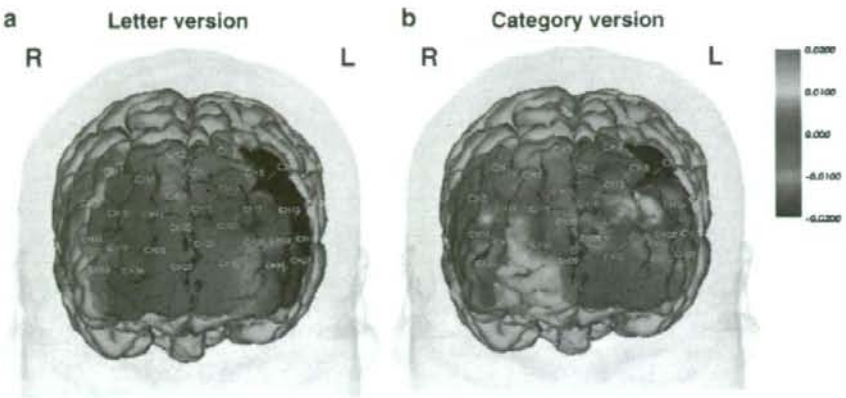

Fig. 1. Superimposed images on 3-D magnetic resonance imaging showing the differences between high- and low-SPQ groups (i.e., high-SPQ minus low-SPQ group) in oxy-Hb concentration changes during the task period (from pretask baseline) across 31 channels, by the letter (left side, a) and the category (right side, b) fluency tasks. The color bar indicates oxy-Hb concentration (mM × cm). Digits represent the numbers of the 31 channels and lines adjacent to the corresponding digits indicate the exact positions of the channels; channels # 1, 5, 6, 10, 14, 15, 19, 23, 24 and 28 were averaged into the RTmean, and # 4, 8, 9, 13, 17, 18, 22, 26, 27 and 31 into the LTmean.

examine the distribution of the data, and non-parametrical analyses, i.e. Mann–Whitney *U*-test to compare means and Spearman's ρ to examine correlations, were used where the data violated the assumptions for parametrical testing; otherwise, the *t*-test to compare means and Pearson's *r* to examine correlations were used. Specifically, age, estimated IQ, total SPQ score, numbers of words generated for both letter and category VFTs, and NIRS data for oxy-Hb, but not handedness, most of the typicality indices (*t*), most of the SPQ subscales, or NIRS data for deoxy-Hb, satisfied the assumptions for parametrical testing. Of the variables examined including demographic characteristics, estimated-IQ and VFT performance (i.e., the number of words generated), those variables that were significantly different between the two SPQ groups and significantly affected the NIRS data for oxy-Hb were considered as potential confounders. Since age has been shown to significantly influence the NIRS data (e.g., Herrmann et al., 2006; Kameyama et al., 2004), this variable was considered as a confounder regardless of the present data. Based on several previous NIRS studies that included the analysis of hemispheric laterality (Hori et al., 2008b; Kubota et al., 2005; Kuwabara et al., 2006), the NIRS data for oxy-Hb were first analyzed by the three-way repeated-measures analysis of covariance (ANCOVA), in which SPQ group was used as the between-subject factor, task condition and hemisphere as the within-subject factors, and the confounders as the covariates. When group-by-hemisphere interaction was significant in this analysis, then the hemispheric laterality was considered to be different between the two groups. Secondly, to examine the relationship between schizotypy and laterality *within* each VFT, the two-way repeated-measures ANCOVA on oxy-Hb data was performed, separately for letter and category VFTs, with SPQ group as the between-subject factor, hemisphere as the within-subject factor, and the confounders as the covariates. Similarly, to investigate the possible relationship between typicality of responses in category VFT and laterality, the two-way repeated-measures ANCOVA was performed, in which typicality group (i.e., high- and low-typicality groups) was used as the between-subject factor, hemisphere as the within-subject factor, and the confounders as the covariates. A similar ANCOVA was used to examine the potential gender difference in hemispheric laterality. To further examine the correlational relationship of schizotypy with laterality, multiple regression analyses were carried out with right-left difference measures (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy and CFT-RTmean-oxy minus CFT-LTmean-oxy) as dependent variables and the total SPQ score and confounders as independent variables. Statistical significance was set at two-tailed $p<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

3.1. Sample characteristics

Table 1 lists the demographic characteristics, estimated IQ, and SPQ scores of participants stratified by high- and low-SPQ groups. The two SPQ groups significantly differed only in SPQ scores. Compared to the low-SPQ group, the high-SPQ group had greater representation of females with statistical trend; however, gender was not considered a confounder because males and females did not significantly differ in

Table 1
Demographic characteristics and SPQ scores of the participants

Characteristic	Total subjects ($n=32$)	High SPQ ($n=16$)	Low SPQ ($n=16$)	Analyses (high vs. low)	
				Statistics	*p*
Gender, male/female	10/22	2/14	8/8	Fisher's exact	0.054
Age, years: mean ± S.D.	40.6 ± 10.8	41.1 ± 11.8	40.2 ± 10.1	$t=0.23$, $df=30$	0.82
Handedness, Edinburgh score: median (range)	95 (70–100)	100 (78–100)	90 (70–100)	Mann–Whitney $U=102.5$	0.30
Estimated IQ, mean ± S.D.	110.9 ± 10.6[a]	108.3 ± 10.5[b]	114.2 ± 10.4[c]	$t=1.19$, $df=16$	0.25
Total SPQ score, mean ± S.D.	12.9 ± 8.6	19.6 ± 7.3	6.3 ± 2.6	$t=6.85$, $df=30$	<0.001
Cognitive–perceptual factor, median (range)	3 (0–12)	5 (1–12)	2 (0–7)	Mann–Whitney $U=54.0$	0.005
Interpersonal factor, median (range)	4 (0–23)	7.5 (3–23)	3 (0–5)	Mann–Whitney $U=18.5$	<0.001
Disorganized factor, median (range)	3 (0–12)	6.5 (2–12)	1 (0–5)	Mann–Whitney $U=12.0$	<0.001

SPQ, schizotypal personality questionnaire; IQ, intelligence quotient.
[a] $n=18$.
[b] $n=10$.
[c] $n=8$.

Table 2
Behavioral data of the two verbal fluency tasks in the subjects stratified by the SPQ groups

Task performance	Total subjects (n=32)	High SPQ (n=16)	Low SPQ (n=16)	Analyses (high vs. low)	
				Statistics	p
Letter fluency task					
Number of words generated, mean±S.D.	16.3±3.2	15.9±2.8	16.7±3.5	t=0.72, df=30	0.48
Category fluency task					
Number of words generated, mean±S.D.	27.8±5.3	27.6±5.9	27.9±4.7	t=0.16, df=30	0.88
Typicality index t of four-footed animals, median (range)	2.4 (1.4–7.1)	2.4 (1.4–6.3)	2.8 (1.8–7.1)	Mann–Whitney U=84.5	0.16
Typicality index t of vegetables, median (range)	2.5 (1.7–4.3)	2.6 (1.7–4.3)	2.4 (1.8–3.8)	Mann–Whitney U=108.5	0.88
Typicality index t of vehicles, median (range)	2.8 (1.7–6.4)	2.7 (2.0–6.4)	2.8 (1.7–5.0)	Mann–Whitney U=117.0	0.91
Average t, median (range)	2.8 (1.9–4.2)	2.7 (1.9–4.1)	2.9 (2.1–4.2)	Mann–Whitney U=99.0	0.41

SPQ, schizotypal personality questionnaire.

any of the 4 NIRS oxy-Hb measures, i.e., LFT-RTmean-oxy, LFT-LTmean-oxy, CFT-RTmean-oxy and CFT-LTmean-oxy (by t-test: all $p>0.3$) or in hemispheric laterality as revealed by the repeated-measures ANCOVA on the oxy-Hb concentration changes, with gender as the between-subject factor, task and hemisphere as the within-subject factors, and age as the covariate [gender-by-hemisphere interaction: $F(1,29)=0.001$, $p=0.97$].

3.2. Behavioral data

The behavioral data on letter and category VFTs by SPQ groups are presented in Table 2. The behavioral performance as indicated by the numbers of words generated was not significantly different between the two SPQ groups, in either letter or category VFT. Both SPQ groups produced significantly larger numbers of words in category VFT than in letter VFT (by paired t-test: for high-SPQ group, $t=8.77$, $p<0.001$; for low-SPQ group, $t=10.67$, $p<0.001$). The two SPQ groups did not significantly differ in any of the three typicality indices (t) or in their average index. In addition, these behavioral measures including the numbers of words generated and typicality indices were not significantly correlated with any of the 4 oxy-Hb measures or their right-left difference measures (all $p>0.1$). Furthermore, when total subjects were split into two groups by the median of "average t", these two groups did not significantly differ in hemispheric laterality during category VFT, which was revealed by the repeated-measures ANCOVA on the oxy-Hb concentration changes, with the typicality group as the between-subject factor, hemisphere as the within-subject factor, and age as the covariate [i.e., group-by-hemisphere interaction: $F(1,29)=1.53$, $p=0.23$]. These

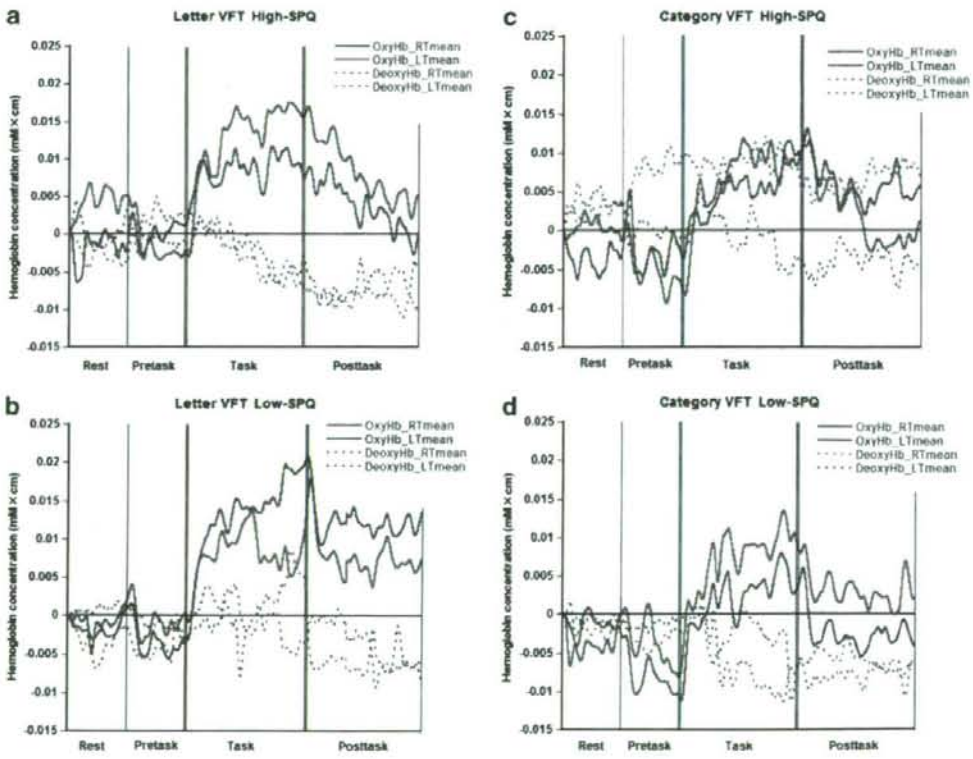

Fig. 2. Overall mean waveforms (oxy-Hb changes in solid lines and deoxy-Hb changes in dotted lines) of RTmean (red) and LTmean (blue) stratified by the SPQ group [high-SPQ group, upper half (a, c); low-SPQ group, lower half (b, d)] and by the fluency task [letter VFT, left side (a, b); category VFT, right side (c, d)].

indicated that typicality of responses did not impact upon schizotypal traits or NIRS data including hemispheric laterality.

3.3. NIRS data

3.3.1. oxy-Hb

Fig. 1 illustrates the differences between high- and low-SPQ groups (defined as high-SPQ minus low-SPQ group) in oxy-Hb concentration changes during the task period (from pretask baseline), by the letter (a) and the category (b) fluency tasks. Compared to the low-SPQ group, the high-SPQ group showed larger right and smaller left hemispheric activation in both VFTs; however, this differential hemispheric dominance pattern between the SPQ groups was more pervasive in letter VFT than in category VFT (Fig. 1). Fig. 2 shows overall mean waveforms of RTmean and LTmean, both for oxy- and deoxy-Hb, during the whole VFT period, by SPQ group and by fluency task. Clear oxy-Hb increases were consistently observed during the task period across SPQ groups, types of fluency tasks, and hemispheres. During the posttask period, both SPQ groups showed an oxy-Hb decrease from the beginning to the end of this period, in both tasks and for both hemispheres.

The initial three-way repeated-measures ANCOVA on the oxy-Hb concentration changes, with SPQ group as the between-subject factor, task condition and hemisphere as the within-subject factors, and age as the covariate, showed no significant main effect of group [$F(1,29)=0.27$, $p=0.61$], task condition [$F(1,29)=0.01$, $p=0.92$], hemisphere [$F(1,29)=1.00$, $p=0.33$], or age [$F(1,29)=0.25$, $p=0.62$]. However, the group-by-hemisphere interaction was significant [$F(1,29)=4.36$, $p=0.046$] and group-by-task-by-hemisphere interaction was at a trend-level significance [$F(1,29)=3.05$, $p=0.091$], while none of the other interactions between group, task, hemisphere and age were significant (all $p>0.3$). Similar results, including the significant group-by-hemisphere interaction [$F(1,29)=6.71$, $p=0.015$], were obtained when summary measures of NIRS data (i.e., RTmean and LTmean) were defined as an average of the lower 4 prefrontal channels. These indicated that the hemispheric laterality was significantly different between the two SPQ groups whereas neither SPQ group nor task condition nor age affected the overall component of NIRS data.

Subsequently, two-way repeated-measures ANCOVA on the oxy-Hb concentration changes was performed separately for letter and category VFTs, with SPQ group as the between-subject factor, hemisphere as the within-subject factor, and age as the covariate. It revealed that group-by-hemisphere interaction was significant for letter VFT [$F(1,29)=21.09$, $p<0.001$], but not so for category VFT [$F(1,29)=0.21$, $p=0.65$]. This analysis for letter VFT further revealed that estimated mean differences (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy), controlling for age, of the high- and low-SPQ groups were 0.0093 ($p=0.003$, 95%CI=0.0034 to 0.015; Fig. 2a) and −0.0095 ($p=0.003$, 95%CI=−0.015 to −0.0036; Fig. 2b), respectively; on the other hand, this analysis for category VFT showed that estimated mean differences (i.e., CFT-RTmean-oxy minus CFT-LTmean-oxy), controlling for age, of the high- and low-SPQ groups were 0.0066 ($p=0.30$, 95%CI=−0.0062 to 0.019; Fig. 2c) and 0.0025 ($p=0.69$, 95%CI=−0.010 to 0.015; Fig. 2d), respectively. This letter VFT-specific hemispheric asymmetry in relation to schizotypy was also found when summary measures of NIRS data were defined as an average of the lower 4 prefrontal channels.

Multiple regression analysis, with the right-left difference measure for letter VFT (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy) as a dependent variable and total SPQ score and age as the independent variables, revealed that SPQ score had a significant effect (β coefficient=0.47, $t=3.05$, $p=0.005$) while age did not (β coefficient=−0.26, $t=-1.69$, $p=0.101$). A similar analysis for category VFT again revealed that SPQ score had a significant effect (β coefficient=0.40, $t=2.35$, $p=0.026$) while age did not (β coefficient=0.004, $t=0.024$, $p=0.98$). These results indicated that the correlational relationship between schizotypy and right hemispheric bias was significant during both letter and category VFTs, which was slightly different from the results yielded by the categorical ANCOVA approach.

3.3.2. deoxy-Hb

As depicted in Fig. 2, deoxy-Hb concentration changes generally showed the opposite pattern to oxy-Hb concentration changes; however, the pattern of deoxy-Hb changes appeared to be more complicated and less consistent, across SPQ groups and tasks, than that of oxy-Hb changes (Fig. 2). Using Spearman's correlation, significant correlations of the total SPQ score were found with LFT-RTmean-deoxy ($\rho=-0.35$, $p=0.048$) and with LFT-RTmean-deoxy minus LFT-LTmean-deoxy ($\rho=-0.52$, $p=0.002$), whereas no significant correlation was found between the total SPQ score and LFT-LTmean-deoxy ($\rho=0.29$, $p=0.10$), CFT-RTmean-deoxy ($\rho=-0.046$, $p=0.80$), CFT-LTmean-deoxy ($\rho=0.16$, $p=0.39$), or CFT-RTmean-deoxy minus CFT-LTmean-deoxy ($\rho=-0.19$, $p=0.30$). These results generally corresponded to the results for oxy-Hb although the directions of the association were opposite.

4. Discussion

In the present study we examined the association between schizotypal traits in a non-clinical population and functional laterality during letter and category versions of VFT, using NIRS. We found that non-clinical schizotypy was significantly related to the right-greater-than-left asymmetry of prefrontal activation pattern during letter VFT. This relationship between schizotypy and right hemispheric bias was less clear during category VFT. There was no significant association of typicality of responses in category VFT with schizotypy or with hemispheric laterality.

The positive association of schizotypal traits with right prefrontal dominance in letter VFT as revealed by the repeated-measures ANCOVA was a replication of the finding from our previous study (Hori et al., 2008a), which had included only female volunteers from community, employed other phonological cues (/a/, /ka/ and /sa/), and used another NIRS machine (Hitachi ETG-100). This association was confirmed with the regression analysis, which may be more suited than the categorical analysis using ANCOVA for testing the study hypothesis of dimensional model of schizotypy. Moreover, in the present study the two SPQ groups were almost identical to each other in terms of age and behavioral performance on letter VFT (indexed by the number of words generated), both of which could be potential confounders in interpreting NIRS data. Therefore, the present finding, coupled with our previous one, has corroborated the relationship between schizotypy and right prefrontal dominance during letter VFT, further suggesting that schizotypy would be associated with the same *qualitative* alteration (i.e., right-greater-than-left asymmetry) as schizophrenia is. On the other hand, the relation of schizotypal traits to overall prefrontal activation during the performance of the letter VFT, as revealed by the main effect of SPQ group on the NIRS data in the repeated-measures ANCOVA, was not significant, indicating that schizotypy at a non-clinical level does not affect the overall extent of PFC activation. Taking into account that a number of NIRS studies have demonstrated that patients with schizophrenia display apparently reduced prefrontal activation during this task (Ehlis et al., 2007; Kubota et al., 2005; Takizawa et al., 2008; Watanabe and Kato, 2004), the present finding of preserved overall PFC activation in the high-SPQ group could be considered as supplementary evidence supporting the notion that schizotypal traits at a non-clinical level would be *quantitatively* different from schizophrenia.

With respect to the prefrontal activation during category VFT, like letter VFT, schizotypy was not significantly associated with overall extent of PFC activation. On the other hand, concerning the association between schizotypy and laterality during this task, the ANCOVA and regression analysis yielded somewhat different results; that is, only regression analysis revealed the significant relation of schizotypy to right hemispheric preference. It may be that schizotypy is associated

with right hemispheric dominance during category VFT, albeit to a lesser degree than during letter VFT. As for patients with schizophrenia, previous NIRS studies have yielded mixed results for the overall extent of prefrontal activation during category VFT (Ehlis et al., 2007; Kubota et al., 2005). In the study of Kubota et al. (2005) schizophrenia patients exhibited preserved or even increased PFC activation during this task compared to healthy controls while Ehlis et al. (2007) reported that patients showed reduced activation in restricted areas of PFC relative to controls. Nevertheless, these two precedent studies were compatible with each other regarding more marked prefrontal hypoactivation during letter vis-à-vis category VFT in patients. Therefore, the present results, combined with these previous findings of NIRS studies in schizophrenia, may suggest that letter VFT is more sensitive than category VFT in detecting functional alterations in PFC associated with schizotypy and/or schizophrenia spectrum, including hemispheric lateralization and overall prefrontal activation. Viewed from another angle, given the putative association between creativity and schizotypy, these differential associations of schizotypy with functional laterality between letter and category VFTs observed here might be attributed to the distinct degrees to which these two VFTs require divergent thinking (i.e., letter VFT requires it more than category VFT does), thereby the right prefrontal dominance in relation to schizotypy would be more clearly seen during letter VFT.

With regard to behavioral performance in the VFTs, the current findings were in line with those of numerous studies in that significantly more words were generated in category than letter VFT, demonstrating that semantic fluency would be easier to perform than letter fluency. The equivalent numbers of words generated between the two SPQ groups in both VFTs indicate that non-clinical schizotypy would not be related to apparent alteration in the level of performance on word fluency, which was also consistent with previous studies (Hori et al., 2008a; Kiang and Kutas, 2006). Regarding the other behavioral measure, namely the typicality of responses in category VFT, schizotypy was not associated with any of the typicality indices examined, which was not in agreement with the prior study (Kiang and Kutas, 2006). This discrepancy between the present and prior studies may result from the differences in semantic cues employed or from differential sample characteristics between studies. In the prior study four semantic cues (fruits, four-footed animals, articles of clothing, vehicles) were used and schizotypy was significantly related to abnormal typicality only for the fruit category; while in the present study we did not include the fruit category in the semantic cues. Concerning sample characteristics, the study of Kiang and Kutas (2006) included 60 English speakers, most of whom were undergraduates, whereas the present sample consisted of 32 adult Japanese speakers. Further investigations are therefore required to elucidate the association between schizotypy and typicality of responses.

Several limitations to the current study should be noted. First, NIRS measurement has some methodological disadvantages, as detailed elsewhere (Suto et al., 2004; Takizawa et al., 2008). Second, since each version of VFT was conducted only once, with only one epoch of the activation condition being included, quality of the NIRS data might have been lowered due to certain artifacts. The third limitation relates to the relatively low mean total SPQ score of 12.9 (S.D. 8.6) of the present sample, given that the mean total SPQ scores of non-clinical samples have been reported to be around 20 in the majority of prior studies, most of which were conducted in Western countries. This discrepancy is likely to have derived from ethnic differences (Western vs. Japanese/Asian). Actually, mean total SPQ scores in healthy Japanese populations are consistently shown to be approximately 10 (Hori et al., 2008a; Noguchi et al., in press; Someya et al., 1994; Wang et al., 2004), which means that the mean total SPQ score of the present sample was within the range of the mean total SPQ scores in healthy populations described in prior reports from Japan. Another plausible explanation for the differential SPQ scores between these samples might be the difference in mean age of participants between studies, i.e., college students in most of the precedent studies vs. adults in the present study. In support of this, a prior study, in which hundreds of Taiwanese adolescents and adults were enrolled, showed that mean SPQ scores of adolescents and adults were 20.6 and 12.9, respectively (Chen et al., 1997). Taken together, it is conceivable that the SPQ score is influenced substantially by ethnicity and to some extent by aging (i.e., lower scores in Asian and older populations) and that the present sample would be highly representative of non-clinical Asian adults; still, the present results would require confirmation by future studies employing non-clinical subjects with even higher schizotypal traits. On the other hand, from the viewpoint of the dimensional notion of schizotypy, the fact that even such degree of difference in schizotypal traits yielded the statistically significant result regarding hemispheric laterality may be regarded as the evidence in support of the continuum model of schizotypy. Fourth, we adopted the category production norms compiled more than 30 years ago (Ogawa, 1972) as there don't exist any newer versions to our knowledge, which may have erroneously caused high typicality indices. However, the problems of high typicality indices due to this issue may be subtle, if any, since typicality indices reported herein were generally lower than those described in the prior study (Kiang and Kutas, 2006). Fifth, as the sample size was not very large, possibilities of type II errors cannot be ruled out for those results which did not reach statistical significance. Finally, since the present study included only non-clinical subjects, further neuroimaging studies that investigate the association of schizotypal traits with hemispheric laterality among clinical populations (e.g., SPD patients with and without family history of schizophrenia) are needed to fully clarify the neurocognitive dimensionality of schizotypy.

5. Conclusion

Our results indicate that schizotypal traits at a non-clinical level are associated with right prefrontal dominance during the letter VFT rather than the category VFT, suggesting that the association between non-clinical schizotypy and functional laterality varies depending on cognitive activation tasks in the manner that this association might be better observed when the cognitive domain tapped by an activation task is more closely linked to the neurocognitive pathology involved in schizophrenia spectrum.

Acknowledgement

This study was supported by Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health) and the Research Grants for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare (H.K.). Sincere appreciation is extended to Mr. Masao Inoue, Yoshitaka Shiomi and Koji Morishima, employees of Shimadzu Corporation, for temporal rental of the NIRS machine and technical support. We would like to thank Ms. Miyako Hashikura, Yukiko Kinoshita and Mikako Kubo for helping with the performance of NIRS.

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